THE INVESTIGATION OF COUNTERMEASURES AGAINST SPACEFLIGHT-INDUCED JOINT DEGRADATION

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LIST OF ABBREVIATIONS

ARED	Advanced Resistive Exercise Device
BMD	Bone Mineral Density
OA	Osteoarthritis
MHU	Mouse Habitat Unit
μg	Microgravity
ISS	International Space Station
NRF2	Nuclear Factor Erythroid 2-Related Factor 2
SOD	Superoxide Dismutase
QCT	Quantitative Computed Tomography
HLU	Hind Limb Unloading
EIF2α	Eukaryotic Translation Initiation Factor 2A
mTOR	Mammalian Target of Rapamycin
RR9	Rodent Research-9
GAG	Glycosaminoglycans
GPx	Glutathione Peroxidase
IL	Interleukins
TNF-α	Tumor Necrosis Factor-α
ROS	Reactive Oxygen Species
MMP	Matrix Metalloproteinases
AG	Artificial Gravity
JAXA	Japanese Aerospace Exploration Agency

BuOE	MnTnBuOE-2-PyP5+
RR18	Rodent Research 18
LAR	Live Animal Return
CRS	Commercial Resupply Services
KSC	Kennedy Space Center
L	Launch
NASA	National Aeronautics and Space Administration
REC	Recovery
EDTA	Ethylenediaminetetraacetic acid
TRAP	Tartrate resistant alkaline phosphatase
H&E	Hematoxylin and Eosin
ADAMTS	A Disintegrin and Metalloproteinase with Thrombospondin Motifs
4HNE	4-Hydroxynonenal
OARSI	Osteoarthritis Research Society International
ELISA	Enzyme-Linked Immunosorbent Assay
RNA-seq	RNA Sequencing
DEGs	Differentially Expressed Genes
FDR	False Discovery Rate
IPA	Ingenuity Pathway Analysis
ANOVA	Analysis of Variance
AOX	Antioxidant
MicroCT	Microcomputed Tomography
OC. N	Osteoclast Number

BS	Bone Surface
TV	Trabecular Volume
BV	Bone Volume
Conn. D	Connectivity Density
Ct. BV	Cortical Bone Volume
LEO	Low Earth Orbit
GC	Ground Control
VIV	Vivarium Control
MARS	Mouse Artificial Gravity System
RFID	Radio-frequency Identification
HCU	Habitat Cage Units
ACF	Animal Care Facility
SANS	Space Flight-associated Neuro-ocular Syndrome
RR	Rodent Research
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
HGC	Habitat Ground Control
VGC	Vivarium Ground Control
ECM	Extracellular Matrix
PDK4	Pyruvate Dehydrogenase Kinase 4
IMNT	Indolethylamine N-Methyltransferase
Spock2	SPARC (osteonectin), cwcv and kazal like domains proteoglycan 2
Gpnmb	Transmembrane glycoprotein NMB

Dpep1	Dipeptidase 1
Sgk1	Serum and glucocorticoid-regulated kinase 1
Galnt15	Polypeptide N-Acetylgalactosaminyltransferase 15
Cdo1	Cysteine Dioxygenase Type 1
Hif1α	Hypoxia Inducible Factor 1 Subunit Alpha
Col15A1	Collagen Type XV Alpha 1 Chain
SerpinH	Serine protease inhibitor H
Ifitm3	Interferon Induced Transmembrane Protein 3
Crabp2	Cellular Retinoic Acid Binding Protein 2
C1qTnf1	C1q and Tumor Necrosis Factor Related Protein 1
Gabarapl1	GABA Type A Receptor Associated Protein Like 1
Pip3ip1	Phosphoinositide-3-Kinase Interacting Protein 1
Tpp3	Tubulin Polymerization Promoting Protein Family Member 3
Slc6a2	Solute carrier family 6 member 2
Dio3	Iodothyronine deiodinase 3
Mpo	Myeloperoxidase
Penk	Proenkephalin
TRAPP	TRAnsport Protein Particle
P53	Tumor protein p53
PI3K	Phosphatidylinositol 3-kinase
AKT	AKT Serine/Threonine Kinase 1
MAPK	Mitogen-activated protein kinases
USP18	Ubiquitin specific peptidase 18

KL	Klotho
DYSF	DYSF dysferlin
SIRT1	Silent mating type information regulation 2 homolog
AMPK	AMP-activated protein kinase
ER-α	Estrogen Receptor Alpha Gene
ET-1	Endothelin 1
PTEN	Phosphatase and tensin homolog
DDB1	Damaged DNA binding protein 1
AHR	Aryl Hydrocarbon Receptor
CASP8	Cysteine-aspartic acid protease 8
UPR	Unfolded protein response
RHOA	Ras Homolog Family Member A
NCF1	Neutrophil cytosolic factor 1
CS	Citrate synthases
DS	Dissociator
PDXN	PDX1 pancreatic and duodenal homeobox 1
CCR2	C-C motif chemokine receptor 2
VitC	Vitamin C
RSP27a	Ribosomal Protein S27a
SNRPA1	Small Nuclear Ribonucleoprotein Polypeptide A'
GSTP1-1	Glutathione S-Transferase P1
R-Ras	Ras Related
RPL32	Ribosomal protein L32

EIF2	Eukaryotic translation initiation factor 2A
RUNX3	RUNX family transcription factor 3
FOXO1	Forkhead box protein O1
PPAR	Peroxisome proliferator-activated receptor
IGF-1	Insulin-like growth factor-1
g	gravity

ABSTRACT

Introduction: The National Aeronautics and Space Administration has outlined a Moon to Mars program with the plan to have long-term manned deep space travel missions outside of low Earth orbit. However, NASA's Human Research Program has pinpointed five hazards that astronauts will encounter on their journeys, chief among them being space radiation and decreased loadbearing outside of Earth's gravity. While their effects have been studied in the musculoskeletal and neuromuscular system, it remains relatively unclear as to its effect on joint soft tissue and on neuromotor function. The purpose of this dissertation is to determine if elevated loads applied across the joints of mice aboard the ISS via centrifugation will prevent an OA phenotype and neuromotor deficits in a gravitational load-dependent manner, and if the SOD mimetic MnTnBuOE-2-PyP5+ (BuOE) administration delivered weekly over the course of 35 and 60 days of spaceflight on the ISS will protect against musculoskeletal degradation during and after spaceflight.

Research Problem: Does spaceflight environment affect joint health and neuromotor function? What are the health effects of partial gravity environments during spaceflight? Does 1g artificial gravity during spaceflight have protective effects? What is the efficacy of antioxidant pharmaceutical countermeasures for the prevention of spaceflight-induced musculoskeletal degradation?

Methods: We performed studies evaluating the efficacy of artificial gravity via centrifugation and antioxidant treatment for the prevention of spaceflight-induced musculoskeletal degradation in rodent models flown aboard the ISS. This study

implements microCT to identify morphometric properties, histological, immunohistochemical, transcriptomic, and proteomic analysis to explore potential mechanisms, and gait analysis to characterize neuromotor deficits caused by spaceflight conditions.

Conclusions: Treatment with BuOE preserved articular cartilage, menisci, and trabecular bone volume during short duration spaceflight and downregulated pro-arthritic pathways in the joint soft tissue. Exposure to prolonged microgravity aboard the ISS, or partial gravitational loading preserved gait patterns relative to both preflight and controls in a gravity-dose dependent manner. Finally, artificial gravity via centrifugation failed to preserve meniscal volume and articular cartilage thickness, however, 1g AG maintained meniscal ECM synthesis and sulfated GAG expression compared to controls.

Future Directions: Further investigation is still needed to validate the protective effects of BuOE on the musculoskeletal system during spaceflight. There is a critical need to characterize these neuromotor deficits in the microgravity and partial gravity environments to better mitigate the risks associated with long-duration spaceflight both in transit and upon reaching a destination, as we plan for missions outside of LEO.

GENERAL INTRODUCTION

An Introduction on the Musculoskeletal Complications During Spaceflight, Current Countermeasures, and Rationale for Study

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Key words: Osteoarthritis, Superoxide Dismutase Mimetics, Artificial Gravity, Spaceflight

Introduction

Maintaining musculoskeletal health and performance is vital for mission success during a long-duration spaceflight, as well as for preserving quality of life post-mission. Prolonged exposure to reduced weight bearing in microgravity during spaceflight is well-documented to cause muscle atrophy [1–3] and bone loss [4–6], with more recent studies identifying degradation of soft-tissue structures in both the knee (e.g., cartilage, menisci) and hip (e.g., cartilage) joints [7, 8]. It is estimated that with a mission to Mars, astronauts will be exposed to approximately 8 to 10 months of weightlessness total during transit to and from Mars, and approximately 12 to 18 months of partial gravity exposure while on surface [9]. Recent studies have shown that neither bisphosphonate treatment [10–12] or the, use of the advanced resistive exercise device (ARED) prevent spaceflight-induced bone mineral density (BMD) decline and bone turnover[13, 14], with limited studies having been conducted on the load-sensitive soft tissues of the knee. There is a critical need to investigate novel countermeasures to preserve astronaut joint health during and after deep space travel such as artificial gravity to counteract the deleterious effects of reduced gravity, as well as, pharmaceutical interventions to counteract spaceflight-induced osteoarthritis (OA).

Intermittent or continuous exposure to elevated gravitational loading is a possible countermeasure to prevent spaceflight-induced decline in musculoskeletal function and performance [15,16]. Prior work has established that the deficits in bone and muscle following exposure to partial gravity, simulated by partial weight bearing in mice, are directly proportional to the degree of unloading [17]. A previous study utilizing the mouse habitat unit (MHU) has demonstrated significant decreases in femur bone density

and the soleus/gastrocnemius muscle weights of μg mice, whereas artificial 1g mice maintained the same bone density and muscle weight as the ground control animals after 35 days aboard the International Space Station (ISS), indicating that artificial gravity plays a preserves musculoskeletal health during spaceflight [18-20]. Yet, the ability of partial gravity applied to rodent limbs while in orbit using the existing Kibo module as a means to prevent osteoarthritic changes are unstudied [21]. Furthermore, studies in our lab have demonstrated that the OA phenotype generated in the knees of mice during spaceflight aboard both the Space Shuttle and ISS is characterized by increased oxidative stress and redox signaling (e.g., lower NRF2 signaling coincident with reduced superoxide dismutases (SOD)) [7]. A disruption in SOD activity has been established as a driver of OA progression[17, 18] and potential therapeutic target in the prevention of OA [19–21].

