Assessment of Lumbar Intervertebral Disc Glycosaminoglycan Content by Gadolinium-Enhanced MRI before and after 21-Days of Head-Down-Tilt Bedrest

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Abstract

During spaceflight, it has been shown that intervertebral discs (IVDs) increase in height, causing elongation of the spine up to several centimeters. Astronauts frequently report dull lower back pain that is most likely of discogenic origin and may result from IVD expansion. It is unknown whether disc volume solely increases by water influx, or if the content of glycosaminoglycans also changes in microgravity. Aim of this pilot study was to investigate effects of the spaceflight analog bedrest on the glycosaminoglycan content of human lumbar IVDs. Five healthy, non-smoking, male human subjects of European descent were immobilized in 6° head-down-tilt bedrest for 21 days. Subjects remained in bed 24 h a day with at least one shoulder on the mattress. Magnetic Resonance Imaging (MRI) scans were taken according to the delayed gadolinium-enhanced magnetic resonance imaging (dGEMRIC) protocol before and after bedrest. The outcome measures were T1 and ΔT1. Scans were performed before and after administration of the contrast agent Gd-DOTA, and differences between T1-values of both scans (ΔT1) were computed. ΔT1 is the longitudinal relaxation time in the tissue and inversely related to the glycosaminoglycan-content. For data analysis, IVDs L1/2 to L4/5 were semi-automatically segmented. Zones were defined and analyzed separately. Results show a highly significant decrease in ΔT1 (p<0.001) after bedrest in all IVDs, and in all areas of the IVDs. The ΔT1-decrease was most prominent in the nucleus pulposus and in L4/5, and was expressed slightly more in the posterior than anterior IVD. Unexpected negative ΔT1-values were found in Pfirrmann-grade 2 discs after bedrest. Significantly lower T1 before contrast agent application was found after bedrest compared to before bedrest. According to the dGEMRIC-literature, the decrease in ΔT1 may be interpreted as an increase in glycosaminoglycans in healthy IVDs during bedrest. This interpretation seems contradictory to previous findings in IVD unloading.

Introduction

Chronic lower back pain (CLBP) is a widespread disease in the population and also occurs in astronauts and cosmonauts [1,2]. In many symptomatic cases, no structural cause is found in conventional MRI, and up to 85% of patients with CLBP do not have any visible anatomical anomalies [3]. Causes for back pain seem to be multifactorial [3], and degeneration of intervertebral discs (IVDs) is one known cause for discogenic pain [4,5]. During spaceflight, IVDs gain height and cause elongation of the spine up to several centimeters [2,6]. Astronauts frequently report moderate to severe, dull lower back pain that is most likely of discogenic origin and may result from IVD expansion [1,6]. It is unknown whether the increase in disc volume is caused solely by water influx, or if the amount of glycosaminoglycans (GAGs) changes in microgravity [7,8]. Furthermore, astronauts may have an increased risk for herniated nucleus pulposus, particularly in the immediate post-flight period [9]. Possible changes in IVD morphology are discussed as causing factors in the literature, however it is unknown what exactly happens within the IVD. The same type of back pain experienced in spaceflight was reported in bedrest studies with 6° head down tilt [10]. Bedrest studies have proven to be a good analog for changes in intervertebral disc morphology [7,11]. Findings from space flight and bedrest might help to better understand the pathophysiology and treatment options for patients suffering from chronic lower back pain.

