# Lumbar muscle atrophy and increased relative intramuscular lipid concentration are not mitigated by daily artificial gravity following 60-day head-down tilt bed rest

Enrico De Martino[1], Julie Hides[2], James M. Elliott[3,4], Mark Hoggarth[3,5], Jochen Zange[6], Kirsty Lindsay[1], Dorothée Debuse[1], Andrew Winnard[1], David Beard[7], Jonathan A Cook[7,8], Sauro Salomoni[9], Tobias Weber[10,11], Jonathan Scott[10,11], Paul W. Hodges[9], Nick Caplan[1]

1. Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK
2. School of Allied Health Sciences, Griffith University, Nathan Campus, Brisbane, Australia
3. Northwestern University, Feinberg School of Medicine Department of Physical Therapy and Human Movement Sciences, Chicago, Illinois, USA
4. Northern Sydney Local Health District & The University of Sydney, Faculty of Medicine and Health, The Kolling Research Institute Sydney, Australia
5. Northwestern University, McCormick School of Engineering, Department of Biomedical Engineering, Evanston, Illinois, USA
6. Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany
7. NIHR Oxford Biomedical Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
8.Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
9. The University of Queensland, NHMRC Centre for Clinical Research Excellence in Spinal Pain, Injury and Health, School of Health and Rehabilitation Sciences, Brisbane, Australia
10. European Astronaut Centre, Space Medicine Team (HRE-OM), European Space Agency (ESA), Cologne, Germany
11. KBR GmbH, Cologne, Germany

**Corresponding author:** Nick Caplan

Aerospace Medicine and Rehabilitation Laboratory
Faculty of Health and Life Sciences
Northumbria University
Newcastle upon Tyne
NE1 8ST
United Kingdom
Email: nick.caplan@northumbria.ac.uk

Supplemental Material Tables available at:
URL: https://figshare.com/s/4af3e4c2cb237bf65d6c

DOI: https://doi.org/10.6084/m9.figshare.13242119

Supplemental Material Figures available at:
URL: https://figshare.com/s/3e633bde4cc5a4af476f

DOI: https://doi.org/10.6084/m9.figshare.14213870

**Abstract**

Exposure to axial unloading induces adaptations in paraspinal muscles, as shown after spaceflights. This study investigated whether daily exposure to artificial gravity (AG) mitigated lumbar spine flattening and muscle atrophy associated with 60-day head-down tilt (HDT) bed rest (Earth-based space analogue). Twenty-four healthy individuals participated in the study: Eight received 30 minutes continuous AG; eight received 6x5 minutes AG, interspersed with rest periods; eight received no AG exposure (control group). Magnetic Resonance Imaging (MRI) of the lumbopelvic region was conducted at baseline (BDC) and at day 59 of HDT (HDT59). T1-weighted images were used to assess morphology of the lumbar spine (spinal length, intervertebral disc angles, disc area) and volumes of the lumbar multifidus (LM), lumbar erector spinae (LES), quadratus lumborum (QL), and psoas major (PM) muscles from L1/L2 to L5/S1 vertebral levels. A chemical shift-based 2-point lipid/water Dixon sequence was used to evaluate muscle composition. Results showed that: spinal length and disc area increased ($P<0.05$); intervertebral disc angles ($P<0.05$) and muscle volumes of LM, LES, and QL reduced ($P<0.01$); and lipid/water ratio for the LM and LES muscles increased ($P<0.01$) after HDT59 in all groups. Neither of the AG protocols mitigated the lumbar spinae deconditioning induced by HDT bed rest. The increase in lipid/water ratio in LM and LES muscles indicates an increased relative intramuscular lipid concentration. Altered muscle composition in atrophied muscles may impair lumbar spine function after body unloading, which could increase injury risk to vulnerable soft tissues. This relationship needs further investigation.

**New & noteworthy**

This study presents novel insights into the morphological adaptations occurring in the lumbar spine following 60-day head-down bed rest and potential role of artificial gravity (AG) to mitigate them. Results demonstrated no protective effect of AG protocols used in this study. In atrophied paraspinal muscles, the ratio of lipids versus intramuscular water increased in the postural lumbar muscles, which could impair muscle function during upright standing. These findings have relevance for future space explorations.

**Keywords:** Short-arm centrifugation, paraspinal muscles, immobilization, magnetic resonance imaging.

## 1. Introduction

Exposure to microgravity reduces the functional capacity of multiple body systems (17). Among those deteriorations, flattening of spinal curvature and atrophy of lumbar musculature have been documented in astronauts (4). Understanding the effects of prolonged axial unloading on the spinal curvature and lumbar musculature can provide unique information that could be used to design interventions not only for space missions but also for people with low back pain on Earth (28), which is the leading cause of disability worldwide (26).

Exposure to microgravity during spaceflight provokes rapid adaptation in lumbar spine morphology (for review, see (24)). Increased spinal length, loss of lumbar lordosis, vertebral osteopenia, and atrophy of the lumbar multifidus (LM) at L4 and L5 vertebral levels have been observed following space missions (4, 11, 30). Recently, using computed tomography (CT), attenuation values in the LM, lumbar erector spinae (LES), and quadratus lumborum (QL) have been described in astronauts after 6-month of spaceflight (9, 51). Radiodensity attenuation values determined by CT in skeletal muscles indicate an increase in intramuscular lipid concentration (ILC), as shown in individuals with obesity and type 2 diabetes mellitus (23). Similar changes in lumbar muscles and spinal curvatures are observed in many individuals with low back pain on Earth at different stages of the condition. For instance, acute low back pain (less than 6 weeks) is associated with localized atrophy of LM muscle, subacute low back pain (between 6 and 12 weeks) with elevated ILC and fibrosis without muscle atrophy of LM muscle, and chronic low back pain (more than 12 weeks) with diffuse atrophy, fibrosis, and elevated ILC of LM muscle (for review, see (33))

As there are few astronauts to study, an alternative is Earth-based analogue studies, such as strict head-down tilt (HDT) bed rest, to understand the effect of prolonged gravitational unloading upon the spinal curvature and lumbar musculature. Without any countermeasures, bed rest induces changes, such as spinal elongation, loss of the lumbar lordosis, intervertebral disc expansion, and atrophy of LM, LES, and QL muscles (6, 47). Importantly, atrophy of the LM is thought to be related to the loss of lumbar lordosis and, consequently, the lumbar distribution of the axial load when in weight-bearing positions (4). No studies have investigated whether ILC is increased in the lumbar paraspinal muscles following HDT bed rest or which muscles are more sensitive to such adaptation. Animal studies have shown that an intervertebral disc lesion at the L3/L4 vertebral level triggers degeneration in LM muscle that crosses the injured segment that commences within days and develops to express fibrosis, ILC, and muscle fiber-type transformation over weeks to months (34, 37). The presence of increased ILC in atrophied LM and LES muscles at the L4 and L5 vertebral levels in elderly individuals is

strongly correlated with an increased sagittal vertical axis measured using Magnetic Resonance Imaging (MRI) (56). The increased sagittal vertical axis has been proposed to contribute to compromised quality of life, independence, and a higher risk of falls due to a more anteriorly placed body center of mass (57). In the bent spine syndrome (camptocormia), paraspinal muscles are fully replaced by adipose tissue (43). In this condition, patients are unable to extend the lumbar spine in relation to the pelvis, and canes are indispensable in upright standing and walking (44).

When applied to HDT bed rest, exercise-based countermeasures, such as lower body negative pressure treadmill exercise (10), high-load resistive vibration exercise (5, 7), and low-magnitude vibration (35), achieve partial protection against lumbar spine changes. However, only high-load resistive vibration exercise performed three days per week mitigated atrophy of the LM muscle (5, 7), likely due to the high mechanical axial compressive forces applied to the lumbar region. Centrifugal acceleration on a short-arm human centrifuge, commonly referred to as artificial gravity (AG) (12), has been shown to mitigate some of the deconditioning effects on the human body in situations where axial loading is diminished (13, 42). Results from HDT bed rest studies suggest that daily exposure to AG mitigated some deconditioning of the cardiovascular, musculoskeletal, and neurovestibular systems (12, 36). For example, daily AG maintains aerobic power and blood volumes, resulting in less orthostatic intolerance during the tilt test after 5-day and 21-day HDT (18, 48, 62).

Whether daily AG mitigates the deconditioning of soft-tissues of the lumbar spine following 60-day HDT bed rest has not been explored. Investigation of the effects of daily AG on the lumbar spine region may reveal which muscles and structures are most sensitive to this countermeasure. Furthermore, it is unknown whether the effects of multiple daily centrifugation sessions would be more efficient as a countermeasure compared with a single bout of centrifugation. This knowledge is an essential step towards understanding the potential impacts of AG on the lumbar spine and may underpin the development of tailored countermeasures to maintain lumbar spine function and prevent spinal injuries in astronauts during and after long-duration space missions.

The first aim of the current study was to investigate whether 2 paradigms of daily AG could mitigate the effects of 60-day strict HDT bed rest on the lumbar spine. As AG is associated with a large acceleration gradient along the body axes ($1G_z$ at the center of mass), we hypothesized that the increased mechanical compressive force to the lumbar spine would stimulate the paraspinal muscle cells and mitigate muscle catabolism of paraspinal muscles. The second aim was to investigate whether 60-day HDT bed rest induces increased ILC of the

lumbar paraspinal muscles and, if so, whether cyclic mechanical compressive force produced by intermittent AG would prevent this increase. This would be expected if intermitted AG inhibited the differentiation from myoblasts to adipocytes (1, 41) caused by body unloading.

## 2. Methods

### *2.1 Participants*

The Artificial Gravity Bed Rest – European Space Agency study (AGBRESA study) was undertaken at the ":envihab" facility in Cologne (Germany) (40) from March to December 2019. Twenty-four participants (8 females) attended the facility for the baseline data collection (BDC) 14 days before undergoing a 60-day strict 6° HDT bed rest period. They remained in the facility for 13 days post-HDT bed rest for a reconditioning period. Participants were pain-free at the time of BDC testing, and they did not report having a history of chronic or acute musculoskeletal or other medical disorders that would affect the measures being collected in the study. After collection of baseline data, participants were allocated to one of three HDT bed rest intervention groups (n=8 for each): a group that underwent 30 minutes continuous centrifugation/day (cAG), a group that underwent six sets of 5 minutes centrifugation/day (iAG) interspersed by rest (3-minute breaks), and a group that was not exposed to AG (controls, CTRL). Participants were pseudo-randomly assigned to groups with regards to sex due to the drop out of three women and subsequent replacement during the campaign (3). The gender, age, height, and weight of the participant groups were comparable (CRTL – 2 females, 32±7 years, 177±7 cm, 79±13 kg; cAG – 3 females, 34±11 years, 172±8 cm, 72±10 kg; iAG – 3 females, 34±10 years, 174±11 cm, 71±5kg) (3).

