Intradiurnal Fluctuations of Off-Resonance Saturation Effects in Healthy Human Achilles Tendons Assessed with a 3D Ultrashort Echo Time MRI Sequence at 3 Tesla

Tageszeitabhängige Schwankungen von Magnetisierungstransfer- (MT-) Effekten in Achillessehnen von gesunden Probanden: Quantitative Auswertung mittels einer 3D-Ultrashort-echo-time Sequenz bei 3T

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Key words
tendons
MR imaging
tissue characterization

Abstract

Purpose: The purpose of this study was to evaluate whether gravitational interstitial fluid accumulation in healthy subjects has an impact on off-resonance saturation ratios (OSR) or the volume of the Achilles tendon after a prolonged time of reduced levels of physical activity.

Materials and Methods: 7 healthy volunteers were repeatedly investigated on 3 consecutive days on a 3 T whole body MR scanner using an ultrashort echo time (UTE) imaging sequence with a Gaussian off-resonance saturation pulse at a frequency offset of 2000 Hz to calculate OSR values. For accurate volumetric quantification of the Achilles tendon, a newly developed contour detection snake algorithm was applied on high-resolution isotropic T2 SPACE sequences. Single-measure intraclass correlation coefficients (ICC) were calculated to estimate test-retest reliability.

Results: For OSR and tendon volume measurements on three consecutive days, excellent reproducibility could be achieved with ICC values above 0.96 and 0.97, respectively. Comparing the results of all three days, a statistically significant mean individual percentage decrease (−4.1 ± 1.5%; p = 0.001) of calculated tendon OSR values was found for the evening measurements. No statistically significant difference between tendon volumes in the morning and the evening could be detected (p = 0.589).

Conclusion: The results of this in-vivo study demonstrate a significant influence of gravitational interstitial fluid accumulation after reduced physical activity on OSR values in the Achilles tendon, but not on tendon volume. Taken together with the demonstrated excellent reproducibility, these findings are important for future studies investigating temporal changes of the Achilles tendon microstructure.

Zusammenfassung

Ziel: In dieser Studie an gesunden Probanden sollten die Auswirkungen des orthostatischen Ödems durch reduzierte körperliche Aktivität auf intratendinöse Magnetisierungstransfer- (MT-) Effekte (oder allgemeiner Off-Resonanz-Sättigungs-Relationen („OSR“)) und das Volumen der Achillessehne untersucht werden.


Ergebnisse: Sowohl für die OSR-Werte als auch das Sehnenvolumen konnte eine ermittelte Reproduzierbarkeit mit ICC-Werten größer 0,96 bzw. 0,97 über die drei untersuchten aufeinander folgenden Messstage gezeigt werden. Bei der Betrachtung aller Messzeitpunkte wurde eine statistisch signifikante Abnahme der intratendinösen OSR-Werte (−4,1 ± 1,5% ; p = 0,001) am Abend gefunden, während das ermittelte Sehnenvolumen hier keine signifikante Änderung zeigte (p = 0,589).

Chronic degenerative alteration of the tendon structure (tendinopathy) is a main risk factor for tendon rupture and responsible for marked morbidity, both in physically active and sedentary populations [1–3]. Due to a better understanding of the pathogenesis of tendinopathy, recent research in magnetic resonance (MR) as well as ultrasound imaging has focused on quantitative and semi-quantitative assessment of healthy tendon structures in comparison to early-stage tendon disorders before subsequent partial or complete tendon tears in more advanced stages of tendon disorders occur [4–9]. In this context, various MR specific parameters (namely longitudinal relaxation time (T1), mono- or multi-exponential analysis of reverse relaxation times (T2*), magnetization transfer (MT) effects or the previously described more generic “off-resonance saturation ratios” (OSR)) have been used in vivo as well as ex vivo to quantitatively evaluate healthy Achilles tendons as well as early stages of tendinopathic tendons [5, 7–15]. T2* and particularly OSR values have proven to be highly sensitive to detect early pathological alterations of the tendon structure, being characterized by an increase of free water protons and breakdown of the highly organized macromolecular content [14]. It is well known that even in healthy subjects (with a fully functional phlebo-lymphatic apparatus), fluids and macromolecules accumulate in the phlebo-lymphatic sector as well as the interstitium after a prolonged time of standing or sitting [16, 17]. This gravitational interstitial fluid accumulation has the potential to influence highly sensitive and quantitative measures such as OSR or tendon volume. Therefore, the aim of this study was to evaluate whether occupational leg swelling in healthy subjects has an impact on the calculated OSR values or the volume of the Achilles tendon after a prolonged time of reduced levels of physical activity.

