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MRI-biochemical follow up measurements of lumbar intervertebral disc in patients with leg length discrepancy: Is it possible to alter cartilage damage with conservative therapy?

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ABSTRACT

Objective: Effect of conservative therapy on intervertebral discs (IVD) in patients with leg-length-discrepancy (LLD).
M&M: Seventy lumbar IVDs of 14 participants (five with LLD 10–20 mm) were examined using a 3T-MRI-scanner. Morphological (Pfirrmann) and molecular (glycosaminoglycan-chemical-exchange-saturation-transfer, gagCEST) grading was assessed before and after a four-month therapy (physiotherapy and shoe inlays).
Results: Significantly lower GAG values in patients with LLD were found (L5/S1, $p = 0.02$). After therapy, a trend towards higher gagCEST values in patients with LLD was observed ($2.48 \pm 1.77\%$ vs. $1.79 \pm 0.79\%$; $p > 0.05$).
Conclusion: LLD represents a risk factor for molecular alterations of lumbar IVDs. Only minor effects of conservative therapy on these alterations could be found.

1. Introduction

Intervertebral disc (IVD) degeneration is a multifactorial disorder and associated with low back pain (LBP) that has become one of the most common diseases with a profound individual and socio-economic impact.[1] Multiple risk factors were identified for IVD degeneration like obesity, age, genetic predisposition or lifting.[1–4]

Leg length discrepancy (LLD) is also marked as a predisposing factor for IVD degeneration, functional changes of facet joints and LBP.[5–8] LLD is defined as a condition in which paired limbs are noticeably unequal.[9] It has been suggested to divide limb length inequality intro three categories: mild (0–30 mm), moderate (30–60 mm) and severe (> 60 mm).[9] Mild cases from 10 mm should be treated non-surgically with shoe inlays and physiotherapy. Moderate cases should be individually addressed and may be treated with surgical intervention, whereas severe LLD should be corrected surgically.[9] Previous studies confirmed relief of LLD-associated symptoms like LBP after therapy with shoe inlays for 3 respectively 4 months.[8,10] There is still no consent on the effects caused by LLD. However, there is evidence that untreated LLD can even lead to structural spinal damage such as scoliosis, concavities in the vertebral body endplates, wedging of the 5th lumbar vertebra and traction spurs.[11,12]

Glycosaminoglycan Chemical exchange saturation transfer (gagCEST) is a MR imaging technique without the need of intravenous contrast agent that allows the detection of early biochemical IVD alterations before morphological changes can be visible.[13–15] Contrast agent free imaging techniques have become even more important because gadolinium-based contrast agents (GBCA) potentially lead to gadolinium depositions in patients' brains.[16] Various authors independently found gadolinium depositions in the dentate nucleus and globus pallidus in patients that received GBCA.[17] For this reason, the European Medicine Agency (EMA) suspended several linear GBCA from human use. Even though macrocyclic GBCA are still approved for diagnostic use, their application should be strictly limited. Hence, there is a great and still rising need for MR imaging techniques that do not depend on GBCA. GagCEST imaging uses GAGs of the matrix of IVDs as an endogenous contrast agent.[15] GAGs are essential to maintain IVD tissue fluid, whereas a deficit of GAG is associated with one of the first steps in the development of IVD degeneration.[18]

The aim of our study is to investigate possible therapy effects on

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Abbreviations

AF	Anulus fibrosus
ASIS	Anterior superior iliac spine
CESL	GagCEST sequence using Spin-Lock technique
DJD	Degenerative joint disease
GAG	Glycosaminoglycans
GagCEST	Glycosaminoglycan chemical exchange saturation transfer
GBCA	Gadolinium-based contrast agent
GT	Great trochanter
IVD	Intervertebral disc
LLD	Leg length discrepancy
LBP	Low back pain
LM	Lateral malleolus
MM	Medial malleolus
MRI	Magnetic resonance imaging
NP	Nucleus pulposus
ROI	Region of interest
ROM	Range of motion
WASABI	Water saturation and B1

lumbar IVDs using gadolinium free molecular MR imaging, gagCEST, in patients with LLD.

2. Materials and methods

2.1. Subjects

The study was approved by the institutional review board and written informed consent was obtained from all individual participants included in the study. 14 participants (4 female; 10 male; mean age: 24.8 ± 3.73 years; range: 21–30 years) without any history of lumbar spine disease, examined by two orthopaedic surgeons with 5 and 7 years of experience in spine pathologies of our spine center, were prospectively included in this study. Five of 14 participants showed an LLD greater than 10 mm. All participants underwent a baseline and a follow-up 3T MRI scan after four months (average 4,9 months/152 days; minimum 96 days, maximum 235 days). The participants with LLD > 10 mm received conservative therapy for four months in the form of physiotherapy once a week and shoe implants for the site of the shorter leg to balance leg length discrepancy.

