Determining repeatability and precision of sodium concentration in calf muscle of healthy human test subjects with ^{23}Na-MRI

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Master's project

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Abstract

Background: Increased sodium storage has been found chronic kidney disease patients. Sodium concentration is therefore proposed to be assessed as clinical biomarker in kidney failure intervention studies. To implement this, sodium concentration needs to be quantified. The goal of this study was to inspect the feasibility and repeatability of quantifying sodium concentration in muscle and skin of healthy subjects with ^{23}Na-MRI at 7T.

Methods: 10 healthy participants were scanned on a 7T MRI using a double-tuned transmit/receive RF coil with two sodium and two proton channels. 4 ROIs were analysed: m. gastrocnemius, m. soleus, m. tibialis anterior and the skin. Quantification was done using reference phantoms with known sodium concentrations. Intersession and intrasession repeatability were assessed in 15 min 3D FFE and 5 min 3D FFE sodium scans with Bland-Altman plots and the coefficient of repeatability. Accuracy was evaluated by comparison to literature. An exercise test was performed as a proof of concept to test physiological differences in a short time frame using multiple 5 min scans.

Results: 15 min scan found mean sodium concentrations of 16.7±3.8 mmol/L (gastrocnemius), 15.6±4.1 mmol/L (soleus), 10.8±2.4 mmol/L (tibialis anterior) and 15.5±2.8 (skin). Coefficient of repeatability for gastrocnemius, soleus, tibialis anterior and skin in 15 min scans are respectively: 51.8%, 48.9%, 25.3% and 49.7%. 5 min scan found mean sodium concentrations of 17.3±3.0 mmol/L (gastrocnemius), 13.6±3.7 mmol/L (soleus), 7.4±2.1 mmol/L (tibialis anterior) and 15.2±2.1 mmol/L (skin). Coefficient of repeatability for gastrocnemius, soleus, tibialis anterior and skin in 5 min scans are respectively: 59.2%, 24.0%, 34.4% and 32.6%.

Conclusion: Proposed quantification method showed good accuracy in the 15 min scan, but showed inadequate repeatability for determining physiological differences in renal disease patients. The 5 min scan showed both poor accuracy and repeatability. For this method to be implemented in intervention studies, improvements in repeatability and accuracy should be made.

Introduction

Chronic kidney disease (CKD) is one of the lead causes of mortality, an estimated 800 million people are affected worldwide and no cure has yet been found [1]. There are different stages in CKD, once diagnosed there are treatment options to prevent the disease from evolving into the next stage. In final stages of CKD, end-stage kidney disease (ESKD), the prime treatment options are haemodialysis (HD), peritoneal dialysis (PD) or a kidney transplant.

One of the main factors playing a role in kidney failure is sodium; increased sodium intake can lead to many toxic side effects in renal disease patients. Recent research has shown that increased sodium storage in the skin has been linked to several clinical biomarkers and may have adverse effects on many biological processes such as inflammatory response and osmoregulation in the human body [2,3]. Excess sodium storage has been found in the muscles and skin of the lower leg in maintenance HD and PD patients [4], as well as general CKD patients [5]. Another study showed sodium storage in muscle tissue has been linked to local and systemic inflammation, which is associated with risk for cardiovascular diseases and consumes protein-energy of end-stage kidney disease (ESKD) patients [6]. These findings show that sodium accumulation in the body is a possible side effect of kidney failure. Sodium can therefore be used as a clinical biomarker in measuring the effects of interventions such as medication, dialysis and diet in renal disease patients. To be able to implement this clinically, sodium concentrations in the human body need to be quantified.

Quantification of sodium deposition in the body can be non-invasively imaged and quantified at tissue level with ^{23}Na-MRI. In clinical research ^{23}Na-MRI is used to provide quantitative information on biochemical processes in the human body, adding to the anatomical data provided by conventionally used proton MRI. However, there are a few obstacles with implementing ^{23}Na-MRI in a clinical setting. The main factor is that sodium gives a significantly lower signal than ^{1}H-MRI scans. Signal-to-noise is much lower in ^{23}Na-MRI compared to ^{1}H-MRI because of 1) a lower natural abundance of sodium in the body (~10.000x less than hydrogen), 2) a lower gyromagnetic ratio (approximately ¼ of that of H) and 3) a fast, bi-exponential decay of the sodium signal due to the quadrupolar nature of the sodium nucleus (spin I = 3/2) [7,8]. This bi-exponential decay means there are two T2* relaxation time constants: the short T2* with ~ 0.5-3 ms and the long T2* with ~ 7-10 ms [9]. It is therefore important in sodium imaging that a short echo time (TE) is used to get enough signal to create an image [10]. With the arrival of ultra-high field scanners (7 Tesla) TE can be as short as a few milliseconds and enough sodium signal can be acquired to create an image. To use ^{23}Na-MRI to image sodium in the muscles and skin, the resolution of a scan should be good enough to resolve these tissues, thus concentrations in these different tissues can be quantified. In previous studies this has been done by comparing tissue groups to phantoms with known sodium concentrations [11]. A trade-off is made between resolution and signal intensity, this is especially prevalent in sodium imaging. Smaller voxel sizes increase resolution but significantly decrease signal intensity per voxel, resulting in too little signal to quantify the sodium concentration. Resolution should therefore be carefully chosen.

The goal of this study is to set up a method to quantify sodium concentrations of muscle groups and the skin in the calf using ^{23}Na-MRI at 7T. The method is validated by comparing precision and accuracy to literature of previous intervention studies. If proven sufficient, this method can be used to determine the effect of future intervention studies in CKD, HD and PD patients.

Methods and materials

All measurements were performed on a 7-T MRI scanner (Philips Healthcare, Best, The Netherlands). A double-tuned transmit/receive RF coil with two sodium and two proton channels was used.

Study participants

In total ten healthy test subjects were included. The age of the study participants were between 24 - 76 years, of whom five were men and three were women, their BMI ranging between 23.6 – 26.4 kg/m^2. In all participants four regions of interest (ROI) were analysed: three muscle groups (m. gastrocnemius, m. soleus and m. tibialis) and one small selected area of skin. The ROIs were drawn in a similar way to a study by Zaric et al, see figure 1.

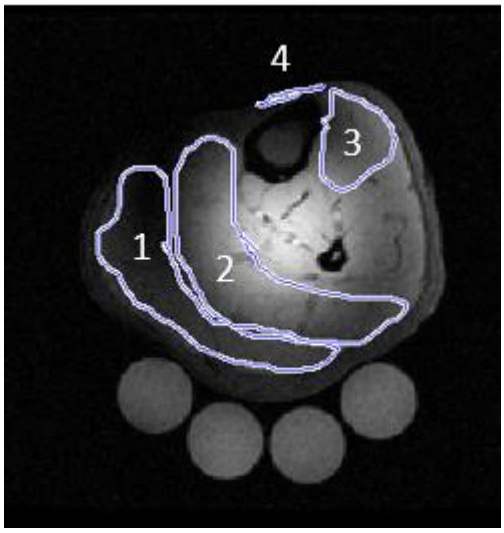


Figure 1: An example of the ROIs drawn on a FFE proton scan. 1 = m. gastrocnemius, 2 = m. soleus, 3 = m. tibialis anterior and 4 = skin.

Standard scan routine

The total scan routine for acquiring the proton and sodium scans took approximately 1 hour, including 10 minutes positioning the participant in the coil. The standard scan routine is shown in figure 2 below.

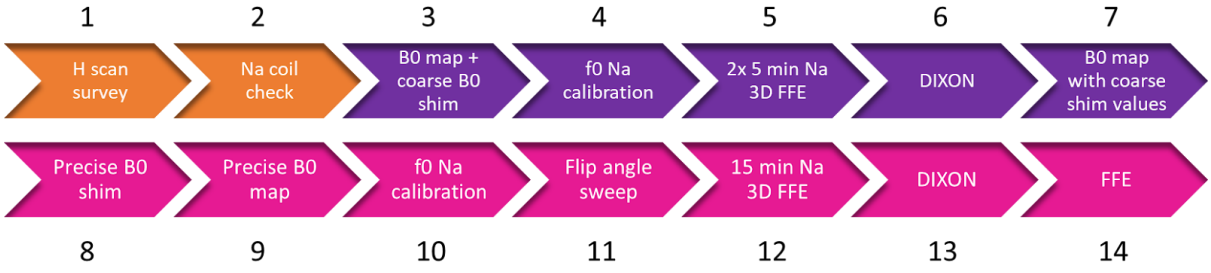


Figure 2: Flow chart of the standard scan routine (exam card) per session to acquire sodium and proton scans per participant.

1. *Proton scan survey*

At first a proton scan was made as a survey to see if the phantoms were correctly positioned in respect to the lower leg. The phantoms should be placed next to the upper part of the lower leg, so all muscles are visualised in the scan.

2. *Sodium coil check*

Only a small part of the sodium coil is sensitive, so a quick sodium scan was made to ensure the phantoms were placed in the sensitive area.

3. *B0 map and coarsely drawn B0 shim*

A B0 map was made to see the homogeneity of the magnetic field. Then an ROI with the phantoms and lower leg was coarsely drawn (a generic circle around all the objects) throughout all the slices (creating a VOI) with an MR code tool to acquire B0 shim values. These values were then applied in the scans from step 4 to 7.

4. *f0 sodium calibration*

The centre frequency of sodium needs to be tweaked to the precise frequency the sodium nuclei are resonating at.

