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RESEARCH ARTICLE

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

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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 analog). Twenty-four healthy individuals participated in the study: 8 received 30-min continuous AG; 8 received 6 × 5-min AG interspersed with rest periods; and 8 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). Longitudinal relaxation time (T1)-weighted images were used to assess morphology of the lumbar spine (spinal length, intervertebral disk angles, disk area) and volumes of the lumbar multifidus (LM), lumbar erector spinae (LES), quadratus lumborum (QL), and psoas major (PM) muscles from L_1/L_2 to L_5/S_1 vertebral levels. A chemical shift-based two-point lipid/water Dixon sequence was used to evaluate muscle composition. Results showed that spinal length and disk area increased ($P < 0.05$); intervertebral disk angles ($P < 0.05$) and muscle volumes of LM, LES, and QL reduced ($P < 0.01$); and lipid-to-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-to-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 after 60-day head-down bed rest and the 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.

AGBRESA; immobilization; magnetic resonance imaging; paraspinal muscles; short-arm centrifugation

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INTRODUCTION

Exposure to microgravity reduces the functional capacity of multiple body systems (1). Among those deteriorations, flattening of spinal curvature and atrophy of lumbar musculature have been documented in astronauts (2). 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 (3), which is the leading cause of disability worldwide (4).

Exposure to microgravity during spaceflight provokes rapid adaptation in lumbar spine morphology (for review, see Ref. 5). Increased spinal length, loss of lumbar lordosis, vertebral osteopenia, and atrophy of the lumbar multifidus (LM) at L_4 and L_5 vertebral levels have been observed after space missions (2, 6, 7). Recently, with computed tomography (CT), attenuation values in the LM, lumbar erector spinae (LES), and quadratus lumborum (QL) have been described in astronauts after 6 mo of spaceflight (8, 9). 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 (10). 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 (<6 wk) is associated with localized atrophy of LM muscle, subacute low back pain (between 6 and 12 wk) with elevated ILC and fibrosis without muscle atrophy of LM muscle, and chronic low back pain (>12 wk) with diffuse atrophy, fibrosis, and elevated ILC of LM muscle (for review, see Ref. 11).

As there are few astronauts to study, an alternative is Earth-based analog 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 disk expansion, and atrophy of LM, LES, and QL muscles (12, 13). 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 (2). No studies have investigated whether ILC is increased in the lumbar paraspinal muscles after HDT bed rest or which muscles are more sensitive to such adaptation. Animal studies have shown that an intervertebral disk lesion at the L_3/L_4 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 (14, 15). The presence of increased ILC in atrophied LM and LES muscles at the L_4 and L_5 vertebral levels in elderly individuals is strongly correlated with an increased sagittal vertical axis measured by magnetic resonance imaging (MRI) (16). The increased sagittal vertical axis has been proposed to contribute to compromised quality of life and independence and a higher risk of falls due to a more anteriorly placed body center of mass (17). In the bent spine syndrome (camptocormia), paraspinal muscles are fully replaced by adipose tissue (18). 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 (19).

When applied to HDT bed rest, exercise-based countermeasures, such as lower body negative pressure treadmill exercise (20), high-load resistive vibration exercise (21, 22), and low-magnitude vibration (23), achieve partial protection against lumbar spine changes. However, only high-load resistive vibration exercise performed 3 days/wk mitigated atrophy of the LM muscle (21, 22), likely because of 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) (24), has been shown to mitigate some of the deconditioning effects on the human body in situations where axial loading is diminished (25, 26). Results from HDT bed rest studies suggest that daily exposure to AG mitigated some deconditioning of the cardiovascular, musculoskeletal, and neurovestibular systems (24, 27). 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 (28–30).

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 toward 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 present study was to investigate whether two 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 (1 G_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 intermittent AG inhibited the differentiation from myoblasts to adipocytes (31, 32) caused by body unloading.

METHODS

Participants

The Artificial Gravity Bed Rest–European Space Agency study (AGBRESA study) was undertaken at the ":envihab" facility in Cologne, Germany (33) 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-day 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 who underwent 30-min continuous centrifugation/day (cAG), a group who underwent six sets of 5-min centrifugation/day (iAG) interspersed by rest (3-min breaks), and a group who were not exposed to AG (control, CTRL). Participants were pseudorandomly assigned to groups with regard to sex because of the dropout of three women and subsequent replacement during the campaign (34). The sex, age, height, and weight of the participant groups were comparable (CRTL: 2 females, 32 ± 7 yr, 177 ± 7 cm, 79 ± 13 kg; cAG: 3 females, 34 ± 11 yr, 172 ± 8 cm, 72 ± 10 kg; iAG: 3 females, 34 ± 10 yr, 174 ± 11 cm, 71 ± 5 kg) (34).

All participants completed the 60 days of HDT bed rest, and participants performed all activities, including hygiene, in 6° HDT and 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 AM wake-up and lights-out at 11 PM. 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. Written informed consent was obtained before study commencement.

Artificial Gravity

During the 60-day HDT bed rest, participants were transferred on a 6° HDT gurney to the centrifuge facility and placed on the centrifuge arm (6° HDT). 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 1 Gz at the center of mass (35). Participants' cardiorespiratory parameters were continuously monitored by trained medical personnel. The participants could perform antiorthostatic maneuvers, 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.

