Repeatability assessment of sodium ($^{23}$Na) MRI at 7.0 T in healthy human calf muscle and preliminary results on tissue sodium concentrations in subjects with Addison’s disease

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Abstract

Objectives: To determine the relaxation times of the sodium nucleus, and to investigate the repeatability of quantitative, in vivo TSC measurements using sodium magnetic resonance imaging ($^{23}$Na-MRI) in human skeletal muscle and explore the discriminatory value of the method by comparing TSCs between healthy subjects and patients with Addison's disease.

Materials and methods: In this prospective study, ten healthy subjects and five patients with Addison's disease were involved. $^{23}$Na-MRI data sets were acquired using a density-adapted, three-dimensional radial projection reconstruction pulse sequence (DA-3DPR) with a modification for the relaxation times measurements. Differences in TSC between muscle groups and between healthy participants were analysed using a nonparametric Friedman ANOVA test. An interclass correlation coefficient (ICC) was used as the repeatability index. Wilcoxon rank sum test was used for evaluation of differences in TSC between study participants.

Results: The mean $T_1$ in the gastrocnemius medialis (GM), the tibialis anterior (TA), and the soleus (S) was $25.9 \pm 2.0$ ms, $27.6 \pm 2.0$ ms, and $28.2 \pm 2.0$ ms, respectively. The mean short component of $T_2^*$, $T_2^*_{\text{short}}$ were GM: $3.6 \pm 0.5$ ms; TA: $3.2 \pm 0.5$ ms; and S: $3.0 \pm 1.0$ ms, and the mean long component of $T_2^*$, $T_2^*_{\text{long}}$ were GM: $12.9 \pm 0.9$ ms; TA: $12.8 \pm 0.7$ ms; and S: $12.9 \pm 2.0$ ms, respectively. In healthy volunteers, TSC values in the GM were $19.9 \pm 0.1$ mmol/L, $13.8 \pm 0.2$ mmol/L in TA, and $12.6 \pm 0.2$ mmol/L in S, and were significantly different ($p = 0.0005$). The ICCs for GM, TA and S were 0.784, 0.818, 0.807, respectively. In patients with Addison's disease, TSC in GC, TA, and S were $10.2 \pm 0.1$ mmol/L, $8.4 \pm 0.6$ mmol/L, and $7.2 \pm 0.1$ mmol/L, respectively.

Conclusions: TSC quantification in a healthy subject's calf at 7.0 T is reliable; the technique is able to distinguish sodium level differences between muscles and between healthy subjects and Addison's disease patients.

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Introduction
Over the last two decades, clinical research has demonstrated that biochemical information on disease progression and treatment efficacy may be obtained with a non-invasive metabolic imaging modality called sodium magnetic resonance imaging ($^{23}$Na-MRI) [1]. The method enables quantification of tissue sodium concentration (TSC), which has been demonstrated to be a useful imaging marker for the investigation of different pathological conditions in almost every part of the human body [2]. Currently, the number of preclinical and clinical $^{23}$Na-MRI studies at 7.0 T is increasing, mainly because of the substantial gain in signal-to-noise ratio (SNR) at ultra-high fields [3–7]. An optimal SNR substantially improves image quality and reduces a patient’s scan time, thus confirming TSC as a marker for disease diagnosis and monitoring. However, an accurate sodium concentration quantification at ultra-high fields is challenging and depends on many factors, such as relaxation times (T1 and T2*) of the tissue of interest. Unfortunately, the knowledge about sodium relaxation times at 7.0 T of the muscle is still limited [8].

Sodium ($^{23}$Na) is the primary cation in the human body and its concentration is connected to the physiological state of a cell and early changes in human homeostasis. Body hormonal imbalance or certain diseases, such as diabetes mellitus, hypertension, and acute heart failure, may cause an increase or a decrease of the sodium concentration in blood and manifest as conditions known as hypernatremia or hyponatremia [9, 10]. Changes of $^{23}$Na concentrations in the calf muscle, on the other hand, may be closely related to the molecular pathophysiology of the muscle tissue, which also may be common in rare diseases, such as muscle channelopathy [11] and dystrophy [5, 12–14]. Addison’s disease is another rare disorder characterized by impaired production of the steroid hormones cortisol and aldosterone, which may affect the sodium and potassium ion equilibrium (electrolyte imbalance) in the tissue [15]. Similar to the above-mentioned rare diseases, in subjects with Addison’s disease, sodium concentration level disturbances in a tissue may be potentially assessed using $^{23}$Na-MRI.