5. *2x 5 min sodium 3D FFE*

A 5 minute sodium scan is made twice using a 3D Fast Field Echo (FFE), resolution 5 x 5 x 25 mm3, FOV 350 × 194.4 × 125 mm3, TR = 90 ms, TE = 2.8 ms, NSA = 30. Kspace is filled by Cartesian sampling.

6. *DIXON*

A DIXON scan is made with the coarsely acquired B0 shim.

7. *B0 map with coarsely acquired shim values*

The B0 map with the new shim values acquired by the coarsely drawn ROI is now made. This step is performed at this point in the scan routine to minimise the time between step 4 and 5 to be able to scan as quickly as possible in case an exercise protocol was performed.

8. *Precise B0 shim*

A more precisely drawn B0 shim is made by drawing an ROI closely around the phantoms and the lower leg through all the slices of the scan. Hereby new B0 shim values were obtained, which were applied in the scans from step 9 to 14.

9. *B0 map with more precisely acquired shim values*

The B0 map with the new shim values acquired by the more precisely drawn ROI is now made.

10. *f0 calibration for sodium*

The centre frequency is set to the right resonance frequency again, because of the slight changes in B0 shim.

11. *Flip angle sweep*

A manual power optimization is done.

12. *15 min sodium 3D FFE*

A 15 minute sodium scan is made using the 3D FFE, resolution 4 x 4 x 25 mm3, FOV 350 × 175 × 125 mm3, TR = 90 ms, TE = 3.3 ms, NSA = 80. Kspace is filled by Cartesian sampling.

13. DIXON

A DIXON scan is made with the more precisely acquired B0 shim. The DIXON scan is used to clearly see the difference between the water and fat signal, which then makes it easier to localise the position of the skin in the scans.

14. FFE

A FFE H scan is made with the more precisely acquired B0 shim. The FFE is used as the anatomical reference for drawing the ROIs of the muscle groups and the skin.

Acquiring sodium concentrations

To quantify sodium concentration of muscle and skin in the calf with ^{23}Na-MRI, phantoms with known sodium concentrations were used as reference, principle based on previous studies [11]. The quantification is done using Matlab (MATLAB version: 9.10 (R2021a), Natick, Massachusetts: The MathWorks Inc.), the steps of this process can be found in the flowchart in figure 3 and the code can be found in Appendix A.

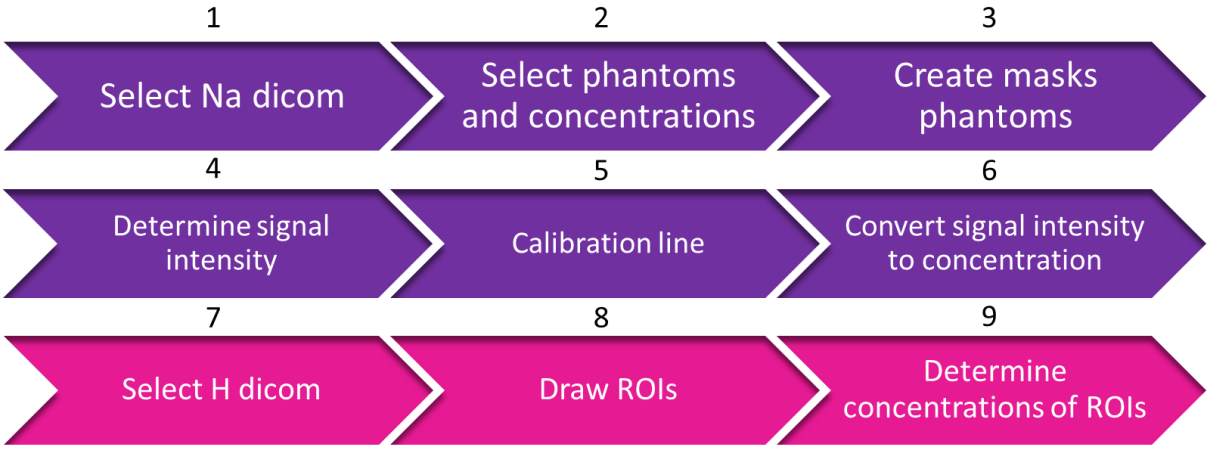


Figure 3: Flowchart of determining the sodium concentration in ROIs.

1. Select the sodium scan with the highest signal intensity of the slices.
2. Select the amount of phantoms used in the scan and their concentrations.
3. Create masks of the phantoms on the sodium scan. Histograms of signal intensities within each mask are made.
4. Determine the mean signal intensity of masks.
5. Create a calibration line linking the signal intensity to the known concentrations of the phantoms used.
6. Convert the signal intensity values of the sodium scan to sodium concentrations, creating a new image with a sodium concentration map.
7. Select the proton scan slice correlated to the position of the sodium scan.

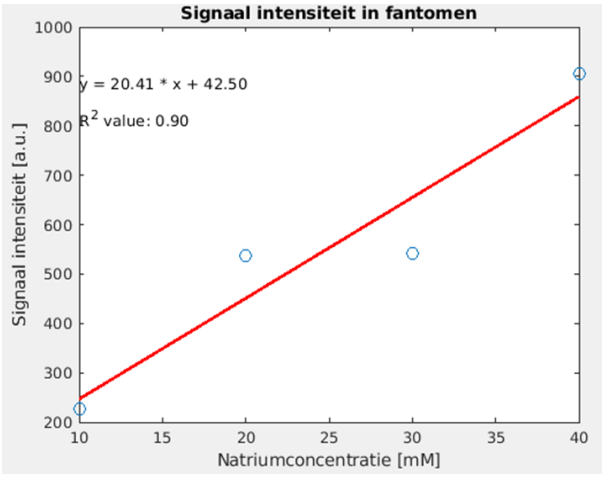
8. Draw ROIs of the muscle groups and skin. Histograms of sodium concentrations within each ROI are made.
9. Determine the mean sodium concentration of the ROIs with the calibration line.

Four phantoms with NaCl solution dissolved in an agar gel were used, with sodium concentrations of 10 mmol/L, 20 mmol/L, 30 mmol/L and 40 mmol/L (figure 4).

Figure 4: Reference phantoms of 10, 20, 30 and 40 mmol/L sodium in agar gel, respectively.

An agar gel is used instead of a liquid solution so the phantoms have similar relaxation properties as the tissue scanned. When a liquid solution is used for reference phantoms, the calibration line acquired will not be based on reality. To demonstrate this, a test scan was made with a phantom containing a solution with 3.0 g/L sodium concentration (~51.33 mmol/L) and the previously mentioned reference phantoms. The results are shown in figure 5 below.

(a)



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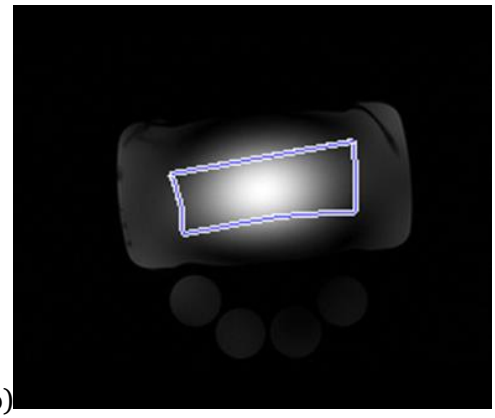

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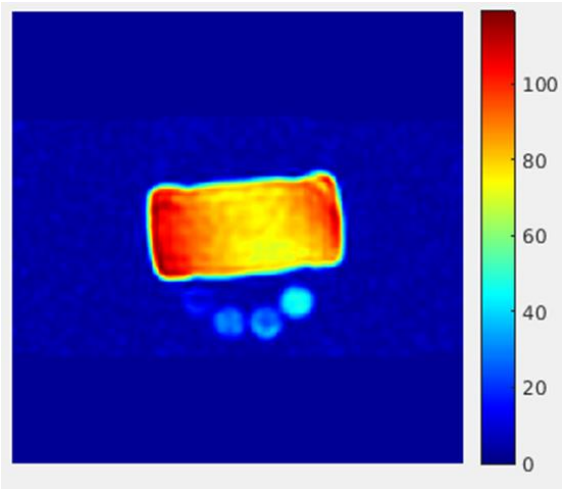


Figure 5: Results of the liquid solution test scan with the reference phantoms. (a) calibration line for converting the signal intensity to sodium concentration in mmol/L (step 5 in figure 3), (b) ROI in the liquid solution drawn on the proton scan (step 8 in figure 3), (c) sodium concentration map converted from the signal intensity with the calibration line (step 6 in figure 3).

With the calibration line the mean value of the ROI drawn in the liquid solution is calculated to be ~80.3 mmol/L. This is an overestimation due to the liquid solution having a longer T2 constant than the reference phantoms. The T2 constant of sodium in agar gel is closer to the T2 constant of human tissue, therefore the reference phantoms with agar gel sodium solution are used.

Repeatability and accuracy

To assess the quality of the quantification method, repeatability and accuracy were determined for each of the ROIs. A distinction was made between the intersession and intrasession repeatability, which was tested in eight out of ten participants. These participants were scanned twice per standard scan routine. Intersession repeatability was assessed in eight participants by comparing the 15 min 3D FFE sodium scans of both routines, intrasession repeatability was assessed in four participants by comparing the 5 min 3D FFE sodium scan made consecutively during the first routine. To test the accuracy of the 5 min 3D FFE, it was compared to the 15 min by performing a paired t-test.