Magnetic Resonance Imaging

MRIs of the lumbar spine were acquired with a 3 T Magnetom Vision system (Siemens, Erlangen, Germany). Participants were positioned on the scanning bed in supine position lying with their knees and hips supported in slight flexion by a pillow. Imaging was conducted on the 2 days before HDT (BDC) and on the 59th day of HDT bed rest (HDT59). Images were collected at the same time of the day for each individual (between 6 PM and 8 PM) and stored for off-line 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 = 3 s) and a series of 12 echoes with echo times (TEs) spanning from 20 ms to 200 ms. A set of 64 transverse images was acquired from the T_{12} vertebra to sacrum [longitudinal relaxation time (T1)-weighted Dixon sequence, total acquisition time = 5 min; slice thickness = 4 mm; distance factor = 20%, TR = 7.02 ms, TE1 = 2.46 ms, TE2 = 3.69 ms, flip angle = 5°; field of view = 400 mm × 400 mm at 1.0 mm × 1.0 mm pixel size). Images were obtained with the signal of carbonyl protons (–CH_2–) 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 (W) images were reconstructed.

The measurement of MRIs was performed; images were assigned a random code to blind the operator to time points and participant groups.

Lumbar Spine Morphology

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

1) Lumbar spinal length: the distance between the dorsorostral corners of S_1 and the L_1, L_2, L_3, L_4, and L_5 vertebral bodies (21),
2) Intervertebral angles: the angles between each adjacent vertebra L_1 through S_1 (22) and the lumbar lordosis (angle between the superior end plate of L_1 and S_1) (21),
3) Intervertebral disk cross-sectional area (CSA): the area of each disk from L_1/L_2 through L_5/S_1 (21).

Lumbar Muscle Volume and Intramuscular Lipid Concentration

Regions of interest (ROIs) were manually traced (bilaterally, with the starting side randomly selected) over the lumbar paravertebral muscles with a semiautomated MATLAB-based program (The MathWorks, Inc., Natick, MA) (36, 37). Muscle chemical proprieties were calculated as the ratio of pixel intensities from the F and W images:

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

This technique to evaluate ILC has been validated in pig and rabbit models with the reference standard biopsy/histology (38), and reliability has been demonstrated in humans for the distribution of fat content in the lumbar paravertebral muscles in the transverse plane (37). The technique used in the present 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 (39).

The volumes and ILC of the LM, LES, QL, and psoas major (PM) muscles were extracted from each image from the top of the L_1/L_2 intervertebral disk to the bottom of the L_5/S_1

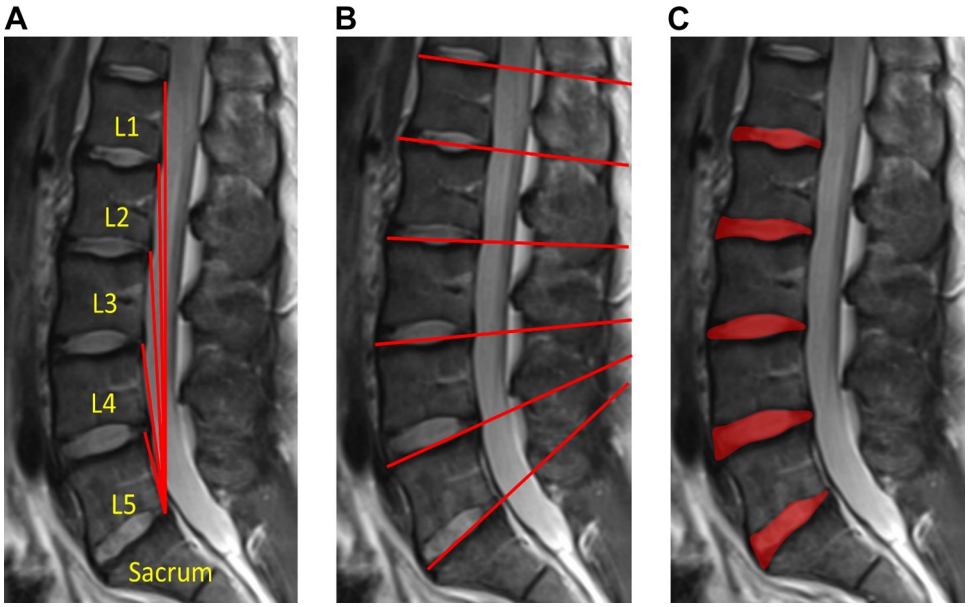

Figure 1. Measurements of spinal morphology on the sagittal image. *A*: spinal length measured from the dorso-rostral corners of each lumbar vertebra to the sacrum. *B*: intervertebral angle calculated between lines drawn at the superior border of each vertebra. *C*: disk cross-sectional area indicated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

disk. To accurately delineate the LM and the LES muscles, the fascial border separating the two muscles was used as an anatomical landmark (40). The following nine lumbar regions were identified: L_1/L_2 intervertebral disk, L_2 vertebral body, L_2/L_3 intervertebral disk, L_3 vertebral body, L_3/L_4 intervertebral disk, L_4 vertebral body, L_4/L_5 intervertebral disk, L_5 vertebral body, and L_5/S_1 intervertebral disk (Fig. 2). Measures of muscle volume and ILC at each lumbar region were averaged, and left- and right-sided measurements were averaged (21). Five regions were selected for the statistical analysis to reduce the number of similar comparisons: L_1/L_2 intervertebral disk, L_2/L_3 intervertebral disk, L_3/L_4 intervertebral disk, L_4/L_5 intervertebral disk, and L_5/S_1 intervertebral disk.