To be used as a reliable tool for different diagnostic and research investigations, this method requires an evaluation of repeatability and reproducibility, which should establish sodium tissue concentration assessments with sufficiently high accuracy.

The purpose of our study was to determine the relaxation properties of sodium nuclei in healthy calf muscle, and second, to perform reproducibility and repeatability tests of the sodium quantification measurements applicable in lower leg muscle investigations at 7.0 T. Further, we aimed to investigate whether regional differences in TSC of muscle are detectable and to explore the feasibility and discriminatory value of this method in patients with Addison’s disease.

Materials and methods
All measurements were performed between July 2020 and June 2021 and were approved by the local research ethics committee. Written, informed consent was obtained from all participants in the study. All methods were performed in accordance with the Declaration of Helsinki.

Hardware
MRI was performed on a whole-body 7.0 T MR scanner with a 70 mT/m gradient amplitude and a 200 mT/m/ms slew rate (Magnetom, Siemens Healthcare, Erlangen, Germany). Study participants were scanned in the supine, feet-first position. A dual-tuned sodium and proton knee coil (14 $^{23}$Na channels and a single $^1$H channel; Stark Contrast, Germany) was used.

Phantoms
A cylindrical phantom made of poly methyl methacrylate (PMMA) filling completely the knee coil was filled with a mixture of agarose (4%) (Agarose, Sigma-Aldrich, USA) and a saline solution of 61.6 mmol/L, and was used for field mapping and corrections.

A set of calibration phantoms was made of 4% agarose and saline solution with four different sodium concentrations: 15.4, 30.8, 46.2, and 61.6 mmol/L, doped with 1% nickel sulfate. Concentration phantoms were attached to the inner side of the coil and were used for calibration curve derivation. To match the T1 value of the phantom and muscle tissue, we added 2.9 g/L copper sulfate to the mixture.

Imaging protocol
All $^{23}$Na multi-channel data sets were acquired using a density-adapted, isotropic three-dimensional radial projection reconstruction pulse sequence (DA-3DPR) [16]. For T1 relaxation time measurements, a modified DA-3DPR with an inversion recovery (IR) pulse was included and five different inversion times (TI) were used. T2* mapping was performed employing the same DA-3DPR sequence with 20 different echo times (TE).
A proton ($^1$H) double echo steady state (DESS) sequence was acquired for partial volume effect (PVE) correction. Sodium imaging was performed using DA-3DPR followed by noise-only scans (no radio frequency [RF] power). More details on the sequences used can be found in Table 1.

**Study participants**

Ten healthy subjects were recruited. In four of ten participants, relaxation time measurements were performed. Repeatability tests were performed in all ten participants and planned in such a way that subjects underwent three lower leg muscle MRI examinations organized as two separate visits. Between the two scans (M1, M2), during one visit, the subject left the scanner for a five-minute-long break. Seven to eight days after the first visit, the same subject was scanned third time (M3). Volunteers were placed in a similar position according to anatomic landmarks from proton ($^1$H) morphological imaging for the next scan. In addition, five participants with a diagnosis of Addison’s disease were scanned. Demographic data on study participants are shown in Table 2.

Healthy subjects with no contraindications for MRI exam, such as cardiac implantable electronic devices, metallic intraocular foreign bodies, drug infusion pumps, implantable neurostimulation systems, cochlear/ear implant, drug infusion pumps, orthodontic braces, metallic particles, or items in or on the subject's body, piercing and/or tattoo, and tendency towards claustrophobia were included in this study. The additional inclusion criteria for patients were that they had biochemically confirmed primary adrenal insufficiency and were on a stable hormone replacement therapy for at least three months prior the study according to routine clinical practice guidelines.