In its early stages, IVD degeneration involves a decrease in GAG content [12,13]. While GAGs are known to decrease in IVD degeneration, an increase over a period of time in turn indicates recovery [14]. Standard MRI-imaging techniques are unable to...
detect early stages of IVD-degeneration [15], and so far microstructural changes and degeneration of lumbar intervertebral discs have not been assessed following space flight or bedrest. A magnetic resonance imaging (MRI) method has been established that can quantify the loss of GAGs and detect degeneration (a decrease in GAG concentration) and recovery (an increase in GAG concentration). Called “delayed Gadolinium Enhanced MRI of Cartilage” (dGEMRIC), it was first applied to joint cartilage [16–19] and has recently been successfully utilized for the assessment of IVD degeneration [13,20–22]. Standard MRI hardware is used for dGEMRIC imaging and measurements are performed before and after administration of a contrast agent that degrades and distributes in IVD tissue reciprocal to the amount of GAGs [12]. The longitudinal relaxation time (T1) in the tissue is shortened by the contrast agent. The effect intensity depends on the amount of contrast agent within the tissue. Thus changes in GAG-content of the IVD can indirectly be assessed through an analysis of T1-times in the MRI.

The aim of the present pilot study was to assess the GAG content of lumbar intervertebral discs before and after bedrest using the dGEMRIC protocol to investigate if the increase in IVD thickness during bed rest might be related to changes in GAG content. The hypothesis was that bedrest does not affect the amount of GAGs in the IVD. The longitudinal relaxation time (T1) in the tissue is shortened by the contrast agent. The effect intensity depends on the amount of contrast agent within the tissue. Thus changes in GAG-content of the IVD can indirectly be assessed through an analysis of T1-times in the MRI.

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Materials and Methods

Ethics Statement

The study was approved by the ethics committee of North Rhine Medical Association (Arzteteam Nordrhein, application number 2007405), and was designed and performed in compliance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Study setting

The experiment presented here was part of a large clinical trial (the NUC-Study), performed during one of the two campaigns, and it is by itself therefore not a clinical trial as defined in the CONSORT or TREND guidelines. The NUC-study was registered with the Clinical Trials Registry http://www.clinicaltrials.gov (Number: NCT01509456). It was also registered in the ESA Erasmus Experiment Archive http://eea.spaceflight.esa.int/portal/ (Experiment record no. 9389). The NUC-study took place in the Institute of Aerospace Medicine of the German Aerospace Center, Cologne, Germany.

Study design

In the NUC-study, seven healthy, non-smoking, male human subjects were immobilized in 6° head-down-tilt bedrest for 21 days (HDT-1 to HDT-21) in a cross-over design. Five of these subjects were included in the presented investigation of IVDs. The entire bedrest-study included two campaigns for each subject, with a wash-out period of 154 days in between. The aim was to investigate a nutritional countermeasure for bone loss (oral application of potassium bicarbonate 30 mmol/tablet three times a day) in a cross-over design [7,23,24]. The dGEMRIC measurements presented here were conducted before and after the second campaign of the NUC-study beginning August 16th and ending October 15th, 2010 (study schedule: Figure 1). For our experiment, it was anticipated that the nutritional countermeasure of the NUC-study would not have major effects on the formation of GAGs in the IVDs. Due to the small number of subjects, smaller potential effects could not have been found. During baseline and recovery data collection, subjects could move free inside the lab (baseline: 7 days, BDC-7 to BDC-1; recovery: 6 days, R+0 to R+5). Reambulation from bedrest took place in the morning of R+0. Throughout bedrest, subjects remained in bed 24 h/day with at least one shoulder on the mattress at any time. All hygienic procedures, food intake and experiments took place in this position without exception. Compliance with the protocol was ensured by video surveillance and by staff. Subjects did not undergo exercise or training. Psychological support was given by psychologists and medical doctors looked after the subjects in daily ward rounds. The dietary intake was strictly controlled by weighing all

Figure 1. Time schedule of the study campaign. BDC = Baseline Data Collection, HDT = Head Down Tilt, R = Recovery. The MRI measurements presented here were performed five days before bedrest (pre-bedrest) and three days after (post-bedrest).

Figure 2. Protocol for dGEMRIC measurements.
ingredients, food items and beverages for each test subject to prevent changes in bodyweight.