2.2. Physical examination

Each participant was examined by two orthopaedic surgeons using two clinical methods for LLD measurement: 1) An indirect method: visualizing the pelvic level using a spirit level (Beckenwasserwaage, Schein Orthopädie Service KG, Remscheid, Germany), which is clipped on the anterior superior iliac spine (ASIS). The degree of LLD is quantified by placing small heel lifts under the shorter leg. 2) A direct method: measuring LLD with measuring tape using bony landmarks. In this study, the distance from ASIS to the medial malleolus (MM) and ASIS to the lateral malleolus (LM) were evaluated. In order to exclude an apparent LLD caused by an asymmetric hypoplastic iliac bone, the distance from the greater trochanter (GT) to MM and GT to LM were determined. To exclude functional LLD caused by contractures, the range of motion (ROM) of the cervical-, thoracic-, lumbar-spine and of the lower limbs (hip-, knee-, upper-/lower-ankle joint), using a double-armed goniometer, was obtained.

2.3. Magnetic resonance imaging protocol: gagCEST and T2-weighted sequences

All participants were examined with a whole-body 3T MR system (Magnetom Trio, A Tim System, Siemens Healthineers, Forchheim, Germany) in supine position. For signal reception, four channel body matrix coils and a 24-channel spine matrix coil were used. The protocol included a localizer, a T2-weighted imaging in sagittal and transversal orientation. Biochemical imaging was performed with a novel gagCEST sequence using the Spin-Lock technique (CESL). WASABI (Water Saturation and B1) method was performed to correct B0 and B1 field inhomogenities.[19] For gagCEST imaging, one reference image without saturation and multiple images with presaturation pulses at different offset frequencies around the bulk water resonance were obtained. The residual signal normalized to the reference image as a function of the offset frequencies (z-spectrum) can be used to determine and quantify the CEST effect according to magnetization transfer asymmetry ratio (= spin-lock ratio; SLRasym) values with respect to the water resonance due to the OH protons of GAG (0.9–1.9 ppm) from the water resonance.[20] Table 1 and Table 2 give detailed information about the sequence parameters. To suppress artefacts caused by abdominal wall or bowel movement a saturation band was applied anterior to the spine.

Table 1
Detailed sequence parameters of T2-weighted images.

	T2-weighted imaging (sagittal)	T2-weighted imaging (transversal)
Sequence type	Turbo spin echo	Turbo spin echo
Turbo factor	31	18
TR/TE [ms]	3100/105	4510/113
Field of View (FOV) [mm^2]	300 × 300	240 × 240
In-pane resulution [mm^2]	1.2 × 1.2	0.8 × 0.6
Slice thickness [mm]	3.0	3.0
Flip angle [°]	160	140
Averages	2	1
Basic resolution	256 × 256	384 × 307
Number of slices	15	54
Acquisition duration [min:sec]	3:39	5:13

2.4. Data analysis

One board certified radiologist with six years of experience in musculoskeletal radiology blinded to the gagCEST values scored all lumbar intervertebral discs according to the Pfirrmann scoring system.[21] A region-of-interest (ROI) analysis was performed for SLRasym evaluation of the NP and annulus fibrosus AF. All ROIs were selected by an in-house developed automatic image processing algorithm based on the MATLAB software (The Mathworks, Inc., Natick, MA, R2012b[22]). The disc segmentation was based on Bayes classification to divide bone and ligament from disc tissue of the lumbar spine. Every automatically positioned ROI was visually checked by one radiologist with six years of experience in IVD segmentation, blinded to

Table 2
Detailed sequence parameters for spin-lock CEST (three pulses with B1 amplitude of 1.0, 1.5 and 2.0 μT) and B0-/B1-field inhomogeneity correction (WASABI).