**Image reconstruction and inhomogeneity correction**

Sodium images were reconstructed using an adaptive combining method (ADC) implemented in three dimensions to combine the complex images of the individual coils, with an interpolation factor of two and an overlap of eight pixels [17, 18]. SNR maps were calculated using a multiple replica approach [19]. The cylindrical homogeneous phantom which filled the knee coil and was used for $B_0$ and $B_1^+$ maps generation, and it was scanned with the same geometry as subjects. The local transmitter $B_1^+$ field was mapped with a phase-sensitive method that applies a pulse combination of nominal flip angles of $\alpha_x = 180°$ about the x-axis and $\alpha_y = 90°$ about the y-axis [20]. External $B_0$ field inhomogeneity correction was achieved by generating a data set with two different TEs; the first data set was acquired for off-resonance map calculations due to the resultant phase differences of two different echo times, and, in a second step, the data set was reconstructed multiple times with the corresponding off-resonance for each pixel to correct for $B_0$ inhomogeneity. Sequence parameters are presented

**Table 1** Imaging parameters of imaging sequences used in this study

| Measurement          | TR (ms) | TE (ms) | Matrix size | In-plane resolution (mm$^2$) | FA (%) | Slice thickness (mm) | Inversion Time (ms) | Number of projections | Pulse duration (ms) | Acquisition time (min:s) |
|----------------------|---------|---------|-------------|-------------------------------|--------|----------------------|---------------------|-----------------------|----------------------|-------------------------|
| $B_0$ mapping        | 139     | 0.60/1.60 | 128 × 128   | 4.0 × 4.0                     | 90     | 4.0                  | /                   | /                     | 1.0                  | 9:16                    |
| $B_1^+$ mapping      | 150     | 1.0     | 128 × 128   | 4.0 × 4.0                     | 90     | 4.0                  | /                   | /                     | 2.0                  | 10:00                   |
| T1 relaxation time   | 253–370 | 0.80    | 128 × 128   | 6.5 × 6.5                     | 90     | 6.5                 | 3.0–120             | 2600                  | 1.5                  | 64:03                   |
| T2$^*$ relaxation time | 65     | 0.4–5.00 | 128 × 128   | 5.8 × 5.8                     | 90     | 5.8                 | /                   | /                     | 0.68                 | 48:45                   |
| Proton imaging       | 9.32    | 2.55    | 320 × 320   | 0.5 × 0.5                     | 20     | 1.0                 | /                   | /                     | /                   | /                      |
| Sodium imaging       | 100     | 0.55    | 128 × 128   | 2.5 × 2.5                     | 90     | 2.5                 | /                   | 10,000                | 0.5                  | 16:40                   |

$B_0$ external field, $B_1^+$ local transmitter field, FA Flip angle, IT Inversion time, T1 longitudinal relaxation time, T2$^*$ Transverse "observed" relaxation time, TE echo time, TR repetition time

**Table 2** Demographic data on participants involved in the study

| Subject number | Age | Gender | Diagnosis           | BMI  | Laterality |
|----------------|-----|--------|---------------------|------|------------|
| 1              | 27  | Male   | Healthy             | 19.7 | Right      |
| 2              | 20  | Male   | Healthy             | 20.1 | Right      |
| 3              | 26  | Female | Healthy             | 21.9 | Right      |
| 4              | 28  | Female | Healthy             | 20.5 | Right      |
| 5              | 32  | Male   | Healthy             | 22.5 | Left       |
| 6              | 35  | Male   | Healthy             | 23.0 | Left       |
| 7              | 34  | Female | Healthy             | 20.8 | Left       |
| 8              | 25  | Female | Healthy             | 19.9 | Left       |
| 9              | 36  | Male   | Healthy             | 21.0 | Left       |
| 10             | 40  | Female | Healthy             | 22.0 | Right      |
| 11             | 33  | Male   | Addison's disease   | 22.3 | Right      |
| 12             | 30  | Female | Addison's disease   | 23.8 | Right      |
| 13             | 49  | Female | Addison's disease   | 22.9 | Right      |
| 14             | 54  | Female | Addison's disease   | 21.0 | Right      |
| 15             | 40  | Male   | Addison's disease   | 22.6 | Right      |
in Table 1. The correction factors derived from the cylindrical phantom were applied on in vivo data similar to previous work [21]. The feasibility of the applied method was evaluated on a set of agarose phantoms with different sodium concentrations comparing prepared sodium concentrations of calibration phantoms with the concentrations calculated after image corrections were applied [22] (supplementary material).