Statistical Analysis

Statistical analysis used the Statistical Package for Social Sciences (SPSS, version 25; IBM, Chicago, IL). Results are presented as means and standard deviations (SDs). Statistical significance was set at a two-sided 5% significance level. All parameters were assessed for normality with visual inspection

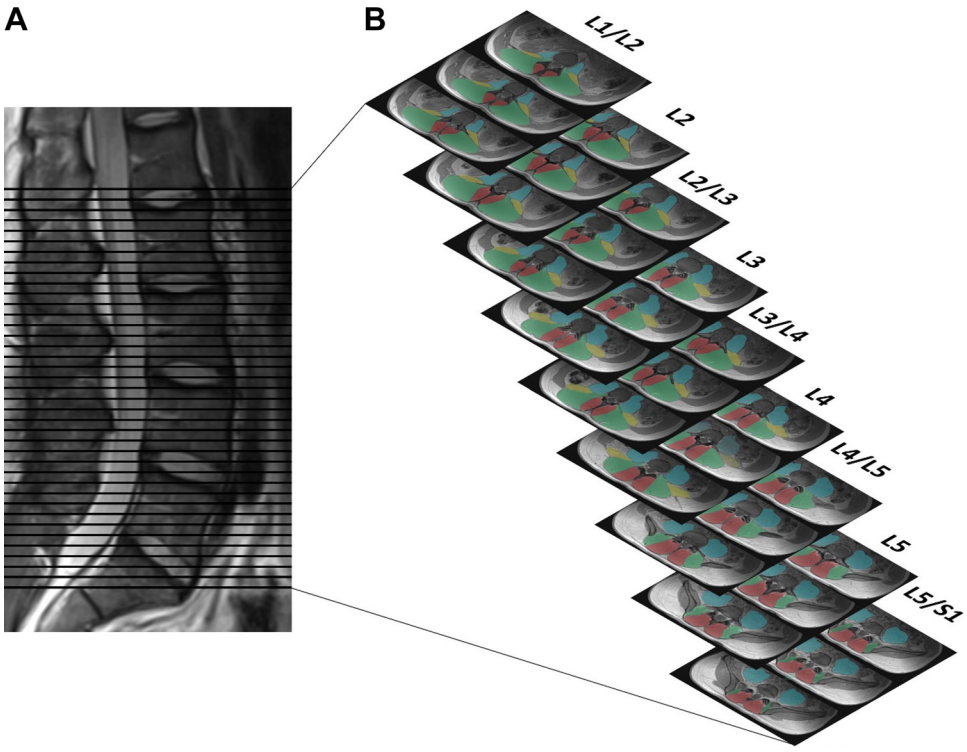

Figure 2. *A*: section of the spine on sagittal images used for the analysis. *B*: characteristic location of lumbar paraspinal muscles identified for the area measurement on axial images (slide thickness = 4 mm; slice gap = 20%). The muscle volume of the lumbar multifidus (red shaded area), lumbar erector spinae (green shaded area), quadratus lumborum (yellow shaded area), and psoas major (blue shaded area) was calculated bilaterally from L_1/L_2 to L_5/S_1 disk level. Each of the 9 lumbar regions was composed of 3–5 slides, depending on the participant's height. Five regions were selected for the statistical analysis: L_1/L_2 intervertebral disk, L_2/L_3 intervertebral disk, L_3/L_4 intervertebral disk, L_4/L_5 intervertebral disk, and L_5/S_1 intervertebral disk; however, the results of all regions are reported in the Supplemental Materials.

of histograms and Q–Q plots. Spinal morphology variables (spinal length, intervertebral angles, and intervertebral disk 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 with 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).

RESULTS

Participants

Data were successfully collected from all participants at all time points. 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) or Group × 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 intrarater reliability of the operator performing the sagittal and transverse plane image measurements was evaluated 1 mo 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].

Lumbar Spine Morphology

Significant Time effects were found for the lumbar spine length between L_1 and S_1, L_2 and S_1, L_3 and S_1, L_4 and S_1, and L_5 and S_1 [all: $F_{1,21} > 10$, $P \leq 0.001$, $\eta^2_{partial} > 0.3$] [Fig. 3*A*, L_1/S_1 length; Supplemental Fig. S1, data for all levels (all Supplemental Figures are available at https://doi.org/