Mechanical loading is important for musculoskeletal health, playing a critical role in the maintenance of skeletal density and structure [27, 28], and prevention of muscle atrophy [29, 30]. Reduced weight bearing is associated with cartilage and meniscal degradation [31–33]. These deleterious effects of reduced weight-bearing have been observed in clinical, large animal, and rodent studies, including spaceflight [34–38]. These changes are associated with elevated redox status in joint tissues, characterized by lowered antioxidant defenses and increased pathways associated with oxidative stress [7, 39–42]. Thus, increased loading conditions through artificial gravity, and / or antioxidant countermeasures, may protect against skeletal deficits both during and after spaceflight. Before exploring the studies performed in later chapters, reviewing and exploring the functions of tissues in the knee joint, potential risks of osteoarthritis, and uses of the

rodent gait model must first be understood. A better understanding of the tissues in the joints, osteoarthritis, spaceflight environment, gait and reasons for studying is necessary for addressing these concerns.

Mechanical loading and the musculoskeletal system health

It has been well established that mechanical loading is required for maintenance of the musculoskeletal system [27–30]. Thus, exposure to spaceflight or reduced mechanical loading on Earth induces marked bone loss, muscle atrophy and degradation of soft-tissue structures in both the knee (e.g., cartilage, menisci, and ligaments) and hip (e.g., cartilage) joints [31–37]. Moreover, complex functions, such as gait [8] and balance [54], which depend on several physiologic systems, are compromised following prolonged exposure to weightlessness. Additionally, spaceflight alters mechanisms related to redox signaling, oxidative stress, and tissue dysfunction which has been implicated in spaceflight-induced muscle atrophy and bone loss [39–42]

The combined effects of spaceflight on musculoskeletal health poses a major concern for astronauts during and after long-duration spaceflight, as there may an increased risk for reduced performance, bone fractures, and both early-onset osteoporosis and arthritis. However, despite advances in exercise, and pharmacologic countermeasures like bisphosphonates, designed to mitigate musculoskeletal deterioration, the protection is incomplete and variable [10, 11, 39]. Thus, deficiencies in musculoskeletal structure, function and neuromotor performance remain hallmarks of long-duration spaceflight [40–43]. It is estimated that with a mission to Mars, astronauts will be exposed to approximately 8 to 10 months of weightlessness total during transit to and from Mars,

and approximately 12 to 18 months of partial gravity exposure while on surface [9]. Without further advances in countermeasures, astronauts' risk serious short- and long-term musculoskeletal debilitation during and after such long-duration spaceflight missions.

Spaceflight, bone loss and skeletal fragility

Spaceflight-induced bone loss has been well documented since the early days of the Gemini program [64]. For the most part, despite rigorous exercise protocols, bone loss in weightbearing bones (1.0-1.6% per month) was nearly 10-fold higher than the rate seen in postmenopausal women [65]. Further, femoral strength predictions by finite element analysis of QCT data declined an alarming 2.6% per month in astronauts who spent 4 to 6 months on the International Space Station [66]. The increased fracture risk that accompanies this bone loss may be substantial [53], as a 20% decrease in femoral neck BMD during a year of spaceflight corresponds to 30 years of age-related bone loss in a postmenopausal woman, and an unacceptable 20–40% increase in fracture risk [51, 52].

Although bisphosphonate treatment inhibits bone loss due to spaceflight [11], treatment with this class of drugs may not be an adequate solution since several study subjects needed to discontinue the medication due to drug-related side effects. Moreover, there remain concerns about use of bisphosphonates in young astronauts due to the long-term retention of bisphosphonates in the skeleton and potential negative effects of prolonged suppression of bone turnover [71]. A second study reported that in ten astronauts, use of the ARED alone, only partially attenuated declines in bone mass but

did not suppress biomarkers for bone resorption or prevent trabecular bone loss [13]. This incomplete protection provided by current countermeasures, indicates that complications related to skeletal health with long duration spaceflight remain unsolved. Thus, there is strong rationale for further investigations to identify possible countermeasures for mitigating negative effects of spaceflight on bone health.

Risk of cartilage degradation during spaceflight

While it has been well-established that microgravity during spaceflight can damage musculoskeletal tissues [56–58], relatively little is known on the effects of spaceflight on the mechanically-sensitive soft tissue structures of the knee joint. Among these tissues are the articular cartilage and the menisci, which serve to distribute loads and maintain joint stability, protecting the knee from failure during dynamic loading [59–61]. On Earth, normal loading applied across the knee joint is needed to maintain structural integrity of the cartilage, proper joint function, and joint homeostasis [62, 63]. Articular cartilage degradation in particular can lead to severe joint pain, symptomatic arthritis, and impaired mobility [64–66]. Thus, degradation of the knee joint during long duration spaceflight could reduce both an astronaut's performance, risking mission success, and quality of life upon return to full weight-bearing. While the damaging effects of prolonged unloading during spaceflight on joint soft tissues still remain to be elucidated, clinical and preclinical evidence indicates that joint cartilage degradation occurs with reduced loading [62, 67–69]. Studies have shown that after periods of prescribed reduced weight bearing in spinal cord injury patients, structural degradation occurs in the joint soft tissues [65, 67, 68]. Furthermore, we have identified cartilage

degradation in male rat knees after short periods (13 days) of hind limb unloading (HLU), and have identified that the pro-arthritic signaling pathway EIF2α is increased in cartilage after 30 days of HLU, while the anabolic pathway mTOR is reduced [85]. Moreover, data from our Rodent Research-9 (RR9) mission showed a volumetric decline in both the articular cartilage (-25.4%) and menisci (-9.5%) in flight animals compared to ground animals after 35 days of spaceflight suggesting that prolonged exposure to microgravity conditions negatively impacts joint soft tissue structures [7]. These data also demonstrated a decrease in sulfated GAG content and matrix proteins and a subsequent increase in metalloproteinases in the joint soft tissues of the flight animals indicating that decreased loading during spaceflight damages the microarchitecture of the joint [7]. As limited evidence exists indicating reduced weight-bearing damages joint cartilage and structural soft tissues, the threshold for joint damage with decreasing gravitational loads applied across the knee remains unknown, particularly for longer time periods of microgravity (e.g., 60 days), or upon a return to normal loading. This study will address this concern, and will identify if and how gravitational loading can protect against knee and hip joint degradation.

Spaceflight and oxidative stress

Elevated oxidative stress leads to muscle atrophy [86], alters bone remodeling process causing an unbalance between osteoclast and osteoblast activity [87], and increased expression of matrix metalloproteinases followed by increased catabolic function of chondrocytes in the cartilage during period of disuse [88]. A study reported that levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) in the blood of

osteoarthritic patients (OA) were decreased compared to controls indicating that a disruption in redox homeostasis could be linked in OA progression [89]. Our metabolomic data from RR9 mice hindlimb muscles identifies mitochondrial dysfunction. Moreover, decreased concentration and activity of antioxidant defenses such as superoxide dismutase (e.g., SOD1, SOD3) can accelerate skeletal muscle atrophy [90]. Furthermore, analysis of musculoskeletal tissues, such as the menisci, from the RR9 mission exhibited reduced antioxidant defenses in response to spaceflight, including lowered SOD1, SOD3, and GPX1 expression [7]. Studies have shown that the utilization of SOD mimetics reduce articular pain and slow osteoarthritic progression [79–81]. A reduction in SOD's have been implicated in osteoarthritic development, specifically a study found a reduction in SOD2 expression in the medial tibial chondyle cartilage, the point at which the highest compressive load is passed cross the joint [94, 95]. Inflammatory mediators, such as interleukins (IL-1β, IL-6, IL-15, IL-17, and IL-18) and tumor necrosis factor-α (TNF-α) and are highly upregulated in OA joints and induce reactive oxygen species (ROS) production and expression of matrix degrading proteases (MMP1, MMP3, MMP13) leading to matrix degradation and joint dysfunction [96–99]. Increased oxidative stress combined with a decrease in antioxidant defenses can cause cartilage and joint degradation.

Thus, reducing oxidative stress through the actions of an SOD mimetic represents a countermeasure strategy to prevent atrophy of muscle and other musculoskeletal tissues during spaceflight.

Artificial gravity to counter deleterious effects of weightlessness

Intermittent or continuous exposure to artificial gravity is a possible approach to mitigate declines in musculoskeletal function and performance [100]. Previous studies have established that the deficits in bone and muscle following exposure to partial gravity, simulated by partial weight bearing in mice, are proportional to the degree of unloading [101]. Yet, the ability of partial gravity, induced by centrifugation, to inhibit adverse musculoskeletal changes remains poorly understood. The 2017 International Roadmap for Artificial Gravity Research recommends spaceflight-based studies are needed to define the relationship between gravitational dose and physiologic response by assessing gravity levels ranging from 0 to 1g [102]. The studies proposed here will address this need at evaluate the impact of gravitational ranges commensurate with long-term destinations in the moon and mars.

While many studies have examined the effects of spaceflight and microgravity on physiologic systems in rodents, only recently was hardware that allows investigation of the effects of partial gravity by centrifugal force on the ISS developed and tested [70, 73]. Japanese investigators have developed the mouse habitat unit for ISS with a centrifuge capable of spinning 6 single house cages capable of creating artificial gravity (AG) ranging from 0.1 to 4g via changes in angular velocity. Using this system, investigators from the Japanese Aerospace Exploration Agency (JAXA) recently reported results from 12 C57Bl/6J male mice exposed to either 0g or 1g via the rotating cage system for 34 days aboard the ISS [103]. The mice exposed to microgravity either gained or maintained their preflight weight, whereas the mice exposed to AG consistently gained weight, an observation suggesting minimal stress imposed by the artificial gravity environment. Most notably, the AG appeared to prevent spaceflight-induced bone loss and muscle

atrophy, with both soleus and gastrocnemius muscle mass having been higher in the AG compared to the microgravity-exposed mice, and the same as ground-based control values. Similarly, trabecular bone volume of the distal femur was maintained in the AG mice, whereas, as expected there was significant deterioration in the microgravity mice, These findings provide strong rationale for further studies to delineate the effects of artificial gravity across the partial gravity continuum to gain insight into basic physiologic responses and to establish guidance for the level of partial gravity loading that may be effective in mitigating deleterious effects of spaceflight on the musculoskeletal system.