Relaxation time calculations

T₁ and T₂* relaxation time maps were derived by fitting the 23Na signal evolution to a mono-exponential function (T₁) or a bi-exponential function (fast T₂ and slow T₂*) components using IDL software (version 6.3, Research Systems, Boulder, CO, USA) with mpfit function [23]. For T₁ calculation five different TIs were used: 3, 15, 30, 60, and 120 ms and for T₂* 20 different TEs were taken: 0.4, 0.6, 1.0, 2.0, 5.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 24.0, 26.0, 29.0, 32.0, 36.0, 40.0, 45.0 and 50.0 ms. For T₂*, bi-exponential fitting procedure was performed on all MR data sets on a ROI-by-ROI basis ROI has been selected on the image acquired at the lowest TE (0.4 ms) and subsequently transferred to the rest of images. Mean sodium signal intensity has been stored for each ROI.

For bi-exponential fitting, a five-parametric function was used to fit the signal intensity:

\[
SI = C_{0s}e^{-\frac{TE}{T_{2s}}} + C_{0l}e^{-\frac{TE}{T_{2l}}} + \text{offset} \tag{1}
\]

where T₂s* corresponds to the short component of T₂*, T₂l* corresponds to the long component of T₂*, C₀s and C₀l are the component ratios expressed further as a percentage value of C₀s + C₀l: F₁ = 100* C₀s / (C₀s + C₀l) and F₁ = 100* C₀l / (C₀s + C₀l). Offset is again determined primarily by the noise in the image.

For T₁ mapping, the following formula has been used:

\[
SI = \text{abs}(M_m \left(1 - 2e^{-\frac{TI}{T_1}}\right)) + \text{offset} \tag{2}
\]

where M_m is the maximum magnetization at full recovery, TI is the set of inversion times, and the offset reflects the noise.

PVE corrections

Segmentation masks were created on morphological images acquired with DESS sequence with the parameters mentioned above and using a combination of automatic and manual segmentation (Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (v6.0, Analysis Group, FMRIB, Oxford, UK, the FMRIB's Automated Segmentation Tool (FAST) [24]) and ITK-SNAP, v3.8.0, Penn Image Computing and Science Laboratory (PIECS), University of Pennsylvania, Scientific Computing and Imaging Institute (SCI), University of Utah). Three different tissue compartments (delineated as regions of interests, ROIs) covering the certain muscle type (GM, S, TA) were used for PVE correction. The segmentation masks derived for three muscles and were co-registered with sodium images previously corrected for B₀ and B₁+ inhomogeneity. PVE calculation was performed according to a method proposed by Niesporek et al. [25], based on the geometric transfer matrix (GTM) method for PVE correction. The structural information was used in combination with a simulated point spread function (PSF) to calculate the corresponding region spread functions (RSF) via convolution. For the PSF simulation and relaxation correction of the 23Na data sets, we used values that we obtained in healthy subjects.

TSC assessments

Sodium data sets from every volunteer were reconstructed using an in-house-written MATLAB (R2020b, MathWorks Inc., Natick, MA) script. Values for 23Na-concentration in millimoles per liter (mmol/L) were calculated according to the formula (3) taking sodium signal intensities from selected ROIs and including them in the following formula:

\[
\left[Na_{in\,vivo}\right] = \frac{I_{corr\,in\,vivo}}{I_{corr\,phant}} \left[Na_{ref}\right] \tag{3}
\]

where [Na_{in\,vivo}] and [Na_{ref}] are sodium concentrations in muscle of interest and reference concentration of 25 mmol/l [13, 26]. I_{corr\,in\,vivo} is signal intensity derived from 23Na images previously corrected for PVE, and B₀ and B₁+ inhomogeneity and I_{corr\,phant} is corrected signal derived from Table 3