All participants completed the 60 days of HDT bed rest, and participants performed all activities, including hygiene, in 6 degrees HDT, and they were discouraged from moving excessively or unnecessarily. Twenty-four hour video surveillance and wearable motion sensors monitored their activities. The participants' diet was controlled during the entire study and consisted of five daily menus with three meals and two snacks. Caloric intake was balanced with the measured individual metabolic energy consumption to keep body mass throughout bed rest, and participants were required to eat all food items served to them. Smoking and the consumption of alcohol or caffeinated drinks were not allowed during the study. Participants followed a day-night cycle of 7 a.m. wakeup and lights-out at 11 p.m. The study was approved by the ethics committee (2018143) of the North Rhine Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany, and was registered in the German Clinical Trials Register

(DRKS-ID: DRKS00015677). All procedures performed in this study were in accordance with the latest version of the declaration of Helsinki.

*2.2 Artificial gravity*

During the 60-day HDT bed rest, participants were transferred on a 6° head-down tilt gurney to the centrifuge facility, and they were placed on the centrifuge arm (6° head-down tilt). AG was produced by rotating the participants on a centrifuge arm with a 3.0-m radius. The rotational speed of the centrifuge was calculated individually based upon each participant's anthropometry to produce 1Gz at the center of mass (22). Participants' cardiorespiratory parameters were continuously monitored by trained medical personnel. The participants could perform anti-orthostatic manoeuvres, such as heel raises and shallow knee bends, to avoid calf pain and maintain the circulation while spinning but were otherwise instructed to remain still.

*2.3 Magnetic Resonance Imaging*

MRIs of the lumbar spine were acquired using a 3 Tesla Magnetom Vision system (Siemens, Erlangen, Germany). Participants were positioned on the scanning bed in supine lying with their knees and hips supported in slight flexion by a pillow. Imaging was conducted on the two days prior to HDT (BDC) and on the 59$^{th}$ day of HDT bed rest (HDT59). Images were collected at the same time of the day for each individual (between 6 p.m. and 8 p.m.) and stored for offline analysis. MRIs were performed in the transverse plane to image lumbar muscles. A set of 12 sagittal images was collected to investigate lumbar spine morphology using a multi-spin-echo sequence with a long repetition time (TR=3s) and a series of 12 echoes with echo times (TE) spanning from 20 ms to 200 ms. A set of 64 transverse images was acquired from the T12 vertebra to sacrum (T1 weighted Dixon sequence, total acquisition time = 5 minutes; slice thickness = 4 mm; distance factor = 20%, TR = 7.02 ms, TE1 = 2.46 ms, TE2 = 3.69 ms, flip angle = 5 deg; field of view = 400 mm x 400 mm at 1.0 mm x 1.0 mm pixel size). Images were obtained with the signal of carbonyl protons (-CH2-) from lipids and water protons in-phase and out of phase; then, so-called fat (F) images representing the amplitude of only carbonyl protons and water images (W) were reconstructed.

Before performing the measurement of MRIs, images were assigned a random code to blind the operator to time-points and participant groups.

*2.4 Lumbar spine morphology*

On sagittal images, the slice placed closest to the center of the lumbar spine was selected (Figure 1), and the following measurements were manually extracted using OsiriX MD software (v.10.0.5, Pixmeo SARL, Bernex, Switzerland):

1. Lumbar spinal length: the distance between the dorso-rostral corners of S1 and the L1, L2, L3, L4, and L5 vertebral bodies (5).
2. Intervertebral angles: the angles between each adjacent vertebra L1 through to S1 (7) and the lumbar lordosis (angle between the superior endplate of L1 and S1) (5).
3. Intervertebral disc cross-sectional area (CSA): the area of each disc from L1/L2 through to L5/S1 (5).

*2.5 Lumbar muscle volume and intramuscular lipid concentration*

Regions of interest (ROI) were manually traced (bilaterally, with the starting side randomly selected) over the lumbar paravertebral muscles using a semi-automated Matlab-based program (The MathWorks, Inc, Natick, MA, USA) (15, 52). Muscle chemical proprieties were calculated as the ratio of pixel intensities from the F and W images:

$$\mathrm{ILC} = \frac{\mathrm{F}}{(\mathrm{W} + \mathrm{F})}\ x\ 100$$

This technique to evaluate ILC has been validated in pig and rabbit models using the reference standard biopsy/histology (60), and reliability has been demonstrated in humans for the distribution fat content in the lumbar paravertebral muscles in the transverse plane (52). The technique used in the current study was not capable of separating between intra- and extramyocellular lipid compartments; however, chemical shift MR imaging methods can produce fat-signal fractions representative of lumbar muscle fat content similar to those obtained from spectroscopy (20).

The volumes and ILC of the LM, LES, QL, and psoas major (PM) muscles were extracted from each image from the top of the L1/L2 intervertebral disc to the bottom of the L5/S1 disc. To accurately delineate the LM and the LES muscles, the fascial border separating the two muscles was used as an anatomical landmark (14). The following nine lumbar regions were identified: L1/L2 intervertebral disc, L2 vertebral body, L2/L3 intervertebral disc, L3 vertebral body, L3/L4 intervertebral disc, L4 vertebral body, L4/L5 intervertebral disc, L5 vertebral body, and L5/S1 intervertebral disc (Figure 2). Measures of muscle volume and ILC at each lumbar region were averaged, and left- and right-sided measurements were averaged

(5). Five regions were selected for the statistical analysis to reduce the number of similar comparisons: L1/L2 intervertebral disc, L2/L3 intervertebral disc, L3/L4 intervertebral disc, L4/L5 intervertebral disc, and L5/S1 intervertebral disc.

*2.6 Statistical analysis*

Statistical analysis used the Statistical Package for Social Sciences (SPSS; Version 25, IBM, Chicago, IL, USA). Results are presented as means and standard deviations (SD). Statistical significance was set at a 2-sided 5% significance level. All parameters were assessed for normality using visual inspection of histograms and Q–Q plots. Spinal morphology variables (spinal length, intervertebral angles, and intervertebral disc CSA), muscle volume, and ILC at each lumbar region were analyzed by mixed-model repeated-measures analysis of variance (RMANOVA) with Group (CRTL, cAG, and iAG) as the between-group factor and Time (BDC and HDT59) as the within-subject factor. An interaction effect of Group and Time was included. Effect sizes (partial eta-squared: $\eta^2_{partial}$) were calculated. Where appropriate, post hoc pairwise analyses were performed using Bonferroni corrected multiple comparisons (with corresponding confidence intervals generated).

A RMANOVA with Group (CRTL, cAG, and iAG) as the between-group factor and Time (BDC and HDT59) as the within-subject factor was performed to evaluate the changes in body stature and body weight between baseline and the first day of recovery (R+0, before standing).

## 3. Results

*3.1 Participants*

Data were successfully collected from all participants at all timepoints. The RMANOVA revealed a main effect of Time for body stature and weight, with an increase of 2.0±1.2 cm (1.2±0.7%) ($F_{1,21} = 75$, $P < 0.001$, $\eta^2_{partial} = 0.8$) and a decrease of 1.8±1.3 kg (2.5±1.7%) ($F_{1,21} = 49$, $P < 0.001$, $\eta^2_{partial} = 0.7$), respectively, at R+0 relative to BDC. There were no significant main effects of Group ($F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.2$ for body height and weight) and Group by Time interactions ($F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$ for body height and weight).

One operator conducted all MRI measurements, and the intra-rater reliability of the operator performing the sagittal and transverse plane image measurements was evaluated one month before starting the analyses of all obtained images. The measurements were taken twice

with a minimum of 5 days apart (intraclass correlation coefficients: $ICC_{2,1}$: range of 0.916-0.998; 95% confidence interval (CI):0.780-0.999).

*3.2 Lumbar spine morphology*

Significant Time effects were found for the lumbar spine length between L1 and S1, L2 and S1, L3 and S1, L4 and S1, and L5 and S1 (all: $F_{1,21} > 10$, $P \leq 0.001$, $\eta^2_{partial} > 0.3$) (Figure 3A - L1/S1 length; Supplementary Figure 1 – data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.7$, $\eta^2_{partial} < 0.1$) and Group by Time interactions (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 1). The length of the entire lumbar spine (between L1 and S1) increased at HDT59 in the CTRL, cAG, and iAG groups by 0.58±0.19 cm (3.54±1.23%), 0.57±0.24 cm (3.58±1.36%), and 0.51±0.26 cm (2.97±1.46%), respectively. At HDT59, pairwise contrasts showed a mean difference of 0.85 cm (95% CI [-1.09, 1.26]) between CRTL and cAG, of -0.28 cm (95% CI [-1.21, 1.15]) between CRTL and iAG, and of -0.11 cm (95% CI [-1.3, 1.06]) between cAG and iAG.

There was a main effect of Time for the lumbar lordosis (angle between the superior endplate of L1 and S1) ($F_{1,21} = 21.8$, $P < 0.001$, $\eta^2_{partial} = 0.5$). There was no significant main effect of Group ($F_{2,21} = 0.4$, $P = 0.65$, $\eta^2_{partial} = 0.04$) and Group by Time interaction ($F_{2,21} = 0.8$, $P = 0.47$, $\eta^2_{partial} = 0.07$). The lumbar lordosis decreased in the CTRL, cAG, and iAG groups by 4.01±3.15º (7.79±5.88%), 3.34±3.92º (5.80±8.07%), and 2.01±2.61º (3.94±5.13%), respectively (Figure 3B - L1/S1 angle; Supplementary Figure 2 – data for all levels). At HDT59, the pairwise contrasts showed a mean difference of -0.22º (95% CI [-10.93, 10.49]) between CRTL and cAG, of -4.21º (95% CI [-14.92, 6.50]) between CRTL and iAG, and of -3.99º (95% CI [-14.70, 6.72]) between cAG and iAG. More specifically, the intervertebral disc angles reduced (less lordosis) between L5/S1 and L5/L4 ($F_{1,21} > 14$, $P < 0.001$, $\eta^2_{partial} > 0.4$ for both analyses) and increased (more kyphosis) between L2/L3 and L1/L2 vertebral levels (both: $F_{1,21} > 5$, $P < 0.05$, $\eta^2_{partial} > 0.2$ for both analyses). There were no significant main effects of Group (both: $F_{2,21} < 2$, $P > 0.3$, $\eta^2_{partial} < 0.1$) and Group by Time interactions for the intervertebral disc angles (both: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 2). There was no significant main effect of Time ($F_{1,21} = 2.8$, $P = 0.11$, $\eta^2_{partial} = 0.11$), Group ($F_{2,21} = 1.0$, $P = 0.4$, $\eta^2_{partial} = 0.1$) and Group by Time interaction ($F_{2,21} = 0.8$, $P = 0.4$, $\eta^2_{partial} = 0.1$) at L3/L4 vertebral levels.