Rationale for Study

Bone loss, muscle atrophy and joint degradation are deleterious consequences due to reduced mechanical loading both in spaceflight and in conditions on Earth, such as spinal cord injury and stroke. Thus, knowledge about the utility of artificial gravity via centrifugation would have broad benefits in understanding how to combat musculoskeletal disability here on Earth. This project investigated the ability of partial gravity loading via centrifugation to mitigate these deleterious changes and established a dose-response relationship for partial gravity loading in key musculoskeletal tissues. These partial gravity loading thresholds will have physiological relevance as they recapitulate the gravity environments that astronauts will face on planned deep space missions to the moon and mars.

Furthermore, a dysregulation on redox defense mechanisms in the musculoskeletal system have been identified as a key mechanism of osteoporotic

progression both during spaceflight and on Earth [7, 74–77]. Additionally, a comprehensive multi-omics study has identified mitochondrial stress as a central biological phenotype in astronauts due to spaceflight [104]. These findings coupled with studies identifying mitochondrial dysfunction as a key driver in osteoporotic and osteoarthritic progression [82–88], make this a potential target for combating musculoskeletal diseases both on Earth and due to spaceflight. This project investigated the efficacy of MnTnBuOE-2-PyP5+, an antioxidant SOD mimetic metalloporphyrin, as a pharmaceutical countermeasure to prevent joint degradation during and after spaceflight.

Hypothesis and Premise

The combined efforts of these two spaceflight studies investigated novel pharmaceutical and mechanical countermeasures to prevent musculoskeletal degradation on Earth, during and after spaceflight to both improve patient quality of life and increase astronaut mission success.

This dissertation presents evidence that short and long duration spaceflight induces some catabolic responses in the articular cartilage lining the medial tibial plateau, menisci, and tibial bone. This study found evidence supporting treatment with SOD mimetics as a potential countermeasure for the prevention of spaceflight-induced musculoskeletal degradation for short duration flight. The study demonstrates, through data collected from an inflight rodent study aboard the ISS, that treatment with BuOE preserved articular cartilage, menisci, and trabecular bone volume during short duration spaceflight and downregulated pro-arthritic pathways in the joint soft tissue.

Furthermore, the investigations conducted in this dissertation provides evidence that continuous exposure to 1g of gravitational acceleration via centrifugation preserved gait patterns relative to both preflight in rodents. Gravity-dose dependent gait preservation was observed and notably, rodents exposed to μg could not perform at live animal return, with only 50% able to perform in the 0.33g-exposed group. These data suggest that partial and low gravity environments in transit and upon reaching deep space destinations like the Moon and Mars may have deleterious effects on astronaut locomotion and performance. This study suggests that there is a critical need to investigate how sub 1g environments will affect astronaut health and performance, and more specifically identify if there is a gravitational threshold at which deficits are prevented.

Finally, this dissertation demonstrated that continuous exposure to 1g of gravitational acceleration via centrifugation failed to preserve meniscal volume and articular cartilage thickness in rodents. Additionally, proteomic and transcriptomic changes of the joint soft tissue were mitigated in a gravity-dose dependent manner compared to controls; with major differences observed after μg, 0.33g, and 0.67g that were then attenuated at 1g. Mice exposed to artificial 1g maintained meniscal ECM synthesis and sulfated GAG expression compared to controls, ECM synthesis was significantly downregulated in μg, 0.33g, and 0.67g groups. These findings combined suggest that while artificial gravity may not be an effective countermeasure for the prevention of joint soft tissue damage, partial gravity does preserve the cartilage ECM compared to μg.

References

1. Nielsen, R.O.; Schjerling, P.; Tesch, P.; Stål, P.; Langberg, H. Collagen Content in the Vastus Lateralis and the Soleus Muscle Following a 90-Day Bed Rest Period with or without Resistance Exercises. *Muscles. Ligaments Tendons J.* **2015**, *5*, 305, doi:10.11138/MLTJ/2015.5.4.305.
2. Parmenter, B.J.; Raymond, J.; Dinnen, P.J.; Lusby, R.J.; Fiatarone Singh, M.A. Preliminary Evidence That Low Ankle-Brachial Index Is Associated with Reduced Bilateral Hip Extensor Strength and Functional Mobility in Peripheral Arterial Disease. *J. Vasc. Surg.* **2013**, *57*, 963-973.e1, doi:10.1016/J.JVS.2012.08.103.
3. Leblanc, A.; Lin, C.; Shackelford, L.; Sinitsyn, V.; Evans, H.; Belichenko, O.; Schenkman, B.; Kozlovskaya, I.; Oganov, V.; Bakulin, A.; et al. Muscle Volume, MRI Relaxation Times (T2), and Body Composition after Spaceflight. **2000**.
4. Willey, J.S.; Lloyd, S.A.J.; Nelson, G.A.; Bateman, T.A. Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications. *Clin Rev Bone Min. Metab* **2011**, *9*, 54–62, doi:10.1007/s12018-011-9092-8.
5. Willey, J.S.; Lloyd, S.A.J.; Nelson, G.A.; Bateman, T.A. Space Radiation and Bone Loss.
6. Tominari, T.; Ichimaru, R.; Taniguchi, K.; Yumoto, A.; Shirakawa, M.; Matsumoto, C.; Watanabe, K.; Hirata, M.; Itoh, Y.; Shiba, D.; et al. Hypergravity and Microgravity Exhibited Reversal Effects on the Bone and Muscle Mass in Mice. *Sci. Reports 2019 91* **2019**, *9*, 1–10, doi:10.1038/s41598-019-42829-z.
7. Kwok, A.T.; Mohamed, N.S.; Plate, J.F.; Yammani, R.R.; Rosas, S.; Bateman, T.A.; Livingston, E.; Moore, J.E.; Kerr, B.A.; Lee, J.; et al. Spaceflight and Hind

Limb Unloading Induces an Arthritic Phenotype in Knee Articular Cartilage and Menisci of Rodents. *Sci. Rep.* **2021**, *11*, doi:10.1038/S41598-021-90010-2.

8. Kwok, A.; Rosas, S.; Bateman, T.A.; Livingston, E.; Smith, T.L.; Moore, J.; Zawieja, D.C.; Hampton, T.; Mao, X.W.; Delp, M.D.; et al. Altered Rodent Gait Characteristics after ~35 Days in Orbit Aboard the International Space Station. *Life Sci. Sp. Res.* **2020**, *24*, 9–17, doi:10.1016/J.LSSR.2019.10.010.
9. Buckey, J.C. Preparing for Mars: The Physiologic and Medical Challenges. Available online: https://pubmed.ncbi.nlm.nih.gov/10477498/ (accessed on 13 December 2022).
10. Sibonga, J.; Matsumoto, T.; Jones, J.; Shapiro, J.; Lang, T.; Shackelford, L.; Smith, S.M.; Young, M.; Keyak, J.; Kohri, K.; et al. Resistive Exercise in Astronauts on Prolonged Spaceflights Provides Partial Protection against Spaceflight-Induced Bone Loss. *Bone* **2019**, *128*, doi:10.1016/j.bone.2019.07.013.
11. LeBlanc, A.; Matsumoto, T.; Jones, J.; Shapiro, J.; Lang, T.; Shackelford, L.; Smith, S.M.; Evans, H.; Spector, E.; Ploutz-Snyder, R.; et al. Bisphosphonates as a Supplement to Exercise to Protect Bone during Long-Duration Spaceflight. *Osteoporos. Int.* **2013**, *24*, 2105–2114, doi:10.1007/S00198-012-2243-Z.
12. Okada, A.; Matsumoto, T.; Ohshima, H.; Isomura, T.; Koga, T.; Yasui, T.; Kohri, K.; LeBlanc, A.; Spector, E.; Jones, J.; et al. Bisphosphonate Use May Reduce the Risk of Urolithiasis in Astronauts on Long-Term Spaceflights. *JBMR Plus* **2022**, *6*, doi:10.1002/JBM4.10550.
13. Sibonga, J.; Matsumoto, T.; Jones, J.; Shapiro, J.; Lang, T.; Shackelford, L.; Smith, S.M.; Young, M.; Keyak, J.; Kohri, K.; et al. Resistive Exercise in

Astronauts on Prolonged Spaceflights Provides Partial Protection against Spaceflight-Induced Bone Loss. *Bone* **2019**, *128*, doi:10.1016/J.BONE.2019.07.013.

14. Greene, K.A.; Tooze, J.A.; Lenchik, L.; Weaver, A.A. Change in Lumbar Muscle Size and Composition on MRI with Long-Duration Spaceflight. *Ann. Biomed. Eng.* **2022**, *50*, 816–824, doi:10.1007/S10439-022-02968-3.
15. Evans, J.M.; Knapp, C.F.; Goswami, N. Artificial Gravity as a Countermeasure to the Cardiovascular Deconditioning of Spaceflight: Gender Perspectives. *Front. Physiol.* **2018**, *9*, 716, doi:10.3389/FPHYS.2018.00716/BIBTEX.
16. Clément, G.R.; Bukley, A.P.; Paloski, W.H.; Mulavara, A.; Cohen, M.M.; Mcgrath, B. Artificial Gravity as a Countermeasure for Mitigating Physiological Deconditioning during Long-Duration Space Missions. **1961**, doi:10.3389/fnsys.2015.00092.
17. Ellman, R.; Spatz, J.; Cloutier, A.; Palme, R.; Christiansen, B.A.; Bouxsein, M.L. Partial Reductions in Mechanical Loading Yield Proportional Changes in Bone Density, Bone Architecture, and Muscle Mass. *J. Bone Miner. Res.* **2013**, *28*, 875–885, doi:10.1002/JBMR.1814.
18. Shimbo, M.; Kudo, T.; Hamada, M.; Jeon, H.; Imamura, Y.; Asano, K.; Okada, R.; Tsunakawa, Y.; Mizuno, S.; Yagami, K.I.; et al. Ground-Based Assessment of JAXA Mouse Habitat Cage Unit by Mouse Phenotypic Studies. *Exp. Anim.* **2016**, *65*, 175–187, doi:10.1538/EXPANIM.15-0077.
19. Shiba, D.; Mizuno, H.; Yumoto, A.; Shimomura, M.; Kobayashi, H.; Morita, H.; Shimbo, M.; Hamada, M.; Kudo, T.; Shinohara, M.; et al. Development of New

Experimental Platform ‘MARS’—Multiple Artificial-Gravity Research System—to Elucidate the Impacts of Micro/Partial Gravity on Mice. *Sci. Reports 2017 71* **2017**, *7*, 1–10, doi:10.1038/S41598-017-10998-4.