Key Points:
- Tendon volume measurements and intratendinous OSR-values demonstrated a high day-to-day reproducibility.
- Gravitational fluid shifts might partly explain the known variation of OSR-measurements in healthy tendons.
- Physiological interstitial fluid shifts of the lower extremity can influence highly sensitive quantitative MR-parameters (as for example in this study used OSR-measurements) even in healthy individuals.

Materials and Methods

Study Population and Inclusion/Exclusion Criteria

The institutional review board approved the study and written informed consent was obtained from all healthy volunteers prior to enrollment. Seven healthy volunteers were enrolled in this study. Common exclusion criteria for MR examinations were applied to all participants, including ferromagnetic implants, pacemakers and claustrophobia. Further exclusion criteria were similar to previously conducted studies and included previous injuries or operations at the ankle, any tendinopathic tendon alterations as well as partial or complete tears [5]. Prior to the start of the study, all participants were clinically examined and a dynamic ultrasound examination of both Achilles tendons was performed to solely exclude pathological tendon alterations (U.G., 4 years of experience in musculoskeletal ultrasound). Using a multi-D linear array transducer (Acuson Antares, VFX 13–5, Siemens Healthcare, Mountain View, CA), the Achilles tendon was evaluated with dynamic imaging and power Doppler ultrasound (standardized region of interest [ROI] settings: ROI 1.5 × 2.0 cm², focus 2 cm) in longitudinal and transverse orientation. The results of this ultrasound examination are not further evaluated in this article. Furthermore, common risk factors associated with Achilles tendon disorders (rheumatoid arthritis or gout, hypercholesterolemia treated with medication and use of fluorquinolones in the past two years) were also regarded as exclusion criteria. In addition, subjects should neither suffer from clinically evident edema or varicose veins of the lower limb nor report a past medical history of deep venous thrombosis in the lower leg.

All participants refrained from ankle-loading physical activity for at least 72 hours prior to the examination and during the three days of the intradiurnal MR measurements. Participants were examined on three consecutive days in the morning at 7 a.m. before heading to work (specific care was taken that ankle-loading activity prior to the MR examination was kept to the lowest level possible) and at the end of a full working day at 6 p.m. All investigated subjects (resident physicians working in the department of radiology at conventional diagnostic workplaces) reported similarly reduced levels of physical activity during working time with a predominantly sedentary occupation.

Case Record Form (CRF) and Clinical Examination Protocol

Results of clinical examination, anthropometric data and past medical history of all participants were documented using a standardized case record form (CRF). In addition, the last performed physical activity as well as regular sports activities were documented and evaluated regarding their influence on the ankle joint using the ankle activity score (AAS).

MR Examination

The right Achilles tendon of all participants was examined using the posterior part of a 32-channel head coil (3 T Siemens TIM Trio, Siemens Healthcare, Erlangen, Germany) for signal reception, while the body coil of the 3 T whole-body scanner (Magne-
tom Trio, Siemens Healthcare, Erlangen, Germany) was used for RF transmission.

All subjects were positioned supine feet first on the examination table. Planter flexion of the foot within the posterior part of the head coil was adjusted to 10°-15° and maintained throughout the examination by placing sand pads around the ankle. The total examination time was approximately 25 minutes.

Morphological MR imaging of the Achilles tendon included an axially oriented nearly PDw 3D FSE sequence with an isotropic resolution of 0.8 mm (TR = 1200 ms, TE = 12 ms, FA = 120°, FOV = 192 × 192 mm², receiver bandwidth 545 Hz/pixel, number of averages: 1) and a sagittal T1w 2D sequence with an in-plane resolution of 0.6 × 0.5 mm and a slice thickness of 3 mm (TR = 750 ms, TE = 12 ms, FA = 140°, number of averages = 1).