The RMANOVA revealed main effects of Time for the CSA of the L1/L2, L2/L3, L3/L4, L4/L5, and L5/S1 intervertebral discs (all: $F_{1,21} > 11$, $P < 0.005$, $\eta^2_{partial} > 0.3$)(Figure 3C – L5/S1 level; Supplementary Figure 3 – data for all levels). There were no significant main

effects of Group (all: $F_{2,21} < 1$, $P > 0.2$, $\eta^2_{partial} < 0.1$) and Group by Time interactions (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 3). On average (all CSA discs), the intervertebral disc CSA increased at the end of HDT bed rest in the CTRL, cAG, and iAG groups by 0.34±0.21 $cm^2$ (15.59±9.71%), 0.31±0.14 $cm^2$ (13.97±7.36%), and 0.29±0.08 $cm^2$ (12.76±2.56%), respectively. At HDT59, the pairwise contrasts showed a mean difference of 0.29 $cm^2$ (95% CI [-0.52, 1.08]) between CRTL and cAG, of 0.04 $cm^2$ (95% CI [-0.76, 0.86]) between CRTL and iAG, and of -0.14 $cm^2$ (95% CI [-1.03, 0.61]) between cAG and iAG.

*3.3 Lumbar muscle volume*

A significant effect of Time was found in LM muscle volume from L1/L2 intervertebral disc to L5/S1 intervertebral disc (all: $F_{2,21} > 12$, $P < 0.005$, $\eta^2_{partial} > 0.4$) (Figure 4A – L3/L4 level; Supplementary Figure 4 – data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.1$) and Group by Time interactions (all: $F_{2,21} < 1$, $P > 0.3$, $\eta^2_{partial} < 0.1$) (detailed statistical results in Supplementary Table 4). The average reduction in LM muscle volume (all vertebral levels) in the CTRL, cAG, and iAG groups was 250±118 $mm^3$ (6.49±3.52%), 204±111 $mm^3$ (5.77±3.91%), and 212±96 $mm^3$ (6.11±3.47%), respectively. At HDT59, the pairwise contrasts showed a mean difference of 413 $mm^3$ (95% CI [-458, 1117]) between CRTL and cAG, of 372 $mm^3$ (95% CI [-454, 1116]) between CRTL and iAG, and of 2 $mm^3$ (95% CI [-785, 784]) between cAG and iAG.

Significant effects of Time were found in LES muscle volume from L1/L2 intervertebral disc to the L3/L4 intervertebral disc (all: $F_{2,21} > 54$, $P < 0.001$, $\eta^2_{partial} > 0.7$) (Figure 4B – L3/L4 level; Supplementary Figure 5 – data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.6$, $\eta^2_{partial} < 0.1$) and Group by Time interactions (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 5). The average reduction in LES muscle volume from the L1/L2 intervertebral disc to the L4/L3 intervertebral disc in the CTRL, cAG, and iAG groups was 973±370 $mm^3$ (11.49±3.60%), 811±385 $mm^3$ (9.92±4.36%), and 670±492 $mm^3$ (8.56±5.54%), respectively. At HDT59, the pairwise contrasts showed a mean difference of 43 $mm^3$ (95% CI [-2169, 2255]) between CRTL and cAG, and of 383 $mm^3$ (95% CI [-1829, 2596]) between CRTL and iAG, and of 340 $mm^3$ (95% CI [-1871, 2561]) between cAG and iAG. There were no significant main effect of Time ($F_{1,21} = 1.2$, $P = 0.3$, $\eta^2_{partial} = 0.1$), Group ($F_{2,21} = 0.1$, $P = 0.9$, $\eta^2_{partial} < 0.1$) and Group by Time interaction ($F_{2,21} < 0.1$, $P > 0.9$, $\eta^2_{partial} < 0.1$) at L4/L5 intervertebral disc. Contrary to the observation at other levels, LES muscle volume increased at the level of

L5/S1 intervertebral disc ($F_{2,21}$ = 17.3, P < 0.001, $\eta^2_{partial}$ = 0.5). There was no significant main effect of Group ($F_{2,21}$ = 1.0, P = 0.4, $\eta^2_{partial}$ = 0.1) and Group by Time interaction ($F_{2,21}$ = 1.3, P = 0.3, $\eta^2_{partial}$ = 0.1). The average increase in LES muscle volume at the L5/S1 intervertebral disc in the CTRL, cAG, and iAG groups was 164±411 $mm^3$ (7.94±11.98%), 200±258 $mm^3$ (7.53±8.40%), and 324±272 $mm^3$ (9.71±8.60%), respectively. At HDT59, pairwise contrasts showed a mean difference of -435 $mm^3$ (95% CI [-1356, 484]) between CRTL and cAG, and of -552 $mm^3$ (95% CI [-1472, 368]) between CRTL and iAG, and of -116 $mm^3$ (95% CI [-1036, 804]) between cAG and iAG.

Significant effects of Time were found in QL muscle volume from L1/L2 to L3/L4 intervertebral disc (all: $F_{2,21}$ > 18, P < 0.001, $\eta^2_{partial}$ > 0.5) (Figure 4C – L3/L4 level; Supplementary Figure 6 – data for all levels). There were no significant main effects of Group (all: $F_{2,21}$ < 1, P > 0.9, $\eta^2_{partial}$ < 0.1) and Group by Time interactions (all: $F_{2,21}$ < 1, P > 0.5, $\eta^2_{partial}$ < 0.1) (detailed statistical results in Supplementary Table 6). The average reduction in QL muscle volume in the CTRL, cAG, and iAG groups was 202±181 $mm^3$ (10.70±8.60%), 170±157 $mm^3$ (7.29±6.79%), and 200±159 $mm^3$ (10.31±8.15%), respectively. At HDT59, the pairwise contrasts showed a mean difference of -43 $mm^3$ (95% CI [-1825, 739]) between CRTL and cAG, and of -31 $mm^3$ (95% CI [-813, 751) between CRTL and iAG, and of 12 $mm^3$ (95% CI [-770, 794]) between cAG and iAG.

A significant effect of Time was found in PM muscle volume at the L1/L2 intervertebral disc ($F_{1,21}$ = 5.2, P = 0.03, $\eta^2_{partial}$ = 0.20) (Figure 4D – L3/L4 level; Supplementary Figure 7 – data for all levels). There was no significant main effect of Group ($F_{2,21}$ = 0.8, P = 0.46, $\eta^2_{partial}$ = 0.07) and Group by Time interaction ($F_{2,21}$ = 0.6, P = 0.56, $\eta^2_{partial}$ = 0.05) (detailed statistical results in Supplementary Table 7). The average increase in PM muscle volume at L1/L2 intervertebral disc in the CTRL, cAG, and iAG groups was 133±228 $mm^3$ (3.11±6.88%), 97±187 $mm^3$ (3.06±6.76%), and 67±129 $mm^3$ (2.15±5.55%), respectively. At HDT59, the pairwise contrasts showed a mean difference of 624 $mm^3$ (95% CI [-799, 2048]) between CRTL and cAG, and of 599 $mm^3$ (95% CI [-824, 2022]) between CRTL and iAG, and of 25 $mm^3$ (95% CI [-1398, 1449]) between cAG and iAG. Contrary to the observation at the L1/L2 intervertebral disc, there were no significant main effect of Time (all: $F_{1,21}$ < 3, P > 0.1, $\eta^2_{partial}$ < 0.1), Group (all: $F_{2,21}$ < 0.2, P > 0.2, $\eta^2_{partial}$ < 0.1) and Group by Time interaction (all: $F_{2,21}$ < 1, P > 0.1, $\eta^2_{partial}$ < 0.1) at other levels in PM muscle volume.

*3.4 Intramuscular lipid concentration*

Significant effects of Time were found in LM ILC at all levels from L1/L2 to L5/S1 intervertebral disc (all: $F_{1,21} > 10$, $P < 0.01$, $\eta^2_{partial} > 0.3$) (Figure 5A – L3/L4 level; Supplementary Figure 8 – data for all levels). There was no significant main effects of Group (all: $F_{2,21} < 0.3$, $P > 0.7$, $\eta^2_{partial} < 0.1$) and Group by Time interactions were revealed by the RMANOVA (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 8). The average increase in LM ILC in the CTRL, cAG, and iAG groups was 2.3±1.8%, 3.3±2.2%, and 3.3±1.6%, respectively. At HDT59, the pairwise contrasts showed a mean difference of -0.3% (95% CI [-11.4, 11.2]) between CRTL and cAG, of -2.2% (95% CI [-16.8, 9.0]) between CRTL and iAG, and of 2.4% (95% CI [-13.6, 9.0]) between cAG and iAG.

Significant effects of Time were found for LES ILC at all levels L1/L2 to L5/S1 intervertebral disc (all: $F_{2,21} > 16$, $P < 0.001$, $\eta^2_{partial} > 0.4$) (Figure 5B and Supplementary Figure 9 – data for all levels). There was no significant main effects of Group (all: $F_{2,21} < 0.9$, $P > 0.4$, $\eta^2_{partial} < 0.1$) and Group by Time interactions (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplementary Table 9). The average increase in LES ILC in the CTRL, cAG, and iAG groups was 2.2±1.9%, 2.6±2.1%, and 1.4±1.2%, respectively. At HDT59, the pairwise contrasts showed a mean difference of 1.0% (95% CI [-1.9, 3.9]) between CRTL and cAG, of -0.1% (95% CI [-3.0, 2.9]) between CRTL and iAG, and of 2.0% (95% CI [-9.6, 10.1]) between cAG and iAG.

No significant changes in Time, Group, and Group by Time interactions were found in QL and PS ILC ($F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$ for all analyses) (Figure 5C and 5D, Supplementary Figure 10 and 11 – data for all levels; detailed statistical results in Supplementary Table 10 and 11).

