Peer Review File

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Reviewer A:

Major concerns:
Comment 1: Page 2, line 29: The concept of an aggravated endoplasmic reticulum (ER) in cartilage is unknown to me and most likely unknown in literature. Can the authors define what is meant by this?
Reply 1: Thank you for your helpful suggestions.The concept of an aggravated endoplasmic reticulum (ER) in cartilage means that ultrastructural observation of the endoplasmic reticulum network structure disorder in cartilage.(see Page 3, line 50-52)
Changes in the text: An aggravated endoplasmic reticulum (ER) in cartilage was modified to "Transmission electron microscopy revealed changes in the mitochondrial inner membrane structure of cartilage, severe vacuolization, disrupted endoplasmic reticulum"

Comment 2: Page 2, line 36: What is an aggravated cartilage degeneration?
Reply 2: Your comments are greatly appreciated, aggravated cartilage degeneration refers to a series of manifestations of articular cartilage degeneration under long-term weightlessness, such as increased T2 value, mitochondrial structure destruction, increased inflammatory response, and cartilage surface damage

Comment 3: Page 4, line 44: "Weight reduction." The concept of weight reduction is distinct from weightlessness. Better clarification is needed and not to be used interchangeably throughout the manuscript.

Reply 3: Thank you for your valuable insights, The Weight reduction has been amended to weightlessness.

Comment 4: Page 9, line 170- 171: "knee chondrocytes displayed evident signs of early apoptosis." Unless you can show molecular evidence of early apoptosis this statement is not supported by the data in your manuscript.
Reply 4: Thank you very much for your constructive comments,"The early signs of apoptosis of chondrocytes of knee joint were obviously" modified to "the knee cartilage cells showed mitochondrial swelling or complete absence, while the size of the coarse ER decreased and the lumen enlarged"
Changes in the text:knee chondrocytes displayed evident signs of early apoptosis was modified to the knee cartilage cells showed mitochondrial swelling or complete absence, while the size of the coarse ER decreased and the lumen enlarged(see Page 11, line 200-202).

Comment 5: "derecombinant chondrocyte." What is derecombinant?
Reply 5: Your comments are greatly appreciated, derecombinant was misused and has been modified to mean weightlessness.
Changes in the text: Derecombinant chondrocyte was modified to after a period of 4 weeks in a weightless environment, observations revealed a reduction or absence of mitochondrial cristae, evident swelling or disappearance of mitochondria, and a noticeable decrease in the rough surface endoplasmic reticulum(see Page 11, line 202-204).

Comment 6:Page 11, line 216: "expression of chondrocytes was reduced." What expression of chondrocytes or what is meant by this statement?
Reply 6: Thank you for your helpful suggestions, reduced chondrocyte expression means less positive expression of collagen type II in cartilage
Changes in the text: expression of chondrocytes was reduced was modified to the positive expression of collagen type II in cartilage was decreased(see Page 13, line 247-248).

Comment 7: Page 12, line 231: “prolonged braking.” What is does this mean?
Reply 7: Thank you for your valuable insights, prolonged braking means braking for a long time.
Changes in the text: prolonged braking was modified to Braking for a long time of the human body(see Page 14, line 262).

Minor concerns but essential
Comment 8: Page 1, line 21; Elisa – this abbreviation is incorrectly written
Reply 8: Thank you very much for your constructive comments, Elisa has been modified to ELISA.

Comment 9: Page 6, line 87: “3% sevoflurane.” What is the unit of % here and in other places in the manuscript?
Reply 9: Your comments are greatly appreciated. Here, "%" is a percentage unit that represents the percentage of concentration

Comment 10: Page 6, line 93: “1mm x 1mm x 1mm.” Can you not express this dimension as a volume?
Reply 10: Thank you for your helpful suggestions. Here, "1mm x 1mm x 1mm" has been modified to $1mm^3$
Changes in the text: 1mm x 1mm x 1mm was modified to $1mm^3$ (see Page 8, line 123).

Comment 11:Page 10, line 183: ‘GROUND.’ Why is the ground group written in uppercase letters?
Reply 11: Thank you very much for your constructive comments. Here, "GROUND" has been modified to ground.
Changes in the text: GROUND was modified to ground(see Page 12, line 215).

Reviewer B:

Thank you for submitting this interesting paper to our journal. I have some questions to the authors.

