Abstract—Hypertension is linked to disturbed total-body sodium (Na\(^+\)) regulation; however, measuring Na\(^+\) disposition in the body is difficult. We implemented \(^{23}\)Na magnetic resonance spectroscopy (\(^{23}\)Na-MR) and imaging technique (\(^{23}\)Na-MRI) at 9.4T for animals and 3T for humans to quantify Na\(^+\) content in skeletal muscle and skin. We compared \(^{23}\)Na-MRI data with actual tissue Na\(^+\) content measured by chemical analysis in animal and human tissue. We then quantified tissue Na\(^+\) content in normal humans and in patients with primary aldosteronism. We found a 29% increase in muscle Na\(^+\) content in patients with aldosteronism compared with normal women and men. This tissue Na\(^+\) was mobilized after successful treatment without accompanying weight loss. We suggest that, after further refinements, this tool could facilitate understanding the relationships between Na\(^+\) accumulation and hypertension. Furthermore, with additional technical advances, a future clinical use may be possible. (Hypertension. 2012;59:167-172.)

Key Words: aldosterone ■ MRI ■ sodium ■ hypertension

Urine sodium (Na\(^+\)), and initially chloride (Cl\(^-\)), was used to estimate salt (NaCl) balance in the early 20th century.\(^1,2\) Timed urinary Na\(^+\) excretion has commonly been used to reflect dietary adherence in randomized, controlled clinical trials of salt reduction in hypertension.\(^3,4\) These tools have been helpful but are oftentimes unsatisfactory. Long-term balance studies, even in a clinical research center environment, have given results that are difficult to interpret.\(^5,6\) Clinicians have generally equated exchangeable extracellular Na\(^+\) as residing within the extracellular fluid volume, with perhaps some Na\(^+\) storage in bone. We have raised the possibility that other sites, such as skin and muscle, could serve to store Na\(^+\) nonosmotically by binding of the Na\(^+\) ion to proteoglycans.\(^7\) Careful review of the literature subsequently taught us that this idea is not new and was shown to be the case by Russian physiologists >30 years ago.\(^8\) In any event, conducting such measurements requires ashing of tissue and atomic absorption spectrometry, which are not suitable in the clinical setting. Needed is a noninvasive method, suitable for repetitive use in probands and patients, that could show Na\(^+\) storage and also the rate of immobilization with changes in dietary intake or therapy. The Na\(^+\) atom has an uneven atomic number and, therefore, is suitable for detection with magnetic resonance spectrometry and MRI. The sole stable isotope is \(^{23}\)Na. We present preliminary results that this approach promises utility in animals models, normal human subjects, and patients with hypertension.

Methods

Study Design

Local government authorities approved the animal studies according to American Physiological Society guidelines. The University of Erlangen-Nürnberg committee on human subjects approved the human studies (Re-No. 3948), and written informed consent was obtained from all of the participants.

\(^{23}\)Na-MRI Quantification of Tissue Na\(^+\) Content in Animals

Twenty Sprague-Dawley rats were randomly assigned to 4 different groups (n=5 per group). Groups 1 and 2 were untreated control groups, whereas groups 3 and 4 received deoxycorticosterone acetate (DOCA) pellets implanted subcutaneously under methohexital anesthesia. DOCA pellets were replaced after 3 weeks. Groups 1 and 3 received tap water to drink (low salt), whereas groups 2 and 4 received 1% saline (high salt) for 5 consecutive weeks. All of the animals were fed a Chow containing <0.1% NaCl by weight. Directly after euthanizing, both quadriceps muscles from each

