EVALUATION OF AN MRI METHOD TO DETERMINE HYDRATION STATES OF TENDONS

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As water content is a determinant of the material properties of tendons and may affect sports performance and injury risk. The purpose of this pilot study was to evaluate the reliability and sensitivity of an MRI based method to quantify the hydration state of a tendon. For this study twenty porcine digital flexor tendons were utilized. All samples were examined on a MR scanner using three 3D ultra-short echo time sequences. With the applied sequences it was possible to determine a decrease in water content of the tendons. In addition, the methods showed good inter session reliability. Further investigations are needed to improve the upper and lower limit of resolution regarding the physiological hydration state.

KEYWORDS: tendon, hydration, weight cutting, injury prevention, MRI

INTRODUCTION: In sports, as well as activities of daily living, the human body continuously changes the position of its centre of mass, by transferring muscle force via a tendon to the skeletal system. The mechanical properties of the tendons and thus the spring-like behaviour can have a profound effect on the performance and effectiveness of this mechanical chain. Besides the collagen, which is organized in a hierarchic manner, the hydration state also contributes to the tendon's tensile and compressive strength.
While water is the main component of the entire human body (60%-65%) and critical for tissue properties, it is also the component of choice for rapid sports discipline-related weight reduction. In disciplines like running, high and long jumping, body weight is of great importance and limits sport performance. An indirect advantage exists in disciplines like weightlifting, rowing, and martial arts (e.g. judo, boxing wrestling), because athletes are categorized in weight classes and competitors try to be in the upper range of their class to obtain a physical advantage. Many athletes continuously monitor, and if 'necessary' adjust, body weight in a minimum of time. During 'weight cutting', experienced athletes can easily reduce their body mass by 10% in a week. Although such un-physiological weight loss prior to a sporting competition is very common in the world of elite sport, the proportion of water loss in the tendon itself and its consequences on the strength and elasticity of the tendons have never been investigated. Due to the fact that water content is a determinant of the material properties of tendons and therefore might affect sports performance and injury risk, the purpose of this pilot study was to evaluate the reliability and sensitivity of a MRI based method to quantify water content of a tendon.

METHODS: For this pilot study eight porcine digital flexor tendons were obtained. All tendons were dissected from mature pigs sourced from a commercial abattoir. To ensure that the experiments were conducted with fresh tissue, it was important to

prepare it shortly after slaughtering (Du et al. 2009). To prevent dehydration and therefore tissue alteration the limbs were refrigerated prior to dissection. The preparation of the tendon is shown in Figure 1a-d. The tendon was separated from the foot at the distal interphalangeal joint and the flexor digitorum superficialis was cut away from the rest of the digital flexor. Finally, the digital flexor tendons were cut at their origin with the deep digital flexor and at their distal end where it is attached to the distal phalange. During the dissection process the tendon was periodically treated with sodium chloride (NaCl, 0.9% solution) to maintain its moisture (Grosse et al. 2009). After preparation, the tendon was soaked in NaCl, wrapped in gauze bandage and aluminum foil and was frozen at -18° C. Prior to subsequent testing, each tendon was taken from the foil, soaked again in NaCl to thaw at room temperature (Longo et al. 2009, Kristiansen et al. 2014). Attention was paid to choose tendons of similar anatomical structure. All tendons were cut uniformly to a length of approximately 7 cm and to avoid evaporation, tendons were wrapped again into foil and got stored in small sealed tubes at 2° C.