20. Tominari, T.; Ichimaru, R.; Taniguchi, K.; Yumoto, A.; Shirakawa, M.; Matsumoto, C.; Watanabe, K.; Hirata, M.; Itoh, Y.; Shiba, D.; et al. Hypergravity and Microgravity Exhibited Reversal Effects on the Bone and Muscle Mass in Mice. *Sci. Rep.* **2019**, *9*, doi:10.1038/S41598-019-42829-Z.
21. Clément, G. International Roadmap for Artificial Gravity Research. *NPJ microgravity* **2017**, *3*, doi:10.1038/S41526-017-0034-8.
22. Koike, M.; Nojiri, H.; Kanazawa, H.; Yamaguchi, H.; Miyagawa, K.; Nagura, N.; Banno, S.; Iwase, Y.; Kurosawa, H.; Kaneko, K. Superoxide Dismutase Activity Is Significantly Lower in End-Stage Osteoarthritic Cartilage than Non-Osteoarthritic Cartilage. *PLoS One* **2018**, *13*, e0203944, doi:10.1371/JOURNAL.PONE.0203944.
23. Scott, J.L.; Gabrielides, C.; Davidson, R.K.; Swingler, T.E.; Clark, I.M.; Wallis, G.A.; Boot-Handford, R.P.; Kirkwood, T.B.L.; Talyor, R.W.; Young, D.A. Superoxide Dismutase down Regulation in Osteoarthritis Progression and End-Stage Disease. *Ann. Rheum. Dis.* **2010**, *69*, 1502, doi:10.1136/ARD.2009.119966.
24. Gui, T.; Luo, L.; Chhay, B.; Zhong, L.; Wei, Y.; Yao, L.; Yu, W.; Li, J.; Nelson, C.L.; Tsourkas, A.; et al. Superoxide Dismutase-Loaded Porous Polymersomes as Highly Efficient Antioxidant Nanoparticles Targeting Synovium for Osteoarthritis Therapy. *Biomaterials* **2022**, *283*, 121437, doi:10.1016/J.BIOMATERIALS.2022.121437.

25. Di Cesare Mannelli, L.; Bani, D.; Bencini, A.; Brandi, M.L.; Calosi, L.; Cantore, M.; Carossino, A.M.; Ghelardini, C.; Valtancoli, B.; Failli, P. Therapeutic Effects of the Superoxide Dismutase Mimetic Compound MnIIMe2DO2A on Experimental Articular Pain in Rats. *Mediators Inflamm.* **2013**, *2013*, 11, doi:10.1155/2013/905360.

26. Yu, D.H.; Yi, J.K.; Yuh, H.S.; Park, S.J.; Kim, H.J.; Bae, K.B.; Ji, Y.R.; Kim, N.R.; Park, S.J.; Kim, D.H.; et al. Over-Expression of Extracellular Superoxide Dismutase in Mouse Synovial Tissue Attenuates the Inflammatory Arthritis. *Exp. Mol. Med. 2012 449* **2012**, *44*, 529–535, doi:10.3858/emm.2012.44.9.060.

27. Aguilar-Agon, K.W.; Capel, A.J.; Fleming, J.W.; Player, D.J.; Martin, N.R.W.; Lewis, M.P. Mechanical Loading of Tissue Engineered Skeletal Muscle Prevents Dexamethasone Induced Myotube Atrophy. *J. Muscle Res. Cell Motil.* **2021**, *42*, 149–159, doi:10.1007/S10974-020-09589-0/FIGURES/6.

28. Klein-Nulend, J.; Bacabac, R.G.; Bakker, A.D. Mechanical Loading and How It Affects Bone Cells: The Role of the Osteocyte Cytoskeleton in Maintaining Our Skeleton. *Eur. Cell. Mater.* **2012**, *24*, 278–291, doi:10.22203/ECM.V024A20.

29. Fu, S.; Thompson, C.L.; Ali, A.; Wang, W.; Chapple, J.P.; Mitchison, H.M.; Beales, P.L.; Wann, A.K.T.; Knight, M.M. Mechanical Loading Inhibits Cartilage Inflammatory Signalling via an HDAC6 and IFT-Dependent Mechanism Regulating Primary Cilia Elongation. *Osteoarthr. Cartil.* **2019**, *27*, 1064–1074, doi:10.1016/J.JOCA.2019.03.003.

30. Aguilar-Agon, K.W.; Capel, A.J.; Martin, N.R.W.; Player, D.J.; Lewis, M.P. Mechanical Loading Stimulates Hypertrophy in Tissue-engineered Skeletal

Muscle: Molecular and Phenotypic Responses. *J. Cell. Physiol.* **2019**, *234*, 23547, doi:10.1002/JCP.28923.

31. Sun, H.B. Mechanical Loading, Cartilage Degradation, and Arthritis. *Ann. N. Y. Acad. Sci.* **2010**, *1211*, 37–50, doi:10.1111/J.1749-6632.2010.05808.X.
32. Musumeci, G. The Effect of Mechanical Loading on Articular Cartilage. *J. Funct. Morphol. Kinesiol. 2016, Vol. 1, Pages 154-161* **2016**, *1*, 154–161, doi:10.3390/JFMK1020154.
33. Jørgensen, A.E.M.; Kjær, M.; Heinemeier, K.M. The Effect of Aging and Mechanical Loading on the Metabolism of Articular Cartilage. *J. Rheumatol.* **2017**, *44*, 410–417, doi:10.3899/JRHEUM.160226.
34. Chu, C.R.; Szczodry, M.; Bruno, S. Animal Models for Cartilage Regeneration and Repair. *Tissue Eng. Part B. Rev.* **2010**, *16*, 105, doi:10.1089/TEN.TEB.2009.0452.
35. Green, D.M.; Noble, P.C.; Bocell, J.R.; Ahuero, J.S.; Poteet, B.A.; Birdsall, H.H. Effect of Early Full Weight-Bearing after Joint Injury on Inflammation and Cartilage Degradation. *J. Bone Joint Surg. Am.* **2006**, *88*, 2201–2209, doi:10.2106/JBJS.E.00812.
36. Bove, S.E.; Calcaterra, S.L.; Brooker, R.M.; Huber, C.M.; Guzman, R.E.; Juneau, P.L.; Schrier, D.J.; Kilgore, K.S. Weight Bearing as a Measure of Disease Progression and Efficacy of Anti-Inflammatory Compounds in a Model of Monosodium Iodoacetate-Induced Osteoarthritis. *Osteoarthr. Cartil.* **2003**, *11*, 821–830, doi:10.1016/S1063-4584(03)00163-8.
37. Messner, K.; Maletius, W. The Long-Term Prognosis for Severe Damage to Weight-Bearing Cartilage in the Knee: A 14-Year Clinical and Radiographic

Follow-up in 28 Young Athletes. *Acta Orthop. Scand.* **1996**, *67*, 165–168, doi:10.3109/17453679608994664.

38. Takahashi, I.; Matsuzaki, T.; Yoshida, S.; Kitade, I.; Hoso, M. Differences in Cartilage Repair between Loading and Unloading Environments in the Rat Knee. *J. Japanese Phys. Ther. Assoc.* **2014**, *17*, 22, doi:10.1298/JJPTA.VOL17_004.
39. Poulet, B.; Beier, F. Targeting Oxidative Stress to Reduce Osteoarthritis. *Arthritis Res. Ther.* **2016**, *18*, 1–2, doi:10.1186/S13075-015-0908-7/METRICS.
40. Ansari, M.Y.; Ahmad, N.; Haqqi, T.M. Oxidative Stress and Inflammation in Osteoarthritis Pathogenesis: Role of Polyphenols. *Biomed. Pharmacother.* **2020**, *129*, 110452, doi:10.1016/J.BIOPHA.2020.110452.
41. Lepetsos, P.; Papavassiliou, A.G. ROS/Oxidative Stress Signaling in Osteoarthritis. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2016**, *1862*, 576–591, doi:10.1016/J.BBADIS.2016.01.003.
42. Zahan, O.M.; Serban, O.; Gherman, C.; Fodor, D. The Evaluation of Oxidative Stress in Osteoarthritis. *Med. Pharm. Reports* **2020**, *93*, 12, doi:10.15386/MPR-1422.
43. Kokkinias, A.D.; Kokkinias, S.-O.D. Mechanical Loading Effect to the Functional Bone Adaptation. *J. Frailty, Sarcopenia Falls* **2017**, *2*, 62.
44. Bergmann, P.; Body, J.J.; Boonen, S.; Boutsen, Y.; Devogelaer, J.P.; Goemaere, S.; Kaufman, J.; Reginster, J.Y.; Rozenberg, S. Loading and Skeletal Development and Maintenance. *J. Osteoporos.* **2011**, *2011*, 1–15, doi:10.4061/2011/786752.
45. Fritton, J.C.; Myers, E.R.; Wright, T.M.; Van Der Meulen, M.C.H. Loading Induces Site-Specific Increases in Mineral Content Assessed by Microcomputed

Tomography of the Mouse Tibia. *Bone* **2005**, *36*, 1030–1038, doi:10.1016/J.BONE.2005.02.013.