		CEST	WASABI
T_R/T_E	[ms]/[ms]	14/3.64	14/3.64
Field of view	[mm^2]	300 × 300	300 × 300
In-plane resolution	[mm^2]	2.3 × 2.3	2.3 × 2.3
Slice thickness	[mm]	5	5
Flip angle	[°]	10	10
Averages		1	1
Basic resolution		128 × 128	128 × 128
Number of slices		1	1
Acquisition duration	[min:sec]	9:51	3:10

Pfirrmann classification analysis and clinical information. None of the ROIs was repositioned. For data analysis, an in-house developed MATLAB software (The Mathworks, Inc., Natick, MA, R2012b[23]) was generated. A reduction of image noise was performed using an in-plane 3 × 3 Gaussian filter. Z-spectra of the WASABI B0 and B1 maps were shifted pixel-wise according to the obtained frequency offset maps. SLRasym maps were calculated by averaging the asymmetry effect in the offset frequency range of GAG resonances (0.9–1.9 ppm). GAG values of the lumbar IVDs correspond to SLRasym and were given in %.[24]

2.5. Statistical analysis

Statistical analysis was performed using MATLAB (MathWorks, Natick, MA, R2015a). The mean and standard deviations for physical examination of LLD, NP- and AF-gagCEST were calculated. Morphological IVD grading was illustrated according to Pfirrmann score.[21] Kolmogorow-Smirnow-Lilliefors tests were used to assess normal distribution. Univariate analysis of variance (ANOVA) and Kruskal-Wallis tests were performed to assess statistical differences of the means of the gagCEST values. P values < 0.05 were assumed to be statistically significant.

3. Results

3.1. Physical examination

The average LLD was 12 mm ± 4 mm. No participant had contractures or a loss of ROM of the cervical-, thoracic-, lumbar-spine or of the lower limbs (hip-, knee-, upper-/lower-ankle joint).

3.2. Morphological IVD analysis

70 IVDs (L1 - S1) of 14 young participants were successfully imaged at baseline (T0) and after 4-month follow up (T1). No IVD had to be excluded due to motion artefacts. Morphological IVD grading according to Pfirrmann classification revealed at baseline MRI 20 IVDs with Pfirrmann score 1, 49 IVDs Pfirrmann grade 2 and 1 IVDs Pfirrmann grade 3. No degenerated discs, Pfirrmann grade 4 and 5, were found. In level L1/2, all 14 discs were scored Pfirrmann grade 2. In level L2/3 and L3/4, 4 discs were graded Pfirrmann score 1 and 10 IVDs were scored Pfirrmann grade 2. In level L4/5, 4 IVDs were scored Pfirrmann grade 1, 9 discs Pfirrmann grade 2 and 1 IVD Pfirrmann grade 3. In Level L5/S1, 2 IVDs were graded Pfirrmann score 1 and 12 discs Pfirrmann grade 2.

No significant difference in morphological Pfirrmann grading was found between LLD patients and healthy controls ($p > 0.05$) (Fig. 1). In follow-up measurements (T1) no changes in morphological Pfirrmann grading were found (Fig. 2).

3.3. Biochemical IVD analysis

At baseline (T0), mean NP-gagCEST values of L5/S1 were significantly lower in patients with LLD greater than 10 mm compared to participants without LLD (1.79 ± 0.78% vs. 3.93 ± 2.11%; $p = 0.02$). All other disc levels showed no significant difference between participants with and without LLD ($p > 0.05$). Additionally, no significant difference between the two groups was found for AF (Fig. 3).

At follow-up (T1), a trend to higher GAG values was found in participants with LLD compared to baseline measurements (2.48 ± 1.77% vs. 1.79 ± 0.79%; $p > 0.05$). Contrary to baseline analysis, after therapy, participants with LLD greater 10 mm showed no significant lower gagCEST values compared to participants without LLD (2.48 ± 1.77% vs. 3.82 ± 0.94%; $p = 0.17$) (Fig. 4).