## 4. Discussion

We hypothesized that exposure to daily AG would mitigate the effects of exposure to 60-day HDT bed rest on lumbar spine muscle deconditioning, intervertebral disc expansion, and loss in lumbar lordosis. The rationale was that the compressive force produced by AG would be sufficient to stimulate the paraspinal muscles and intervertebral discs, resulting in the mitigation of muscle cell catabolism and maintenance of the shape of the lumbar spine. Contrary to our hypothesis, we found no significant protective effects from the daily AG exposure on any lumbar spine parameter.

We also aimed to investigate whether 60-day HDT bed rest induced an increase in ILC in the lumbar paraspinal musculature. Accumulation of relative ILC was found to be selective

to the LM and LES muscles, which are primarily postural/antigravity muscles. This novel finding supports similar observations with aging and chronic low back pain. ILC is an additional potentially modifiable target for reconditioning upon cessation of prolonged body unloading.

*4.1 Lumbar spinal morphology*

Contrary to our hypothesis, the AG protocols used in the current study did not significantly mitigate the effects of HDT bed rest on elongation of the lumbar spine, reduction in the lumbar lordosis, and expansion of the intervertebral discs. In contrast to these results, the application of other countermeasures, such as lower body negative pressure treadmill exercise (10), resistive vibration exercise (5, 7), and low-magnitude vibration (35), have reduced expansion of the intervertebral discs. Similar to the current investigation results, exposure to flywheel and spinal mobilization interventions (8) did not mitigate intervertebral discs expansion, lumbar spine flattering, and lumbar elongation. One possible explanation for different effects between AG and other countermeasures is that lower body negative pressure treadmill exercise, resistive vibration exercise, and low-magnitude vibration involve both the compressive force applied to the spine by elastic cords (~60–65% body weight) plus dynamic forces produced by the exercises, such as walking or squatting, which were included in these interventions. It is possible that the application of passive forces to the spine without any dynamic force loads to the lumbar spine is insufficient to mitigate lumbar spine adaptations. The compressive force of $1G_z$ to the lumbar spine used in the current study can be considered a passive force as the only movements performed by the participants were submaximal heel raises and shallow knee bends, which were undertaken to maintain circulation.

An alternative explanation is that the AG protocol with a duration of only 30 min/day of $1G_z$ centrifugation may have been an insufficient dose to produce a protective effect on the lumbar spine, considering that 'normal' exposure times to gravity loading through everyday activities is substantially longer (50). Future studies could investigate the application of dynamic lumbar spine movement, longer duration of exposure to AG, or a combination.

Similar to results of 60-day HDT bed rest studies, flattening of the lumbar lordosis and increased spinal length have been reported in astronauts after space flight (4, 47, 63). However, a recent study reported only negligible changes in disc height and swelling after prolonged exposure to microgravity (4). Although the explanation for the lack of changes in disc properties is not clear, it may be related to adoption of in-flight exercise protocols, which load the spine in the axial direction.

*4.2 Muscle volume*

Daily AG was also insufficient to mitigate atrophy of the paraspinal musculature. Muscle volume was primarily decreased in the lower lumbar spine region for the LM and QL muscles, and in the upper lumbar spine for the LES. These regions relate to the largest volume of those muscles and the regions where they generate their greatest extension moments to maintain the upright posture of the spine when challenged by gravity (16). Our data of reduced muscle volume and reduced lumbar lordosis agree with the strong association between these parameters identified previously in astronauts (4). There are two possible explanations: muscle atrophy could reduce control of the lordosis, or stretch of the muscle with a flatter lordosis could result in smaller muscle cross-sectional area measures. Regardless of the causal relationship, the flatter lumbar lordosis and reduced muscle capacity might increase the risk of intervertebral disc injury because of greater compressive disc loads and maintenance of end range of flexion (59).

Similar to the present results, lower body negative pressure treadmill exercise (10), low-magnitude vibration (35), flywheel, and spinal mobilization (8) have all failed to mitigate lumbar muscle atrophy following HDT bed rest. The only intervention that has shown success in preventing LM, LES, and QL atrophy has been high-load resistive vibration exercise (5, 7). This suggests that high-intensity exercise is necessary to reduce or prevent the catabolic cascade. However, this is not straightforward as participants performing high-load training found it difficult to maintain the lumbar lordosis, and they reported more days of lower back pain than the control group. This complicates the application of the intervention as the flexed lumbar posture might further loading the spine, and if muscle composition changes persist, this might increase the risk of soft-tissue injury (59).

The increased muscle volume of the LES muscle at the L5/S1 intervertebral disc represents a new finding. Although previous studies have not investigated the LES muscle at this vertebral level (5, 7, 10, 35), a reduction of 4% (7) and 8% (5) at the L5 vertebral body level has been previously reported in individuals exposed to bed rest, without any countermeasure. Increased muscle volume in our study may be explained by the high percentage of ILC observed at the distal portion of the LES muscle at baseline. The percentage of ILC was three times higher in the lower lumbar spine (~30±10% ILC) than in the upper lumbar spine (~10±5% ILC). The high percentage of adipose tissue at those vertebral levels may induce an increase in the volume that is not related to muscle, but instead to expansion of

the intermuscular adipose cells or intercellular connective tissues during the HDT bed rest. This would mask and changes to muscle tissue, as shown in other studies (21).

Finally, the increase in the volume of the psoas major muscle observed in the current investigation is in agreement with results of previous bed rest studies (5, 29, 58). Although speculative, recruitment of this muscle in tasks such as daily hygiene activities in bed rest might explain the observed increases in muscle volume (58). Further, changes in the lumbar erector spinae and quadratus lumborum muscles are minimal after exposure to bed rest (29). These findings contrast, the reduced volume of the psoas major, quadratus lumborum, and lumbar erector spinae muscles after spaceflight (11, 46). These differences highlight that although bed rest studies serve as a useful analogue for spaceflight, in that axial loading of the spine is decreased, there are inherent differences between the two conditions. For example, astronauts can move around freely in space, and in bed rest studies, the axis of gravity is shifted 90 degrees rather than eliminated as occurs in microgravity conditions.

*4.3 Intramuscular lipid concentration*

This study provides the first evidence of increased ILC in the LM and LES muscles at the end of 60 days of HDT bed rest, similar to that observed with aging, low back pain patients and astronauts (9, 39, 51). Unfortunately, AG protocols used in this investigation did not mitigate these changes. Increased percentage of ILC implies lipid deposition in these postural muscles, as a consequence of prolonged muscle unloading, or a related decrease in water and muscle protein. Recent long-duration spaceflights have reported a ~5% radiodensity attenuation of LM, LES, and QL muscles in computed tomography scans at the L2 vertebral body (9, 51), particularly in astronauts with less exposure to resistive exercise equipment (51). Radiodensity attenuation of muscles on CT scan has been associated with increased ILC (23) and is related to reduced trunk muscle function (27). Increased ILC has also been described after 3-day dry immersion, another simulated microgravity model, in the quadriceps muscle, indicating that a short period of severe inactivity is sufficient to induce accumulation of ILC in the antigravity muscles, concomitant with muscle deconditioning (55). Moreover, patients with low back pain with functional disabilities often show a high percentage of ILC in the LM muscle at the L5 vertebral level (32), particularly during the subacute (without atrophy) or chronic (with atrophy) pain stages (33).

Accumulation of ILC may arise from either excess triacylglycerols from food intake or reduced fat oxidation in these postural muscles. In contrast with previous bed-rest studies, energy uptake by nutrition was tailored to maintain a constant body mass in the current study.

Although participants' weight reduced by approximately 2 kg, an excess of diet-enhanced triacylglycerols may partially explain the increase in ILC. Downregulation of mitochondrial oxidoreductase activity has been observed in the vastus lateralis and soleus muscles following bed rest (19, 31). Importantly, muscles with greater oxidative power prior to HDT bed rest have a higher propensity to accumulate ILC, and a relationship between the oxidative capacity of a muscle and the development of ILC has been described (49). As LM and LES muscles have high proportions of slow-twitch fibres, high capillarization, and high concentration of oxidative muscle enzymes (~60%, (54)), consistent with their tonic role in maintenance of upright posture (38), the increase in ILC in LM and LES in the current study might be explained by a downregulation of the oxidative activity. In contrast, PM muscle has fewer slow-twitch fibres (~40%, (2)), and a low oxidative capacity may explain less accumulation of ILC in this muscle. One challenge to this argument is that rodent studies have shown high oxidative capacities in QL similar to LM and LES (61), yet ILC in QL did not show any change in our study. Species differences cannot be discounted.

An alternative explanation for increased relative ICL in the LM and LES muscles in the current study may also be related to fluid and electrolyte shifts between intra- and extracellular compartments, which accompany HDT bed rest and spaceflight (25, 45). The degradation of protein in muscle results in loss of intracellular water and, if the amount of lipid remains constant or decreases less than the amount of water, the net loss of water may cause an increase in ILC without a net uptake of adipose tissue.

*4.4 Operational relevance for planetary surface explorations and terrestrial populations*

One of the goals of NASA/ESA's Human Research Roadmap is to develop optimal countermeasures to mitigate the deconditioning effect of mechanical unloading to the lumbar spine. Atrophied and weakened spinal musculature is likely to reduce the crew's ability to perform critical tasks after landing and working on a planetary surface. It is plausible that this could also increase the risk of spinal injury (53) after prolonged spaceflight. Our results showed and confirmed that unloading leads to lumbar muscle deconditioning, but daily AG protocols did not significantly protect the tissue from these effects. Future studies could consider whether other AG paradigms are effective, such as prolonged exposure, higher compressive $G_z$, or combining AG with dynamic exercise. As highlighted above, dynamic exercise with loading can impact disc expansion (5, 7), and addition of dynamic movements with other paradigms of AG might produce additional protective effects on the lumbar spine.

A new finding of this study is the increase in ILC in the atrophied LM and LES following HDT bed rest, which may alter trunk muscle function. The specificity of changes at different vertebral lumbar levels might have important implications for selection of appropriate countermeasures to targeting the affected muscles during reconditioning. If countermeasures can be identified, this is not only relevant for tailoring of interventions for astronauts but also for bedridden individuals and patients with low back pain (28).