46. Frost, H.M. From Wolff's Law to the Utah Paradigm: Insights about Bone Physiology and Its Clinical Applications. *Anat. Rec.* **2001**, *262*, 398–419, doi:10.1002/AR.1049.

47. Fitzgerald, J. Cartilage Breakdown in Microgravity—a Problem for Long-Term Spaceflight? *npj Regen. Med. 2017 21* **2017**, *2*, 1–2, doi:10.1038/s41536-017-0016-1.

48. Ramachandran, V.; Wang, R.; Ramachandran, S.S.; Ahmed, A.S.; Phan, K.; Antonsen, E.L. Effects of Spaceflight on Cartilage: Implications on Spinal Physiology. *J. Spine Surg.* **2018**, *4*, 433, doi:10.21037/JSS.2018.04.07.

49. Ganse, B.; Cucchiarini, M.; Madry, H. Joint Cartilage in Long-Duration Spaceflight. *Biomedicines* **2022**, *10*, doi:10.3390/BIOMEDICINES10061356.

50. Juhl, O.J.; Buettmann, E.G.; Friedman, M.A.; DeNapoli, R.C.; Hoppock, G.A.; Donahue, H.J. Update on the Effects of Microgravity on the Musculoskeletal System. *npj Microgravity* **2021**, *7*, doi:10.1038/S41526-021-00158-4.

51. Lee, P.H.U.; Chung, M.; Ren, Z.; Mair, D.B.; Kim, D.H. Factors Mediating Spaceflight-Induced Skeletal Muscle Atrophy. *Am. J. Physiol. Cell Physiol.* **2022**, *322*, C567–C580, doi:10.1152/AJPCELL.00203.2021.

52. Radugina, E.A.; Almeida, E.A.C.; Blaber, E.; Poplinskaya, V.A.; Markitantova, Y. V.; Grigoryan, E.N. Exposure to Microgravity for 30 Days Onboard Bion M1 Caused Muscle Atrophy and Impaired Regeneration in Murine Femoral Quadriceps. *Life Sci. Sp. Res.* **2018**, *16*, 18–25, doi:10.1016/j.lssr.2017.08.005.

53. Vandenburgh, H.; Chromiak, J.; Shansky, J.; Del Tatto, M.; Lemaire, J. Space Travel Directly Induces Skeletal Muscle Atrophy. *FASEB J.* **1999**, *13*, 1031–1038, doi:10.1096/FASEBJ.13.9.1031.

54. Tays, G.D.; Hupfeld, K.E.; McGregor, H.R.; Salazar, A.P.; De Dios, Y.E.; Beltran, N.E.; Reuter-Lorenz, P.A.; Kofman, I.S.; Wood, S.J.; Bloomberg, J.J.; et al. The Effects of Long Duration Spaceflight on Sensorimotor Control and Cognition. *Front. Neural Circuits* **2021**, *15*, 110, doi:10.3389/FNCIR.2021.723504/BIBTEX.

55. Yang, J.; Zhang, G.; Dong, D.; Shang, P. Effects of Iron Overload and Oxidative Damage on the Musculoskeletal System in the Space Environment: Data from Spaceflights and Ground-Based Simulation Models. *Int. J. Mol. Sci. 2018, Vol. 19, Page 2608* **2018**, *19*, 2608, doi:10.3390/IJMS19092608.

56. Kumar, A.; Tahimic, C.G.T.; Almeida, E.A.C.; Globus, R.K. Spaceflight Modulates the Expression of Key Oxidative Stress and Cell Cycle Related Genes in Heart. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/IJMS22169088/S1.

57. Stein, T.P. Space Flight and Oxidative Stress. *Nutrition* **2002**, *18*, 867–871, doi:10.1016/S0899-9007(02)00938-3.

58. Tahimic, C.G.T.; Globus, R.K. Redox Signaling and Its Impact on Skeletal and Vascular Responses to Spaceflight. *Int. J. Mol. Sci.* **2017**, *18*, doi:10.3390/IJMS18102153.

59. Smith, S.M.; Heer, M.A.; Shackelford, L.C.; Sibonga, J.D.; Ploutz-Snyder, L.; Zwart, S.R. Benefits for Bone from Resistance Exercise and Nutrition in Long-Duration Spaceflight: Evidence from Biochemistry and Densitometry. *J. Bone Miner. Res.* **2012**, *27*, 1896–1906, doi:10.1002/jbmr.1647.

60. Vico, L.; van Rietbergen, B.; Vilayphiou, N.; Linossier, M.T.; Locrelle, H.; Normand, M.; Zouch, M.; Gerbaix, M.; Bonnet, N.; Novikov, V.; et al. Cortical and Trabecular Bone Microstructure Did Not Recover at Weight-Bearing Skeletal Sites and Progressively Deteriorated at Non-Weight-Bearing Sites During the Year Following International Space Station Missions. *J. Bone Miner. Res.* **2017**, *32*, 2010–2021, doi:10.1002/JBMR.3188.

61. Cohen, H.S.; Kimball, K.T.; Mulavara, A.P.; Bloomberg, J.J.; Paloski, W.H. Posturography and Locomotor Tests of Dynamic Balance after Long-Duration Spaceflight. *J. Vestib. Res.* **2012**, *22*, 191–196, doi:10.3233/VES-2012-0456.

62. English, K.L.; Lee, S.M.C.; Loehr, J.A.; Ploutz-Snyder, R.J.; Ploutz-Snyder, L.L.; Reeves, J.M. Isokinetic Strength Changes Following Long-Duration Spaceflight on the ISS. *Aerosp. Med. Hum. Perform.* **2015**, *86*, A68–A77, doi:10.3357/AMHP.EC09.2015.

63. Petersen, N.; Lambrecht, G.; Scott, J.; Hirsch, N.; Stokes, M.; Mester, J. Postflight Reconditioning for European Astronauts - A Case Report of Recovery after Six Months in Space. *Musculoskelet. Sci. Pract.* **2017**, *27 Suppl 1*, S23–S31, doi:10.1016/J.MSKSP.2016.12.010.

64. PAt, B.; Beery Mack, L.; Lacuaxce, P.A.; Vose, G.P.; Vogt, F.B. AMERICAN JOURNAL OF ROENTGENOLOGY RADIUM THERAPY AND NUCLEAR MEDICINE BONE DEMINERALIZATION OF FOOT AND HAND OF GEMINI-TITAN IV, V AND VII ASTRO-NAUTS DURING ORBITAL FLIGHT*.

65. Orwoll, E.S.; Adler, R.A.; Amin, S.; Binkley, N.; Lewiecki, E.M.; Petak, S.M.;

Shapses, S.A.; Sinaki, M.; Watts, N.B.; Sibonga, J.D. Skeletal Health in Long-Duration Astronauts: Nature, Assessment, and Management Recommendations from the NASA Bone Summit. *J. Bone Miner. Res.* **2013**, *28*, 1243–1255, doi:10.1002/JBMR.1948.

66. Sibonga, J.D.; Evans, H.J.; Sung, H.G.; Spector, E.R.; Lang, T.F.; Oganov, V.S.; Bakulin, A. V.; Shackelford, L.C.; LeBlanc, A.D. Recovery of Spaceflight-Induced Bone Loss: Bone Mineral Density after Long-Duration Missions as Fitted with an Exponential Function. *Bone* **2007**, *41*, 973–978, doi:10.1016/J.BONE.2007.08.022.

67. Stavnichuk, M.; Mikolajewicz, N.; Corlett, T.; Morris, M.; Komarova, S. V. A Systematic Review and Meta-Analysis of Bone Loss in Space Travelers. *npj Microgravity 2020 61* **2020**, *6*, 1–9, doi:10.1038/s41526-020-0103-2.

68. Cappellesso, R.; Nicole, L.; Guido, A.; Pizzol, D. Spaceflight Osteoporosis: Current State and Future Perspective. *Endocr. Regul.* **2015**, *49*, 231–239, doi:10.4149/ENDO_2015_04_231.

69. Gabel, L.; Liphardt, A.M.; Hulme, P.A.; Heer, M.; Zwart, S.R.; Sibonga, J.D.; Smith, S.M.; Boyd, S.K. Incomplete Recovery of Bone Strength and Trabecular Microarchitecture at the Distal Tibia 1 Year after Return from Long Duration Spaceflight. *Sci. Reports 2022 121* **2022**, *12*, 1–13, doi:10.1038/s41598-022-13461-1.

70. Davis, S.A.; Davis, B.L. Exercise Equipment Used in Microgravity: Challenges and Opportunities. *Curr. Sports Med. Rep.* **2012**, *11*, 142–147, doi:10.1249/JSR.0B013E3182578FC3.

71. Smith, S.M.; Heer, M.A.; Shackelford, L.C.; Sibonga, J.D.; Ploutz-Snyder, L.; Zwart, S.R. Benefits for Bone from Resistance Exercise and Nutrition in Long-Duration Spaceflight: Evidence from Biochemistry and Densitometry. *J. Bone Miner. Res.* **2012**, *27*, 1896–1906, doi:10.1002/JBMR.1647.

72. Hinterwimmer, S.; Krammer, M.; Krötz, M.; Glaser, C.; Baumgart, R.; Reiser, M.; Eckstein, F. Cartilage Atrophy in the Knees of Patients after Seven Weeks of Partial Load Bearing. *Arthritis Rheum.* **2004**, *50*, 2516–2520, doi:10.1002/ART.20378.

73. Luan, H.Q.; Sun, L.W.; Huang, Y.F.; Wu, X.T.; Niu, H.; Liu, H.; Fan, Y.B. Use of Micro-Computed Tomography to Evaluate the Effects of Exercise on Preventing the Degeneration of Articular Cartilage in Tail-Suspended Rats. *Life Sci. Sp. Res.* **2015**, *6*, 15–20, doi:10.1016/J.LSSR.2015.06.001.

74. Souza, R.B.; Baum, T.; Wu, S.; Feeley, B.T.; Kadel, N.; Li, X.; Link, T.M.; Majumdar, S. Effects of Unloading on Knee Articular Cartilage T1rho and T2 Magnetic Resonance Imaging Relaxation Times: A Case Series. *J. Orthop. Sports Phys. Ther.* **2012**, *42*, 511–520, doi:10.2519/JOSPT.2012.3975.