The repeatability is quantitively analysed with Bland-Altman plots and the coefficient of repeatability. The coefficient of repeatability is defined in this study as

$$Coefficient\ of\ repeatability = \left(\frac{1.96*SD}{mean}\right) * 100\% .$$

The accuracy of sodium concentration in vivo is hard to measure, since there is no reference with the actual sodium tissue concentration without performing a biopsy. The accuracy will therefore be determined by comparing the sodium concentrations of the muscle groups and skin to previously published literature. To determine if there is a significant difference between the sodium concentrations of the four ROIs, a non-parametric Friedman test is performed.

Proof of concept: exercise test

The purpose of the exercise protocol is to measure a physiological difference in sodium concentration before and after exercise, to simulate an intervention (e.g. medication or diet) in a much shorter timeframe. This protocol was tested in two participants as a proof of concept. The effect of the exercise on sodium levels can be measured up until 30 minutes after the exercise [12], thus the time between exercise and measuring sodium levels should be optimized and sodium scan times should be shortened to be able to measure an effect over time. To accommodate this, the 5 min 3D FFE sodium scan was implemented.

The exercise protocol was as follows:

1. 2x 15 heel raises as warm-up with 30 seconds break in between
2. 4x 50 heel raises with 1 min breaks in between
3. Final set of heel raises until exhaustion

The hypothesis for the exercise protocol is that the muscles active during the exercise have increased sodium concentrations and will decrease over time. Previous studies have found a 8-13% increase in the gastrocnemius and soleus, while the tibialis anterior showed no significant difference in sodium concentration [13].

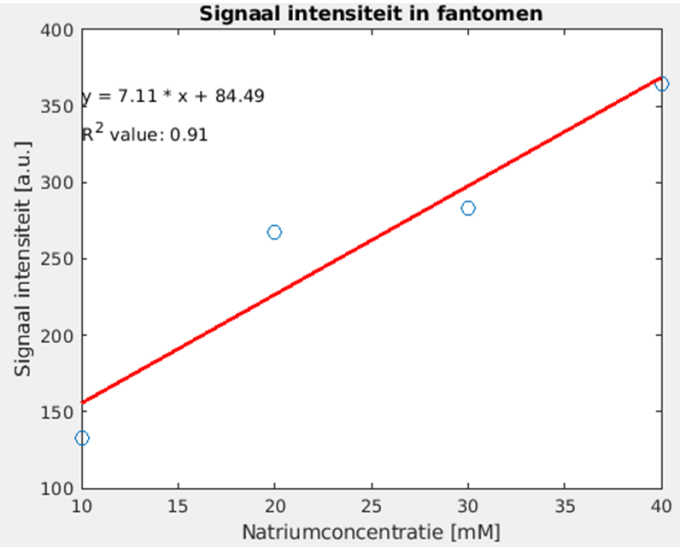
The participant was scanned per standard scan routine (see figure 2), then taken out of the scanner to do the exercise protocol. The participant was scanned immediately after finishing the exercise protocol, repeating step 1-4 of the standard scan routine. Then 9 dynamics (5 min 3d FFE sodium scans) were made consecutively with resolution 5 x 5 x 25 mm3, FOV 350 × 194.4 × 125 mm3, TR = 90 ms, TE = 2.8 ms, NSA = 30. Finally step 13, 14 and 7 of the standard scan routine were executed respectively. The time between the exercise protocol and the start of the first sodium scan was approximately 15 min.

Results

Intersession repeatability - 15 min 3D FFE sodium scan

All participants were analysed with the method described in figure 3. An example of the determination of the sodium concentrations of one participant, sub002, is shown in figure 6.

a)



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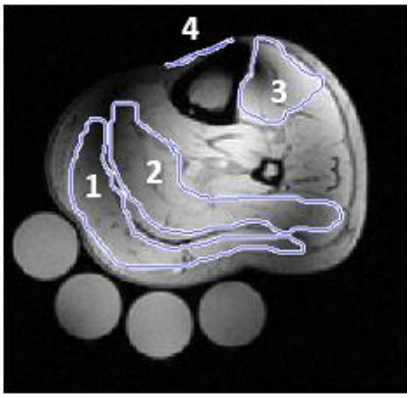

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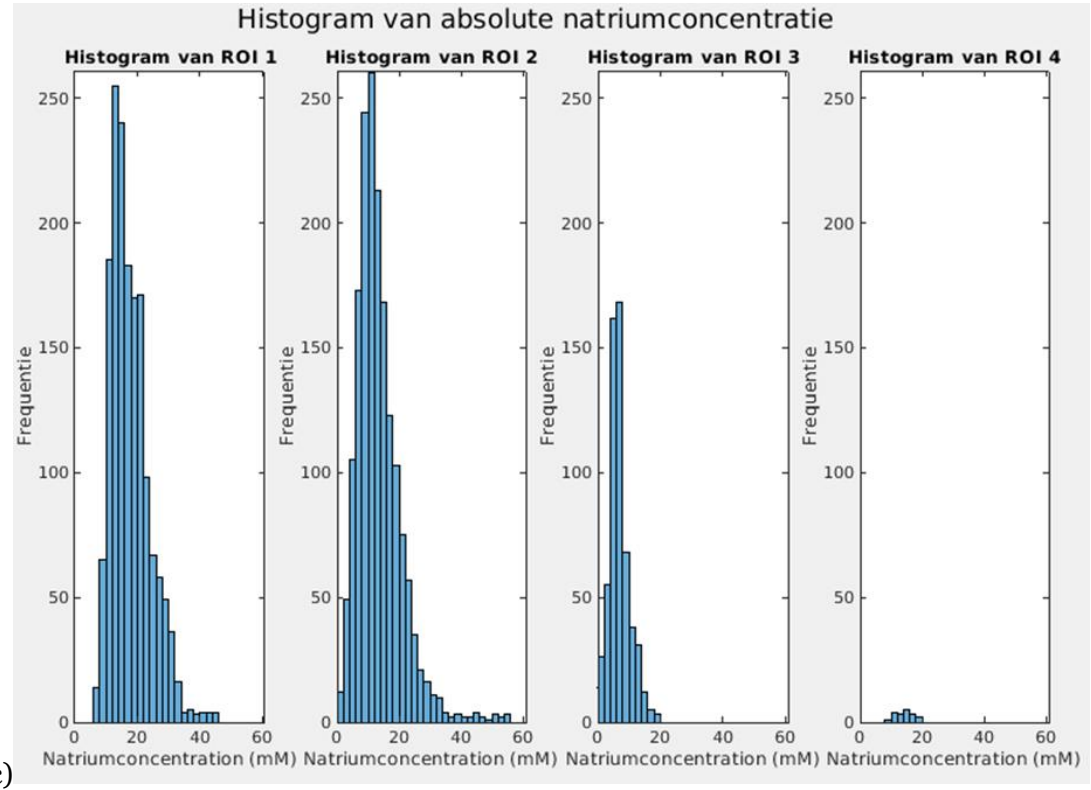


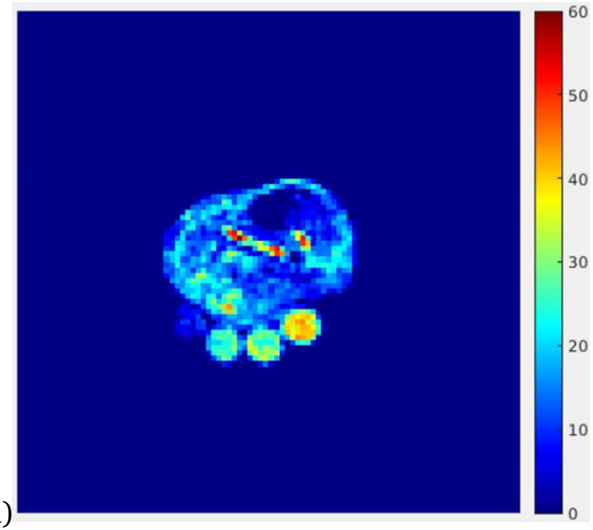
d) 

Figure 6: The results of quantifying sodium concentrations in sub002: a) calibration line correlating the signal intensity to the sodium concentration (step 5 in figure 3), b) the ROIs of the muscle groups and skin drawn in the FFE proton scan (step 8 in figure 3), c) histograms of the sodium concentrations measured in each ROI, d) the sodium concentration map with signal intensities converted to sodium concentrations (step 6 in figure 3).

The mean sodium concentrations of the gastrocnemius, soleus, tibialis anterior and skin (n=8) are 16.7±3.8 mmol/L, 15.6±4.1 mmol/L, 10.8±2.4 mmol/L and 15.5±2.8 mmol/L, respectively. A nonparametric Friedman test was performed to see if there is a significant difference between the sodium concentrations of the 4 different ROIs. It showed that there was a significant difference between all 4 ROIs (p=0.0005).

To determine the precision and repeatability of the sodium quantification, the data was assessed using Bland-Altman plots of each muscle group and skin (figure 7). Coefficient of repeatability for gastrocnemius, soleus, tibialis anterior and skin are respectively: 51.8%, 48.9%, 25.3% and 49.7%.