For quantification of tendon volume, an axially oriented T2w 3D fast spin echo (FSE) sequence with an isotropic resolution of 0.8 mm (TR = 3200 ms, TE = 359 ms, FA = 120°, FOV = 192 × 192 mm², receiver bandwidth = 745 Hz/pixel, number of averages = 1) was used.

To quantify off-resonance saturation effects of the Achilles tendons, a 3D ultrashort echo time (UTE) sequence with radial k-space sampling (FOV = 192 mm, isotropic spatial resolution = 1.0 mm, TR = 11 ms, TE = 0.07 ms, excitation flip angle = 12°; bandwidth = 2370 Hz/pixel) was applied. The minimal echo time (TE) was 0.07 ms (time interval between the center of the rectangular RF pulse and the time when central k-space data are recorded) and is given by the sum of half the RF pulse duration (½ TRF = 0.05 ms) and the switching time of the RF system from transmit to receive mode (0.02 ms). For analysis of the tendon signal reduction due to off-resonance saturation (leading to both magnetization transfer and direct saturation effects), a 5120 ms Gaussian-shaped saturation pulse, i.e., magnetization transfer (MT) pulse, was additionally applied prior to each excitation pulse at an off-resonance frequency of 2000 Hz up-field of the normal resonance frequency at 3T (approximately 123 244 MHz). No parallel imaging techniques were used and further imaging parameters of the applied 3D UTE sequence are given in Table 1.

Off-resonance saturation ratio (OSR) maps were calculated pixel-wise, similarly to the commonly used MT ratio with the “Image Calculator” plugin of the image processing software ImageJ (NIH, Bethesda, MD). This was done according to equation (1), where S0 denotes the pixel intensity in an image acquired with an off-resonance saturation pulse at 2000 Hz, while S0 represents the pixel intensity in an image acquired without a preparation pulse:

$$\text{OSR} = \frac{S_0 - S_{\text{corr}}}{S_0}$$

Data Analysis

Before the pixelwise analysis, image data with and without off-resonance saturation pulse was motion-corrected with the realignment and re-slice tool of the MATLAB software package Statistical Parametric Mapping 8 (Matlab R2011b, The Mathworks inc., Matick, MA; SPM 8) using a least squares approach and a six parameter rigid-body spatial transformation with fourth degree B-spline interpolation.

Similar to previously performed studies, a quadratic noise correction (2) was applied on the measured signal intensities before data evaluation according to the following equation, where Scorr and Suncorr represent the noise corrected and uncorrected signal intensity, respectively [18]:

$$S_{\text{corr}} = \sqrt{S_{\text{uncorr}}^2 - (S_{\text{noise}})^2}$$

Snoise represents the arithmetic mean of the noise background and was calculated from a region of interest (ROI) containing approximately 100 pixels in an object-free homogeneous area without any visible artifacts. It should be mentioned here that the noise level was uniform in all parts of MR images, since no parallel imaging techniques were applied.

In the resulting OSR maps, representative ROIs containing the whole tendon cross-sectional area were analyzed at the following locations: 0.5 cm (“caudal mid-portion area”, [CA]) and 3.0 cm above the most cranial part of the tuber calcanei (“cranial mid-portion area”, [CR], i.e. 5 to 6 cm cranial of the insertional area depending on tendon length). At each location, five consecutive slices were used to calculate the mean OSR values using ImageJ.

Multiplanar reconstructions (MPR) of the T2-weighted 3D SPACE sequence with a slice thickness of 0.8 mm were used to assess the tendon volume of the Achilles tendon. An automated contour detection algorithm (AVAT), which was self-written using Matlab (Matlab 7.3.0, The Mathworks Inc., Natick, MA) and based on an established automatic procedure with an implemented snake algorithm (active contour model), was used to assess the volume of the Achilles tendon [mm3] [19, 20]. Measurements started from the cranial border of the calcaneal tuberosity and the fixed end point of the measurement in all volunteers was 3 cm proximal to the starting point (at the site of the cranial OSR measurement). Thus, the tendon volume measurement included the representative mid-portion of the tendon, which is mostly affected by tendon disorders. The starting point was set by an experienced radiologist (RS: 6 years of experience in musculoskeletal imaging). All further contour detection was subsequently performed automatically. Results of the automatic contour detection algorithm were visually approved and manually corrected as indicated.