\(^{23}\)Na Magnetic Resonance Imaging of Tissue Sodium

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animal were removed. Na$^+$ concentration of the left quadriceps was detected by chemical analysis, whereas Na$^+$ content of the right muscle was analyzed by $^{23}$Na-MR spectroscopy. Na$^+$ content of the right muscle was analyzed by $^{23}$Na-MR spectroscopy with a 9.4T vertical bore magnet (Bruker, Karlsruhe, Germany) equipped with a microimaging gradient system. Two grams of quadriceps tissue were placed in test tubes. A 50-µL glass capillary containing 150 mmol/L of Na$^+$ and 5 mmol/L of shift reagent (Na$_2$HTmDOTP, M-155, Macrocyclcs, Dallas, TX), centered within the muscle samples, was used as an external concentration standard. $^{23}$Na spectra were obtained as 128 time-averaged, free-induction decay response signals using a repetition time of 1.5 seconds. Analysis of the $^{23}$Na spectra was performed with the Bruker XWIN-NMR software package. Total muscle Na$^+$ content (in millimoles per liter) was calculated by integrating the area under its signal, in relation to the integrated area of the shifted Na$^+$ signal of the reference solution. Muscle water content was determined gravimetrically after desiccation of the tissue. Na$^+$ concentration of muscle was obtained by generating the ratio of total muscle Na$^+$ content (MR spectroscopy) and tissue water (desiccation).

**Ashing Procedure and Chemical Analysis**

Tissues were weighted (wet weight) and then desiccated at 80°C for 72 hours (dry weight). The difference between wet weight and dry weight was considered as tissue water content. After desiccation, the tissues were ashed at 200°C, 400°C, and 600°C for 24 hours at each temperature level and then dissolved in 5% HNO$_3$. ANa concentration of muscle was obtained by chemical analysis (atomic absorption spectrometry) after ashing. DOCA + high-salt increased muscle Na$^+$ content; MR spectrometry detected this increase in the same rats. Relationship between muscle Na$^+$ content measured noninvasively with MR spectrometry (abscissa) and chemical analysis (ordinate) in the same animal. †P<0.05 vs control; †*P<0.05 vs tap water.

**Human Studies**

Patients requiring extremity amputation (malignancy and diabetic complications) were asked preoperatively whether they would allow their extremities to be analyzed by $^{23}$Na-MRI and ashing. For the muscle measurements, the extremities were placed inside the scanner along with standards (below). For the skin $^{23}$Na-MRI measurements, technical difficulties were encountered with the amputation specimens that precluded reproducible determinations in this nonviable tissue. Therefore, the skin was removed from the specimens, and skin Na$^+$ content was measured separately in test tubes.

Normotensive men and women were recruited for noninvasive $^{23}$Na-MRI measurements, as were 5 patients with primary aldosteronism, 4 with adenomas, and 1 with bilateral hyperplasia. The primary aldosteronism patients were diagnosed based on plasma renin activity, aldosterone values, and imaging. Subjects of the control groups took no antihypertensive medication. Before $^{23}$Na-MRI measurements, blood and urine samples were obtained, and blood pressure was measured in a seated position with the Riva-Rocci method after 5 to 10 minutes of rest. Average blood pressure was calculated after 3 consecutive measurements. Tissue Na$^+$ content in patients with aldosteronism was determined before and ~4 weeks after adrenal adenoma surgery or ≥2 weeks after initiation of spironolactone treatment.

**$^{23}$Na-MRI Quantification of Tissue Na$^+$ Content in Humans**

Tissue Na$^+$ content was assessed noninvasively with a 3.0T clinical MR system (Magnetom Tim Trio, Siemens Healthcare, Erlangen, Germany). $^{23}$Na-MRI was performed with a gradient echo sequence (D-FLASH [Fast Low Angle SHot], total acquisition time: 13.7 minutes, echo time: 2.7 ms [ampatuated legs]/echo time: 2.07 ms [in vivo lower legs], repetition time: 100 ms, flip angle: 90°, 128 averages, resolution: 3x3x30 mm$^3$) and a frequency-adapted monoresonant transmit/receive birdcage knee coil (32.6 MHz, Stark-Contrast, Erlangen, Germany). $^1$H imaging was performed with the body coil of the scanner using the scout sequence of the system (123.2 MHz, 2D-FLASH, total acquisition time: 4 s, echo time: 4 ms, repetition time: 8.6 ms, flip angle: 20°, 2 averages, resolution: 0.375x0.375x7 mm$^3$). For ex vivo imaging, slices from amputated lower legs were fixed in a polystyrene holder including 50-mL test tubes (Falcon) with 10, 20, 30, 40, and 50 mmol/L of Na$^+$ (as NaCl) as calibration solution. For in vivo measurements, subjects placed their lower legs in the center of the $^{23}$Na knee coil. $^{23}$Na-MRI grayscale measurements of standard solutions with increasing Na$^+$ concentration (10, 20, 30, and 40 mmol/L) served to calibrate relative tissue Na$^+$. **Data Analysis**