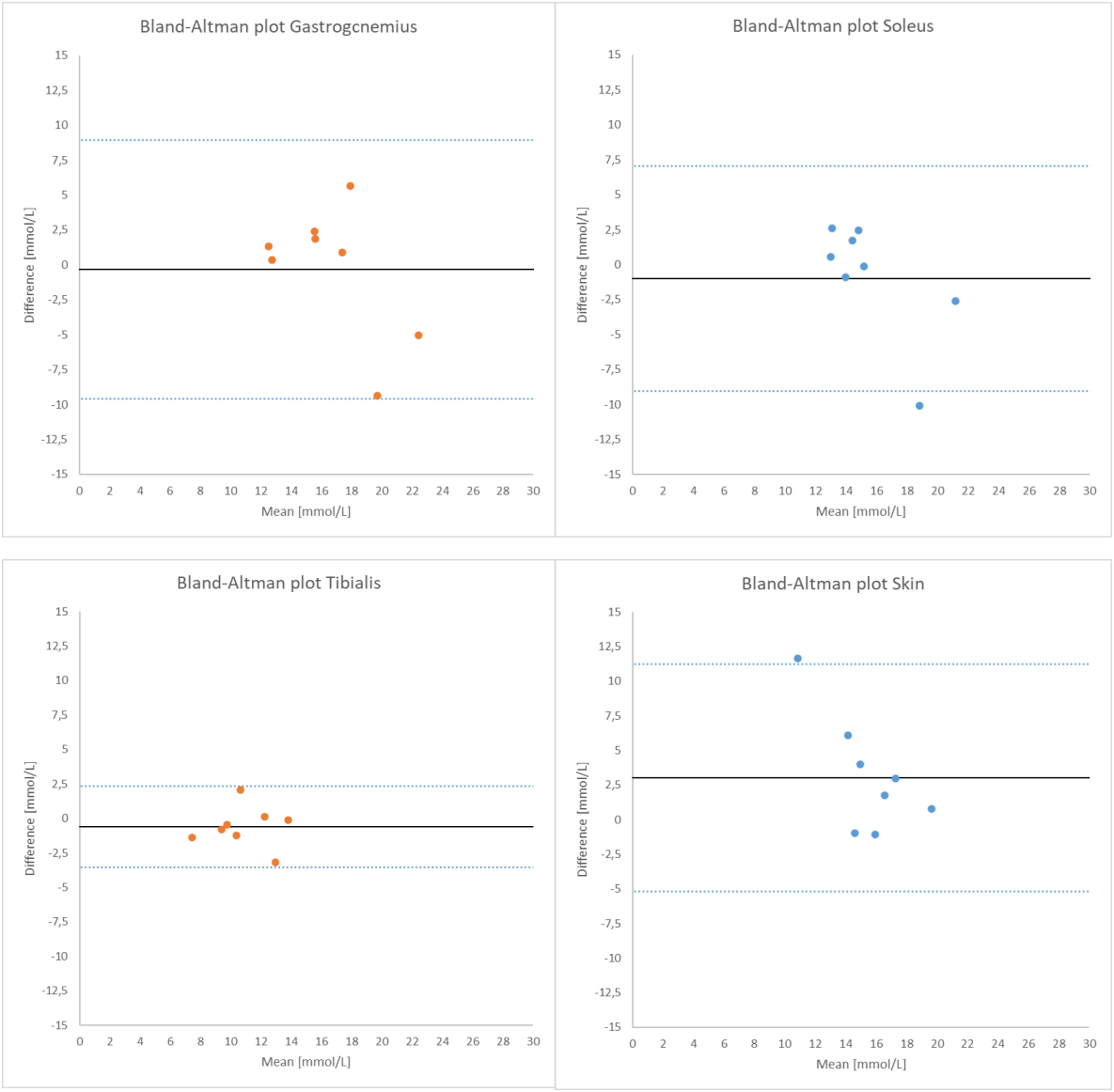


Figure 7: Bland-Altman plots of the muscle groups (gastrocnemius, soleus and tibialis anterior) and the skin of the 15 min FFE 3D sodium scan. Each datapoint in a Bland-Altman is one participant. The horizontal axis shows the mean of the sodium concentration measured in the two scans made per participant, the vertical axis shows the difference between the sodium concentration of the two scans. The straight black line is the mean difference, the dotted blue lines are the upper and lower bounds of the 95% confidence interval.

Intrasession repeatability - 5 min 3D FFE sodium scan

The mean sodium concentrations of the gastrocnemius, soleus, tibialis anterior and skin are 17.3±3.0 mmol/L, 13.6±3.7 mmol/L, 7.4±2.1 mmol/L and 15.2±2.1 mmol/L, respectively. A nonparametric Friedman test was performed to see if there is a significant difference between the sodium concentrations of the 4 different ROIs. It showed that there was a significant difference between all 4 ROIs (p=0.019).

The Bland-Altman plots of each muscle group and skin are shown in figure 8. Coefficient of repeatability for gastrocnemius, soleus, tibialis anterior and skin are respectively: 59.2%, 24.0%, 34.4% and 32.6%. The zero difference value falls within the 95% confidence intervals of each of the ROIs.

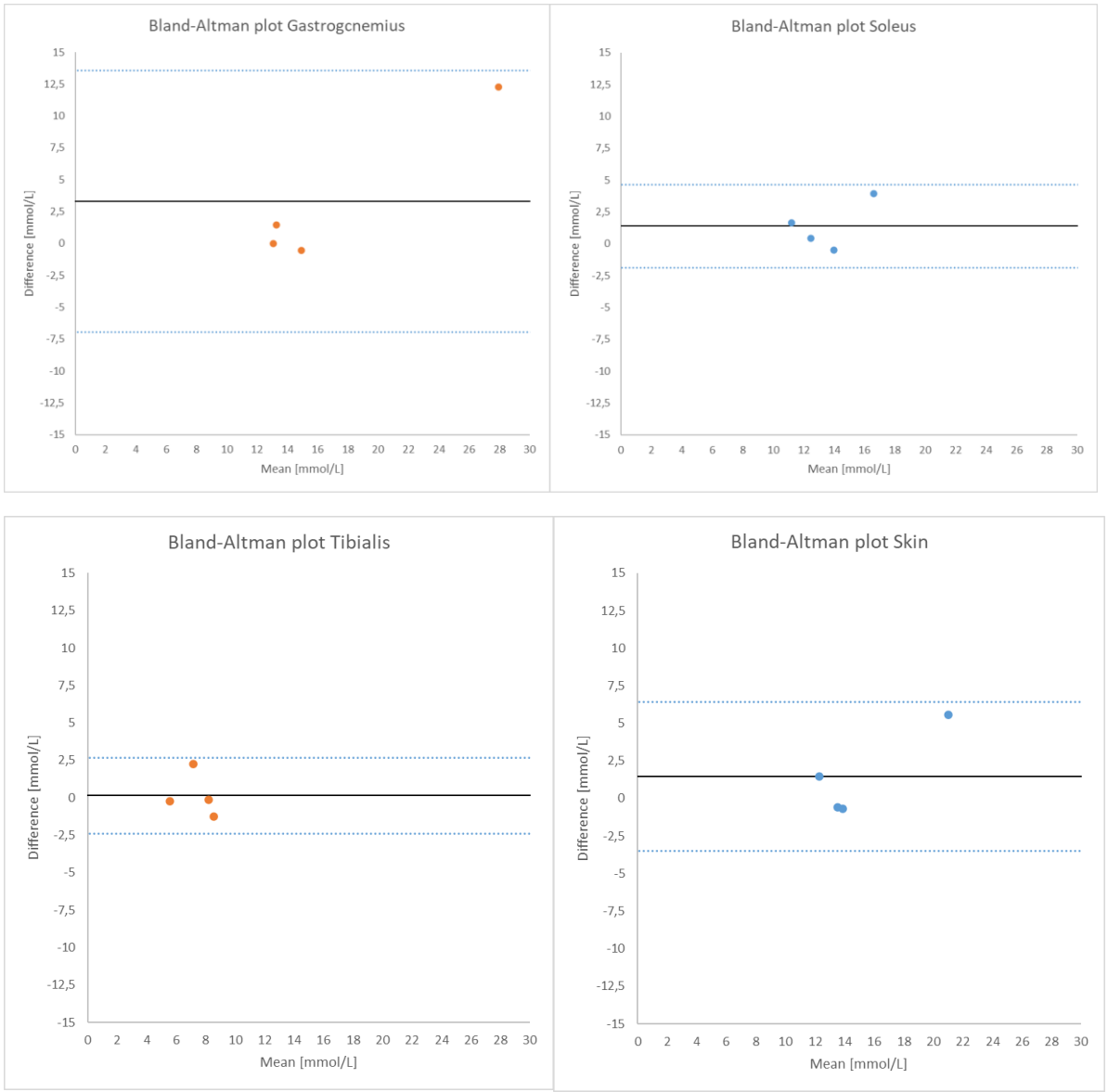


Figure 8: Bland-Altman plots of the muscle groups (gastrocnemius, soleus and tibialis anterior) and the skin of the 5 min FFE 3D sodium scan. Each datapoint in a Bland-Altman is one participant. The horizontal axis shows the mean of the sodium concentration measured in the two scans made per participant, the vertical axis shows the difference between the sodium concentration of the two scans. The straight black line is the mean difference, the dotted blue lines are the upper and lower bounds of the 95% confidence interval.

Comparison 5 min - 15 min scans

To see if there is a significant difference between the sodium concentrations obtained with the 15 min and 5 min 3D FFE scans, a paired t-test was performed for each of the muscle groups

and skin. The p-values were 0.434 (gastrocnemius), 0.860 (soleus), 0.028 (tibialis anterior) and 0.704 (skin). The Bland-Altman plots of each muscle group and skin are shown in figure 9. Coefficient of repeatability for gastrocnemius, soleus, tibialis anterior and skin are respectively: 67.5%, 29.2%, 24.9% and 57.1%. The zero difference value falls within the 95% confidence intervals of each of the ROIs except for the tibialis anterior (confidence interval of 0.39 – 4.73 mmol/L).