### *4.5 Limitations*

There are some limitations to the current study. First, the sample size was small due to the intrinsically complex nature and expense associated with prolonged bed rest studies. Many linked and similar outcomes have been analyzed, given the rare opportunity to measure these factors. The sample size limited the analytic options available to address complexity in the data. Previous 60-day HDT bed rest studies have used a similar number of groups and participants, and some have identified differences by applying the same statistical model as the current study (5, 7). A consideration is that, because of the small sample size, only large effect sizes from countermeasures can be detected (5, 7), and more subtle effects are likely overlooked. Another weakness of the sample is the uneven sex distribution (16 male, 8 female). Inclusion of equal numbers of males and females in future studies would allow investigation of potential sex differences in lumbopelvic muscles and the response to bed rest.

There are some measurement issues. First, although manual segmentation of volumes of lumbar spine muscles is rater-dependent and time-consuming, this technique is reliable and valid (52). Second, changes in spinal curvature and spinal length may influence MRI measurements of muscle as the cross-sections were not perfectly perpendicular to each muscle's axis. For this reason, the muscle volume of each trunk muscle was extracted from the top of the L1/L2 intervertebral disc to the bottom of the L5/S1 intervertebral disc (supplementary tables).

Finally, the re-distribution of fluids occurring during bed rest requires appropriate investigation since it may play an important role in paraspinal muscle size changes and the percentage of intramuscular lipids.

### *4.6 Conclusion*

This study found no effects of daily intermittent or continuous AG on mitigation of the degradation of the paraspinal muscles induced by 60-day HDT bed rest. An increase of relative ILC was observed in the atrophied LM and LES muscles. Further studies are needed to explore

alternative strategies to counteract the deteriorating effects of severe physical inactivity on the lumbopelvic musculature, such as the combination of AG with exercise.

**5. Acknowledgement:**

The AGBRESA study was funded by the German Aerospace Center, the European Space Agency (contract number: 4000113871/15/NL/PG), and the National Aeronautics and Space Administration (contract number: 80JSC018P0078). The study was performed at the: ENVIHAB research facility of the DLR Institute of Aerospace Medicine. Funding for this selected project (ESA-HSO-U-LE-0629) was received from the STFC/UK Space Agency. This study was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC). PH is funded by a Fellowship from the National Health and Medical Research Council of Australia (NHMRC: APP1102905). SS is supported by a Program grant from the NHMRC awarded to PH (APP1091302).

**6. Author contribution:**

NC, EDM, TW, JS, DD, JH, PH, SS, and GL contributed to the initial conception of the design of the research. NC, EDM, KL, TW, JZ, JE, and JH contributed to the development refinement of the methodology. JZ contributed to the acquisition of MRI data. JE and MH developed the software to analyze the data. EDM analyzed the MRI data. DB and JC provided statistical expertise. EDM wrote the draft of the manuscript. All authors provided critical revision to the draft of the manuscript. All authors approved the final version of the manuscript.

**7. Conflict of interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## 8. References


1. **Akimoto T**, **Ushida T**, **Miyaki S**, **Akaogi H**, **Tsuchiya K**, **Yan Z**, **Williams RS**, **Tateishi T**. Mechanical stretch inhibits myoblast-to-adipocyte differentiation through Wnt signaling. *Biochem Biophys Res Commun* 329: 381–385, 2005. doi: 10.1016/j.bbrc.2005.01.136.
2. **Arbanas J**, **Klasan GS**, **Nikolic M**, **Jerkovic R**, **Miljanovic I**, **Malnar D**. Fibre type composition of the human psoas major muscle with regard to the level of its origin. *J Anat Anat* 215: 636–641, 2009. doi: 10.1111/j.1469-7580.2009.01155.x.
3. **Attias J**, **Grassi A**, **Bosutti A**, **Ganse B**, **Degens H**, **Drey M**. Head-down tilt bed rest with or without artificial gravity is not associated with motor unit remodeling. *Eur J Appl Physiol* , 2020. doi: 10.1007/s00421-020-04458-7.
4. **Bailey JF**, **Miller SL**, **Khieu K**, **Neill CWO**, **Healey RM**, **Coughlin DG**, **Sayson J V**, **Chang DG**, **Hargens AR**, **Lotz JC**. From the international space station to the clinic : how prolonged unloading may disrupt lumbar spine stability. *Spine J* 18: 7–14, 2018. doi: 10.1016/j.spinee.2017.08.261.
5. **Belavý DL**, **Armbrecht G**, **Gast U**, **Richardson CA**, **Hides JA**, **Felsenberg D**. Countermeasures against lumbar spine deconditioning in prolonged bed rest : resistive exercise with and without whole body vibration. *J Appl Physiol* 109: 1801–1811, 2010. doi: 10.1152/japplphysiol.00707.2010.
6. **Belavý DL**, **Armbrecht G**, **Richardson CA**, **Felsenberg D**, **Hides JA**. Muscle Atrophy and Changes in Spinal. *Spine (Phila Pa 1976)* 36: 137–145, 2011. doi: 10.1097/BRS.0b013e3181cc93e8.
7. **Belavý DL**, **Hides JA**, **Wilson SJ**, **Stanton W**, **Dimeo FC**, **Rittweger J**, **Felsenberg D**, **Richardson CA**. Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bed-rest. *Spine (Phila Pa 1976)* 33: 121–131, 2008. doi: 10.1097/BRS.0b013e3181657f98.
8. **Belavy DL**, **Ohshima H**, **Bareille M**, **Rittweger J**, **Felsenberg D**. Limited effect of fly-wheel and spinal mobilization exercise countermeasures on lumbar spine deconditioning during 90 d bed-rest in the Toulouse LTBR study. *Acta Astronaut* 69: 406–419, 2011. doi: 10.1016/j.actaastro.2011.05.015.
9. **Burkhart K**, **Allaire B**, **Bouxsein ML**. Negative Effects of Long-duration Spaceflight on Paraspinal Muscle Morphology. *Spine (Phila Pa 1976)* 44: 879–886, 2019. doi: 10.1097/BRS.0000000000002959.
10. **Cao P**, **Kimura S**, **Macias BR**, **Ueno T**, **Watenpaugh DE**, **Hargens AR**. Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest. *J Appl Physiol* 99: 39–44, 2005. doi: 10.1152/japplphysiol.01400.2004.
11. **Chang DG**, **Healey RM**, **Snyder AJ**, **Sayson J V.**, **Macias BR**, **Coughlin DG**, **Bailey JF**, **Parazynski SE**, **Lotz JC**, **Hargens AR**. Lumbar spine paraspinal muscle and intervertebral disc height changes in astronauts after long-duration spaceflight on the International Space Station. *Spine (Phila Pa 1976)* 41: 1917–1924, 2016. doi: 10.1097/BRS.0000000000001873.
12. **Clément G**. International roadmap for artificial gravity research. *Microgravity* 3: 1–7, 2017. doi: 10.1038/s41526-017-0034-8.
13. **Clément GR**, **Bukley AP**, **Paloski WH**. Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions. *Front Syst Neurosci* 9: 1–11, 2015. doi: 10.3389/fnsys.2015.00092.
14. **Crawford RJ**, **Cornwall J**, **Abbott R**, **Elliott JM**. Manually defining regions of interest when quantifying paravertebral muscles fatty infiltration from axial magnetic resonance imaging : a proposed method for the lumbar spine with anatomical cross- reference. *BMC Musculoskelet Disord*. 2017; 18: 25.
15. **Crawford RJ**, **Volken ÃT**, **Elliott J.**, **Hoggarth MA**, **Dpt MS**, **Samartzis D**. Geography of Lumbar Paravertebral Muscle. *Spine (Phila Pa 1976)* 44: 1294–1302, 2019. doi: 10.1097/BRS.0000000000003060.
16. **De Martino E**, **Salomoni SE**, **Winnard A**, **Mccarty K**, **Lindsay K**, **Riazati S**, **Weber T**, **Scott J**, **Green DA**, **Hides J**, **Debuse D**, **Hodges PW**, **Van Dieën JH**, **Caplan N**. Hypogravity reduces trunk admittance and lumbar muscle activation in response to external perturbations. *J Appl Physiol* : 1044–1055, 2020. doi: 10.1152/japplphysiol.00756.2019.
17. **Demontis GC**, **Germani MM**, **Caiani EG**, **Barravecchia I**, **Passino C**, **Angeloni D**. Human pathophysiological adaptations to the space environment. *Front Physiol* 8: 1–17, 2017. doi: 10.3389/fphys.2017.00547.
18. **Diaz-Artiles A**, **Heldt T**, **Young LR**. Short-term cardiovascular response to short-radius centrifugation with and without ergometer exercise. *Front Physiol* 9: 1–16, 2018. doi: 10.3389/fphys.2018.01492.
19. **Ferretti G**, **Antonutto G**, **Denis C**, **Hoppeler H**, **Minetti AE**, **Narici M V.**, **Desplanches D**. The interplay of central and peripheral factors in limiting maximal O2 consumption in man after prolonged bed rest. *J Physiol* 501: 677–686, 1997. doi: 10.1111/j.1469-7793.1997.677bm.x.
20. **Fischer MA**, **Nanz D**, **Shimakawa A**, **Schirmer T**, **Guggenberger R**, **Chhabra A**, **Carrino JA**, **Andreisek G**. Quantification of muscle fat in patients with low back pain: Comparison of multi-echo MR

imaging with single-voxel MR spectroscopy. *Radiology* 266: 555–563, 2013. doi: 10.1148/radiol.12120399.