**Subject selection**

Subject recruitment was announced on the institution’s website as well as by flyers and posters in several universities and research institutes. Only male subjects were recruited for this study because the European Space Agency expected hormonal variations in female subjects to mimic effects in some of the experiments. Subject selection included a variety of clinical and laboratory tests as well as a psychological examination. Inclusion criteria were: male gender, age 20–45 years, body mass index 20–26 kg/m², body height 158–190 cm (62–75 inches), body weight 65–85 kg and willingness to participate in the entire study. Exclusion criteria included abuse of drugs, medicine, nicotine or alcohol, regular medication, vegetarians and vegans, history of mental illness, rheumatic diseases, chronic hypertension, diabetes, obesity, arthritis, hyperlipidaemia, renal dysfunction, thyroid dysfunction, hepatic disease, disorders of calcium and bone metabolism, exercising more than four times a week, chronic back pain, a history of intervertebral disc prolapse, muscle and joint disease, family history of thrombosis and blood clotting disorders (Tests performed included AT III, Lupus-PTT, ferritin, Factors II, IV and V Leiden).

**The dGEMRIC protocol**

MRI scans of the longitudinal relaxation time were taken according to the dGEMRIC protocol as described in the literature [14,20–22]. Each subject underwent MRI scans of the lumbar spine pre- and post-bedrest on BDC-5 and R+3 (Figure 1). MRI scans were taken with a phased-array back coil in a 3T MRI scanner (Achieva, Phillips Medical Systems). A fast spin spin-echo inversion recovery sequence was applied which allows the pixel-wise calculation of T1 from a series of seven images recorded with fixed TR (1800 ms) and TE (13 ms) and variable inversion-recovery times (TI 50, 150, 350, 700, 1050, 1400 and 2000 ms,

![Figure 3. Selection of the intervertebral disc (left), calculated T1-map for subject D (right). doi:10.1371/journal.pone.0112104.g003](image)

![Figure 4. ΔT1-maps. Subject A, L1/2 divided into sectors, before (left) and after (right) bedrest. doi:10.1371/journal.pone.0112104.g004](image)
respectively). For each examined intervertebral disc, two series of cross-sectional images were individually planned based on sagittal pilot images of the spine. The images came from two consecutive slices recorded with 300 mm field of view, an acquisition matrix of 512 x 512 pixels and 3 mm slice thickness. Each time, MRI scans were performed before and after i.v.-administration of the gadolinium-based contrast agent Gd-DOTA (Dotarem, Gadoteric acid, 0.4 mg/kg bodyweight). The schedule of Gd-DOTA administration, subject handling, and the measurements followed the protocol of Niikima¨ki et al. [20] and is shown in Figure 2.

Calculation of $T_1$-values

The automatic normalization of signal intensity was accidentally not disabled during some of the measurements before bedrest. In this case the signal intensity of the seven images in a series must be corrected prior to the calculation of $T_1$. For the correction of signal intensities, a normalization-factor was calculated using manually chosen points in the subcutaneous fat tissue. $T_1$-values of fat were known from the correctly recorded images and were proven to remain constant after the application of contrast agent. All post-bedrest measurements were performed without this technical error.

Finally, $T_1$ was calculated for selected regions in the disc by fitting the signal intensities of selected pixels in the seven images and the corresponding inversion recovery times to the Nelder-Mead equation as published by Vaga et al. [21] using multidimensional unconstrained nonlinear minimization.

Segmentation

$T_1$-maps were calculated for L1/2 to L4/5. The IVD region was segmented semiautomatically (Figure 3). An ellipse was fitted to eight points that were set manually. The ellipse was then subdivided into 60 sectors (Figure 4). For each sector, means and SD of the $T_1$-times, as well as $\Delta T_1$ were computed. Rings and zones were defined and separately analyzed (Figure 5).