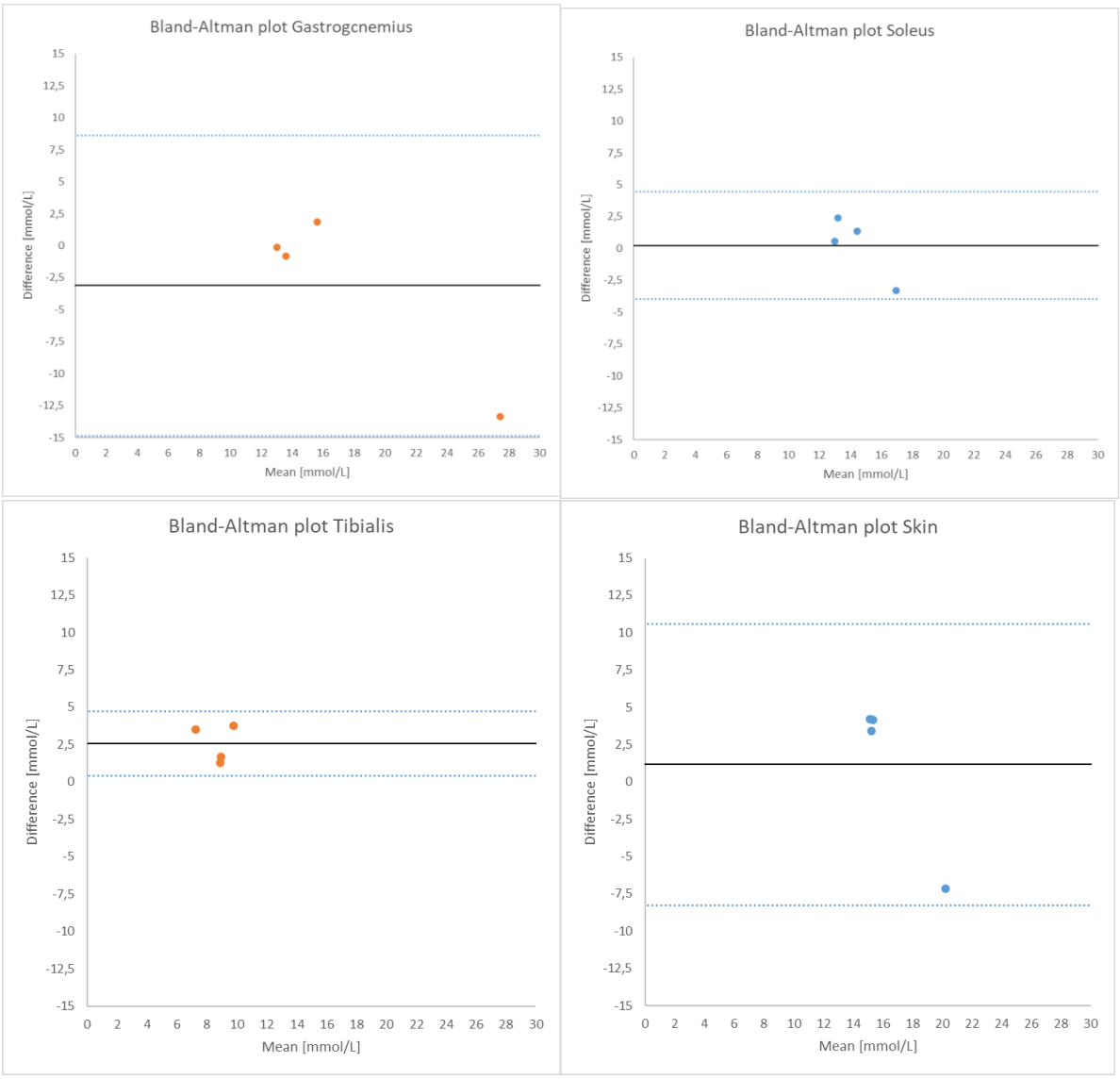
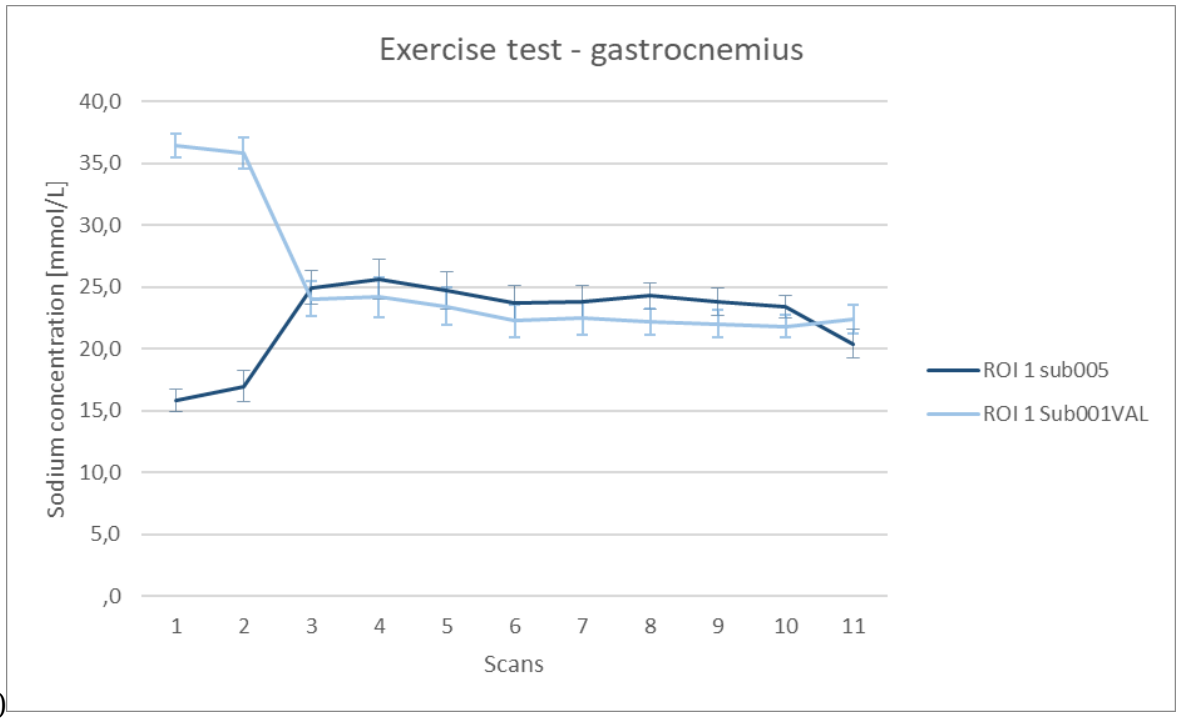


Figure 9: Bland-Altman plots of the muscle groups (gastrocnemius, soleus and tibialis anterior) and the skin of the 15 and 5 min FFE 3D sodium scan. Each datapoint in a Bland-Altman is one participant. The horizontal axis shows the mean of the sodium concentration measured in the two scans (one 5 min and one 15 min scan) made per participant, the vertical axis shows the difference between the sodium concentration of the two scans. The straight black line is the mean difference, the dotted blue lines are the upper and lower bounds of the 95% confidence interval.

Proof of concept - exercise test

The sodium concentration per muscle group/skin over time of the two participants is shown in figure 10. The first two points are reference scans from before the exercise protocol, points 3-11 are the concentrations derived from the 9 dynamics consecutively made after the exercise protocol. All data points in figure 10 are obtained by consecutively made 5 min 3D FFE sodium scans.



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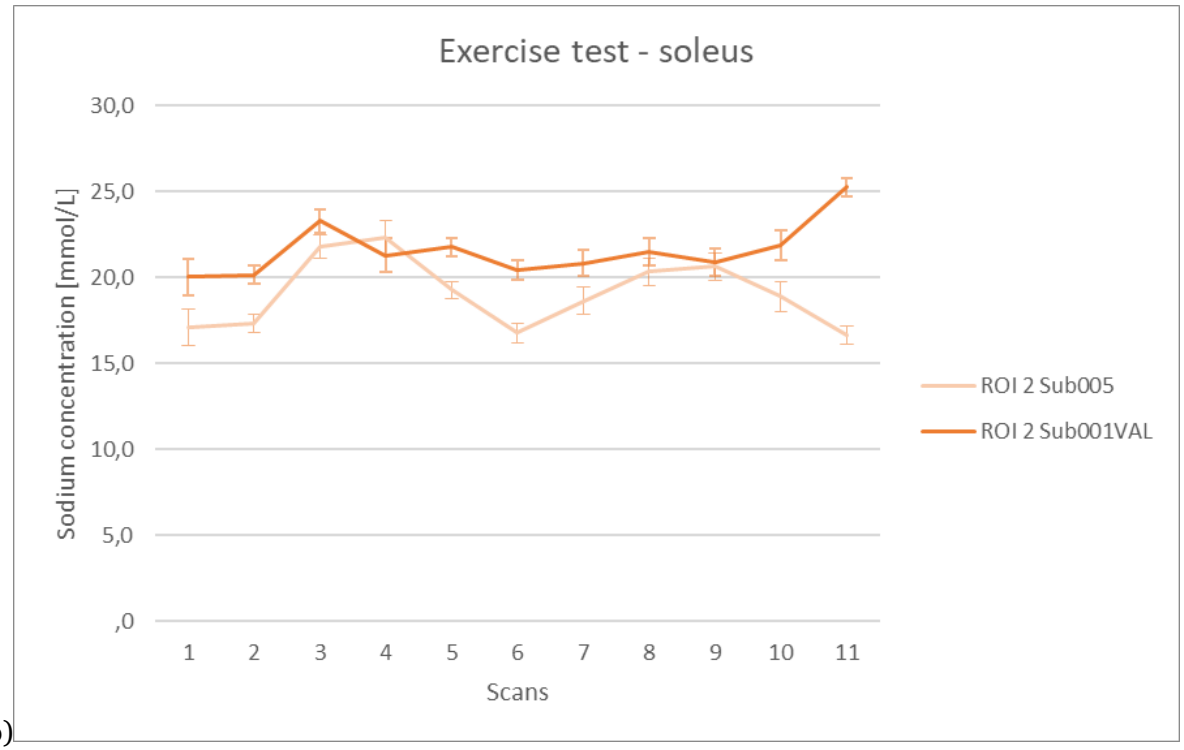