75. Liphardt, A.M.; Mündermann, A.; Koo, S.; Bäcker, N.; Andriacchi, T.P.; Zange, J.; Mester, J.; Heer, M. Vibration Training Intervention to Maintain Cartilage Thickness and Serum Concentrations of Cartilage Oligometric Matrix Protein (COMP) during Immobilization. *Osteoarthr. Cartil.* **2009**, *17*, 1598–1603, doi:10.1016/J.JOCA.2009.07.007.

76. Stone, A. V.; Loeser, R.F.; Vanderman, K.S.; Long, D.L.; Clark, S.C.; Ferguson, C.M. Pro-Inflammatory Stimulation of Meniscus Cells Increases Production of

Matrix Metalloproteinases and Additional Catabolic Factors Involved in Osteoarthritis Pathogenesis. *Osteoarthr. Cartil.* **2014**, *22*, 264–274, doi:10.1016/J.JOCA.2013.11.002.

77. Felson, D.T. Osteoarthritis as a Disease of Mechanics. *Osteoarthr. Cartil.* **2013**, *21*, 10–15, doi:10.1016/J.JOCA.2012.09.012.

78. Williams, G.N.; Chmielewski, T.; Rudolph, K.S.; Buchanan, T.S.; Snyder-Mackler, L. Dynamic Knee Stability: Current Theory and Implications for Clinicians and Scientists. *J. Orthop. Sports Phys. Ther.* **2001**, *31*, 546–566, doi:10.2519/JOSPT.2001.31.10.546.

79. Bader, D.L.; Salter, D.M.; Chowdhury, T.T. Biomechanical Influence of Cartilage Homeostasis in Health and Disease. *Arthritis* **2011**, *2011*, 979032, doi:10.1155/2011/979032.

80. Vanwanseele, B.; Eckstein, F.; Knecht, H.; Spaepen, A.; Stüssis, E. Longitudinal Analysis of Cartilage Atrophy in the Knees of Patients with Spinal Cord Injury. *Arthritis Rheum.* **2003**, *48*, 3377–3381, doi:10.1002/ART.11367.

81. Yercan, H.S.; Ait Si Selmi, T.; Sugun, T.S.; Neyret, P. Tibiofemoral Instability in Primary Total Knee Replacement: A Review, Part 1: Basic Principles and Classification. *Knee* **2005**, *12*, 257–266, doi:10.1016/J.KNEE.2005.01.004.

82. Sun, H.B. Mechanical Loading, Cartilage Degradation, and Arthritis. *Ann. N. Y. Acad. Sci.* **2010**, *1211*, 37–50, doi:10.1111/J.1749-6632.2010.05808.X.

83. Lohmander, L.S.; Englund, P.M.; Dahl, L.L.; Roos, E.M. The Long-Term Consequence of Anterior Cruciate Ligament and Meniscus Injuries: Osteoarthritis. *Am. J. Sports Med.* **2007**, *35*, 1756–1769, doi:10.1177/0363546507307396.

84. Leong, D.J.; Gu, X.I.; Li, Y.; Lee, J.Y.; Laudier, D.M.; Majeska, R.J.; Schaffler, M.B.; Cardoso, L.; Sun, H.B. Matrix Metalloproteinase-3 in Articular Cartilage Is Upregulated by Joint Immobilization and Suppressed by Passive Joint Motion. *Matrix Biol.* **2010**, *29*, 420–426, doi:10.1016/J.MATBIO.2010.02.004.

85. Willey, J.S.; Lloyd, S.A.J.; Nelson, G.A.; Bateman, T.A. Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications. *Clin. Rev. Bone Miner. Metab.* **2011**, *9*, 54–62, doi:10.1007/S12018-011-9092-8.

86. Lian, D.; Chen, M.M.; Wu, H.; Deng, S.; Hu, X. The Role of Oxidative Stress in Skeletal Muscle Myogenesis and Muscle Disease. *Antioxidants* **2022**, *11*, doi:10.3390/ANTIOX11040755.

87. Domazetovic, V.; Marcucci, G.; Iantomasi, T.; Brandi, M.L.; Vincenzini, M.T. Oxidative Stress in Bone Remodeling: Role of Antioxidants. *Clin. Cases Miner. Bone Metab.* **2017**, *14*, 209, doi:10.11138/CCMBM/2017.14.1.209.

88. Ziskoven, C.; Jäger, M.; Zilkens, C.; Bloch, W.; Brixius, K.; Krauspe, R. Oxidative Stress in Secondary Osteoarthritis: From Cartilage Destruction to Clinical Presentation? *Orthop. Rev. (Pavia).* **2010**, *2*, 23, doi:10.4081/OR.2010.E23.

89. Paździor, M.; Kiełczykowska, M.; Kurzepa, J.; Luchowska-kocot, D.; Kocot, J.; Musik, I. The Oxidative Stress in Knee Osteoarthritis Patients. An Attempt of Evaluation of Possible Compensatory Effects Occurring in the Disease Development. *Medicina (Kaunas).* **2019**, *55*, doi:10.3390/MEDICINA55050150.

90. Capozzi, A.; Saucier, C.; Bisbal, C.; Lambert, K. Grape Polyphenols in the Treatment of Human Skeletal Muscle Damage Due to Inflammation and Oxidative

Stress during Obesity and Aging: Early Outcomes and Promises. *Molecules* **2022**, *27*, doi:10.3390/MOLECULES27196594.

91. Scott, J.L.; Gabrielides, C.; Davidson, R.K.; Swingler, T.E.; Clark, I.M.; Wallis, G.A.; Boot-Handford, R.P.; Kirkwood, T.B.L.; Talyor, R.W.; Young, D.A. Superoxide Dismutase down Regulation in Osteoarthritis Progression and End-Stage Disease. *Ann. Rheum. Dis.* **2010**, *69*, 1502, doi:10.1136/ARD.2009.119966.

92. Di Cesare Mannelli, L.; Bani, D.; Bencini, A.; Brandi, M.L.; Calosi, L.; Cantore, M.; Carossino, A.M.; Ghelardini, C.; Valtancoli, B.; Failli, P. Therapeutic Effects of the Superoxide Dismutase Mimetic Compound MnIIMe2DO2A on Experimental Articular Pain in Rats. *Mediators Inflamm.* **2013**, *2013*, 11, doi:10.1155/2013/905360.

93. LEE, J.Y.; LEE, S.U.; LIM, T.; CHOI, S.H. Healing Effects and Superoxide Dismutase Activity of Diode/Ga-As Lasers in a Rabbit Model of Osteoarthritis. *In Vivo (Brooklyn).* **2014**, *28*.

94. Regan, E.; Flannelly, J.; Bowler, R.; Tran, K.; Nicks, M.; Carbone, B.D.; Glueck, D.; Heijnen, H.; Mason, R.; Crapo, J. Extracellular Superoxide Dismutase and Oxidant Damage in Osteoarthritis. *Arthritis Rheum.* **2005**, *52*, 3479–3491, doi:10.1002/ART.21387.

95. Scott, J.L.; Gabrielides, C.; Davidson, R.K.; Swingler, T.E.; Clark, I.M.; Wallis, G.A.; Boot-Handford, R.P.; Kirkwood, T.B.L.; Talyor, R.W.; Young, D.A. Superoxide Dismutase down Regulation in Osteoarthritis Progression and End-Stage Disease. *Ann. Rheum. Dis.* **2010**, *69*, 1502, doi:10.1136/ARD.2009.119966.

96. Regan, E.A.; Bowler, R.P.; Crapo, J.D. Joint Fluid Antioxidants Are Decreased in

Osteoarthritic Joints Compared to Joints with Macroscopically Intact Cartilage and Subacute Injury. *Osteoarthr. Cartil.* **2008**, *16*, 515–521, doi:10.1016/J.JOCA.2007.09.001.

97. Mobasheri, A.; Biesalski, H.K.; Shakibaei, M.; Henrotin, Y. Antioxidants and Osteoarthritis. *Syst. Biol. Free Radicals Antioxidants* **2014**, *9783642300189*, 2997–3026, doi:10.1007/978-3-642-30018-9_130/COVER.

98. Grover, A.K.; Samson, S.E. Benefits of Antioxidant Supplements for Knee Osteoarthritis: Rationale and Reality. *Nutr. J.* **2016**, *15*, doi:10.1186/S12937-015-0115-Z.

99. Ansari, M.Y.; Ahmad, N.; Haqqi, T.M. Oxidative Stress and Inflammation in Osteoarthritis Pathogenesis: Role of Polyphenols. *Biomed. Pharmacother.* **2020**, *129*, 110452, doi:10.1016/J.BIOPHA.2020.110452.

100. Shimbo, M.; Kudo, T.; Hamada, M.; Jeon, H.; Imamura, Y.; Asano, K.; Okada, R.; Tsunakawa, Y.; Mizuno, S.; Yagami, K.I.; et al. Ground-Based Assessment of JAXA Mouse Habitat Cage Unit by Mouse Phenotypic Studies. *Exp. Anim.* **2016**, *65*, 175–187, doi:10.1538/EXPANIM.15-0077.

101. Ellman, R.; Spatz, J.; Cloutier, A.; Palme, R.; Christiansen, B.A.; Bouxsein, M.L. Partial Reductions in Mechanical Loading Yield Proportional Changes in Bone Density, Bone Architecture, and Muscle Mass. *J. Bone Miner. Res.* **2013**, *28*, 875, doi:10.1002/JBMR.1814.

102. Clément, G. International Roadmap for Artificial Gravity Research. *npj Microgravity 2017 31* **2017**, *3*, 1–7, doi:10.1038/s41526-017-0034-8.

103. Shiba, D.; Mizuno, H.; Yumoto, A.; Shimomura, M.; Kobayashi, H.; Morita, H.;

Shimbo, M.; Hamada, M.; Kudo, T.; Shinohara, M.; et al. Development of New Experimental Platform 'MARS'-Multiple Artificial-Gravity Research System-to Elucidate the Impacts of Micro/Partial Gravity on Mice. *Sci. Rep.* **2017**, *7*, doi:10.1038/S41598-017-10998-4.