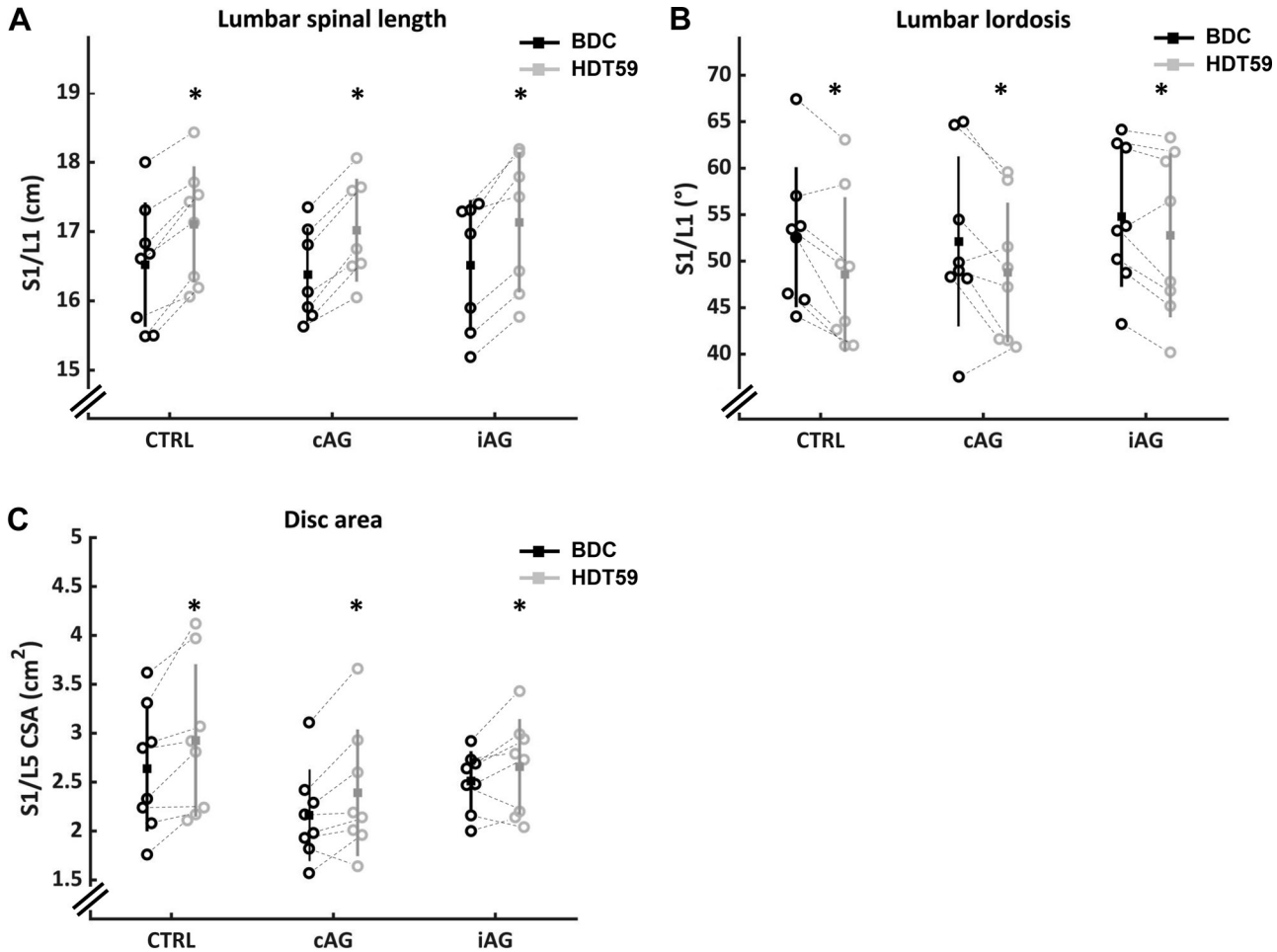

Figure 3. Lumbar spinal morphology at baseline data collection (BDC) and day 59 of head-down tilt bed rest (HDT59) for participants in the control (CTRL, $n = 8$), continuous artificial gravity (cAG, $n = 8$), and intermittent artificial gravity (iAG, $n = 8$) groups. Each open circle represents a participant, the group mean is a filled square, and the standard deviation is vertical lines. The black vertical line is BDC and the gray vertical line HDT59. *A*: lumbar spinal length from S_1 to L_1. *B*: the angle between S_1 and L_1 (lumbar lordosis). *C*: disk cross-sectional area (CSA) for a representative level: L_5/S_1. Significantly higher lumbar spinal length at BDC compared with HDT59 (*$P < 0.05$).*Significant main effect of Time ($P < 0.05$).

10.6084/m9.figshare.14213870)]. There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.7$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S1; all Supplemental Tables are available at https://doi.org/10.6084/m9.figshare.13242119). The length of the entire lumbar spine (between L_1 and S_1) 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 end plate of L_1 and S_1) ($F_{1,21} = 21.8$, $P < 0.001$, $\eta^2_{partial} = 0.5$). There was no significant main effect of Group ($F_{2,21} = 0.4$, $P = 0.65$, $\eta^2_{partial} = 0.04$) or Group × 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 ± 3.15° (7.79 ± 5.88%), 3.34 ± 3.92° (5.80 ± 8.07%), and 2.01 ± 2.61° (3.94 ± 5.13%), respectively (Fig. 3*B*, L_1/S_1 angle; Supplemental Fig. S2, data for all levels). At HDT59, the pairwise contrasts showed a mean difference of −0.22° (95% CI [−10.93, 10.49]) between CRTL and cAG, of −4.21° (95% CI [−14.92, 6.50]) between CRTL and iAG, and of −3.99° (95% CI [−14.70, 6.72]) between cAG and iAG. More specifically, the intervertebral disk angles reduced (less lordosis) between L_5/S_1 and L_5/L_4 ($F_{1,21} > 14$, $P < 0.001$, $\eta^2_{partial} > 0.4$ for both analyses) and increased (more kyphosis) between L_2/L_3 and L_1/L_2 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: $F_{2,21} < 2$, $P > 0.3$, $\eta^2_{partial} < 0.1$) or Group × Time interactions for the intervertebral disk angles (both: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S2). There was no significant main effect of Time ($F_{1,21} = 2.8$, $P = 0.11$, $\eta^2_{partial} = 0.11$) or Group ($F_{2,21} = 1.0$, $P = 0.4$, $\eta^2_{partial} = 0.1$) or Group × Time interaction ($F_{2,21} = 0.8$, $P = 0.4$, $\eta^2_{partial} = 0.1$) at L_3/L_4 vertebral levels.