Table 1 Imaging parameters of the applied 3D ultrashort echo time (UTE) sequence with MT preparation pulse at 2000 Hz off-resonance are listed.

<table>
<thead>
<tr>
<th>parameters</th>
<th>values</th>
</tr>
</thead>
<tbody>
<tr>
<td>echo time (TE)</td>
<td>0.07 ms</td>
</tr>
<tr>
<td>time of repetition (TR)</td>
<td>11 ms</td>
</tr>
<tr>
<td>field of view (FOV)</td>
<td>192 mm</td>
</tr>
<tr>
<td>resolution</td>
<td>1 mm isotropic</td>
</tr>
<tr>
<td>readout bandwidth</td>
<td>2370 Hz/pixel</td>
</tr>
<tr>
<td>projections</td>
<td>18 432</td>
</tr>
<tr>
<td>excitation flip angle</td>
<td>12°</td>
</tr>
<tr>
<td>excitation pulse duration</td>
<td>100 µs</td>
</tr>
<tr>
<td>MT pulse: Off-resonance frequency</td>
<td>2000 Hz</td>
</tr>
<tr>
<td>MT pulse: Duration</td>
<td>5120 µs</td>
</tr>
<tr>
<td>MT pulse: Flip angle</td>
<td>220°</td>
</tr>
<tr>
<td>acquisition time</td>
<td>3:23 min</td>
</tr>
</tbody>
</table>
bility of OSR values and tendon volume in the morning and in the evening.

A repeated-measures univariate analysis of variance (ANOVA) was performed to test for a statistically significant influence of the time of measurement (morning or evening), tendon section (CR or CA) or measurement repetition on OSR values and tendon volume. Bonferroni correction was performed for multiple testing and a corrected p-value of p < 0.05 was considered significant.

Results ▼

All seven participants (all male; age at examination: 30.4 ± 2.0; BMI 22.8 ± 1.5 kg/m²) did not report any episode of posterior heel pain in their past. Neither clinical examination nor dynamic ultrasound examination revealed any signs of an Achilles tendon disorder. Regular moderate physical activity of all participants resulted in an AAS score of 4.9 ± 0.9. According to inclusion/exclusion criteria of this study, all participants reported no ankle loading activities during the last 7 days prior to the examination. In contrast to the OSR measurements, there was no statistically significant difference (p = 0.589) between tendon volume in the morning and the evening (Fig. 1 and Table 2).

Discussion ▼

The results of this in-vivo study demonstrate a significant influence of gravitational interstitial fluid accumulation after 11 hours of reduced physical activity on OSR values, but not on tendon volume. This represents an important finding for future studies investigating temporal changes of the Achilles tendon microstructure using magnetization transfer effects.

Several previous studies used the possibility of obtaining signal from healthy tendinous tissue by using UTE sequences and thereby investigated the Achilles tendon microstructure with different MR-specific parameters such as T1, mono or multi-exponential T2 relaxation times and magnetization transfer effects (Fig. 2 and Table 2). A statistically significant influence on OSR measurements was demonstrated a better sensitivity and specificity for detecting mild and severe stages of tendinopathy when compared to mono-exponential T2 and particularly when compared to T1 relaxation times [14]. Furthermore, OSR measurements at 2000 Hz off-resonance showed the highest repeatability compared to OSR values at other off-resonance frequencies [20]. As a consequence, in this study a 2000 Hz off-resonance saturation in addition to tendon volume measurements was used to analyze potential intradiurnal changes of the tendon water content. Note in this context that both measurement parameters have already been successfully used in several previous published studies investigating tendinopathic tendon alterations [5, 9, 14, 19 – 21].

In the presented study, excellent reproducibility of OSR and tendon volume measurements on three consecutive days (separately for morning and evening measurements) was found. The highest reproducibility, i.e. highest ICC, was found for the morning OSR measurements. The ICC values for the tendon volume measurements in the evening were slightly lower, but still showed excellent reproducibility. This most likely demonstrates the repeated overnight return to normal tendon structure after a day of prolonged sedentary occupation. The demonstrated reproducibility of this quantitative MRI method has marked implications for future studies determining the tendon volume and OSR values.