4. Discussion

This study deals with the post-therapeutic changes of the intervertebral disc cartilage in subjects with LLD, as it is not yet clear to what extent therapy with shoe implants and physiotherapy influences cartilage integrity. In 2009 Burstein found higher GAG and proteoglycan values in patients with exercise intervention after surgery.[25] He used the biochemical MR imaging technique dGEMRIC (delayed gadolinium-enhanced magnetic resonance imaging of cartilage) that requires the application of intravenous GBCA. In this study we applied gagCEST, a modern non-invasive, contrast agent free diagnostic MRI technique for the evaluation of biochemical IVD degeneration. This is all the more important due to potentially negative effects of gadolinium-based contrast agent with brain deposits after intravenous administration.[17,26,27] With gagCEST imaging, it is possible to visualize low concentration molecules, for example GAGs, in the millimolar (mM) range by using the signal of water protons in the molar (M) range as an indirect sensor.[27,28] In IVDs, molecular GAG loss has been suspected to be a predictor of early biochemical IVD degeneration. Especially in the NP, prior morphological disc alterations are visible with conventional MRI.[14,18,22,29,30] This is in accordance with our results. In baseline and in follow-up measurements we did not find any significant difference in morphological disc analysis between participants with and without LLD. Furthermore, no IVD showed disc degeneration, scored Pfirrmann grade 4 or 5. However, in biochemical IVD analysis with gagCEST, we found significantly lower GAG values in participants with LLD > 10 mm compared to participants without LLD. These morphological healthy but molecular altered IVDs can be explained by the young age of our participants. Especially in early stages of IVD degeneration, only molecular imaging has the potential to visualize disc alterations.[14,31] Those molecular disc changes could only be illustrated in level L5/S1 in our study. The low average age of our subjects could be the cause, as IVD herniation of L5/S1 occurs at younger ages, whereas IVD herniation of L4/L5 and especially L3/L4 occur at older ages.[32] Tayler et al. made the conclusion that the proteoglycans of L5/S1 turned over faster than the proteoglycans of the adjacent lumbar discs because of its proximity to the rigid segment of the sacrum.[33] Arguably, this could be a reason why a compensation for LLD and pelvic obliquity is affecting L5/S1 first. To the best of our knowledge, there is no consensus regarding the amount of LLD that should be treated by physical therapy and shoe inlays.[34] Newer studies suggest that mild LLD

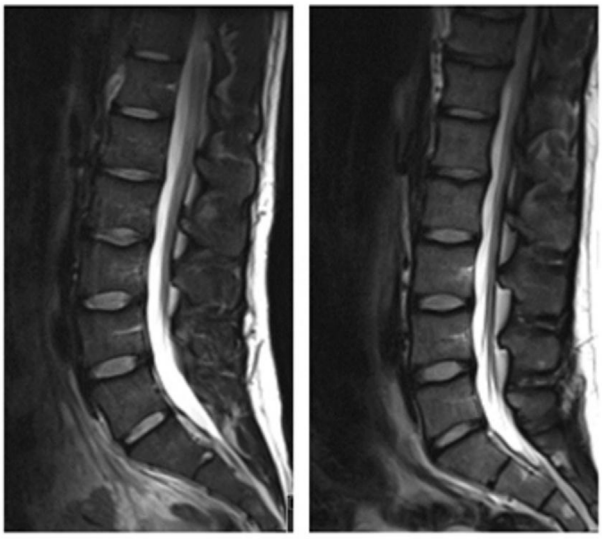

Fig. 1. T2-images at baseline of one exemplary patient of the control group (left) and one LLD patient (right). Pfirrmann grading illustrated no morphological disc degeneration with scores of 1 or 2.

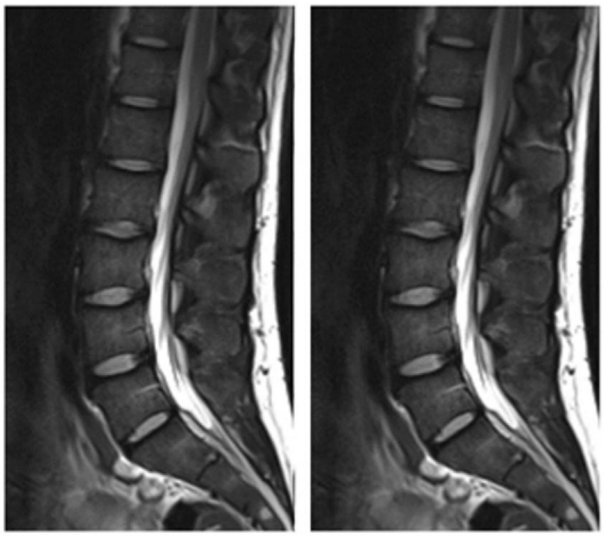

Fig. 2. T2-images at follow up of one patient of the control group (left) and one LLD patient (right).