Pfirrmann-grading

Sagittal $T_2$ (spin-spin relaxation time) weighted multi echo images of the spine were acquired using a 1.5 T MRI scanner (Sonata, Siemens Medical Systems, Erlangen) with TR: 2500 ms, 15$\times$TE: 10,3 ms–164,8 ms, 256$\times$256 pixels in FOV: 330 mm$\times$330 mm, 3 mm slice thickness, 6 mm interslice gap with a dedicated spine coil. The images were evaluated for Pfirrmann-grading of IVD degeneration by a radiologist [25]. Pfirrmann-grades were correlated with findings from dGEMRIC measurements.

Statistical analysis

Using factorial ANOVAs (Statistica 10, Statsoft, Tulsa, OK, USA) native $T_1$ values, $T_1$-values after administration of the contrast agent, and $\Delta T_1$ were tested for group effects caused by bedrest, differences between regions within each disc and differences between different discs. Significance was assumed at $p<0.05$. Data are presented as counts and percentages, and as means and their sd. Exclusion conditions were $T_1$ times <400 ms and >1500 ms. Where significance was found, a Tukey’s post-hoc test was performed. SigmaPlot was used for plotting of data. $\Delta T_1$ was the primary outcome measure of this study.

Results

Five out of seven subjects completed the entire experiment (Table 1). Two subjects were excluded from the analysis due to incomplete data sets (loss of data due to a software problem). There was no adverse event in connection with the dGEMRIC measurements.

Table 1. Details of the subjects.

| Subject | Age (years) | Weight (kg) | Height (cm) |
|---------|-------------|-------------|-------------|
| A       | 27          | 79.2        | 185         |
| B       | 26          | 71.5        | 182         |
| C       | 30          | 88.8        | 178         |
| D       | 23          | 74.4        | 179         |
| F       | 33          | 85.5        | 186         |
| Mean (sd) | 27.8 (3.8) | 79.9 (7.3) | 182 (3.5)  |

doi:10.1371/journal.pone.0112104.t001
The main findings of this study were: 1. a decrease in $\Delta T_1$ after bedrest compared to before, 2. negative $\Delta T_1$-values, particularly in Pfirrmann-grade 2-discs after bedrest and in L4/5, and 3. significantly lower native $T_1$ values after bedrest than before bedrest.

Average $\Delta T_1$ value of all intervertebral discs was $104.87 \text{ ms (sd 7.64 ms)}$ pre-bedrest and $-20.20 \text{ ms (sd 4.70 ms)}$ post-bedrest. This difference is highly significant ($p < 0.001$). Table 2 gives an overview of data, showing average values and standard deviations.

### Differences between IVDs

Figures 6 and 7 compare native and Gd-affected $T_1$-values (Figure 6) and $\Delta T_1$-values (Figure 7) in different IVDs before and after bedrest.

Regarding native $T_1$-values, no significant differences were found between IVDs comparing before and after bedrest. After the application of the contrast agent, a significant difference occurred between L1/2 and L4/5 pre-bedrest ($p = 0.006$), but there was no significant difference between IVDs post-bedrest. The effect of bedrest on pre-contrast $T_1$ was significant for all discs (L1/2, L2/3 and L3/4: $p < 0.001$ and L4/5: $p = 0.021$). The effect of bedrest on Gd-affected $T_1$ was not significant for L1/2, L2/3 and L3/4, but for L4/5 ($p = 0.002$).

Before bedrest, positive $\Delta T_1$-values were found as anticipated effects of the contrast agent in all IVDs. Surprisingly, after bedrest, negative $\Delta T_1$-values were found in L2/3, L3/4 and L4/5. Negative $\Delta T_1$-values resulted from a longer $T_1$ after the application of the contrast agent, which is physically incompatible.