These results are in accordance with previously published data by Syha et al., who reported excellent repeatability of OSR measurements after removing, repositioning and rescanning the same patient [20]. However, the slightly lower ICC values for the evening measurements may be attributed to a less standardized influence of a day-long predominantly sedentary occupation compared to a relatively standardized rest during sleep overnight. Nevertheless, the ICC for both OSR measurements and tendon volume also show good reproducibility with values around 0.96 (CI: 0.86 – 0.99). The OSR measurements in this study are within the range of previously reported measurement values for the mid-portion Achilles tendon area (for example Grosse et al. who investigated 8 healthy female as well as 9 healthy male participants and reported mean OSR values of 0.32 ± 0.03 at 2000 Hz off-resonance) [5]. However, it is worth mentioning that OSR values cannot be considered as absolute biomarkers and are dependent on several external sequence-specific as well as tissue-dependent factors [7]. Hence, the observed decrease of OSR values in the evening measurements can be either attributed to an increased amount
of free water protons or a decrease in the number of bound protons (consequently decreasing the OSR numerator) or a combination of both factors. In our opinion, the decrease of OSR values in the evening can be most likely explained by the infiltration of the Achilles tendon by free water protons caused by gravitational interstitial fluid accumulation. This hypothesis is in our view not contradicted by the fact that no statistically significant increase of tendon volume in the evening was observed, since the differences in tendon volume (e.g. swelling of the tendon accompanying the intra-tendinous fluid accumulation) might be too subtle to be depicted by measurements based on a 0.8 mm isotropic MR sequence. A change in the macromolecular content or in the amount of bound water protons is also less likely to be the cause, due to the repeated return to normal overnight. This finding is supported in Fig. 2, which shows, additionally to the decrease of the OSR in the Achilles tendon, a decrease of the OSR values in the surrounding dermis, known to feature marked magnetization transfer effects. This is in particular true for the epidermis and less marked for the subcutis [22].

It is important to notice that not only in pathological conditions, but also in healthy young subjects with a fully functional phlebo-lymphatic apparatus fluids and macromolecules are known to accumulate in the interstitium of the lower extremity after a prolonged time of standing or sitting [16, 17]. This has been extensively studied assessing the lower leg volume using either water plethysmography or other 3D measurement methods. For example, Noddeland et al. reported a 5.7% volume increase after 8 hours of sitting, while Tessari et al. stated absolute values and
An increase of the lower leg volume in participants after 8 hours of standing of about 87.5 to 90 ml was reported by Noddeland et al. in participants who keep their feet spontaneously active. A less marked increase of foot volume of 2.7% was reported by Noddeland et al. in participants who keep their feet spontaneously active.

### Table 2
Mean OSR values with standard deviations at 2000 Hz off-resonance averaged over all participants are given at the cranial and caudal mid-portion area as well as tendon volume (mean and standard deviation) for each of the consecutive measurement days (morning and evening measurements).

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning</td>
<td>Evening</td>
<td>Morning</td>
</tr>
<tr>
<td>OSR caudal (CA)</td>
<td>0.28 ± 0.03 (104 ± 18)</td>
<td>0.27 ± 0.03 (109 ± 14)</td>
<td>0.28 ± 0.03 (101 ± 15)</td>
</tr>
<tr>
<td>OSR cranial (CR)</td>
<td>0.30 ± 0.02 (55 ± 10)</td>
<td>0.29 ± 0.02 (53 ± 16)</td>
<td>0.30 ± 0.02 (53 ± 14)</td>
</tr>
<tr>
<td>Tendon volume [mm³]</td>
<td>2378 ± 406</td>
<td>2374 ± 369</td>
<td>2391 ± 386</td>
</tr>
</tbody>
</table>

For all OSR values the number of pixels in the used ROI for OSR calculation is given in brackets. Please note, that only the intra-individual course of OSR values of all participants was evaluated in the statistical analysis and showed a statistically significant difference between morning and evening measurements.

Zusätzlich steht in Klammern für alle OSR-Werte die gemittelte Anzahl an Pixeln mit Standardabweichung der benutzten ROI. Hierbei ist zu beachten, dass nur die intra-individuellen OSR-Veränderungen im Tagesverlauf einen statistisch signifikanten Unterschied zwischen morgendlichen und abendlichen Messergebnissen aufwiesen.