Data are expressed as average ± SD. Data from chemical analysis, Na$^+$ spectroscopy, $^{23}$Na-MRI measurements, and characteristics of the study population were analyzed by multivariate analysis (general linear model) and paired t test. SPSS software was used for statistical analysis (version 17.0).
Results

Figure 1A shows results of $^{23}$Na-MR spectrometry in rats. MR spectrograms from 5 rat muscle samples are shown (right peak). Calibration signal derived from a 150-mmol/L Na$^+$ standard with shift reagent resulted in the shifted calibration signal (left peak) from which the Na$^+$ muscle content was calculated. Figure 1B shows the $^{23}$Na-MR spectrometric results in control and DOCA rats given tap water or 1% saline to drink. DOCA increased muscle Na$^+$ content, and 1% saline increased the concentration further ($P<0.05$). With direct ashing, Na$^+$ content in response to DOCA and 1% saline showed the same increases ($P<0.05$), although the absolute values were higher with ashing than with $^{23}$Na-MR spectrometry. Figure 1C shows the robust direct relationship between $^{23}$Na-MR spectrometry results and ashing ($R^2=0.70$). $^{23}$Na-MRI methodology was used for noninvasive quantification. Na$^+$ content by referencing signal intensities was compared with standard test tubes with increasing Na$^+$ concentrations in muscle and skin. Figure 2A shows the 5 standards containing 10, 20, 30, 40, and 50 mmol/L of Na$^+$ grouped around the MR image of a cross-section from an amputated lower leg. The image was used for the muscle concentration estimates. Figure 2B shows the image used for the skin Na$^+$ content estimates. The 4 standards are grouped around the skin specimen, which was placed within a test tube. Figures 2C and 2D show the robust relationships between MRI estimates of Na$^+$ content and results obtained by ashing in muscle ($R^2=0.87$) and skin ($R^2=0.82$). The Na$^+$ content was quite variable, as shown by the chemical analysis (skin Na$^+$ content: 77±16 mmol/kg of wet weight; muscle Na$^+$ content: 57±15 mmol/kg of wet weight; $n=21$), and exhibited a broad range. In contrast, plasma Na$^+$ concentrations in the same patients were stable within a very narrow range (138±4 mmol/L). Again, the $^{23}$Na-MRI measurements of human tissue Na$^+$ were lower than direct measurements; however, there was a close correlation between both methods. Furthermore, repetitive $^{23}$Na-MRI measurements of the same cross-section showed a high intramethod precision with an SD of 1.4% for both, muscle and skin tissue ($n=5$; data not shown), respectively.

The Table shows demographic data from control normal women and men and from the 5 patients with primary aldosteronism, before and after treatment. Figure 3A shows $^{23}$Na-MRI estimates of Na$^+$ content in muscle, whereas Figure 3B shows the same estimates for skin. In muscle, women and men had similar Na$^+$ content. Patients with primary aldosteronism had decidedly higher muscle values (19.6±2.7 mmol/L; $n=17$ [men control] versus 18.9±1.9 mmol/L; $n=13$ [women control] versus 26.1±3.1 mmol/L; $n=5$ [primary aldosteronism]; $P_{aldosterone}<0.05$). In the skin, men had higher Na$^+$ content values than women (24.1±5.6 versus 17.5±2.6 mmol/L; $P_{aldosterone}<0.05$). The 5 aldosteronism patients had skin values that were numerically higher than normal, albeit not statistically significant.