### Table 2. Average values and standard deviations.

|          | Pre-contrast pre-bedrest | Pre-contrast post-bedrest | Post-contrast pre-bedrest | Post-contrast post-bedrest | Delta T1 pre-bedrest | Delta T1 post-bedrest |
|----------|--------------------------|---------------------------|---------------------------|---------------------------|----------------------|----------------------|
| L1/2     | 934.41 (SD 19.54)        | 813.23 (SD 19.04)         | 784.66 (SD 17.03)         | 794.80 (SD 19.46)         | 149.76 (SD 15.83)   | 18.43 (SD 16.13)     |
| L2/3     | 884.06 (SD 17.81)        | 774.13 (SD 15.55)         | 780.38 (SD 15.45)         | 796.16 (SD 15.75)         | 103.69 (SD 11.95)   | -22.03 (SD 11.39)    |
| L3/4     | 866.15 (SD 18.26)        | 788.02 (SD 17.01)         | 766.18 (SD 17.51)         | 805.20 (SD 18.60)         | 99.97 (SD 12.06)    | -17.19 (SD 12.23)    |
| L4/5     | 853.50 (SD 22.35)        | 766.44 (SD 18.35)         | 705.00 (SD 17.86)         | 814.75 (SD 16.69)         | 148.51 (SD 18.14)   | -48.31 (SD 16.21)    |
| Ring 1   | 1131.38 (SD 13.39)       | 972.09 (SD 11.92)         | 972.32 (SD 15.82)         | 992.94 (SD 11.13)         | 159.06 (SD 14.60)   | -20.85 (SD 15.58)    |
| Ring 2   | 1007.97 (SD 18.06)       | 872.82 (SD 15.69)         | 864.45 (SD 16.09)         | 904.37 (SD 15.36)         | 143.52 (SD 15.67)   | -31.55 (SD 15.69)    |
| Ring 3   | 916.16 (SD 18.17)        | 789.31 (SD 15.52)         | 756.98 (SD 14.33)         | 799.49 (SD 14.85)         | 159.18 (SD 17.11)   | -10.19 (SD 14.68)    |
| Ring 4   | 680.31 (SD 10.78)        | 591.37 (SD 11.06)         | 602.19 (SD 12.85)         | 603.88 (SD 11.77)         | 78.12 (SD 15.16)    | -12.51 (SD 14.53)    |
| Ring 5   | 532.66 (SD 10.27)        | 490.62 (SD 17.55)         | 495.44 (SD 10.24)         | 512.16 (SD 20.97)         | 37.22 (SD 13.17)    | -21.54 (SD 15.12)    |
| Anterior | 560.13 (SD 20.66)        | 555.28 (SD 30.26)         | 537.03 (SD 19.11)         | 603.61 (SD 34.35)         | 23.10 (SD 24.41)    | -48.33 (SD 36.22)    |
| Posterior| 855.94 (SD 30.70)        | 739.68 (SD 24.41)         | 686.21 (SD 24.02)         | 730.06 (SD 27.48)         | 169.73 (SD 27.68)   | 9.62 (SD 25.93)      |

Figure 6. $T_1$-values before and after administration of contrast agent, pre- and post bedrest for all intervertebral discs.

doi:10.1371/journal.pone.0112104.g006
with a mere effect of Gd uptake into the discs. In terms of the
negative $\Delta T_1$ values found after bedrest, differences between IVDs
were neither significant before nor after bedrest with one
exceptional difference between L1/2 and L4/5 after bedrest
($p=0.049$). The bedrest-induced decrease in $\Delta T_1$ was significant
for all IVDs ($p<0.001$). Changes induced by bedrest were
strongest in L4/5.