21. **Fortin M**, **Lazáry À**, **Varga PP**, **Battié MC**. Association between paraspinal muscle morphology, clinical symptoms and functional status in patients with lumbar spinal stenosis. *Eur Spine J* 26: 2543–2551, 2017. doi: 10.1007/s00586-017-5228-y.
22. **Frett T**, **Green DA**, **Mulder E**, **Noppe A**, **Arz M**, **Pustowalow W**, **Petrat G**, **Tegtbur U**, **Jordan J**. Tolerability of daily intermittent or continuous short-arm centrifugation during 60-day 6o head down bed rest (AGBRESA study). *PLoS One* 15: 1–11, 2020. doi: 10.1371/journal.pone.0239228.
23. **Goodpaster BH**, **Kelley DE**, **Thaete FL**, **He J**, **Ross R**. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol* 89: 104–110, 2000.
24. **Green DA**, **Scott JPR**. Spinal Health during Unloading and Reloading Associated with Spaceflight. *Front Physiol* 8: 1–7, 2018. doi: 10.3389/fphys.2017.01126.
25. **Greenleaf JE**, **Bernauer EM**, **Staley RW**. Fluid and electrolyte shifts with isometric and isotonic during bed rest exercise. *J Appl Physiol* 42: 56–66, 1977.
26. **Hartvigsen J**, **Hancock MJ**, **Kongsted A**, **Louw Q**, **Ferreira ML**, **Genevay S**, **Hoy D**, **Karppinen J**, **Pransky G**, **Sieper J**, **Smeets RJ**, **Underwood M**, **Buchbinder R**, **Cherkin D**, **Foster NE**, **Maher CG**, **van Tulder M**, **Anema JR**, **Chou R**, **Cohen SP**, **Menezes Costa L**, **Croft P**, **Ferreira PH**, **Fritz JM**, **Gross DP**, **Koes BW**, **Öberg B**, **Peul WC**, **Schoene M**, **Turner JA**, **Woolf A**. What low back pain is and why we need to pay attention. *Lancet* 391: 2356–2367, 2018. doi: 10.1016/S0140-6736(18)30480-X.
27. **Hicks GE**, **Simonsick EM**, **Harris TB**, **Newman AB**, **Weiner DK**, **Nevitt MA**, **Tylavsky FA**. Trunk Muscle Composition as a Predictor of Reduced Functional Capacity in the Health , Aging and Body Composition Study : The Moderating Role of Back Pain. *J Gerontol* 60: 1420–1424, 2005.
28. **Hides J**, **Hodges PW**, **Lambrecht G**. State-of-the-Art Exercise Concepts for Lumbopelvic and Spinal Muscles – Transferability to Microgravity. *Front Physiol* 10: 10:837, 2019. doi: 10.3389/fphys.2019.00837.
29. **Hides JA**, **Belavý DL**, **Stanton W**, **Wilson SJ**, **Rittweger J**, **Felsenberg D**, **Richardson CA**. Magnetic resonance imaging assessment of trunk muscles during prolonged bed rest. *Spine (Phila Pa 1976)* 32: 1687–1692, 2007. doi: 10.1097/BRS.0b013e318074c386.
30. **Hides JA**, **Lambrecht G**, **Sexton CT**, **Pruett C**, **Petersen N**, **Jaekel P**, **Rosenberger A**, **Weerts G**. The effects of exposure to microgravity and reconditioning of the lumbar multifidus and anterolateral abdominal muscles: implications for people with LBP. *Spine J* : 1–14, 2020. doi: 10.1016/j.spinee.2020.09.006.
31. **Hikida R**, **Gollnick P**, **Dudley G**, **Convertino V**, **Buchanan P**. Structural and metabolic characteristics of human skeletal muscle following 30 days of simulated microgravity. *Aviat Sp Env Med* 60: 664–70, 1989.
32. **Hildebrandt M**, **Fankhauser G**, **Meichtry A**, **Luomajoki H**. Correlation between lumbar dysfunction and fat infiltration in lumbar multifidus muscles in patients with low back pain. *BMC Musculoskelet Disord.* 2017; 18: 12.
33. **Hodges PW**, **Danneels L**. Changes in Structure and Function of the Back Muscles in Low Back Pain: Different Time Points, Observations, and Mechanisms. *J Orthop Sport Phys Ther* 49: 464–476, 2019. doi: 10.2519/jospt.2019.8827.
34. **Hodges PW**, **James G**, **Blomster L**, **Hall L**, **Schmid A**, **Shu C**, **Little C**, **Melrose J**. Multifidus Muscle Changes after Back Injury Are Characterized by Structural Remodeling of Muscle, Adipose and Connective Tissue, but Not Muscle Atrophy: Molecular and Morphological Evidence. *Spine (Phila Pa 1976)* 40: 1057–1071, 2015. doi: 10.1097/BRS.0000000000000972.
35. **Holguin N**, **Muir J**, **Rubin C**, **Judex S**. Short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. *Spine J* 9: 470–477, 2009. doi: 10.1016/j.spinee.2009.02.009.
36. **Iwase S**. Effectiveness of centrifuge-induced artificial gravity with ergometric exercise as a countermeasure during simulated microgravity exposure in humans. *Acta Astronaut* 57: 75–80, 2005. doi: 10.1016/j.actaastro.2005.03.013.
37. **James G**, **Sluka KA**, **Blomster L**, **Hall L**, **Schmid AB**, **Shu CC**, **Little CB**, **Melrose J**, **Hodges PW**. Macrophage polarization contributes to local inflammation and structural change in the multifidus muscle after intervertebral disc injury. *Eur Spine J* 27: 1744–1756, 2018. doi: 10.1007/s00586-018-5652-7.
38. **Jørgensen K**, **Cand M**, **Nicholaisen T**, **Miya K**. Muscle Fiber Distribution, Capillary Density, and Enzymatic Activities in the Lumbar Paravertebral Muscles of Young Men: Significance for Isometric Endurance. *Spine (Phila Pa 1976)* 18: 1439–1450, 1993.
39. **Kjaer P**, **Bendix T**, **Sorensen JS**, **Korsholm L**, **Leboeuf-Yde C**. Are MRI-defined fat infiltrations in the multifidus muscles associated with low back pain? *BMC Med* 5: 1–10, 2007. doi: 10.1186/1741-7015-5-2.

40. **Koch B**, **Gerzer R**. A research facility for habitation questions to be built at the German Aerospace Center in Cologne: Future challenges of space Medicine. *Hippokratia* 12: 91–96, 2008.
41. **Kook SH**, **Lee HJ**, **Chung WT**, **Hwang IH**, **Lee SA**, **Kim BS**, **Lee JC**. Cyclic mechanical stretch stimulates the proliferation of C2C12 myoblasts and inhibits their differentiation via prolonged activation of p38 MAPK. *Mol Cells* 25: 479–486, 2008.
42. **Kotovskaya AR**. The problem of artificial gravity: The current state and prospects. *Hum Physiol* 36: 780–787, 2010. doi: 10.1134/s0362119710070078.
43. **Laroche M**, **Cintas P**. Bent spine syndrome (camptocormia): A retrospective study of 63 patients. *Jt Bone Spine* 77: 593–596, 2010. doi: 10.1016/j.jbspin.2010.05.012.
44. **Laroche M**, **Delisle MB**, **Aziza R**, **Lagarrigue J**, **Mazieres B**. Is Camptocormia a primary muscle disease? *Spine (Phila Pa 1976)* 20: 1011–1016, 1995.
45. **Leach C**. A review of the consequences of fluid and electrolyte shifts in weightlessnesst. *Acta Astronaut* 6: 1123–1135, 1979.
46. **LeBlanc A**, **Lin C**, **Shackelford L**, **Sinitsyn V**, **Evans H**, **Belichenko O**, **Schenkman B**, **Kozlovskaya I**, **Oganov V**, **Bakulin A**, **Hedrick T**. Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *J Appl Physiol* 89: 2158–2164, 2000. doi: 10.1016/s0957-4271(96)00169-3.
47. **LeBlanc AD**, **Evans HJ**, **Schneider VS**, **Wendt RE**, **Hedrick TD**. Changes in IVD cross sectional area with bedrest and space flight. *Spine (Phila. Pa. 1976).* 19: 812–817, 1994.
48. **Linnarsson D**, **Hughson RL**, **Fraser KS**, **Clément G**, **Karlsson LL**, **Mulder E**, **Paloski WH**, **Rittweger J**, **Wuyts FL**, **Zange J**. Effects of an artificial gravity countermeasure on orthostatic tolerance, blood volumes and aerobic power after short-term bed rest (BR-AG1). *J Appl Physiol* 118: 29–35, 2015. doi: 10.1152/japplphysiol.00061.2014.
49. **Manini TM**, **Clark BC**, **Nalls MA**, **Goodpaster BH**, **Ploutz-Snyder LL**, **Harris TB**. Reduced physical activity increases intermuscular adipose tissue in healthy young adults. *Am J Clin Nutr* 85: 377–384, 2007. doi: 10.1093/ajcn/85.2.377.
50. **Mayorga-Vega D**, **Casado-Robles C**, **Viciana J**, **López-Fernández I**. Daily step-based recommendations related to moderate-to-vigorous physical activity and sedentary behavior in adolescents. *J Sport Sci Med* 18: 586–595, 2019.
51. **McNamara KP**, **Greene KA**, **Moore AM**, **Lenchik L**, **Weaver AA**. Lumbopelvic muscle changes following long-duration spaceflight. *Front Physiol* 10, 2019. doi: 10.3389/fphys.2019.00627.
52. **Mhuiris ÁN**, **Volken T**, **Elliott JM**, **Hoggarth M**, **Samartzis D**, **Crawford RJ**. Reliability of quantifying the spatial distribution of fatty infiltration in lumbar paravertebral muscles using a new segmentation method for T1-weighted MRI. *BMC Musculoskelet Disord* 17: 1–7, 2016. doi: 10.1186/s12891-016-1090-z.
53. **Nelson E**, **Lewandowski B**, **Licata A**, **Myers G**. Development and Validation of a Predictive Bone Fracture Risk Model for Astronauts. *Ann Biomed Eng* 37: 2337–2359, 2009. doi: 10.1007/s10439-009-9779-x.
54. **Ng JKF**, **Richardson CA**, **Kippers V**, **Parnianpour M**. Relationship Between Muscle Fiber composition and Functional Capacity of Back muscles in Healthy Subjects and patients With Back Pain. *JOSPT* 27: 389–402, 1998.
55. **Pagano AF**, **Brioche T**, **Arc-Chagnaud C**, **Demangel R**, **Chopard A**, **Py G**. Short-term disuse promotes fatty acid infiltration into skeletal muscle. *J Cachexia Sarcopenia Muscle* 9: 335–347, 2018. doi: 10.1002/jcsm.12259.
56. **Park JS**, **Soo Y**, **Jaedong P**, **Jeongmin K**, **Dae H**, **Choe H**. Sarcopenia and fatty degeneration of paraspinal muscle associated with increased sagittal vertical axis in the elderly : a cross‐sectional study in 71 female patients. *Eur Spine J* 29: 1353–1361, 2020. doi: 10.1007/s00586-020-06416-5.
57. **Roussouly P**, **Nnadi C**. Sagittal plane deformity : an overview of interpretation and management. *Eur Spine J* : 1824–1836, 2010. doi: 10.1007/s00586-010-1476-9.
58. **Shackelford LC**, **LeBlanc AD**, **Driscoll TB**, **Evans HJ**, **Rianon NJ**, **Smith SM**, **Spector E**, **Feeback DL**, **Lai D**. Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol* 97: 119–129, 2004. doi: 10.1152/japplphysiol.00741.2003.
59. **Shirazi-Adl A**, **Parnianpour M**. Effect of changes in lordosis on mechanics of the lumbar spine-lumbar curvature in lifting. *J Spinal Disord* 12: 426–447, 1999.
60. **Smith AC**, **Parrish TB**, **Abbott R**, **Hoggarth MA**, **Mendoza K**, **Chen YF**, **Elliott JM**. Muscle-fat MRI: 1.5 Tesla and 3.0 Tesla versus histology. *Muscle and Nerve* 50: 170–176, 2014. doi: 10.1002/mus.24255.
61. **Stark H**, **Frober R**, **Schilling N**. Intramuscular architecture of the autochthonous back muscles in humans. *J Anat* 222: 214–222, 2013. doi: 10.1111/joa.12005.
62. **Stenger MB**, **Evans JM**, **Knapp CF**, **Lee SMC**, **Phillips TR**, **Perez SA**, **Moore AD**, **Paloski WH**, **Platts SH**. Artificial gravity training reduces bed rest-induced cardiovascular deconditioning. *Eur J Appl Physiol* 112: 605–616, 2012. doi: 10.1007/s00421-011-2005-1.