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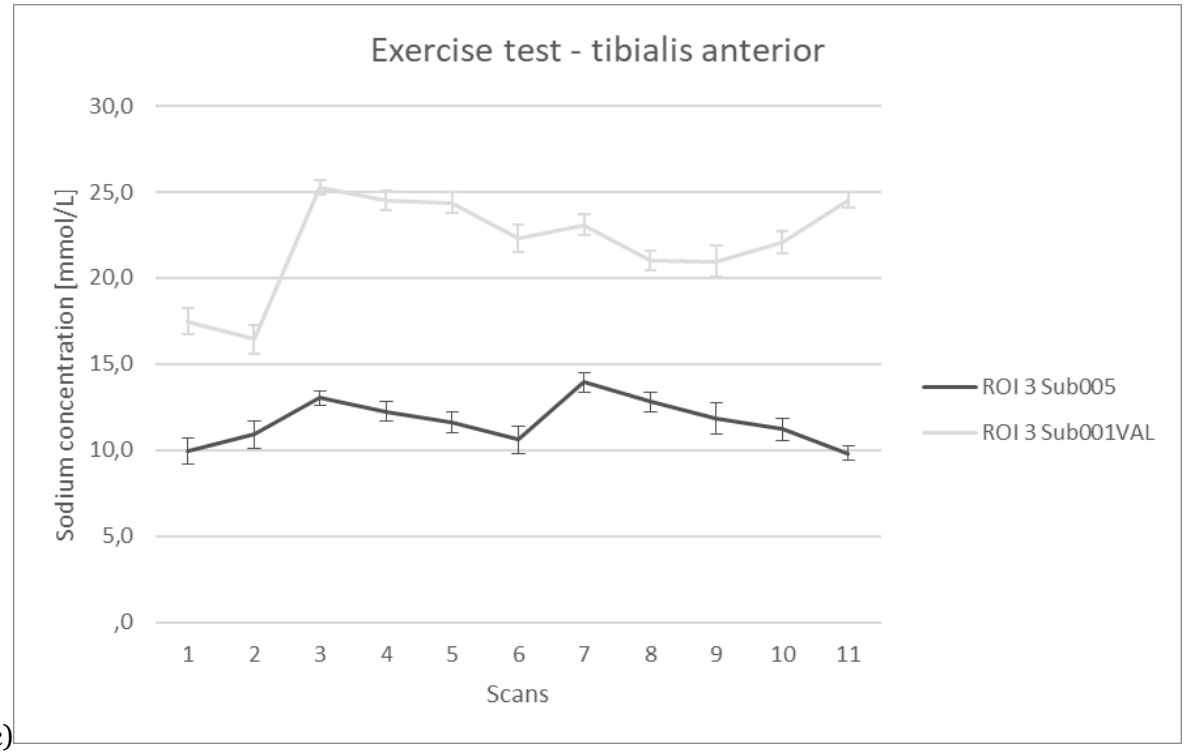

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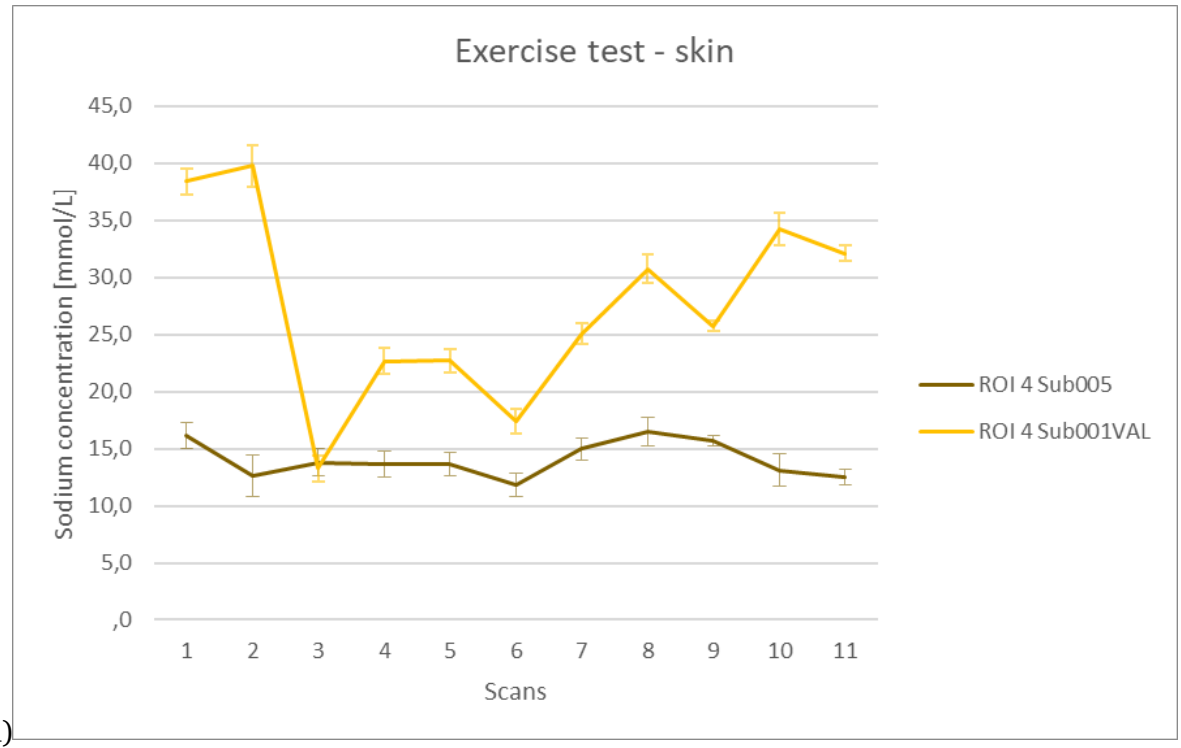


Figure 10: The sodium concentration per scan from before and after the exercise protocol of a) gastrocnemius, b) soleus, c) tibialis anterior and d) skin. Scan 1-2 are the reference scans from before the exercise protocol, scan 3-11 are the 9 dynamics made consecutively after the exercise protocol.

Discussion and conclusion

This study aimed to find the feasibility, repeatability and accuracy of non-invasively quantifying sodium concentrations in muscles and skin in the calf with ^{23}Na-MRI at 7T, with the purpose to implement this method to evaluate future intervention studies in CKD, HD and PD patients.

In all of the participants enough sodium signal could be acquired to create a sodium image, making the quantification with the method described in figure 3 feasible. However, the repeatability and accuracy of this method are not yet adequate enough to implement this method in intervention studies.

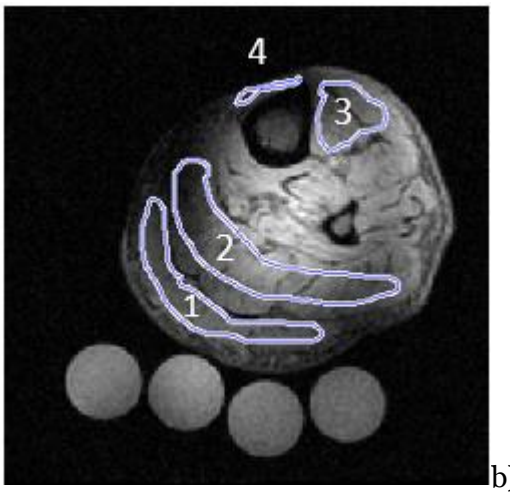
The repeatability coefficient for intersession (15 min 3D FFE) and intrasession (5 min 3D FFE) for all muscle groups and skin were between 25.3%-51.8% and 24.4%-59.2%, respectively. This means that if a scan is repeated in one participant and the difference in sodium concentration between scans is larger than 51.8% in the gastrocnemius, the difference is due to a physiological effect and not a measurement error. To compare this to literature: a study by Kopp et al. showed that sodium concentrations in muscle had decreased 21.4% in patients after haemodialysis [14]. Our technique would not have been precise enough to measure the effect of haemodialysis as a physiological difference in both 15 min and 5 min 3D FFE sodium scan.

Testing the accuracy of a non-invasive quantification method for sodium concentrations in the human body is a challenge because there is no 'gold standard'. When ^{23}Na-MRI was first validated as a method to quantify sodium concentrations, its accuracy was assessed by comparison to amputated extremities in rats and humans [15]. This is difficult to reproduce, thus in this study the accuracy is determined by comparison to previous studies. The mean sodium concentrations of the gastrocnemius, soleus, tibialis anterior and skin measured with the 15 min 3D FFE (n=8) are 16.7±3.8 mmol/L, 15.6±4.1 mmol/L, 10.8±2.4 mmol/L and 15.5±2.8 mmol/L respectively. This is in line with literature for gastrocnemius (17.0±2.2 mmol/L), soleus (18.1±1.4 mmol/L), tibialis anterior (14.3±1.3 mmol/L) and the skin (14.4±3.5 mmol/L) [3, 11]. This remains true for the mean sodium concentrations of the tissue groups measured with the 5 min 3D FFE (n=4) for the gastrocnemius (17.3±3.0 mmol/L) and the skin (15.2±2.1 mmol/L), but not for the soleus (13.6±3.7 mmol/L) and the tibialis anterior (7.4±2.1 mmol/L). An explanation for this phenomenon could be the lower SNR and resolution of the 5 min 3D FFE scan, resulting in lower sodium signal intensities measured.