The patients with primary aldosteronism were studied a second time after treatment. Figure 4A shows muscle Na$^+$...
content determined by $^{23}$Na-MRI before and after treatment. The values decreased in every patient by approximately one third ($P<0.05$). Figure 4B shows little or no effect on body weight by these interventions. The patients had not been given any dietary instructions. Figure 4C shows a representative $^{23}$Na-MRI from a single patient. A dramatic effect is evident in the tissue, although the 4 control tubes appear similar in the 2 studies.

**Discussion**

Our important finding is that body Na$^+$ stores can be monitored noninvasively and longitudinally with $^{23}$Na-MRI. Our $^{23}$Na-MRI measurements suggest that the tissue Na$^+$ content in humans is much more variable than expected. Considerable amounts of Na$^+$ are stored in muscle, and particularly in skin, without apparent accompanying fluid retention or changes in serum Na$^+$ concentration in patients with primary aldosteronism. We are not the first to develop $^{23}$Na-MRI technology. Others have used $^{23}$Na-MRI to assess Na$^+$ content in infarcted myocardium.$^9,10$ The $^{23}$Na-MRI technique has also been used to quantify total Na$^+$ in normal and diseased human skeletal muscle.$^11,12$

The noninvasive quantitative diagnostic approach toward detecting disturbances in Na$^+$ disposition may provide a valuable new tool for patient-oriented research and perhaps patient care. First, measurements of tissue Na$^+$ content could facilitate assessment of the environmental factor, dietary NaCl, on cardiovascular disease in humans. Compared with 24-hour urine collections, direct detection of tissue Na$^+$ with $^{23}$Na-MRI may provide a superior end point for human studies examining potential benefits of diuretic drug treatment or dietary salt restriction. Second, changes in tissue Na$^+$ content in response to dietary interventions could further substantiate the clinical evidence and support population-wide salt reduction as a means to prevent cardiovascular disease.$^{13,14}$ Third, $^{23}$Na-MRI measurements of tissue Na$^+$ content in patients with hypertension might assist in the diagnosis of primary aldosteronism.$^{15}$ $^{23}$Na-MRI quantification could perhaps serve for follow-up of patients with treated aldosteronism. However, we are aware that any implications regarding aldosterone and specific Na$^+$ storage in tissues are premature. Finally, phenotyping Na$^+$ metabolism with $^{23}$Na-MRI quantification of tissue Na$^+$ storage may help us to better understand and monitor the treatment of Na$^+$ and water retention. The latter is particularly relevant for patients with hepatic, cardiac, and renal edema, as well as for dialysis patients.$^{15}$

We and others$^7,8$ have performed earlier studies involving Na$^+$ storage in proteoglycan-rich regions of the body and have presented data on how this storage could contribute to salt-sensitive hypertension. These notions involve local tissue osmolsor sensing, presumably through Na$^+$ content, via macrophages and regulation of lymphatic Na$^+$ storage capacities.$^{16}$ We showed that interrupting osmosensing resulted in faulty Na$^+$ storage and salt-sensitive hypertension. We speculate that $^{23}$Na-MRI may permit studying these issues noninvasively in patients.

We suggest that our findings could have relevance to testing the role of Na$^+$ in hypertension and in assessing long-term cardiovascular risk in populations. Current methods for doing so rely on 24-hour urine estimates of Na$^+$ intake or more commonly on questionnaires concerning dietary habits. Both of these techniques are fraught with problems. Although virtually all guideline committees strongly recommend a reduction in dietary