104. Da Silveira, W.A.; Fazelinia, H.; Rosenthal, S.B.; Mason, C.E.; Costes, S. V; Beheshti, A.; Laiakis, E.C.; Kim, M.S.; Meydan, C.; Kidane, Y.; et al. Comprehensive Multi-Omics Analysis Reveals Mitochondrial Stress as a Central Biological Hub for Spaceflight Impact. *Cell* **2020**, *183*, 1185-1201.e20, doi:10.1016/j.cell.2020.11.002.

105. Angireddy, R.; Kazmi, H.R.; Srinivasan, S.; Sun, L.; Iqbal, J.; Fuchs, S.Y.; Guha, M.; Kijima, T.; Yuen, T.; Zaidi, M.; et al. Cytochrome c Oxidase Dysfunction Enhances Phagocytic Function and Osteoclast Formation in Macrophages. *FASEB J.* **2019**, *33*, 9167–9181, doi:10.1096/FJ.201900010RR.

106. Dobson, P.F.; Dennis, E.P.; Hipps, D.; Reeve, A.; Laude, A.; Bradshaw, C.; Stamp, C.; Smith, A.; Deehan, D.J.; Turnbull, D.M.; et al. Mitochondrial Dysfunction Impairs Osteogenesis, Increases Osteoclast Activity, and Accelerates Age Related Bone Loss. *Sci. Reports 2020 101* **2020**, *10*, 1–14, doi:10.1038/s41598-020-68566-2.

107. Ansari, M.Y.; Ahmad, N.; Voleti, S.; Wase, S.; Malik, M.; Novak, K.; Haqqi, T. Mitochondrial Dysfunction in Osteoarthritis and Aged Cartilage Triggers Inflammatory Response and Matrix Degradation via Ros Mediated Activation of JNK-MAPK/CFos-AP1 Axis in Chondrocytes. *Osteoarthr. Cartil.* **2020**, *28*, S187, doi:10.1016/j.joca.2020.02.304.

108. Blanco, F.J.; López-Armada, M.J.; Maneiro, E. Mitochondrial Dysfunction in Osteoarthritis. *Mitochondrion* **2004**, *4*, 715–728, doi:10.1016/J.MITO.2004.07.022.

109. Mao, X.; Fu, P.; Wang, L.; Xiang, C. Mitochondria: Potential Targets for Osteoarthritis. *Front. Med.* **2020**, *7*, 581402, doi:10.3389/FMED.2020.581402.

110. Gandhi, S.S.; Muraresku, C.; McCormick, E.M.; Falk, M.J.; McCormack, S.E. Risk Factors for Poor Bone Health in Primary Mitochondrial Disease. *J. Inherit. Metab. Dis.* **2017**, *40*, 673, doi:10.1007/S10545-017-0046-2.

CHAPTER I

Treatment with a superoxide dismutase mimetic for joint preservation during 35 and 75 days in orbit aboard the International Space Station, and after 120 days recovery on Earth.

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Key words: International Space Station, Rodent Spaceflight, Osteoarthritis, Osteoporosis, Antioxidant, Countermeasure

Treatment with a superoxide dismutase mimetic for joint preservation during 35 and 75 days in orbit aboard the International Space Station, and after 120 days recovery on Earth.

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Abstract

Reduced weight-bearing during spaceflight has been associated with joint soft tissue degradation that risks astronaut health and performance in transit and upon reaching deep space destinations. Previous rodent experiments aboard the international space station (ISS) have identified that the spaceflight-induced molecular arthritic phenotype was characterized with an increase in oxidative stress. This study evaluated if treatment with a superoxide dismutase (SOD) mimetic in orbit could prevent spaceflight-induced damage to the knee and hip articular cartilage, and the menisci in rodents. Cartilage and meniscal degradation in mice were measured via microCT, histology, and transcriptomics after: (1) ~ 35 days on the ISS, (2) ~ 35 days on the ISS followed by 120 days weight-bearing readaptation on Earth or (3) ~ 75 days on the ISS. The study had a limited sample size, so both significant effects and generalized patterns are reported. After 35 days aboard the ISS, cartilage volume at the tibial-femoral cartilage-cartilage contact point decreased, meniscal volume decreased concurrent with an increase in pro-osteoarthritic signaling in the joint soft tissue. Similarly, a decrease in cortical and trabecular bone volume of the tibia was observed. Treatment with the SOD mimetic preserved the trabecular bone, articular cartilage and the menisci after 35 days aboard the ISS. After 35 days aboard the ISS and 120 days weight-bearing recovery or after 75 days aboard the ISS, treatment with the SOD mimetic failed to preserve the trabecular bone, cortical bone, menisci, and/or the articular cartilage.

Introduction

While degradation of bone and muscle with unloading are well-documented during spaceflight, less is known regarding how reduced weight bearing across joints of the lower limb while in microgravity of the spaceflight environment affects mechanically-sensitive joint soft tissues, especially articular cartilage and menisci within the knee [5]. A degraded joint during and/or after spaceflight (e.g upon reaching a destination) could risk mission success, as pain, and lowered stability can reduce performance of astronauts in multiple manners, such as dynamic loading situations or rapid ingress or egress maneuvers [6]. Previous findings from both actual spaceflight studies [6]–[8] and ground-based analogues for reduced weight-bearing during spaceflight have identified that joint soft tissue degradation does occur, coincident with an osteoarthritic phenotype [9]–[14]. These studies have demonstrated that decreased loading results in elevated pro-arthritic metalloproteinase expression (MMP-3, MMP-13), increased inflammatory markers within the synovial joint (IL-1β, TNF-α, IL-6), and reduced sulfated glycosaminoglycans content [6], [15], [16]. Thus, spaceflight conditions are seemingly disadvantageous to joint health.

Osteoarthritic cartilage has been characterized to exhibit an imbalance between the production and removal of reactive oxygen species (ROS), ultimately leading to matrix degradation and joint dysfunction [17]–[20]. A previous rodent spaceflight mission, Rodent Research 9 (RR9), identified that the spaceflight-induced molecular arthritic phenotype in the menisci was characterized with an increase in oxidative stress: alterations in NRF2 signaling coincident with reduced antioxidant enzymes abundance (e.g., superoxide dismutase (SOD3) and glutathione peroxidase (GPX4) are aligned with elevated oxidative stress [10]. A number of previous rodent studies and astronaut data

have identified that simulated and real spaceflight result in systemic mitochondrial dysfunction characterized by an imbalance between ROS production and antioxidant defense in mitochondria [21]. Intracellular and extracellular SOD1 and SOD3 expression in chondrocytes plays a key role in the regulation of ROS in the articular cartilage [22]–[26]. Mitochondrial dysfunction associated with osteoarthritis is characterized by reduced SOD2 expression, and studies have shown that overexpression of SOD2 in osteoarthritic chondrocytes reduces disease progression [27]–[32]. Thus, chronic oxidative stress during spaceflight could lead to joint degradation and promote an osteoarthritic phenotype.

Antioxidant countermeasures may serve to mitigate an osteoarthritic phenotype during spaceflight. Manganese-centered porphyrins such as MnTE-2-PyP5+, MnTnHex-2-PyP5+, and MnTnBuOE-2-PyP5+ (BuOE) can serve as SOD mimetics by scavenging O_2^-, H_2O_2, and lipid peroxides, thereby limiting ROS-induced cellular and tissue damage[33]–[35]. BuOE is currently in multiple phase 1 and phase 2 human clinical trials and has shown protective effects against radiation-induced damage [36]–[38]. However, the protective effects of SOD mimetics on spaceflight-induced joint soft tissue damage remain to be investigated. For this Rodent Research 18 (RR18) study, mice were launched to the ISS and remained in orbit for 35 (live animal return; LAR) and 75 days (on-orbit dissection); one cohort of mice returned after 35 days were then permitted to recover in full weight bearing. We measured whether BuOE delivered while the mice were on orbit could prevent spaceflight-induced damage to the knee and hip articular cartilage, and the menisci.

Methods and Materials

RR18 Spaceflight Mission: Analyses were performed on male C57BL/6J mice (Jackson Labs) that spent ~35 and 75 days in microgravity aboard ISS as part of the RR18 mission, which launched Dec 2021. The experiment was launched as part of the SpaceX Commercial Resupply Services (CRS)-23 mission from the Kennedy Space Center (KSC) at Cape Canaveral, FL to the ISS. Four weeks prior to launch (L) or enrollment in the study (L-28), mice arrived at the KSC and were maintained on a 12:12 hour dark/light cycle and housed in standard vivarium caging (n=10/cage). At L-7, six cages of mice were placed into the three primary groupings, with 2 cages per group; whole cages were grouped instead of random selection of individuals to avoid complications related to an altered hierarchical structure within cages; this study only had access to two of the 3 groups. BuOE at 1 mg/kg was injected subcutaneously at L-7 and weekly aboard the ISS. The FLIGHT mice were subdivided into two groups saline or BuOE treated. Nine-week-old male mice were grouped as follows: mice launched to the ISS (FLIGHT; n=40); Ground-based habitat controls (Ground Control, GROUND; n=40) placed in environmental chambers replicating the environmental conditions for the FLIGHT mice aboard ISS, including similar NASA Type 12 nutrient-upgraded rodent food bars, temperature, humidity, and CO_2 partial pressure. One group of Vivarium controls were also selected, housed in standard cages in the animal facility, however for this tissue sharing study the vivarium controls were not collected and measured. The FLIGHT group was further subdivided into three experimental cohorts: a 35-day cohort (n=5/per group), a 75-day cohort (n=6-8/per group), and a 35-day recovery (FLIGHT-REC) cohort (n=6-7/per group). At L-1, all 10-week-old FLIGHT mice were loaded into a transporter,

with original cage grouping of n=10 isolated from the others due to a separator in the transporter (again to prevent aggression), and then loaded into the Dragon SpaceX Capsule. Mice were transferred to two Rodent Habitats aboard ISS, n=10 per habitat. Unberthing from ISS and splashdown of live mice occurred on L+34 (LAR), and the live mice were collected by the science teams on L+35 for functional testing and tissue collection at the Roskamp Institute for the 35-day cohort. At L+35 the 35-day FLIGHT REC cohort was transported to Loma Linda University and housed for ~120 days, after which functional testing and tissue collection was performed at Loma Linda University. A corresponding group of GROUND mice (GROUND REC) were also transported to Loma Linda for testing whether full weight bearing for 120 days could lead to recover of any joint degradation or arthritic phenotype in the FLIGHT REC mice that had been observed on Day 35 in the FLIGHT mice. The 75-day cohort was sacrificed on-orbit, returned, and tissues from frozen carcasses stored at -80ºC were collected by the science team at Loma Linda University.