[General comments]
Comment 1: Please define the group name in the text.
Reply 1: Thank you for your valuable insights. Therefore, three groups (10 rats/groups) were established: the ground group, the 14-day hindlimb unloading group, and the 28-day hindlimb unloading group(see Page 7, line 102-104)

Comment 2: Please explain what it would mean to evaluate the structure of mitochondria in muscle. Describe how mitochondria and rough endoplasmic reticulum are altered by muscle atrophy, hypertrophy, contracture, fatty degeneration, and non-weight bearing, respectively. Please also explain how mitochondrial and rough endoplasmic reticulum changes differ depending on the pathology of the muscle.
Reply 2: Mitochondria are energy production centers in cells and are essential for maintaining the function of muscle cells. The changes of muscle mitochondria before and after weightlessness were observed by transmission electron microscopy to provide new insights into muscle atrophy caused by weightlessness.
In atrophy, mitochondria may be reduced in number, smaller in size, and impaired in function. This can lead to insufficient energy supply to muscle cells, affecting muscle function.
During muscle hypertrophy, the number and size of mitochondria may increase to meet increased metabolic demands. This change helps provide more energy and supports the growth and function of muscle cells.
During muscle contracture, mitochondria may be compressed and damaged, resulting in changes in their structure and function. This may affect the energy production of mitochondria within muscle cells and affect muscle contraction and relaxation.
fatty degeneration can cause mitochondria to be affected by lipid accumulation, which affects their function and morphology. This can lead to the energy metabolism of muscle cells being affected and may affect muscle function.
Under non-weight bearing conditions, mitochondrial number and function may be reduced because muscle

cells no longer need to support weight or perform powerful contractile movements, which can lead to mitochondrial degradation.
rough endoplasmic reticulum, similar to mitochondria, is also affected by the various muscle lesions described above. In different muscle lesions, the morphology and function of the trachyplasmic reticulum may change accordingly, depending on the nature and extent of the pathological process. For example, muscle atrophy may lead to a decrease in the rough endoplasmic reticulum, while muscle hypertrophy may promote its increase.
In conclusion, by evaluating the changes of mitochondria and trachyplasmic reticulum in different muscle lesions, we can better understand the impact of these lesions on the structure and function of muscle cells, and provide important references for the diagnosis and treatment of related diseases.

1.Comment 3: This weightlessness model is already in use, but what is new information in this study in assessing muscle and articular cartilage?
Reply 3: Weightlessness models, also known as microgravity models, have been widely used to study the effects of reducing mechanical loads on the musculoskeletal system. However, advances in this study continue to reveal new information and insights for evaluating muscle and articular cartilage in weightless conditions. In this study, ultrastructures of cartilage and muscle were observed by transmission electron microscopy, and biomechanical analysis was performed by nanoindentation, both of which provide new advances in the evaluation of the effects of mechanical load on muscle and articular cartilage. These advances contribute to a more complete understanding of the physiological and pathological responses of musculoskeletal tissue to altered gravity conditions.

[Specific comments]
Comment 4: Line 72-74; What was the reason for using 10 rats per group? You wrote that “the sample size was calculated based on previous experiments to establish tail suspension on rat models.” What was the reason for using 10 rats in the previous study?
Reply 4: Based on statistical considerations and experimental design principles, the decision to use 10 rats per group was based on previous experiments or pilot studies conducted by the researchers, and a sample size of 10 rats per group was necessary to obtain meaningful results and detect the effects of tail suspension on the rat model, providing sufficient statistical power to detect significant differences between the experimental groups.

Comment 5: Line 20; Why did you evaluate muscle mitochondrial activity?
Reply 5: The evaluation of muscle mitochondrial activity is an important part of this study. The aim of this study was to observe the effects of simulated weightlessness on the microstructure of quadriceps and knee cartilage in rats. When exposed to simulated weightlessness, the ultrastructure and activity of muscle mitochondria in rats changed, indicating that the muscle's metabolic capacity and energy production were affected, and the results showed that exposure to simulated weightlessness caused damage to the quadriceps muscle mitochondria. Mitochondria are essential for the production of ATP and play a central role in maintaining muscle function and health. The assessment of mitochondrial activity provides insight into how muscles respond to a weightless environment and the subsequent impact on their metabolic processes.

Comment 6: Line 26 and 31; What exactly was the cartilage “expression” and the cell ”expression”?
Reply 6: the "expression" and the cell "expression" of cartilage meant that cartilage type II positive cells expression.