63. **Wing PC**, **Tsang IK**, **Susak L**, **Gagnon F**, **Gagnon R**, **Potts JE**. Back pain and spinal changes in microgravity. *Orthop Clin North Am* 22: 255–262, 1991.

**Figure**

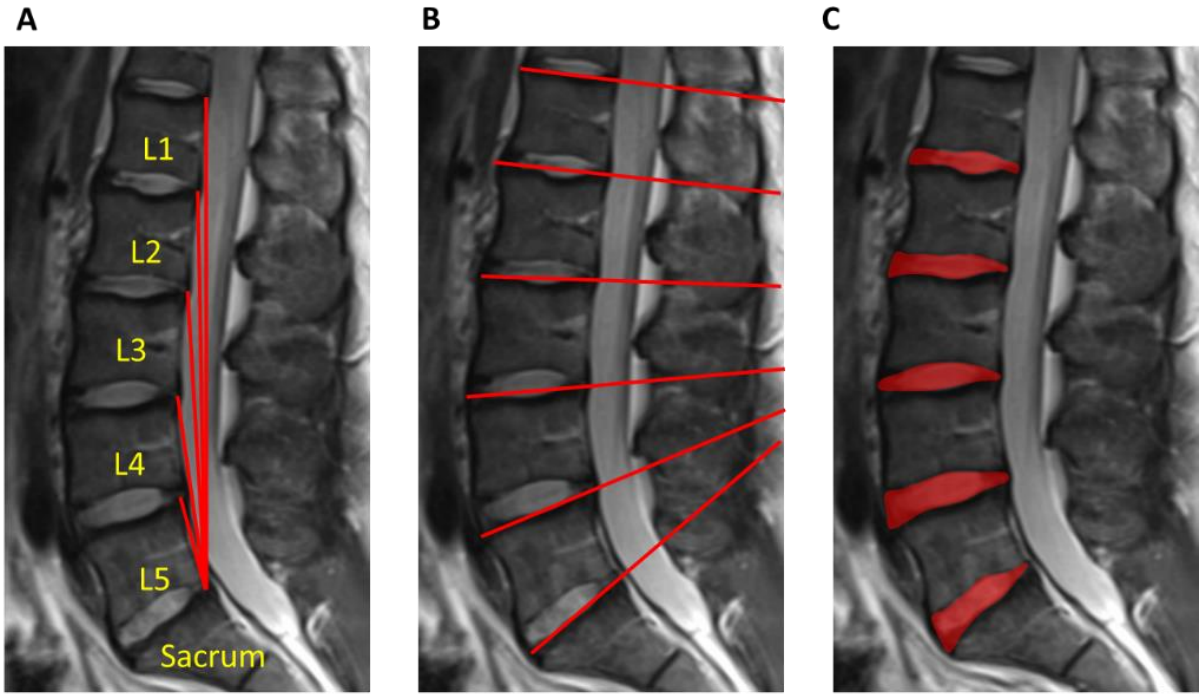


**Figure 1.** Measurements of spinal morphology on the sagittal image. **A:** Spinal length measured from the dorso-rostral corners of each lumbar vertebra to the sacrum; **B:** Intervertebral angle calculated between lines drawn at the superior border of each vertebra; **C:** Disc cross-sectional area indicated by the red shaded area.

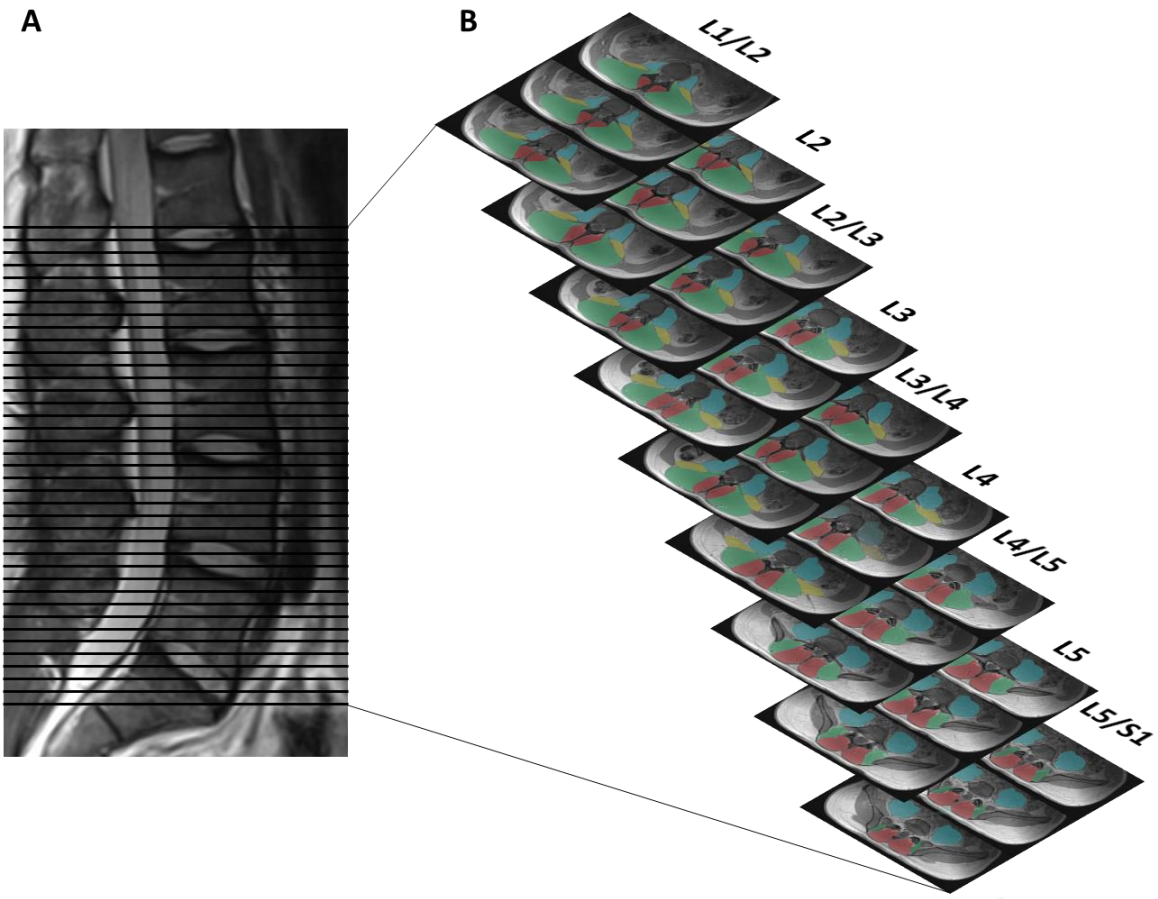


**Figure 2. A:** Section of the spine on sagittal images used for the analysis. **B:** Characteristic location of lumbar paraspinal muscles identified for the area measurement on axial images (slide thickness = 4 mm; Slice gap = 20%). The muscle volume of the lumbar multifidus (red shaded area), lumbar erector spinae (green shaded area), quadratus lumborum (yellow shaded area), and psoas major (blue shaded area) was calculated bilaterally from L1/L2 to L5/S1 disc level. Each of the nine lumbar regions was composed of 3-5 slides, depending on the participant's height. Five regions were selected for the statistical analysis: L1/L2 intervertebral disc, L2/L3 intervertebral disc, L3/L4 intervertebral disc, L4/L5 intervertebral disc, and L5/S1 intervertebral disc; however, the results of all regions are reported in the supplement material.