The t-test comparing the 5 min to the 15 min 3D FFE scan showed that there is significant difference between the two scans in all ROIs ($p>0.05$) except for the tibialis anterior ($p<0.05$). However, the zero difference value in the tibialis anterior plot falls outside of the 95% confidence interval. This means that the 5 min 3D FFE sodium scan systematically underestimates the sodium concentration in the tibialis anterior. Since the accuracy of the 15 min 3D FFE sodium scan is good compared to literature, it can be concluded that the 5 min 3D FFE sodium scan is not adequate enough to accurately measure sodium concentrations.

This means that the proof of concept of the exercise test based on the 5 min 3D FFE sodium scan is not yet accurate enough to draw conclusions from. Furthermore, since the repeatability coefficient between consecutive 5 min scans is high (24.4%-59.2%), the difference between the reference scans and the dynamics made in the exercise test in all muscle groups and skin cannot be categorized as physiological difference, except for the decrease in skin sodium concentration in sub001VAL (66.7% difference, see figure 10). However, the ROIs were drawn again for each dynamic and reference scan, therefore there is a chance the ROI was not drawn the exact same way in both scans, seemingly creating a larger difference in sodium

concentration. Figure 11 shows there is indeed a difference in the ROIs drawn for the skin between the reference scan and the first dynamic. Recommendation for future studies is to draw the ROIs once and copy them for each dynamic, to avoid this effect.

a) 

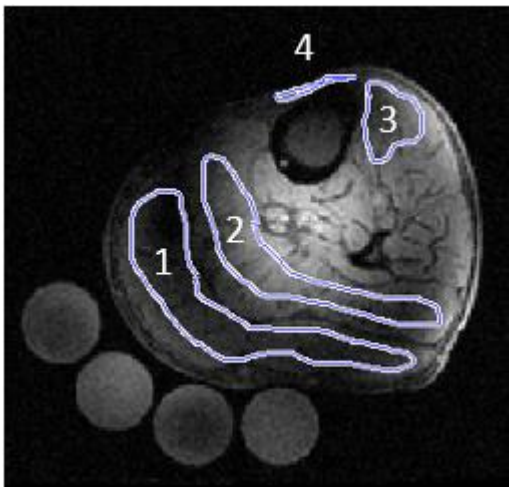
b) 

Figure 11: a) ROIs drawn on the 3D FFE proton reference scan 1 pre-exercise, b) ROIs drawn on the 3D FFE proton dynamic 1 post-exercise. ROI 1 = gastrocnemius, ROI 2 = soleus, ROI 3 = tibialis anterior, ROI 4 = skin.

To conclude, we established the feasibility of non-invasively quantifying sodium concentrations in muscles and the skin in the calf with ^{23}Na-MRI at 7T. The repeatability for both intersession (15 min scan, repeatability coefficient 25.3%-51.8%) and intrasession (5 min scan, repeatability coefficient 24.4%-59.2%) is inadequate to measure physiological difference in sodium concentration in intervention studies. Mean values of sodium concentrations in 15 min scan were in line with literature, indicating good accuracy. The 5 min scan showed inadequate accuracy by comparison with literature. The method is not yet suitable for evaluation of intervention studies.

Recommendations for improvement are applying radial or spiral sampling of Kspace to reduce TE, thereby increasing the sodium signal acquired. Another factor to optimize sodium signal could be the implementation of a B1 shim, to even further reduce inhomogeneities. Furthermore, more data should be analysed to reduce the 95% confidence interval in the Bland-Altman plots, increasing precision of the sodium concentrations measured. The age group of the participants analysed should also include a larger variety, given that sodium concentrations differ with age. Lastly, all data in this study has been analysed by one observer. For future studies it would be interesting to see how interobserver repeatability influences the sodium concentrations obtained per ROI.

Ethics paragraph

In recent years MRI has solidified itself as one of the pioneer imaging techniques within the medical imaging field. Innovations have made MRI more clinically accessible, making it a cornerstone of diagnostic medicine. But with increasing implementation of MRI in the clinic come multiple ethical considerations.

The main ethical issue in MRI research is MR safety. With a constant push for innovation of technology, certain safety concerns and appropriate study design can be easily overlooked to attain the goals of the research [16]. This is especially important in MR safety since high magnetic fields, in this case ultrahigh fields (7T), are involved. There are many exclusion criteria for MRI study participants: pacemakers, ferromagnetic implants, metal shavings etc., but also pregnancy and recent surgery (in the past 6 months). It is important that MR safety comes first in research, even if there is a lot of academic and societal pressure.

With the advance of the digital age one particular ethical issue has been proven difficult to tackle: maintaining the privacy of patients and healthy participants. Data safety management has been become a hot topic, also due to the many data breaches of data servers at Dutch universities in recent years. Data anonymization and multifactor authentication are steps to be taken to prevent data breaches.

Another ethical issue is inclusion of acquaintances in studies. It is most often hard to find study participants for a control group, therefore researchers often include friends, family and fellow researchers as healthy test subjects. This can become a problem when possible diseases/pathology in the participants are found during the research [17]. Researchers are to take on a professional attitude towards study participants, even if they are acquainted. Due to an emotional relationship between the researcher and participant findings of pathology in the participant can interfere with objective clinical decision making. The researcher may not be qualified to relay information about possible pathology, but might not be able to keep the findings to themselves when the participant is an acquaintance. As a result incomplete or false information can be communicated, causing stress and anxiety in the participant. It has been shown that >90% of healthy participants wish to be informed of found abnormalities [18]. However, it should be ensured the relayed information is clinically relevant and communicated by a medical professional.

In this study the previously mentioned ethical concerns have been taken into account before and during the research, to ensure moral integrity of the study. All researchers participating in the study had to pass a MR safety exam, healthy test subjects were screened and informed twice of the risks involved; once beforehand and once on the day of the scan. All study data was anonymized and saved only on a research server managed by the Amsterdam UMC, where data is stored for a limited amount of time. Family members were excluded from participating, friends and fellow researchers were not. A strict protocol was in place in case of findings of clinically relevant abnormalities during the scan: the general practitioner of the test subject would be informed, who in turn would inform the participant. This is both to ensure privacy of the participant and that the information is relayed by a medical professional. The researchers involved in the study were to abstain from comments about potential abnormalities found. The protocol was explained to the participants on the day itself and they agreed to the protocol by signing a form. The decision to include friends and fellow researchers in the control group was made based on the clinical relevance of this study. If this decision had not been made, the study would have taken many months longer to conclude. The delay in progress in a study of which >10% of the population would gain from did not outweigh potential ethical risks, especially when great measures were taken to reduce these risks.

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Appendix A

The Matlab code (MATLAB version: 9.10 (R2021a), Natick, Massachusetts: The MathWorks Inc.) used for quantifying sodium concentration with signal intensity.

```
clear all, close all, clc
%% Specify DICOM file
[file_name, folder_path] = uigetfile('*.dcm', 'Choose a DICOM file');

% Check if the file selection was canceled
if file_name == 0
    fprintf('File selection was canceled.\n');
    return;
end

% Construct the full file path
file_path = fullfile(folder_path, file_name);

% Read the selected DICOM file
data_selected_dicom = double(dicomread(file_path));
dicom_info = dicominfo(file_path);
ss = dicom_info.Private_2005_100e;
si = dicom_info.Private_2005_100d;
data_selected_dicom = (data_selected_dicom - si) ./ (ss * 1000);

scandata.image_data{1} = data_selected_dicom;
%% Number of phantoms
num_phantoms = inputdlg('Enter the number of phantoms:', 'Number of Phantoms', 1);
num_phantoms = str2double(num_phantoms{1});

ref_concentrations = zeros(num_phantoms, 1);
```

```
% prompt to enter all phantom concentrations at once %%AANGEPAST
prompt = {'Enter the concentration of phantom 1:','Enter the concentration of phantom 2','Er
name = 'Phantom concentrations';
numlines = 1;

ref_concentrations = str2double(inputdlg(prompt, name, numlines));

save('ref_concentrations.mat', 'ref_concentrations');
save('num_phantoms.mat', 'num_phantoms');

    scandata.repetition_time = dicom_info.RepetitionTime;
    scandata.number_of_image_slices = dicom_info.Private_2001_1018;

%% Create masks
load('num_phantoms.mat', 'num_phantoms');
max_ref = num_phantoms;
save('max_ref.mat', 'max_ref');

    data_image = scandata.image_data{1};

    mask_all_spheres = zeros(size(data_image,1),size(data_image,1));
    mask_each_sphere = zeros(max_ref,size(data_image,1),size(data_image,1));
    figure();
    imshow(squeeze(data_image(:,:)),[])