| Subject number | Muscle type      | T₁ [ms]        | T₂ long [ms]   | T₂ short [ms] |
|---------------|-----------------|----------------|----------------|---------------|
| 1             | gastrocnemius   | 28.5±0.5       | 14.0±0.5 (40)  | 3.4±0.2 (60)  |
| 2             | tibialis anterior| 29.4±1.0       | 12.5±0.3 (40)  | 3.1±0.2 (60)  |
| 3             | soleus          | 28.9±0.3       | 14.2±0.3 (40)  | 2.5±0.8 (60)  |
| 2             | gastrocnemius   | 24.1±2.0       | 12.6±0.2 (40)  | 2.5±0.3 (60)  |
| 3             | tibialis anterior| 25.4±0.3       | 13.7±0.8 (40)  | 3.0±0.4 (60)  |
| 3             | soleus          | 26.0±0.2       | 12.7±0.3 (55)  | 4.0±0.3 (45)  |
| 4             | gastrocnemius   | 24.4±3.0       | 11.9±0.2 (40)  | 2.7±0.1 (60)  |
| 3             | tibialis anterior| 26.4±0.4       | 12.2±0.2 (45)  | 2.6±0.3 (55)  |
| 3             | soleus          | 27.7±0.8       | 11.0±0.2 (40)  | 2.6±0.1 (60)  |
| 4             | gastrocnemius   | 26.4±0.9       | 13.0±0.2 (40)  | 5.6±0.2 (60)  |
| 3             | tibialis anterior| 29.0±2.0       | 12.7±0.5 (40)  | 3.9±0.2 (60)  |
| 4             | soleus          | 30.1±0.9       | 13.4±2.0 (45)  | 4.5±0.6 (55)  |
form a calibration curve (supplementary material, Table 1). Additionally, $I_{\text{corr}}^{\text{in vivo}}$ and $I_{\text{corr}}^{\text{phan}}$ were corrected for relaxation time differences between muscle tissue and phantoms.

**Statistical analysis**

Statistical analysis was done using IBM SPSS Statistics for Windows, version 22.0 (IBM, Armonk, NY). Metric data are described by means (mean ± SD) and normal distribution of measured values was tested using the Shapiro Wilks test. For categorical data, absolute frequencies and percentages are presented. Differences in TSC between the muscle regions were tested using a nonparametric Friedman ANOVA test. To investigate which pairs of muscles were significantly different in terms of TSC, we used a pairwise Wilcoxon signed-rank test with Bonferroni correction of $P$-values to compensate for multiple comparisons. Wilcoxon rank sum test was used for evaluation of differences in TSC between healthy volunteers and patients. A $P$ value less than 0.05 was considered statistically significant.

The repeatability of measurements was estimated with an intra-class correlation coefficient (ICC) in test–retest analysis using an two-way mixed, absolute agreement model [27]. Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 were indicative of poor, moderate, good, and excellent repeatability, respectively.
Agreements between three measurements (M1, M2, M3) were assessed using Bland–Altman plots.

**Results**

**Relaxation time measurements**
In the subgroup of four healthy volunteers (age: 25±4 years), the mean T1 and T2* relaxation times were measured, calculated, and summarized in Table 3. The mean T1 in GM, TA, and S were: 25.9±2.0 ms, 28.2±2.0 ms, and 27.6±2.0 ms, respectively. The mean short component of T2*, T2 short were GM: 3.6±2.0 ms; TA: 3.2±0.5 ms; and S: 3.0±1.0 ms, and the mean long component of T2*, T2 long were GM: 12.9±0.9 ms; TA: 12.8±0.7 ms; and S: 12.9±2.0 ms, respectively (Fig. 1).

**Repeatability tests**
Flowchart showing the image correction procedure is provided in Fig. 2. For the first measurement, the mean TSC values for GM, TA, and S were: 19.9±0.6 mmol/L, 14.0±0.5 mmol/L, and 12.7±0.7 mmol/L, respectively. For the second scan, we found TSC of 19.9±0.6 mmol/L for GM, 13.8±0.6 mmol/L for TA, and 12.7±0.5 mmol/L for S. The third exam for GM, TA, and S found TSC values of 19.8±0.5 mmol/L, 13.7±0.5 mmol/L and 12.4±0.8 mmol/L, respectively. ICC test–retest analysis showed a good repeatability for each group of muscles individually. The ICC for the gastrocnemius medialis was 0.784 (CI 95%, lower bound =0.468, upper bound =0.951), for the tibialis anterior, the ICC was 0.818 (CI 95%, lower bound =0.435, upper bound =0.948), and, for the soleus, the ICC was 0.807 (CI 95%, lower bound =0.435, upper bound =0.948). Bland–Altman diagrams of reproducibility tests show that majority of points are scattered between the limits of agreement, except for the comparison of M1 with M3 in tibialis anterior, M2 with M3 in tibialis anterior, and M2 with M3 soleus muscle. There is no apparent pattern in the Bland–Altman plot – the difference between measurements does not change as the average of measurements increases and indicates a good agreement between the three measurements performed in the same subject (Fig. 3).
TSC quantification of the calf muscles