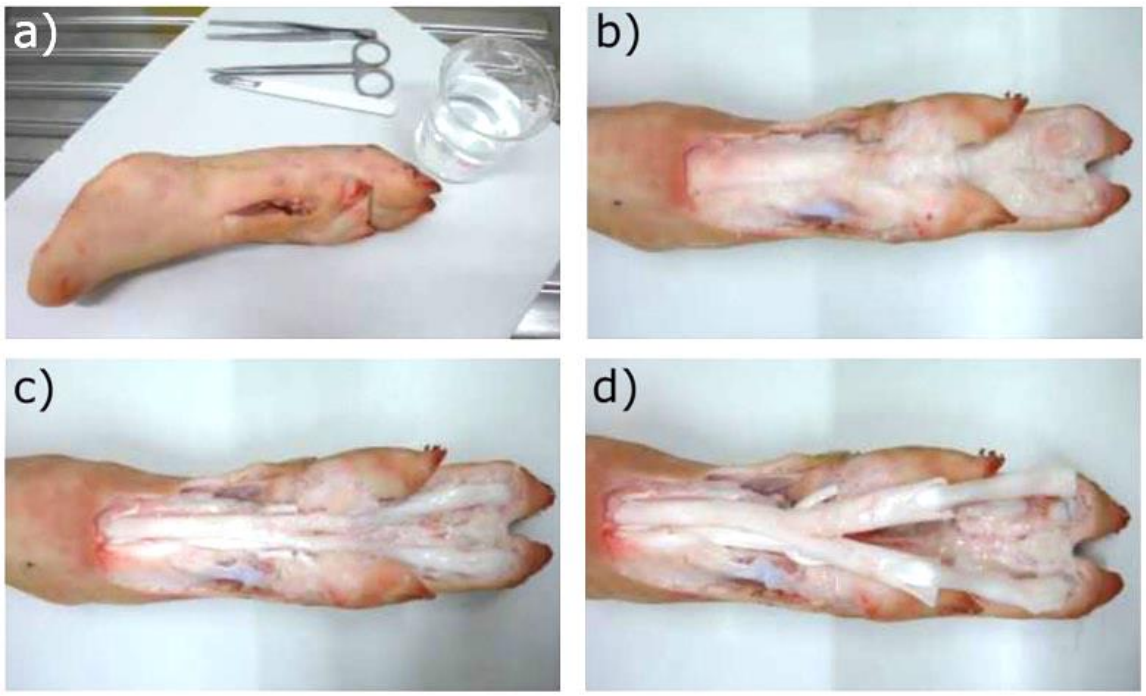

Figure 1: Process of tendon dissection: a) pig trotter before dissection; b) skin and subcutaneous tissue removing; c) digital flexor tendon exposing; d) digital flexor tendon removing from the rest of big trotter.

For MRI measurements a phantom was designed to be small enough to fit in the knee coil of the MR scanner. As shown in Figure 2a-b the designed phantom was made of water-like gel pads (Sonogel, Sonokit Soft 100 mm x 100 mm x 10 mm) and the tendons were alternatively layered between gel pads, and stabilized with thin plastic plates and rubber bands. For internal stability it tendons were staggered as shown in Figure 2c.

Figure 2: Eight tendons: a) alternately layered between water like gel pads; b) stabilized through thin plastic plates and rubber bands; c) cross section sketch of phantom.

All samples were examined on a MR scanner (Philips, Ingenia 3.0T) using a knee coil for signal reception and transmission. For all scans, DICOM images with a reconstructed matrix size of 192×192 were processed. Each scanning session consisted of three 3D ultra-short echo time (UTE) sequences (field of view (FOV) of 192 mm, isotropic spatial resolution 1.0 mm, 435 time of repetition (TR) of 15 ms, echo time (TE) 0.14 ms and a bandwidth of 942 Hz/pixel).
To achieve a maximum tendon signal, a B_0 and B_1 mapping was included in every session and image flip angle was set to 12°
For reproducibility testing eight tendons were dried within a 15 min. drying treatment (hot air) to obtain an initial reduction of mass of about 10-15% as a individual baseline for every tendon. The first and second scanning session were executed without moving the phantom. To avoid heating induced errors and during a 5 minute break between the two scans, the phantom's temperature was checked using an infra-red thermometer (Etekcity, Lasergrip 774). Third scanning session (repositioned phantom) was performed after another 5 minutes break.
For the hydration state measurements five scanning sessions were necessary. The initial scan with a reduction in mass of 0% was followed by four additional drying treatments to reduce additional mass to a total of 4%, 8% 12% and 16%, where the percentage of mass reduction was normalized to the tendons' initial mass.
All tendons were segmented (mid of tendon $\pm$ 20 frames) and cross-sectional areas and OSR values were determined (Matlab, Mathworks 2016) according to Syha et al. (2014) and Grosse et al. (2015).
For both, reproducibility and hydration state measurement, Gaussian normal distribution was verified with the D'Agostino-Pearson normally test. An one-way ANOVA for multiple comparisons was performed to test for statistically significant (α=0.05) changes in water content, and where significant effects were found a Bonferroni post-hoc test was conducted. Reproducibility was evaluated using a paired t-test. Pearsons' correlation coefficient was calculated to measure the linear correlation between variables. Statistical outlies were identified and removed by the ROUT method, which combines robust non-linear regression and outlier removal. Coefficient Q, which determines how aggressively the ROUT method defines outlines, was set to 1%.

RESULTS:
For the reproducibility measurement (no repositioning), a significant correlation for both frequencies, 2 kHz (r=0.989) and 3 kHz (r=0.985) was found (Figure 3). For the repositioned condition, which aims to mimic an *in-vivo* pre-post testing session of an athlete, a correlation coefficient of r=0.946 for 2 kHz and r=0.768 for 3 kHz where found.
The change in tendon OSR (ΔOSR) values for the 41 MRI frames per tendon (mid of tendon $\pm$ 20 frames), showed no significant differences for the 2 kHz protocol.
The 3 kHz protocol only showed significant differences between the initial baseline (0% mass reduction) and the 12% mass reduction condition. Even a more mass reduced and therefore dryer condition (16%) did not show any significant differences in ΔOSR values. The analysed cross sectional areas of every tendon corresponded to approximately 65%-70% of the entire tendon length.