The RMANOVA revealed main effects of Time for the CSA of the L_1/L_2, L_2/L_3, L_3/L_4, L_4/L_5, and L_5/S_1 intervertebral disks (all: $F_{1,21} > 11$, $P < 0.005$, $\eta^2_{partial} > 0.3$) (Fig. 3*C*, L_5/S_1 level; Supplemental Fig. S3, data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.2$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S3). On average (all CSA disks), the intervertebral disk 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.

Lumbar Muscle Volume

A significant effect of Time was found in LM muscle volume from the L_1/L_2 intervertebral disk to the L_5/S_1 intervertebral disk (all: $F_{2,21} > 12$, $P < 0.005$, $\eta^2_{partial} > 0.4$) (Fig. 4*A*, L_3/L_4 level; Supplemental Fig. S4, data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 1$, $P > 0.3$, $\eta^2_{partial} < 0.1$) (detailed statistical results in Supplemental Table S4). 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, 1,117]) between CRTL and cAG, of 372 mm^3 (95% CI [−454, 1,116]) 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 the L_1/L_2 intervertebral disk to the L_3/L_4 intervertebral disk (all: $F_{2,21} > 54$, $P < 0.001$, $\eta^2_{partial} > 0.7$) (Fig. 4*B*, L_3/L_4 level; Supplemental Fig. S5, data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.6$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 2$, $P > 0.2$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S5). The average reduction in LES muscle volume from the L_1/L_2 intervertebral disk to the L_3/L_4 intervertebral disk 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 [−2,169, 2,255]) between CRTL and cAG, of 383 mm^3 (95% CI [−1,829, 2,596]) between CRTL and iAG, and of 340 mm^3 (95% CI [−1,871, 2,561]) between cAG and iAG. There was no significant main effect of Time ($F_{1,21} = 1.2$, $P = 0.3$, $\eta^2_{partial} = 0.1$) or Group ($F_{2,21} = 0.1$, $P = 0.9$, $\eta^2_{partial} < 0.1$) or Group × Time interaction ($F_{2,21} < 0.1$, $P > 0.9$, $\eta^2_{partial} < 0.1$) at the L_4/L_5 intervertebral disk. Contrary to the observation at other levels, LES muscle volume increased at the level of the L_5/S_1 intervertebral disk ($F_{2,21} = 17.3$, $P < 0.001$, $\eta^2_{partial} = 0.5$). There was no significant main effect of Group ($F_{2,21} = 1.0$, $P = 0.4$, $\eta^2_{partial} = 0.1$) or Group × Time interaction ($F_{2,21} = 1.3$, $P = 0.3$, $\eta^2_{partial} = 0.1$). The average increase in LES muscle volume at the L_5/S_1 intervertebral disk 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 [−1,356, 484]) between CRTL and cAG, of −552 mm^3 (95% CI [−1,472, 368]) between CRTL and iAG, and of −116 mm^3 (95% CI [−1,036, 804]) between cAG and iAG.

Significant effects of Time were found in QL muscle volume from L_1/L_2 to L_3/L_4 intervertebral disk (all: $F_{2,21} > 18$, $P < 0.001$, $\eta^2_{partial} > 0.5$) (Fig. 4*C*, L_3/L_4 level; Supplemental Fig. S6, data for all levels). There were no significant main effects of Group (all: $F_{2,21} < 1$, $P > 0.9$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 1$, $P > 0.5$, $\eta^2_{partial} < 0.1$) (detailed statistical results in Supplemental Table S6). 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 [−1,825, 739]) between CRTL and cAG, 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 L_1/L_2 intervertebral disk ($F_{1,21} = 5.2$, $P = 0.03$, $\eta^2_{partial} = 0.20$) (Fig. 4*D*, L_3/L_4 level; Supplemental Fig. S7, data for all levels). There was no significant main effect of