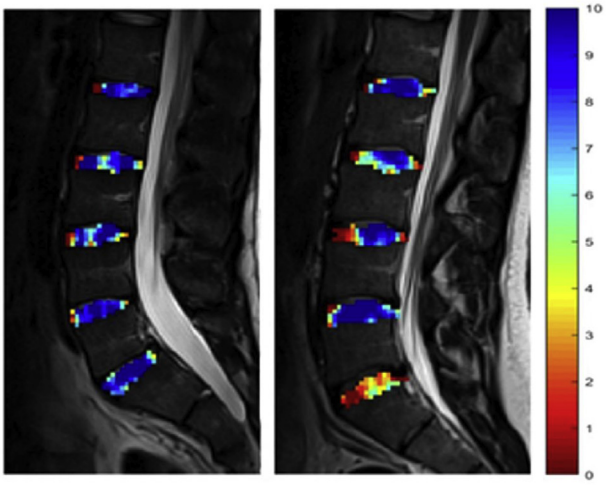

Fig. 3. GagCEST map with high GAG content in blue and low GAG content in red of the lumbar spine (L1 - S1) of a control group patient (left) and a LLD-patient (right). Molecular alteration of the lumbar disc on level L5/S1 are demonstrated with low GAG content in red and orange for the LLD-patient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(< 20 mm) causes compensatory changes during gait to compensate LLD. However, this strategy was unable to prevent effects of mild LLD on pelvic obliquity.[35] Harvey et al. demonstrated that LLD of 10 mm or more is associated with symptomatic knee osteoarthritis in the shorter leg[35,36]. In line with that, Defrin et al. showed that shoe inlays can significantly reduce pain intensity and functional disability in patient with LLD < 10 mm.[37] To our knowledge we were the first who prospectively demonstrated early molecular IVD alterations in participants with mild LLD. Interestingly our results showed positive therapy effects of shoe inlays and physiotherapy in patients with LLD > 10 mm. Therefore, we conclude, that there is a protective impact of shoe inlays and physiotherapy on the biochemical IVD integrity with a trend to higher GAG values and that it is possible to monitor LLD-patients with gagCEST-MRI under therapy. Conservative therapy seems to stop or delay the progress of lumbar IVD degeneration.

Concerning strengths and limitations: The main limitation is the limited number of participants. Nevertheless, the results of this study seem to be promising for further evaluation in a larger population. The

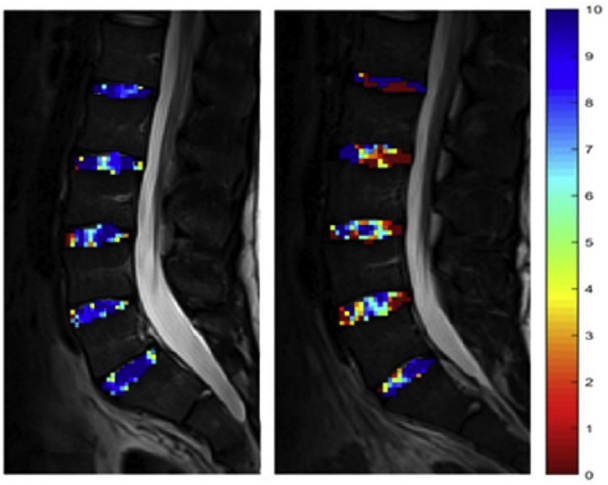

Fig. 4. GagCEST map at follow up of a control group patient (left) and a LLD-patient (right). With rising GagCest values in IVD L5/S1 in LLD patients after therapy, there is no significant difference between control group and LLD patients. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

second limitation is that LLD has only been determined by clinical and not by radiological investigations for radiation protection reasons. We minimized measurement errors by using two clinical methods for LLD assessment (indirect and direct method). Furthermore in daily clinical practice, physical examination is frequently used to determine the clinical LLD, and only in severe cases, radiological examination is mandatory.[38] Therefore, we think this is only a minor limitation. In this study, we tried to elucidate the effect of LLD on GAG content in lumbar IVDs as a predisposing factor for degeneration or even herniation. Therefore, a relatively homogeneous, young patient-collective was examined, since lumbar IVD herniations are uncommon in the first two decades of life, with a peak of prevalence in the fourth decade.[39] For gagCEST and Pfirrmann classification no intra- and inter-observer agreement was performed. However, gagCEST analysis was performed automatically with an established segmentation algorithm, and Pfirrmann classification is known to enable excellent intra- and inter-reader agreement.[22,23] Another limitation is the lack of control over compliance by participants. We tried to compensate for this with questionnaires, which provided an overall positive feedback on the use of physiotherapy and insoles. Finally, with regard to our positive results after therapy, it remains unclear whether conservative therapy stops disc degeneration or only delays its course. Further studies with longer follow-up intervals are needed to clarify this point.

In conclusion biochemical MR imaging with gagCEST showed lower GAG values of NP in young participants with mild LLD > 10 mm indicating that LLD represents a risk factor for the development of early biochemical alterations of lumbar IVDs. After conservative treatment with physical therapy and shoe inlays, a trend towards higher GAG values was observed in LLD patients, suggesting that conservative therapy may stop or delay IVD degeneration. In addition, gagCEST proved to be a possible tool for non-invasive and contrast medium-free monitoring of patients with LLD under therapy.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

There has been no financial funding.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Appendix A. Supplementary data

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