Differences between inner and outer regions of the IVDs

Figures 8 and 9 show $T_1$-values pre- and post-contrast and pre-
and post-bedrest (Figure 8) as well as $\Delta T_1$-values plotted for each
selected ring pre- and post-bedrest (Figure 9). The central region
was numbered as 1 and the following outer rings were numbered
from 2 to 5. The nucleus pulposus of an IVD is covered by region
1 and ring 2, and the annulus fibrosus by rings 3 to 5.
Overall, $T_1$-values were highest in region 1 and lowest in ring 5 with a continuous decrease from the centre of the IVD to the periphery. Differences in $T_1$ between all rings were significant within each of the four categories (pre- and post Gd application, pre and post-bedrest) shown in Figure 8 ($p<0.001$; the only exception is the difference between rings 2 and 3 post-bedrest pre-contrast: $p=0.008$). The bedrest-effect on $T_1$-values pre-contrast is significant in all rings (rings 1–3 $p<0.001$ and ring 4 $p=0.024$) except for ring 5.

Before bedrest $\Delta T_1$ was positive in all rings with an average of $133.23$ ms (sd $7.48$ ms). After bedrest $\Delta T_1$ was negative in all rings with an average of $-19.73$ ms (sd $7.33$ ms). Before bedrest, differences of rings were significant between: rings 1 and 4 ($p<0.001$), rings 1 and 5 ($p<0.001$), rings 2 and 4 ($p<0.001$), rings 2 and 5 ($p<0.001$), rings 3 and 4 ($p=0.005$) and rings 3 and 5 ($p<0.001$), but not between rings 1 to 3. Pre-bedrest $\Delta T_1$-values were higher in the nucleus pulposus than in the annulus fibrosus. Post-bedrest, there were no significant differences between rings. The
bedrest-induced decrease in $\Delta T_1$ was significant in rings 1 to 4 (rings 1–3 $p < 0.001$ and ring 4 $p = 0.006$), but not in ring 5.

Findings in the anterior and posterior sector

Figures 10 and 11 show results from analysis of the anterior and posterior segment of the intervertebral discs (segments are highlighted in Figure 5). The analysis includes $T_1$-values pre-and post-contrast pre- and post-bedrest (Figure 10) and $\Delta T_1$-values (Figure 11).

The difference between anterior and posterior $T_1$-values pre-contrast was significant before ($p < 0.001$) and after ($p = 0.024$) bedrest. Post-contrast, it was only significant before bedrest ($p = 0.029$). The effect of bedrest was neither significant for anterior nor for posterior $T_1$-values.

$\Delta T_1$ was significantly higher in the posterior sector compared to the anterior sector pre-bedrest ($p < 0.001$) but not post-bedrest ($p = 0.500$). The difference between $\Delta T_1$ pre- compared to post-bedrest was significant in the posterior ($P = 0.004$) but not in the anterior segment.

Pfirrmann-grading

Pfirrmann-grades of IVDs are shown in Table 3. Intervertebral discs with a Pfirrmann-grade $\geq$2 were excluded from the following analysis because there was only one case each. Statistical analysis reveals a significant difference between Pfirrmann-grade and $\Delta T_1$ ($p = 0.006$), but not between Pfirrmann-grade and $T_1$ ($p = 0.125$). $\Delta T_1$ post-bedrest is 12.42 (sd 10.36) ms in Pfirrmann-grade 1 and -27.94 (sd 10.32) ms Pfirrmann-grade 2. Therefore the unexpected occurrence of negative $\Delta T_1$ post-bedrest corresponds with disc degeneration.

Discussion

The aim of this pilot study was to indirectly assess the GAG content of the lumbar intervertebral discs L1/2 to L4/5 before and after 21 days of bedrest using the dGEMRIC protocol to investigate if changes can be found. Results showed

1. A highly significant decrease in $\Delta T_1$ induced by the bedrest-intervention in L1/2 to L4/5 before and after 21 days of bedrest using the dGEMRIC protocol to investigate if changes can be found. Results showed

2. Unexpected negative $\Delta T_1$-values were found in Pfirrmann-grade 2-discs after bedrest and in L4/5.

3. Significantly lower $T_1$ before contrast agent application after bedrest compared to before bedrest.

The dGEMRIC protocol is a reliable method to measure changes in GAG-content of cartilage and IVDs [13,16–22]. It has

Table 3. Pfirrmann-grades.

| Subject | L1/2 | L2/3 | L3/4 | L4/5 |
|---------|------|------|------|------|
| A       | 2    | 2    | 1    | 1    |
| B       | 2    | 2    | 1    | 1    |
| C       | 2    | 2    | 2    | 2    |
| D       | 4    | 2    | 3    | 2    |
| F       | 2    | 2    | 1    | 2    |

There were no changes in values throughout bedrest.