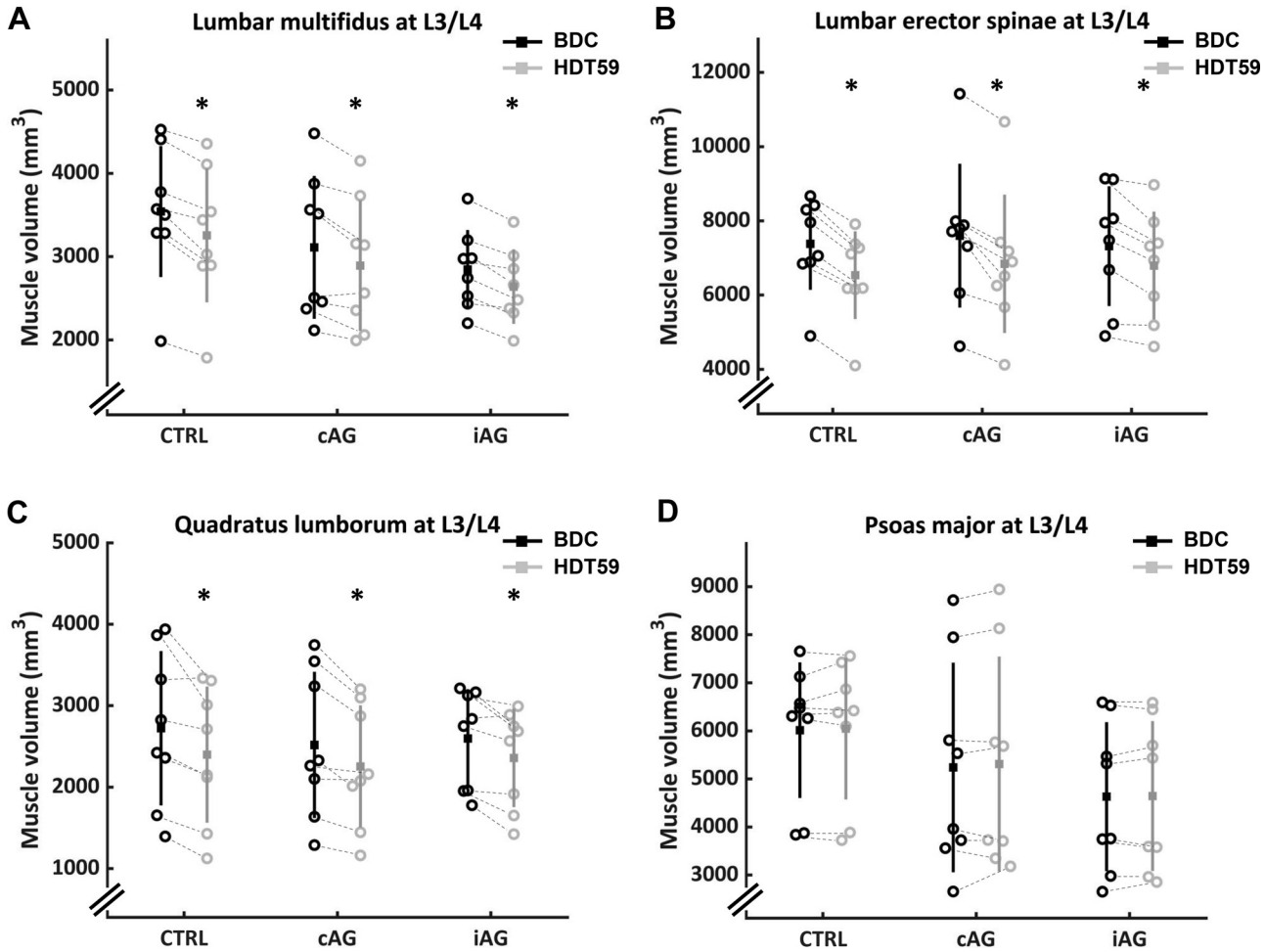

Figure 4. Lumbar muscle volume at a representative level (L_3/L_4 intervertebral disk) at baseline data collection (BDC) and day 59 of head-down tilt bed rest (HDT59) for participants in the control (CTRL, $n = 8$), continuous artificial gravity (cAG, $n = 8$), and intermittent artificial gravity (iAG, $n = 8$) groups. 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 the 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 ($P < 0.05$).

Group ($F_{2,21} = 0.8$, $P = 0.46$, $\eta^2_{partial} = 0.07$) or Group × Time interaction ($F_{2,21} = 0.6$, $P = 0.56$, $\eta^2_{partial} = 0.05$) (detailed statistical results in Supplemental Table S7). The average increase in PM muscle volume at the L_1/L_2 intervertebral disk 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, 2,048]) between CRTL and cAG, of 599 mm^3 (95% CI [−824, 2,022]) between CRTL and iAG, and of 25 mm^3 (95% CI [−1.398, 1,449]) between cAG and iAG. Contrary to the observation at the L_1/L_2 intervertebral disk, there was no significant main effect of Time (all: $F_{1,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.1$) or Group (all: $F_{2,21} < 0.2$, $P > 0.2$, $\eta^2_{partial} < 0.1$) or Group × Time interaction (all: $F_{2,21} < 1$, $P > 0.1$, $\eta^2_{partial} < 0.1$) at other levels in PM muscle volume.