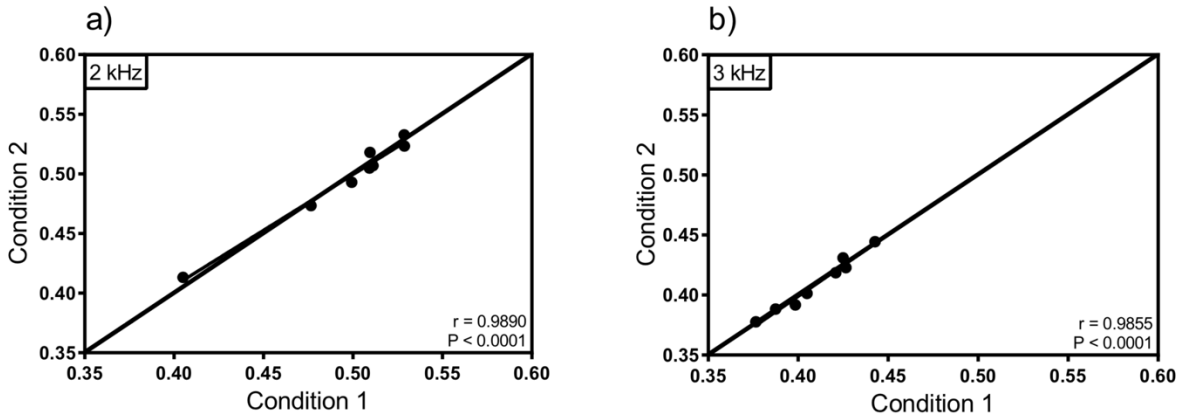

Figure 3: Correlation of condition 1 (OSR of tendons in first position) and condition 2 (OSR of tendons in first position) - all tendons have almost the same hydration state: a) 2 kHz and b) 3 kHz including line of identity and linear regression.

DISCUSSION and CONCLUSION: Based on the twofold methodological approach of this study it has been clearly shown that the investigated phantom-tendon combination delivers the desired results in terms of reproducibility. The first aim of the study could be confirmed, however, the second assumption regarding the detection of the change in water content of a tendon did not show clear results. In general, it was possible to measure a decrease in water content of the tendons (0% to 12%), but the expected measuring limit could not be identified. Additionally, the evaluation of the manual drying process of the tendons also showed a highly linear course of all tendons across all four mass reduced conditions.
This might imply that the used phantom, as well as the manual drying method employed were sufficiently accurate, but the technical limitation of the MR scanner (e.g. spatial resolution), the used scanning protocol and/or the used biological structure itself (e.g. limited cross sectional area of porcine tendons), might be responsible for the unexpected results. Based on these methodological results (phantom with gel pads and drying process), an *in-vivo* study (active metabolism), with an altered MRI protocol might get clearer results regarding the relationship of whole body water loss to tendinous water loss. Further investigations are needed to verify the limits of water content determination of tendons, to better monitor the risk of injuries during weight cutting periods.

REFERENCES

Du, J., Takahashi, A. M., Bydder, M., Chung, C. B., & Bydder, G. M. (2009). Ultrashort TE imaging with off-resonance saturation contrast (UTE-OSC). *Magnetic resonance in medicine*, *62*(2), 527-531.

Grosse U., Syha R., Partovi S. etal. Intradiurnal Fluctuations of Off-Resonance Saturation Effects in Healthy Human Achilles Tendons Assessed with a 3D Ultrashort Echo Time MRI Sequence at 3 Tesla. Fortschr Röntgenstr 2015; 187: 1003–1010

Longo, U. G., Ronga, M., Maffulli, N. (2009). Achilles tendinopathy.*Sports medicine and arthroscopy review*,*17*(2), 112-126.

Kristiansen, M. S., Uhrbrand, A., Hansen, M., Shiguetomi-Medina, J. M., Vissing, K., Stødkilde-Jørgensen, H., & Langberg, H. (2014). Concomitant changes in cross-sectional area and water content in skeletal muscle after resistance exercise. *Scandinavian journal of medicine & science in sports*,*24*(4).

Syha, R., Springer, F., Grözinger, G., Würslin, C., Ipach, I., Ketelsen, D., Schick, F. (2014). Short-term exercise-induced changes in hydration state of healthy achilles tendons can be visualized by effects of off-resonant radiofrequency saturation in a three-dimensional ultrashort echo time MRI sequence applied at 3T. *Journal of Magnetic Resonance Imaging*, *40*(6), 1400-1407.