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Figure 11. $\Delta T_1$-values pre- and post-bedrest for the anterior and posterior segment.
doi:10.1371/journal.pone.0112104.g011

Table 3. Pfirrmann-grades.

| Subject | L1/2 | L2/3 | L3/4 | L4/5 |
|---------|------|------|------|------|
| A       | 2    | 2    | 1    | 1    |
| B       | 2    | 2    | 1    | 1    |
| C       | 2    | 2    | 2    | 2    |
| D       | 4    | 2    | 3    | 2    |
| F       | 2    | 2    | 1    | 2    |

There were no changes in values throughout bedrest.
doi:10.1371/journal.pone.0112104.t003
been used and validated in a number of clinical and experimental studies. It seems to be well established that increased GAG concentration within the IVD will result in a decrease in $\Delta T_1$ [13, 14, 20, 21]. A high GAG-concentration causes a small $\Delta T_1$ during dGEMRIC-measurements because only small amounts of contrast agent shift into the IVD [12]. A low GAG-concentration in turn leads to a high $\Delta T_1$. Increased $\Delta T_1$ after an intervention (as compared to before) has been interpreted as degeneration process in the literature [13, 14].

This study showed a decrease in $\Delta T_1$ after bedrest in the healthy lumbar IVDs (L1/2 to L4/5, Pfirrmann-grade 1), which, according to the literature, might be interpreted as GAG-increase [13, 14]. After bedrest, $T_1$ was already decreased before the administration of contrast agent compared to before bedrest. This finding indicates that the intervention of bedrest had an effect on the IVDs. A theoretical possibility is that contrast agent might have remained within the IVD during bedrest and therefore caused decreased $T_1$ after bedrest. Gd-DOTA, however, is excreted rapidly through the kidneys and concentrations in IVDs show their maximum 210 minutes after injection followed by a speedy decrease as shown by Vaga et al. [21]. As subjects walked normally for 4.5 more days after the injection of the contrast agent and before bedrest, remaining contrast agent in the IVD is very unlikely to explain the particularly short $T_1$-times post-bedrest.

Surprisingly, $\Delta T_1$ showed negative values after bedrest in the IVDs with first signs of degeneration (Pfirrmann grade 2). This phenomenon, to our knowledge, has not been previously reported in studies using the dGEMRIC protocol to determine GAG-content of IVDs [13, 14, 20, 21]. It might be an incidental finding related to the small sample size. In this study, negative $\Delta T_1$-values result from an increase in $T_1$ after injection of the contrast agent post-bedrest. This finding cannot be explained by an increase in GAG-content only. As the contrast agent shortens $T_1$-time, mere increase in GAG-content would not cause longer $T_1$ after Gd-DOTA administration. In case no contrast agent reaches the IVD, $T_1$ should remain unchanged, but there is no way for it to increase just by contrast agent. It can neither be explained by disc degeneration, because a low amount of GAGs results in small $\Delta T_1$-values, but not in negative $\Delta T_1$-values. Therefore, an additional effect might have influenced our findings and led to the increase in $T_1$ in the slightly degenerated IVDs after bedrest. Considering the contrast agent’s chemistry, the Gd-DOTA-complex, due to its inertness is unlikely to interact with the intervertebral disc in a way that might alter $T_1$-time. As free water shows longest $T_1$, an increase in free water within the IVD might be a possible cause. The contrast agent, however, was injected between the two MRI measurements; subjects remained in supine position for 30 minutes and walked around for 60 minutes (Figure 2). In theory, compression of IVDs during walking would decrease the water content and not increase it [26]. Post-bedrest dGEMRIC-measurements were performed in the morning three days after bedrest. During these three days, subjects were already allowed to walk around while having a number of experiments (spirometry, DEXA, pQCT, different MRI measurements, muscle fatigue, eye examinations and ultrasound measurements). However, most of this time was not spent in the upright position, but rather sitting and lying. Therefore, walking for 60 minutes may have changed the composition of the already slightly degenerated IVDs. Processes such as osmosis or a pump mechanism might play a role here, e.g. by changing the content of free water by releasing bound water. Furthermore, it is unclear in how far intra-nuclear fissures and clefts might affect results in disc degeneration as well [27].