```

```
52
53 - for i = 1:max_ref
54 -     fprintf('Adjust the size and radius of the ROI of phantom %d by dragging its corners.\n', i);
55 -     roi_VFA = drawcircle('Center', [50 50], 'Radius', 2.5, 'Color', 'r', 'InteractionsAllowed', 'all'); %
56
57       % Wait for the user to finish adjusting the ROI (doubleclick to select
58       % new ROI)
59 -     wait(roi_VFA);
60
61       % Create masks for the ROI
62 -     mask_sphere_VFA{i} = createMask(roi_VFA);
63 -     mask_all_spheres(mask_sphere_VFA{i}) = 1;
64 -     mask_each_sphere(i, :, :) = squeeze(mask_sphere_VFA{i});
65 - end
66
67 - for idx = 1:max_ref
68 -     scandata.mask_each_sphere{idx} = squeeze(mask_each_sphere(idx, :, :));
69 - end
70 - scandata.mask_all_spheres = mask_all_spheres;
71
72   %% Signal Intensity in ROI
73 -   load('max_ref.mat', 'max_ref');
74 -   load('ref_concentrations.mat', 'ref_concentrations');
75
76 -   nr_of_scans = 1;
```

```
77 -     scandata.mean_signal=zeros(max_ref,nr_of_scans);
78
79 -     for idx_sphere = 1:max_ref
80 -         for idx_scan = 1:nr_of_scans
81 -             mask = scandata.mask_each_sphere{idx_sphere};
82 -             image = double(scandata.image_data{idx_scan});
83
84              % Exclude zeros from mask and image
85 -             nonZeroIndices = mask ~= 0;
86 -             nonZeroMask = mask(nonZeroIndices);
87 -             nonZeroImage = image(nonZeroIndices);
88
89              % Calculate the mean signal over non-zero elements
90 -             mean_signal = mean(nonZeroMask .* nonZeroImage);
91
92 -             scandata.mean_signal(idx_sphere, idx_scan) = mean_signal;
93 -         end
94 - end
95
96   %% Matrix of signal intensity of all concentrations
97 -     for idx_sphere=1:max_ref
98 -         for idx_scan = 1:nr_of_scans
99 -             matrix_data =  nonzeros(scandata.mask_each_sphere{idx_sphere} .* double(scandata.image_data{
100 -            file_name = sprintf('matrix_data_%d.mat', idx_sphere);
```

```
101 -            save(file_name, 'matrix_data');
102 -        end
103 - end
104
105  %% Histogram for all concentrations of signal intensity
106 - fileList = dir('matrix_data_*.mat');
107 - numFiles = numel(fileList);
108
109  % Extract the file names
110 - fileNames = cell(1, numFiles);
111 - for i = 1:numFiles
112 -     fileNames{i} = fileList(i).name;
113 - end
114
115 - figure;
116
117 - maxSignalIntensity = -inf; % Initialize the maximum value
118 - maxValue = -inf; % Initialize the maximum frequency
119
120 - for fileIndex = 1:numFiles
121
122 -     load(fileNames{fileIndex}, 'matrix_data');
123
124 -     maxSignalIntensity = max(maxSignalIntensity, max(matrix_data));
```

```
125
126 -       [frequencies, ~] = histcounts(matrix_data);
127
128 -       maxValue = max(maxValue, max(frequencies));
129
130 -       subplot(1, numFiles, fileIndex);
131
132 -       histogram(matrix_data,'BinWidth',50);
133
134 -       titleText = strrep(fileNames{fileIndex}, '_', ' ');
135 -       title(sprintf('Histogram - %s', titleText));
136 -       xlabel('Signaal intensiteit (a.u.)');
137 -       ylabel('Frequentie');
138 -   end
139
140     % Set the same y-axis limits for all histograms
141 -   for fileIndex = 1:numFiles
142 -       subplot(1, numFiles, fileIndex);
143 -       ylim([0, maxValue+1]);
144 -   end
145
146     % Set the same x-axis limits for all histograms
147 -   for fileIndex = 1:numFiles
148 -       subplot(1, numFiles, fileIndex);
149 -       xlim([0, maxSignalIntensity+40]);
150 -   end
151
152 -   sgtitle('Histogrammen van signaal intensiteit in de ROIs');
153
154
155     %% Plot; signal intensity vs concentration
156 -   x = ref_concentrations;
157 -   y = scandata.mean_signal;
158
159 -   figure;
160 -   plot(x, y, 'o', 'MarkerSize', 8);
161 -   xlabel('Natriumconcentratie [mM]');
162 -   ylabel('Signaal intensiteit [a.u.]');
163 -   title('Signaal intensiteit in fantomen');
164
165 -   coefficients = polyfit(x, y, 1);  % Fit a first-degree polynomial (linear line)
166
167 -   correlationMatrix = corrcoef(x, y);
168 -   R_value = correlationMatrix(1, 2);
169 -   R_squared = R_value^2;
170
171 -   hold on;
172 -   x_fit = min(x):0.1:max(x);  % Generate x values for the line
173 -   y_fit = polyval(coefficients, x_fit);  % Calculate y values using the line equation
174 -   plot(x_fit, y_fit, 'r-', 'LineWidth', 2);
175
176 -   equation = sprintf('y = %.2f * x + %.2f', coefficients(1), coefficients(2));
177 -   text(min(x), max(y), equation, 'HorizontalAlignment', 'left', 'VerticalAlignment', 'top');
178 -   text(min(x), max(y)-0.1*(max(y)-min(y)), sprintf('R^2 value: %.2f', R_squared), 'HorizontalAlignment', '
179 -   hold off;
180
181     %% Rewrite function for absolute sodium concentration
182 -   a = coefficients(1);
183 -   b = coefficients(2);
184
185     %%  Translate signal intensity to absolute sodium concentration
186 -   transformed_image = (data_image - b) / a;
187 -   figure;
188 -   imshow(transformed_image);
189
190     %Add a colorbar and customize the colorbar
191 -   colorbar;
192 -   caxis([min(transformed_image(:)), max(transformed_image(:))]);
193
194     %Enable interactive display of pixel information
195 -   impixelinfo;
196
```

```
%% Specify proton DICOM file
[file_nameH, folder_pathH] = uigetfile('*.dcm', 'Choose a DICOM file');

% Check if the file selection was canceled
if file_nameH == 0
    fprintf('File selection was canceled.\n');
    return;
end

% Construct the full file path
file_pathH = fullfile(folder_pathH, file_nameH);

% Read the selected DICOM file
data_selected_dicomH = double(dicomread(file_pathH));

%% Resize sodium scan to same dimension as proton scan
resized_natrium = imresize(transformed_image, size(data_selected_dicomH));
resized_natrium = squeeze(resized_natrium(:,:));

%% ROI tekenen in proton beeld en signaal meten van absolute natrium concentratie
rois = cell(0);
meanValues = [];
stdValues = [];
%Allow to draw ROIs

figure;
imshow(squeeze(data_selected_dicomH(:,:)),[]) %% AANGEPAST
roiCount = 0;
rois = cell(0);
meanValues = [];
stdValues = [];

while true
    % Allow to draw a custom ROI
    h = imfreehand;
    position = wait(h);
    % Create a binary mask based on the drawn ROI, store the ROI and mask
    roiMask = createMask(h);
    rois{end+1} = roiMask;

    % Extract the region of interest of sodium scan based on the ROI mask of proton scan
    roiImage = resized_natrium .* roiMask;

    meanValue = mean(roiImage(roiMask));
    meanValues(end+1) = meanValue;
    disp(['Mean value of ROI ', num2str(numel(meanValues)), ': ', num2str(meanValue)]);

    stdValue = std(roiImage(roiMask));
    stdValues(end+1) = stdValue;

    disp(['Standard deviation of mean value of ROI ', num2str(numel(stdValues)), ': ', num2str(stdValue)

    roiCount = roiCount + 1; % Increment the ROI count

    roiFileName = ['ROI_', num2str(roiCount-1), '.mat'];
    roiMatrix = double(roiImage);
    save(roiFileName, 'roiMatrix');

    % Ask if you want to draw more ROIs
    choice = questdlg('Do you want to draw more ROIs?', 'Draw More ROIs', 'Yes', 'No', 'No');
    if strcmp(choice, 'No')
        break;
    end

end

save('mean_values.mat', 'meanValues');

%% Histogram of absolute concentration from ROI
% Plot the histograms of the ROIs
figure;
numRois = numel(rois);

maxConcentration = -Inf; % Initialize the maximum concentration
```

```
269 -    maxFrequency = -inf; % Initialize the maximum frequency
270
271 -    for i = 1:numRois
272 -        subplot(1, numRois, i);
273 -        roiMask = rois{i};
274 -        roiImage = resized_natrium .* roiMask;
275 -        roiMatrixNoZeros = roiImage(roiImage ~= 0);
276
277 -        maxConcentration = max(maxConcentration, max(roiMatrixNoZeros));
278 -        [frequencies2, ~] = histcounts(roiMatrixNoZeros);
279 -        maxFrequency = max(maxFrequency, max(frequencies2));
280
281 -        histogram(roiMatrixNoZeros,'BinWidth',2);
282
283 -        title(['Histogram van ROI ', num2str(i)]);
284 -        xlabel('Natriumconcentration (mM)');
285 -        ylabel('Frequentie');
286 -    end
287
288      % Set the same y-axis limits for all histograms
289 -    for i = 1:numRois
290 -        subplot(1, numRois, i);
291 -        ylim([0, maxFrequency+250]);
292 -    end
```

```
293
294      % Set the same x-axis limits for all histograms
295 -    for i = 1:numRois
296 -        subplot(1, numRois, i);
297 -        xlim([0, maxConcentration+5]);
298 -    end
299
300 -    sgtitle('Histogram van absolute natriumconcentratie');
301      %% Convert signal intensity to an absolute sodium concentration map with colorbar
302
303 -    figure;
304 -    imshow(transformed_image, [0 60]);
305
306 -    colorbar;
307 -    colormap(jet)
```