Ten healthy subjects, five men and five women (age: 30 ± 6 years, body mass index (BMI), 21.1 ± 1.0 kg/m²) were involved in repeatability tests. The mean SNR of sodium images of the healthy human calf was 16 ± 3. In subjects with Addison's disease the mean SNR was 13 ± 5. Before corrections were applied, the mean TSC values for GM, TA, and S were 22.7 ± 0.7 mmol/L, 17.8 ± 1.0 mmol/L, and 16.4 ± 0.9 mmol/L and, respectively. After all corrections were applied, the mean TSC of three measurements were: gastrocnemius medialis: 19.9 ± 0.1 mmol/L; tibialis anterior: 13.8 ± 0.2 mmol/L; and soleus: 12.6 ± 0.2 mmol/L (Table 4).

Nonparametric Friedman test showed a significant difference between TSC values for all three types of muscles (P = 0.0005). There was a difference between gastrocnemius medialis and soleus muscle (P = 0.018) and between gastrocnemius medialis and tibialis anterior (P = 0.018). A difference between the soleus and tibialis was found as well (P = 0.018).

In participants with Addison's disease (age: 41 ± 10 years, BMI: 22.5 ± 1.0 kg/m²) the mean TSC measured in the gastrocnemius medialis was 10.2 ± 1.0 mmol/L. In the tibialis anterior and soleus, measured TSC values were 8.4 ± 0.6 mmol/L and 7.2 ± 0.1 mmol/L, respectively (Table 4).
A significant difference in TSC values was found between healthy volunteers and patients for all three muscles: \( P = 0.0007, P = 0.0027 \) and \( P = 0.0007 \) for GM, TA and S, respectively (Fig. 4).

**Discussion**

Tissue sodium concentration can be considered a useful parameter for biochemical investigations of muscular pathologies. At present, TSC values reported in the literature indicate substantial differences between individual reports of this parameter assessed in human calf muscle. This is most probably due to the lack of established MRI quantification protocols available and different quantification methodologies applied. The utilization of ultra-high systems such as 7 T enabled investigation of many different relevant clinical questions in relatively short scanning times, sodium imaging with substantially higher SNR and higher resolution compared to 3 T. This is particularly important for investigation of i.e., endocrinological disorders by quantifying sodium concentrations in skin and muscle as organs where disease can be non-invasively diagnosed and monitored.

Our study was performed at 7.0 T MRI and involved ten healthy subjects and included relaxation time measurements, TSC quantification, and repeatability tests. Relaxation time measurements demonstrated no substantial differences in \( T_1, T_{2\text{long}}, \) and \( T_{2\text{short}} \) of three different muscle types. Sodium concentration quantification in healthy participants showed significant differences in TSCs in three different parts of the calf muscle (gastrocnemius medialis, tibialis anterior, and soleus) \( (P = 0.0005) \). In healthy participants, repeatability tests showed good repeatability of sodium concentration quantification in three different regions of the calf muscle \( (ICC = 0.784–0.948) \). The Bland–Altman plot was uniformly scattered between the limits of agreement, therefore, we could conclude that there was a good agreement between every two pairs of measurements.

In addition, we examined and quantified TSCs in the calf muscle of five subjects with a diagnosis of Addison’s disease and found substantially lower TSC values compared to those in healthy participants. This finding is in line with our expectation and the choice of AGS as a model for \( ^{23}\text{Na}^+ \) imbalances. Glucocorticoids and mineralocorticoids play a major role in the regulation of sodium balance. Interestingly, these differences could be observed despite adequate hormone substitution therapy in patients with adrenal insufficiency. Whether \( ^{23}\text{Na-MRI} \)