Intramuscular Lipid Concentration

Significant effects of Time were found in LM ILC at all levels from L_1/L_2 to L_5/S_1 intervertebral disk (all: $F_{1,21} > 10$, $P < 0.01$, $\eta^2_{partial} > 0.3$) (Fig. 5*A*, L_3/L_4 level; Supplemental Fig. S8, data for all levels). There was no significant main effect of Group (all: $F_{2,21} < 0.3$, $P > 0.7$, $\eta^2_{partial} < 0.1$) and Group × Time interactions were revealed by the RMANOVA (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S8). 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 from L_1/L_2 to L_5/S_1 intervertebral disk (all: $F_{2,21} > 16$, $P < 0.001$, $\eta^2_{partial} > 0.4$) (Fig. 5*B* and Supplemental Fig. S9, data for all levels). There was no significant main effect of Group (all: $F_{2,21} < 0.9$, $P > 0.4$, $\eta^2_{partial} < 0.1$) or Group × Time interactions (all: $F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$) (detailed statistical results in Supplemental Table S9). 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%

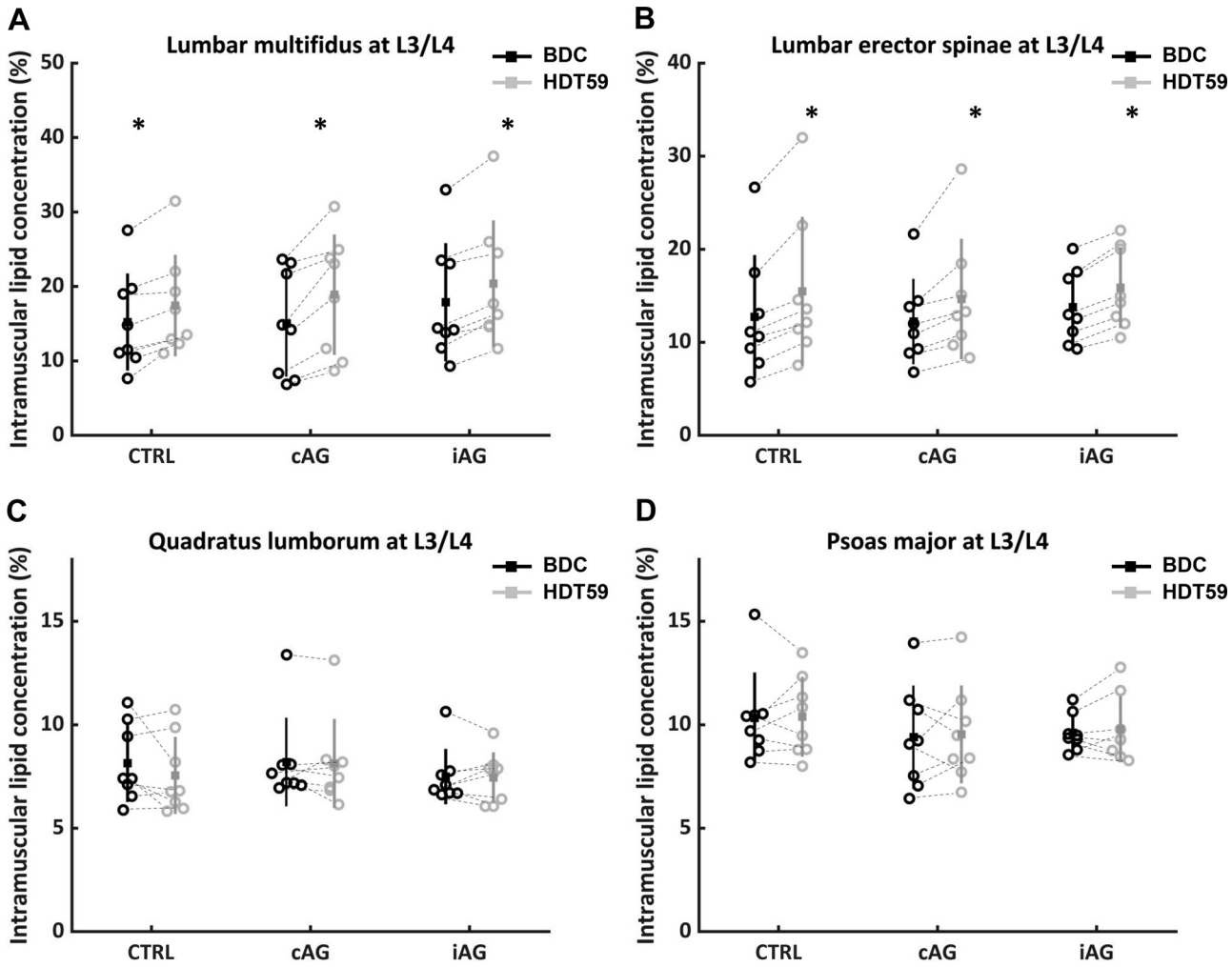

Figure 5. Lumbar muscle intramuscular lipid concentration (ILC) at a representative level (L_3/L_4 intervertebral disk) at baseline data collection (BDC) and day 59 of head-down tilt bed rest (HDT59) for participants in the control (CTRL, $n = 8$), continuous artificial gravity (cAG, $n = 8$), and intermittent artificial gravity (iAG, $n = 8$) groups. 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 the 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 ($P < 0.05$).

(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 or Group or Group × Time interactions were found in QL and PS ILC ($F_{2,21} < 3$, $P > 0.1$, $\eta^2_{partial} < 0.2$ for all analyses) (Fig. 5, *C* and *D*, Supplemental Figs. S10 and 11, data for all levels; detailed statistical results in Supplemental Tables S10 and S11).

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 disk 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 disks, 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.