### Table. Demographic and Laboratory Data on Normotensive Men and Women, as Well as Patients With Primary Aldosteronism (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men (Normotensive)</th>
<th>Women (Normotensive)</th>
<th>Primary Aldosteronism Before</th>
<th>Primary Aldosteronism After</th>
</tr>
</thead>
<tbody>
<tr>
<td>No., n</td>
<td>17</td>
<td>13</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Age, y</td>
<td>62±7</td>
<td>60±7</td>
<td>52±13</td>
<td>52±13</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.1±9.9</td>
<td>66.2±7.5</td>
<td>82.2±8.5</td>
<td>81.6±9.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7±3.0</td>
<td>23.8±3.0</td>
<td>27.0±4.0</td>
<td>26.9±4.5</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>125.5±9.0</td>
<td>119.1±8.8</td>
<td>149±12</td>
<td>133±20</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.4±5.9</td>
<td>74.8±6.4*</td>
<td>84±10</td>
<td>83±8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>95.8±5.9</td>
<td>90.3±4.1*</td>
<td>108±9</td>
<td>101±11</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>31±20</td>
<td>41±23</td>
<td>330±134</td>
<td>43±23†</td>
</tr>
<tr>
<td>Aldosterone-renin ratio</td>
<td></td>
<td></td>
<td>171±50</td>
<td>5±4†</td>
</tr>
<tr>
<td>Cr, mg/dL</td>
<td>0.96±0.11</td>
<td>0.77±0.13*</td>
<td>1.17±0.67</td>
<td>1.39±0.87</td>
</tr>
<tr>
<td>Serum Na$^+$, mmol/L</td>
<td>140±1.6</td>
<td>140±1.2</td>
<td>142±2.3</td>
<td>139±2.7</td>
</tr>
<tr>
<td>Serum K$^+$, mmol/L</td>
<td>3.9±0.2</td>
<td>3.8±0.2</td>
<td>3.0±0.3</td>
<td>4.3±0.6†</td>
</tr>
<tr>
<td>Na$^+$ spot urine, mmol/g of Cr</td>
<td>141±66</td>
<td>157±91</td>
<td>85±107</td>
<td>99±38</td>
</tr>
<tr>
<td>K$^+$ spot urine, mmol/g of Cr</td>
<td>62±22</td>
<td>95±29*</td>
<td>47±15</td>
<td>59±22</td>
</tr>
<tr>
<td>Albumin spot urine, mg/g of Cr</td>
<td>4±2</td>
<td>18±38</td>
<td>48±57</td>
<td>15±21</td>
</tr>
</tbody>
</table>

Cr indicates creatinine; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

* $P_{\text{between}}<0.05$.
† $P_{\text{treatment}}<0.05$. 

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Na\(^+\) as a public health measure, a recent epidemiological analysis suggested that a lower Na\(^+\) intake was associated with a higher cardiovascular disease mortality.\(^{17}\) We suggest that \(^{23}\)Na-MRI determinations could provide greater precision to test such hypotheses.

There are numerous limitations that confront these studies. First, construction of \(^{23}\)Na coils was not trivial. The development also required certification by regulatory agencies. We had available a 9.4T ultrahigh field scanner for our animal work. We performed our human measurements with 3.0T. The resolution for muscle tissue appeared satisfactory; however, we are not yet satisfied with the skin estimates that are important to our central hypothesis about Na\(^+\) storage. We have preliminary data that a 7.0T scanner might help us in that regard (in preparation). Second, although we have no reason for any health-related concerns, regulatory agencies must be convinced before these measurements could be performed on a wide scale. The time required is \(\approx 30\) minutes per measurement, which is a hindrance for population studies. Third, we are aware that, before making further conclusions regarding total body Na\(^+\) regulation, incorporation of careful balance studies in such investigations would be necessary. We were encouraged to find that our aldosterone patients apparently lost Na\(^+\) without losing weight. However, this observation requires confirmation. Finally, in our patients, we measured Na\(^+\) in the lower extremity and not the entire body. We have no reason to believe that Na\(^+\) storage would be heterogeneous; however, we are not certain in that regard.

**Perspectives**

We showed that \(^{23}\)Na-MRI could, in principle, measure Na\(^+\) in muscle and skin noninvasively and repeatedly. With additional technical improvements, this technique could permit novel studies into Na\(^+\) balance in animals and humans.

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Disclosures
None.

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