Regarding the negative $\Delta T_1$ values, it is thought that there is a fluid effect induced by degeneration processes, the number of subjects is too small and the finding is accidental, or the method dGEMRIC reveals its weaknesses in accuracy. The question how bedrest affects the GAG-content of degenerated discs in higher stages of degeneration needs to be addressed in future studies.

The results of this study are in accordance with results from Vaga et al. [14] and Giavarro et al. [13] who both showed a GAG-increase in operatively stabilized lumbar IVDs. Contradictory findings were published by Hutton et al. [28] who found a significant decrease in proteoglycan content of IVDs in rats after four weeks of tail suspension as model for simulated weightlessness. It is however unclear how well IVDs are unloaded during tail suspension. A decrease in proteoglycan concentration was also found in rat-IVDs after 5 days of spaceflight [29]. Results of changes in GAG-content in human IVDs during simulated or actual spaceflight have not been published before. Comparability between species seems to be limited due to differences in cell cytomorphology [30], and biomechanical forces and strains differ between vertical and horizontal spines.

Vaga et al. [21] correlated the biochemistry-derived sGAG-content of IVDs and $\Delta T_1$ assessed by dGEMRIC, performed a linear regression analysis and found a regression function ($y = -1.38x + 238$). Applying this regression function to the average post-bedrest-$\Delta T_1$ of Pfirrmann grade 1 discs found in the present study, a GAG-content of over 250µg/mg is found. This value may be slightly overestimated as Vaga et al. waited for 210 minutes instead of 90 minutes for the second MRI after injection of contrast agent, which would influence $T_1$ by about 50 ms as shown in the same paper. In any way, the GAG-concentration resulting from $\Delta T_1$-values found in this study probably exceeds results from healthy IVDs published in the literature [21].

Regarding CLBP, our study results are in accordance with findings from Arvinen et al. [31] who have found out that an insufficient quantity of sleep is a risk factor for low back pain. Sufficient time in bed might be necessary for the IVDs to recover from the mechanical load and strain of the daily activities. Further studies are required to examine a possible connection between the daily time spent in bed and the IVDs GAG content, as well as CLBP incidence. In addition, further research on changes in composition of IVDs during bedrest needs to be conducted. Though results are highly significant, the present pilot study was performed on a small number of healthy subjects only, and results should be confirmed in a larger cohort and with different approaches.

Acknowledgments

We thank all test subjects for their commitment and willingness to participate. We also acknowledge the support of Dr. Oliver Angerer of ESA, as well as Dr. Petra Frings-Meuthen, Alexandra Noppe, Dr. Joachim Latisch and Dr. Francisca May (all DLR) and the study management team of DLR.

Author Contributions

Conceived and designed the experiments: TK, JZ, JR, RPF, MH, PE, BG. Performed the experiments: TK, JZ, MH, BG. Analyzed the data: BG, JZ, JR, RPF. Contributed reagents/materials/analysis tools: TK, PE, MH, JR. Wrote the paper: TK, JZ, JR, RPF, MH, PE, BG.
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