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**Table 4** Tissue sodium concentration (TSC) determined after external \((B_0)\) and local \((B_1)\) field inhomogeneity corrections and partial volume effect \((PVE)\) in healthy subjects in different muscle regions and during three imaging sessions (measurements), M1, M2, and M3

| Subject number | Muscle type | TSC [mmol/L]—M1 GM | TSC [mmol/L]—M2 GM | TSC [mmol/L]—M3 GM |
|----------------|-------------|---------------------|---------------------|---------------------|
| 1              | TA          | 20.2±0.8            | 21.0±0.8            | 20.0±0.3            |
| 2              | TA          | 20.5±0.9            | 20.8±0.4            | 20.5±1.0            |
| 3              | TA          | 19.9±0.2            | 19.8±0.6            | 19.6±0.8            |
| 4              | TA          | 20.8±0.5            | 20.5±1.0            | 20.0±0.8            |
| 5              | TA          | 20.0±0.8            | 19.6±0.8            | 19.4±1.0            |
| 6              | TA          | 19.5±1.0            | 20.0±2.0            | 20.8±0.9            |
| 7              | TA          | 19.9±0.9            | 19.6±1.0            | 19.7±0.6            |
| 8              | TA          | 19.5±0.8            | 19.5±0.9            | 19.4±2.0            |
| 9              | TA          | 19.0±0.2            | 19.5±1.0            | 19.0±0.5            |
| 10             | TA          | 19.8±0.5            | 19.0±0.7            | 19.8±0.9            |
| 11a            | TA          | 9.6±0.6             | 7.7±0.7             | 7.2±0.8             |
| 12a            | TA          | 10.7±0.8            | 8.0±0.8             | 7.1±1.0             |
| 13a            | TA          | 11.1±0.8            | 9.0±1.0             | 7.1±2.0             |
| 14a            | TA          | 10.8±0.9            | 8.8±2.0             | 7.4±0.5             |
| 15a            | TA          | 8.8±1.0             | 8.7±0.9             | 7.3±0.4             |

GM Gastrocnemius, M1, M2, M3 the first session, the second, and the third imaging session, respectively, S Soleus, TA Tibialis anterior, TSC Tissue sodium concentration

a Addison’s patient
might play a useful role in the clinical evaluation of adequate treatment should be evaluated in future trials.

The relaxation $T_1$ times of $^{23}$Na of the healthy human lower leg muscle at 3.0 T reported in the literature were about 30 ms [26, 28]. Measurements of relaxation times of the $^{23}$Na nucleus of lower leg muscle measured at 7.0 T showed values that were slightly different from those obtained on 3.0 T scanners. The short and long components of $T_2^*$ at 3.0 T were reported to be 0.7 ± 0.1 ms and 13.2 ± 0.2 ms, respectively [29]. In our study, $T_{2\text{short}}^*$ component was higher, most probably due to limitation of imaging sequence and minimum echo time of 0.4 ms that could be used.

Moreover, because of different physiological and pathological contributions (edema, volume regulation, etc.) to the sodium concentration, the $^{23}$Na-MRI measurements in patients may be potentially confounded by $T_1$ and $T_2^*$ relaxation effects, and consequently, the values can actually deviate from the values measured in healthy tissue [14].

Quantitative TSC values of the lower leg muscle determined noninvasively with $^{23}$Na-MRI in healthy male subjects are in good agreement with the results obtained invasively by chemical analysis in patients who require extremity amputation [30]. TSC values reported in the literature are in the range of 13.0 to 25.0 mmol/L [31]. Gast et al. recently reported TSC after $B_0$ corrections for the gastrocnemius medialis, assessed at two sites, as 17.0 ± 2.2 mmol/L for site ≠ 1 and 16.2 ± 1.3 mmol/L for site ≠ 2. For the tibialis anterior, TSC at two sites were 14.3 ± 1.3 mmol/L and 13.7 ± 1.2 mmol/L, respectively. For the soleus, TSC values were 18.1 ± 1.4 mmol/L and 17.5 ± 1.0 mmol/L, respectively [3], confirming significant differences in TSC between GM, TA, and S muscle ($p < 0.0005$). Recently, Ahlulail et al. reported substantially higher TSC values after using $T_2^*$ corrections and found the highest concentrations of sodium in the soleus muscle (34.1 ± 2.2 mmol/L) compared to GM (25.0 ± 2.8 mmol/L) and TA (25.3 ± 2.1 mmol/L) [29]. This discrepancy in the highest sodium content found in different muscles reported in the literature need to be further investigated using standardized protocols and applied on a larger group of subjects.