![Fig. 2](image1.png)

Fig. 2  Transverse OSR maps (2 kHz off-resonance; caudal to cranial from left to right and above to below) acquired in the morning and in the evening of a 31-year-old male participant (BMI 25 kg/m²) are shown. Note the slight decrease of OSR values of the Achilles tendon (Achilles tendon marked with white arrows) in the evening while at the same time a marked decrease of OSR values could be observed in the peritendinous tissue and the adjacent skin (skin marked with black arrowheads) resulting in a better delineation of the tendon.

![Abb. 2](image2.png)

Other authors additionally evaluated the influence of various degrees of exercise on foot volume and found an increase of foot volume of about 1% to 3% depending on the duration and type of activity [23]. Further studies investigated the positive effect of various compression stockings on occupational leg swelling [24, 25]. In addition to these volumetry-based studies, numerous MR studies also examined the morphological MR appearance of lymphedema or phlebothrombosis in the lower extremity [26–28]. Meier et al. reported a marked subcutaneous, perifascicular and even intramuscular fluid accumulation, but only used morphological MR sequences in patients with fluid overload [29]. However, to our knowledge no previous study quantitatively investigated the influence of gravitational interstitial fluid accumulation in tendinous tissue in the lower extremity. One might argue that the mean intra-individual decrease of OSR of 4.1% ± 1.5% in the evening without a significant increase in tendon volume is negligible, but this was demonstrated continuously in all seven volunteers and the results are within the range of previously reported exercise-induced differences in OSR values. For example, a study with comparable parameters by Syha et al. evaluating the effect of 6km cross-country running reported a mean increase of the absolute OSR value in the mid-portion area of the tendon of 0.029, which approximates about 9% [20]. The knowledge of these subtle natural fluctuations of water content in healthy tendons, either exercise-induced or due to prolonged sitting/standing during the day of examination, is a prerequisite for studies quantitatively evaluating the tendon microstructure. This study demonstrates the high sensitivity of 3D UTE sequences for detecting these effects and one has to keep in mind that this interstitial fluid accumulation might be even more pronounced in patients without a functional phlebo-lymphatic apparatus. In addition to the already known variation of tendon relaxation times between different tendon sections, future cross-sectional and longitudinal studies investigating temporal alterations of the tendon microstructure as well as early stages of tendon disorders should be aware of these subtle influencing factors, which might become evident already after a prolonged time of standing (more than 30 minutes) during the day of examination [23]. The main limitation of this pilot study is the relatively small sample size and the missing correlation of our data with lower leg volume measurement methods. Another limitation of the study is that only male participants were examined and OSR measurements were only performed at 2000 Hz off-resonance. Due to the demonstrated high repeatability/reproducibility of OSR measurements at this off-resonance frequency and the only minor observed intradiurnal OSR fluctuations in this study, it is unlikely that less sensitive parameters, such as mono-exponential T2* calculations, might be able to detect these subtle changes of the tendon microstructure. Nevertheless, future studies should investigate the time dependency of gravitational fluid shifts in the different tissues of the lower leg using OSR values, multi-exponential T2* calculations and lower leg volumetry. Additionally, we recommend the quantitative analysis of the tendon with a higher spatial resolution to investigate for possible regional differences.

In conclusion, intradiurnal gravitational fluid shifts in the lower leg after a prolonged time of a sedentary occupation do not seem to markedly affect tendon volume, but cause a decrease of the OSR values in tendinous tissues, which can be most likely explained by an accumulation of free water protons. Though subtle, these findings can be appreciated with 3D UTE sequences demonstrating its high sensitivity and might partly explain the variation of OSR measurements in healthy tendons in previous studies. Therefore, great care regarding both the time of measurement and the previous physical activity should be taken into account in future quantitative studies investigating the Achilles tendon by magnetization transfer effects and other quantitative parameters.

Clinical relevance

- The demonstrated high day-to-day reproducibility of tendon volume and intratendinous OSR values makes them a promising measurement tool for the analysis of temporal alterations of tendon microstructure.
- Gravitational fluid shifts in the lower leg might partly explain the variation of OSR measurements in healthy tendons in previous studies.
- Physiological interstitial fluid shifts even in healthy individuals can influence highly sensitive quantitative MR parameters such as the OSR measurements used in this study.

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