Lumbar Spinal Morphology

Contrary to our hypothesis, the AG protocols used in the present 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 disks. In contrast to these results, the application of other countermeasures, such as lower body negative pressure treadmill exercise (20), resistive vibration exercise (21, 22), and low-magnitude vibration (23), has reduced expansion of

the intervertebral disks. Similar to the present investigation results, exposure to flywheel and spinal mobilization interventions (41) did not mitigate intervertebral disk 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) and dynamic forces produced by the exercises, such as walking or squatting, that 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 1 G_z to the lumbar spine used in the present 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 1 G_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 are substantially longer (42). 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 spaceflight (2, 13, 43). However, a recent study reported only negligible changes in disk height and swelling after prolonged exposure to microgravity (2). Although the explanation for the lack of changes in disk properties is not clear, it may be related to adoption of in-flight exercise protocols, which load the spine in the axial direction.

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 (44). Our data of reduced muscle volume and reduced lumbar lordosis agree with the strong association between these parameters identified previously in astronauts (2). 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 disk injury because of greater compressive disk loads and maintenance of end range of flexion (45).

Similar to the present results, lower body negative pressure treadmill exercise (20), low-magnitude vibration (23), flywheel, and spinal mobilization (41) 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 (21, 22). 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 load the spine, and if muscle composition changes persist this might increase the risk of soft-tissue injury (45).

The increased muscle volume of the LES muscle at the L_5/S_1 intervertebral disk represents a new finding. Although previous studies have not investigated the LES muscle at this vertebral level (20–23), a reduction of 4% (22) and 8% (21) at the L_5 vertebral body level has been reported previously 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 changes to muscle tissue, as shown in other studies (46).

Finally, the increase in the volume of the psoas major muscle observed in the present investigation is in agreement with results of previous bed rest studies (21, 47, 48). Although speculative, recruitment of this muscle in tasks such as daily hygiene activities in bed rest might explain the observed increases in muscle volume (48). Furthermore, changes in the lumbar erector spinae and quadratus lumborum muscles are minimal after exposure to bed rest (47). These findings contrast with the reduced volume of the psoas major, quadratus lumborum, and lumbar erector spinae muscles after spaceflight (6, 49). These differences highlight that although bed rest studies serve as a useful analog 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° rather than eliminated, as occurs in microgravity conditions.

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 (8, 9, 50). 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 an ~5% radiodensity attenuation of LM, LES, and QL muscles in computed tomography scans at the L_2 vertebral body (8, 9), particularly in astronauts with less exposure to resistive exercise equipment (9). Radiodensity attenuation of muscles on CT

scan has been associated with increased ILC (10) and is related to reduced trunk muscle function (51). 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 (52). Moreover, patients with low back pain with functional disabilities often show a high percentage of ILC in the LM muscle at the L_5 vertebral level (53), particularly during the subacute (without atrophy) or chronic (with atrophy) pain stages (11).

Accumulation of ILC may arise from either excess triacylglycerols from food intake or reduced fat oxidation in these postural muscles. In contrast to previous bed rest studies, energy uptake by nutrition was tailored to maintain a constant body mass in the present study. Although participants' weight reduced by ~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 after bed rest (54, 55). Importantly, muscles with greater oxidative power before 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 (56). As LM and LES muscles have high proportions of slow-twitch fibers, high capillarization, and high concentration of oxidative muscle enzymes [~60% (57)], consistent with their tonic role in maintenance of upright posture (58), the increase in ILC in LM and LES in the present study might be explained by a downregulation of the oxidative activity. In contrast, PM muscle has fewer slow-twitch fibers [~40% (59)], 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 (60), yet ILC in QL did not show any change in our study. Species differences cannot be discounted.

An alternative explanation for increased relative ILC in the LM and LES muscles in the present study may also be related to fluid and electrolyte shifts between intra- and extracellular compartments that accompany HDT bed rest and spaceflight (61, 62). 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.

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 a 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 (63) 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 disk expansion (21, 22), 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 target the affected muscles during reconditioning. If countermeasures can be identified, this is relevant for tailoring of interventions not only for astronauts but also for bedridden individuals and patients with low back pain (3).

Limitations

There are some limitations to the present study. First, the sample size was small because of 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 present study (21, 22). A consideration is that, because of the small sample size, only large effect sizes from countermeasures can be detected (21, 22) and more subtle effects are likely overlooked. Another weakness of the sample is the uneven sex distribution (16 males, 8 females). 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 (37). 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 L_1/L_2 intervertebral disk to the bottom of the L_5/S_1 intervertebral disk.

Finally, the redistribution 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.

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.

SUPPLEMENTAL DATA

Supplemental Tables S1–S11: https://doi.org/10.6084/m9.figshare.13242119

Supplemental Figures S1–S11: https://doi.org/10.6084/m9.figshare.14213870

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.D., J.H., K.L., D.D., A.W., S.E.S., T.W., J.S., P.W.H., and N.C. conceived and designed research; J.Z. performed experiments; E.D., J.M.E., and M.H. analyzed data; E.D., J.H., J.M.E., M.H., J.Z., K.L., D.B., J.A.C., T.W., and N.C. interpreted results of experiments; E.D. prepared figures; E.D. drafted manuscript; E.D., J.H., J.M.E., M.H., J.Z., K.L., D.D., A.W., D.B., J.A.C., S.E.S., T.W., J.S., P.W.H., and N.C. edited and revised manuscript; E.D., J.H., J.M.E., M.H., J.Z., K.L., D.D., A.W., D.B., J.A.C., S.E.S., T.W., J.S., P.W.H., and N.C. approved final version of manuscript.

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