The difference in TSC values before and after corrections were from 12 to 23% depending on muscle type. Since that we have good homogeneity of external and
local fields, we assume that this discrepancy mainly originates from PVE correction. The largest contribution of PV correction (34 ± 1) % to the total correction factor was found by Lott et al. who has measured TSC in human heart. In calf muscle tissue of the healthy subjects, Lott et al. found TSC of 20 ± 3 mM at 7.0 T which is in a good agreement with our results [28]. The influence of PVE correction on TSC differences between muscle types should be further investigated.

Repeatability investigations of 23Na-MRI in lower leg [4, 29, 31] or in upper leg muscle [32] have been already reported in the literature; however, measurement conditions for different research centers may be different (i.e. external magnetic field strength, coils, etc.), and repeatability may vary from one to another investigation site. Therefore, we performed our own tests to demonstrate the repeatability of quantification methodology applied in this study. Our study has some limitations. First, our population group was too small to have an appropriate analysis of sex- and age-related differences in TSC. Sodium relaxation times in Addison’s and healthy subjects muscle tissue may be different as well. Moreover, it is known that 23Na concentration in the calf of women can be hormonally dependent, which makes standardization of quantification more challenging [33]. Second, our healthy participants were slightly younger than the Addison’s disease patients. Future studies should include age matching and obese subjects who may have lower TSC values in calf muscle, most probably due to a fat infiltration. In this study, BMIs between the participants were comparable, but it would be interesting to investigate possible TSC differences between obese adults and patients Addison’s disease, as TSC differences between them may have different origin [34].

The post-processing procedure is time-consuming, and further development of the image reconstruction methods is necessary. However, we performed evaluations in the post-processing phase, so they didn’t influence the participant’s scanning time. In favor of reduced workload for the clinicians, we didn’t perform segmentation of calibration tubes and blood vessels, which may influence PVE corrections due to the high Na\(^+\) concentration in blood and phantoms. Similarly, the muscle segmentation we performed on three slices instead of the whole calf volume could influence TSC quantification accuracy and should be the subject of future research.

A short acquisition time is extremely important if measurements must be performed on patients to reduce potential motion artefacts and keep discomfort to a minimum. To investigate further connection of sodium relaxation time and different pathological conditions, the application of sodium magnetic resonance fingerprinting (23Na-MRF) would enable much faster data acquisition for relaxation time measurements [35, 36]. Relaxometry, in this context, may have a crucial role in disease detection and efficacy evaluation of different treatment approaches.

**Conclusion**

Sodium concentrations in the tissue may be reliably assessed using 23Na-MRI at 7.0 T. The technique is quantitative and sensitive enough to distinguish differences in sodium levels between healthy and pathological tissue. Early metabolic changes in the tissue may be evaluated with 23Na-MRI. This might provide information on the effectiveness of glucocorticoid and mineralocorticoid replacement therapy in adrenal insufficiency and could be a useful parameter with which to evaluate ongoing treatment.

**Abbreviations**

AC: Adaptive combine; AUC: Area under the curve; \(B_0\): Static magnetic field; \(B_1\): Combined transmit and receive radiofrequency field; \(B_1^+\): Transmit radiofrequency field; GC: Gastrocnemius medialis; ROI: Region-of-interest; S: Soleus; TA: Tibialis anterior; TSC: Tissue sodium concentration; 23Na-MRI: Sodium magnetic resonance imaging.

**Supplementary Information**

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**Authors’ contributions**

O.Z. was conception and design of the work, data the acquisition, analysis, interpretation of data and manuscript drafting. H.B. worked on data acquisition and analysis. J.V. performed data acquisition and analysis. P.S. was involved in data interpretation and manuscript revision. P.W. performed data interpretation. M.K. did data interpretation and manuscript revision. M.K. made data interpretation and manuscript revision. V.J. did conception and design of the work, data acquisition, analysis and interpretation of data. The author(s) read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated and/or analysed during the current study are not publicly available due their size and complexity but are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Institutional Review Board of Medical University of Vienna approval was obtained.
Written informed consent was obtained from all subjects (patients) in this study. All methods were performed in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
Not applicable.

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