Comment 7: Line 114; How reliable is the resolution of 3T MRI in evaluating articular cartilage in rats? What is the thickness of articular cartilage in rats and special resolution of the MRI?
Reply 7:The reliability of employing a Discovery MR750 3.0T MR scanner for evaluating rat articular cartilage is yet to be fully established. Our study utilized a 0.5 mm slice thickness and a 256 × 256 matrix, yielding high-resolution images that effectively depicted the microstructure and tissue characteristics of rat

articular cartilage. The weight-bearing region of the medial tibiofemoral joint was designated as the region of interest, demonstrating statistically significant differences.
Rat articular cartilage thickness varies across joints and specific locations, typically within the range of a few hundred micrometers.
With a 0.5 mm slice thickness and a 256 × 256 matrix, our study achieved high spatial resolution, enabling the capture of intricate structural details and the visualization of changes in rat articular cartilage. Consequently, based on these observations and previous findings, utilizing the Discovery MR750 3.0T MR scanner for rat articular cartilage assessment holds certain reference significance.

Comment 8: Line 184-185 and Fig. 2D; How was the ROI set up, and is there any tissue other than articular cartilage in the ROI?
Reply 8: the region of interest (ROI) was established by focusing on the weight-bearing area of the tibiofemoral cartilage contact area of the medial knee compartment. The specific setup involved delineating the ROI within the articular cartilage of this region. As for the presence of other tissues within the ROI, our study ensured that only articular cartilage was included in the defined ROI to maintain the specificity of our analysis.

Comment 9: Line188 and 189: Please state the p-value.
Reply 9: Has been in the corresponding contents on the calibration P value
Changes in the text:the ultrastructural analysis of cartilage revealed that knee chondrocytes in the ground group exhibited a normal morphology in terms of mitochondria and rough endoplasmic reticulum. Additionally, the mitochondrial cristae were observed to be intact. However, after a period of 2 weeks in a weightless environment, the knee cartilage cells showed mitochondrial swelling or complete absence, while the size of the coarse ER decreased and the lumen enlarged($p<0.05$). After a period of 4 weeks in a weightless environment, observations revealed a reduction or absence of mitochondrial cristae, evident swelling or disappearance of mitochondria, and a noticeable decrease in the rough surface endoplasmic reticulum($p<0.05$) (Fig. 2A). Compared with the control group, the muscle ultrastructure analysis showed that the mitochondrial ridge was weakened in the weightlessness group for 14 days, weightlessness 28 days, mitochondria swelling, cavitation, mitochondria ridge weakens($p<0.05$) (Fig. 2B).(see Page 12, line 198-207)

Comment 10: Line 321; Which part exactly is the “weight-bearing area of the femoral part”?
Reply 10: Thank you for your question. To clarify, the "weight-bearing area of the femoral part" specifically refers to the weight-bearing area of the tibiofemoral cartilage contact area of the medial knee compartment, which is the region that bears the majority of the load during weight-bearing activities. We have updated the manuscript to specify this more clearly.
Changes in the text:No significant changes were observed in the tibiofemoral cartilage contact area of the medial knee compartment on T2 mapping and conventional sequences at 14 days. On day 28, T2 mapping observed significant changes in the tibia-femoral cartilage contact area in the medial knee joint. The T2 value of HLU rats was slightly higher than that of ground rats, indicating the loss of water and collagen fibers. Cartilage thinning in the medial region was observed with conventional sequences. (see Page 12, line 209-213)

Comment 11: Line 199-201, Fig. 3A, B; Please show the articular cartilage and subchondral bone in each of the femur and tibia.
Reply 11:"Thank you for your comment. In our study, the focus was on the articular cartilage changes of the femur and tibia in the medial region of the knee joint, and the subchondral bone was not involved. At the same magnification at which significant cartilage findings were observed, the subchondral bone region was not analyzed for methodological reasons. Therefore, these areas are not included in our results. In FIG. 3A B, articular cartilage has been indicated using black arrows.

Comment 12: Line 202-204, Fig 3C; Please indicate which is Col II.
Reply 12: Thank you for your valuable feedback. Regarding your question, in this study, Col II (type II collagen) positive cells are indicated by a brown to dark brown color in the immunohistochemical staining. We updated FIG. 3C to indicate positive cells with red arrows in order to clearly label the collagen type II-positive regions.