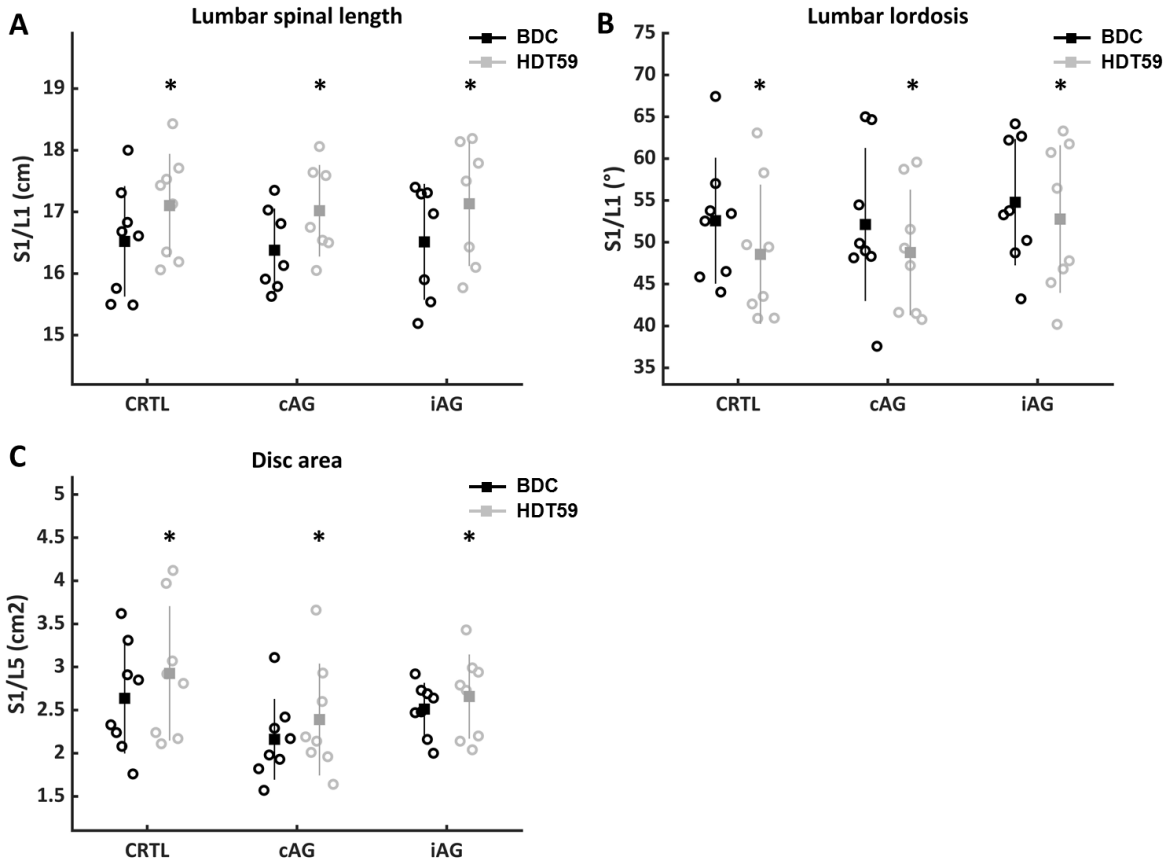


**Figure 3.** Lumbar spinal morphology at BDC and HDT59 for participants in the CRTL (N = 8), cAG (N = 8), iAG (N = 8). Each open circle represents a participant, the group mean is a filled square, and the standard deviation is vertical lines. The black vertical line is BDC and gray vertical line HDT59. **A.** Lumbar spinal length from S1 to L1. **B.** The angle between S1 and L1 (lumbar lordosis). **C.** L5/S1 disc cross-sectional area (CSA). Significantly higher lumbar spinal length at BDC compared with HDT59 (*, $P<0.05$). * = Significant main effect of Time ($P<0.05$).

BDC - Baseline data collection, HDT - head-down tilt bed rest, CRTL – Control, cAG – continuous artificial gravity, iAG – intermittent artificial gravity

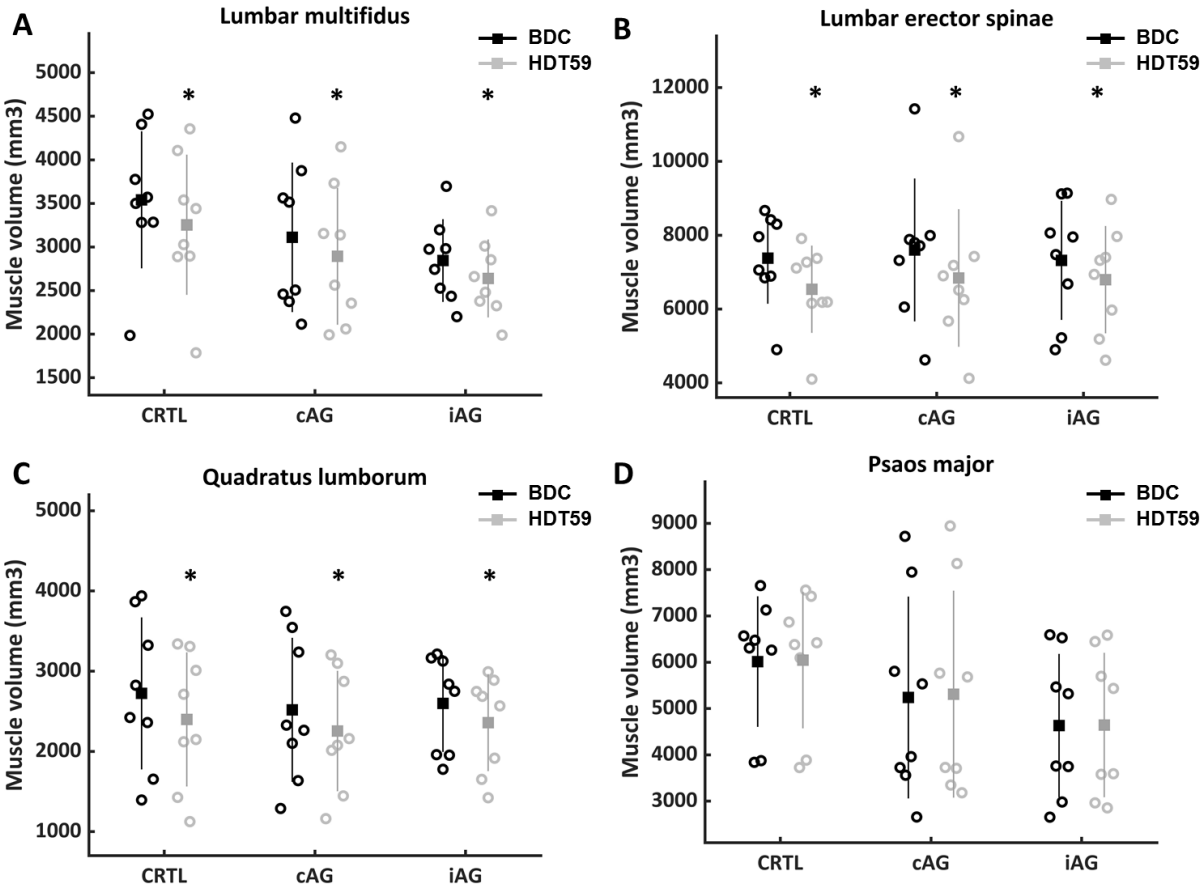


**Figure 4.** Lumbar muscle volume at L3/L4 intervertebral disc at BDC and HDT59 for participants in the CRTL (N = 8), cAG (N = 8), iAG (N = 8). Each open circle represents a participant, the group mean is a filled square, and the standard deviation is vertical lines. The black vertical line is BDC and gray vertical line HDT59. **A.** Muscle volume of the lumbar multifidus. **B.** Muscle volume of the lumbar erector spinae. **C.** Muscle volume of the quadratus lumborum. **D.** Muscle volume of the psoas major. * = Significant main effect of Time ($P<0.05$).

BDC - Baseline data collection, HDT - head-down bed rest, CRTL – Control, cAG – continuous artificial gravity, iAG – intermittent artificial gravity

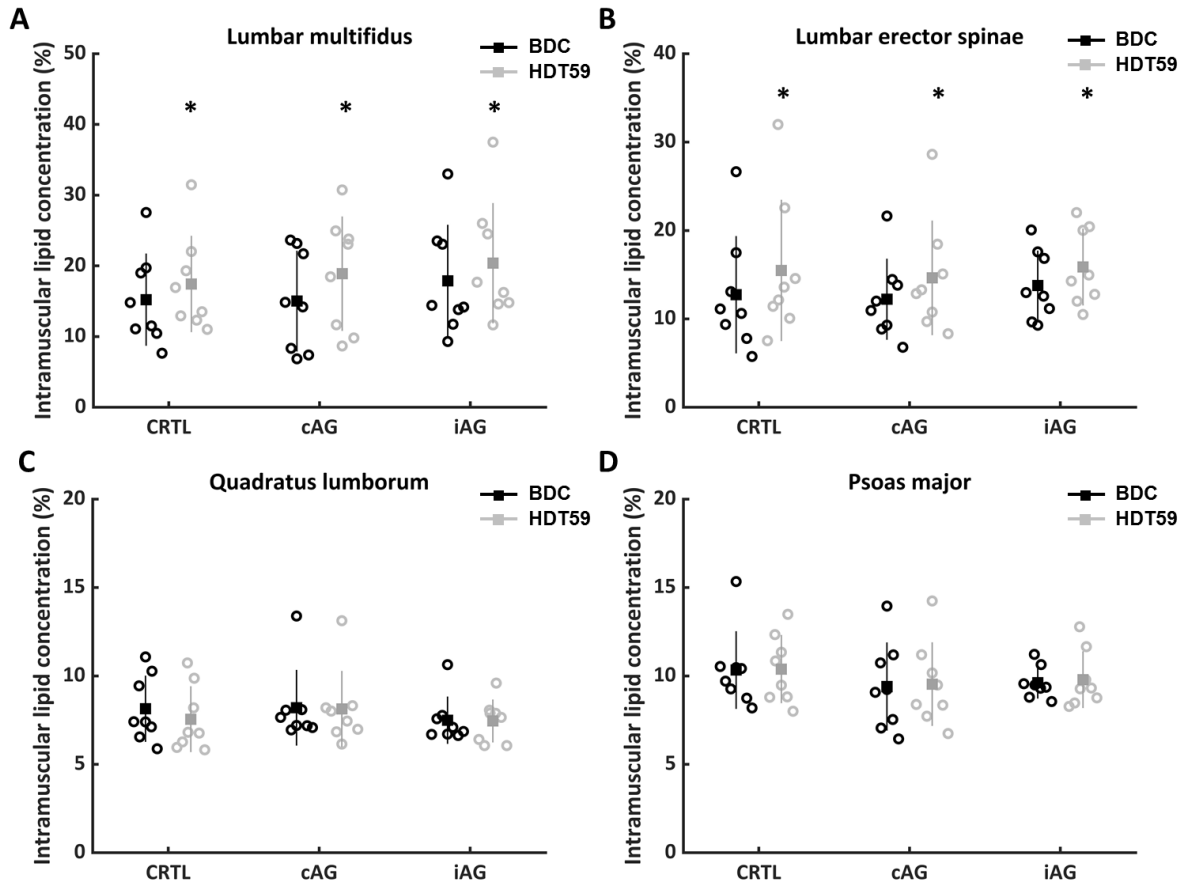


**Figure 5.** Lumbar muscle ILC at L3/L4 intervertebral disc at BDC and HDT59 for participants in the CRTL (N = 8), cAG (N = 8), iAG (N = 8). Each open circle represents a participant, the group mean is a filled square, and the standard deviation is vertical lines. Black vertical line is BDC and gray vertical line HDT59. **A.** ILC into the lumbar multifidus. **B.** ILC into the lumbar erector spinae. **C** ILC into the quadratus lumborum. **D.** ILC into the psoas major. * = Significant main effect of Time ($P<0.05$).

BDC - Baseline data collection, HDT - head-down tilt bed rest, ILC – Intramuscular lipid concentration, CRTL – Control, cAG – continuous artificial gravity, iAG – intermittent artificial gravity.