Tissue Harvest: *35-day LAR and 35-day Recovery Groups*: The right hindlimbs of the mice were harvested and preserved in 10% Neutral-buffered formalin for preservation. After 3 days, the hindlimbs were switched into 70% ethanol. The right proximal femur was isolated, flash frozen in liquid nitrogen, and stored at -80 °C. The femur and tibia from the left hind limb were disarticulated, and the meniscus was micro-dissected, flash frozen in liquid nitrogen, and stored at -80 °C. *75-day Flight Group*: The right hindlimb and proximal femur of the mice were harvested and processed and stored the same as previously described for the other RR18 study cohorts. The left hindlimbs were wrapped in saline-soaked gauze and stored at -20°C. The left proximal femurs were collected and

stored in RNA later for 24 hours at 4°C and then transferred to -80°C for long term storage.

Microcomputed Tomography (MicroCT): To examine the structural alterations of the articular cartilage, menisci, and bone, the intact right knees were be submerged in 1% phosphotungstic acid, a contrast-enhancing agent [39], [40], and were imaged using Bruker Skyscan 1275 instrument, with X-Ray emission parameters of 65 kV and 153 µA, and at a 10µm resolution. The 2D-Xray data was collected as an average of two images at each of 1400 positions, separated by 0.3°. The image output reconstruction was performed with NRecon v1.7.4.2. Mimics 23.0 was utilized to segment the meniscus and cartilage to calculate meniscal volume and cartilage thickness at the medial tibial plateau. The trabecular and cortical bone was separated using Mimics, then imported to ImageJ where the BoneJ plugin will be employed to determine bone volume, bone volume fraction, and thickness (max, mean) for both bone compartments. Thickness mapping was performed using 3-Matics 15.0. Bone morphometric indices are aligned with the standard nomenclature for the American Society for Bone and Mineral Research [41]. Reported indices include Tb. BV; Ct. BV; Tb. BV/TV; and Conn. D.

Finite Element Modeling: Hexahedral 8-point 3D bone meshes, 1mm regions of the proximal tibias were imported from Mimics 23.0 software to FeBio for finite element analysis [42] (Fig 5d). Each of the bone sections were assigned nominal, homogenous bone properties (Young's Modulus of 10 GPa and Poisson's ratio of 0.3). Axial compression was simulated by applying a 0.5% strain downward displacement on the

superior node set of the bone with the inferior node set held at fixed boundary condition (Fig 5e). The resultant force on the inferior node set required to reach the specified displacement was calculated and retrieved through the output data files. Following the completion of the simulation, the parameters of total volume, stiffness, structural efficiency, 10th percentile Von Mises stresses, and 90th percentile Von Mises stresses were calculated.

Histology and Immunostaining: Histology was performed on the intact right knee joint (from the the 35-day LAR and 35-day recovery cohorts) after microCT imaging to characterize degradation of soft tissue structures. The right knees were decalcified in EDTA for 14 days with a solution change at day 7 following previously published protocol [10], [16]. The hindlimbs were then assessed and prepared for embedding in paraffin for sectioning. The sectioning of the knee was completed around the cartilage-cartilage contact point, and 5 µm-thick coronal slices were prepared. Histochemical stains were used to quantify the proteoglycan content (Safranin-O), osteoclastic number and activity (TRAP), and arthritis (H&E). Immunostaining was performed to identify the presence of biomarkers indicating active degradation cartilage, including: matrix metalloproteinase-13 (MMP13) and ADAMTS. Additionally, 4HNE immunostaining was performed to assess the redox status of the knee joint. The Osteoarthritis Research Society International (OARSI) scoring system[43] was used to evaluate arthritis severity. Stained sections were scanned at 20x using the Olympus vs120 and analyzed utilizing the Viziopharm v2023.01 image analysis software.

TRAP5b Serum Enzyme-Linked Immunosorbent Assay (ELISA): TRAP5b serum concentration was measured in duplicate using mouse TRAP5b ELISA kit

(Immunodiagnostics) according to standard protocols.

Bulk RNAseq Analysis: Total RNA was isolated from the homogenized proximal femoral cartilage and menisci, from the 35-day LAR cohort, and RNA integrity was verified by electrophoretic tracing using an Agilent Bioanalyzer. RNA-seq libraries was constructed from ~500 ng of total RNA using the Illumina TruSeq Stranded Total RNA LT kit with Ribo-Zero rRNA depletion. Indexed libraries were sequenced on an Illumina NextSeq 500 DNA sequencer and the quality of raw sequencing reads was evaluated by FASTQC analysis. Read alignment was conducted using the STAR sequence aligner. Differentially expressed genes (DEGs) were verified using the DESeq2 algorithm and statistically significant results defined as $p \leq 0.05$ after adjusting for false discovery (FDR) were analyzed using Ingenuity Pathway Analysis (IPA).

Statistical Analysis: The comparisons for cartilage, meniscal, and bone health were calculated with either a one-way ANOVA with a Tukey post-hoc test for multiple comparisons or an unpaired t-test. For transcriptomics analyses, differentially expressed genes (DEGs) were verified using the DESeq2 algorithm and statistically significant results defined as $p \leq 0.05$ after adjusting for false discovery (FDR) were analyzed using Ingenuity Pathway Analysis (IPA). This study was powered and designed to investigate retinal health [44] as the skeletal responses were the result of forming a self-selected team of investigators approved by NASA, with each examining responses in different tissues. This is important in the context of the reporting and interpretation of results, given that the sample size was likely underpowered for some cartilage and meniscal outcomes.

Results

Joint soft tissue histologiol and immunohistochemical alterations after 35 days in orbit and/or recovery

Sulfated glycosaminoglycans (GAG) in the medial tibial articular cartilage were similar in the 35-day FLIGHT cohorts (FLIGHT and FLIGHT+AOX) vs corresponding GROUND controls (Fig 1a). However, after the 120-day full weight-bearing recovery (REC) on Earth, reduced GAG content was observed from all groups vs at LAR, with a larger magnitude in the FLIGHT REC groups (-63% FLIGHT REC vs GROUND REC, -43% FLIGHT+AOX REC vs GROUND+AOX REC, $p<0.001$) (Fig 1a). Sulfated GAG concentration in the medial menisci was lower after FLIGHT (-17% vs GROUND), but preserved with BuOE (FLIGHT+AOX +4.1% vs GROUND) (Fig 1d). A reduction in meniscal GAG content was observed in all groups after 120-days REC compared to 35-day cohorts, with protective effects observed with BuOE treatment (+239% GROUND+AOX vs GROUND, +140% FLIGHT+AOX vs FLIGHT, $p<0.05$) (Fig. 1d).

4HNE concentration within the medical tibial articular cartilage is a measure of redox stress; 4HNE concentration increased with BuOE treatment in FLIGHT+AOX (+140% vs FLIGHT) and GROUND+AOX groups (+163% vs GROUND) (Fig 1b). However, after 120 REC, 4HNE concentration in the medial tibial articular cartilage was lower in all BuOE treated groups (-12.4% FLIGHT REC vs FLIGHT+AOX REC, -34.3% GROUND REC vs GROUND+AOX REC) (Fig 1b). 4HNE concentration in the medial menisci from FLIGHT was significantly greater at LAR (+296% FLIGHT vs GROUND), BuOE treatment attenuated this response in FLIGHT groups (-13.6%

FLIGHT+AOX vs FLIGHT) (Fig 1e). 4HNE concentration in the medial menisci was lower in all FLIGHT groups after 120 REC vs ground controls (-77.4% FLIGHT vs GROUND, -82.9% FLIGHT+AOX vs GROUND+AOX, $p<0.001$) (Fig 1e).

The concentration of the type II collagen-degrading MMP-13 throughout the cartilage lining the medial tibial plateau was lower in all FLIGHT groups at LAR vs GROUND controls (-41.3% FLIGHT vs GROUND, -28.4% FLIGHT+AOX vs GROUND+AOX) (Fig 1c). After 120-day REC, MMP-13 concentration in the medial tibial plateau was lower in FLIGHT REC vs control (-55% vs GROUND REC), and greater wtih BuOE treatment in FLIGHT+AOX REC (+84.4% vs FLIGHT REC) (Fig 1c). MMP13 concentration in the medial menisci was lower in all groups compared to GROUND: FLIGHT (-12% vs GROUND), FLIGHT+AOX (-21.9% vs GROUND), GROUND+AOX (-48.6% vs GROUND) (Fig 1f). MMP13 concentration in the medial menisci was greater in BuOE treated mice at 120-day REC (+157% FLIGHT+AOX REC vs FLIGHT REC) (Fig 1f).

Joint soft tissue morphometric alterations after 35 days in orbit and/or after recovery

Morphometric analyses via contrast enhanced microCT of the articular cartilage lining the medial tibial plateau identified cartilage thinning at the weight-bearing tibial-femoral cartilage contact point vs in FLIGHT at LAR (-16.5% vs GROUND), and preserved with BuOE treatment (FLIGHT + AOX +7% vs FLIGHT) (Fig. 2a). At 120-day REC, articular cartilage thickness was greater all groups compared to thickness observed at LAR, with no difference between the groups (Fig 2a). Medial meniscal volume was reduced in FLIGHT vs GROUND after 35 days in orbit (FLIGHT -9.4% vs

GROUND), and preserved with BuOE treatment (FLIGHT+AOX -1.2% vs GROUND) (Fig 2b). After 120-day REC, meniscal volume was greater in all groups compared to what was observed at LAR (Fig 2b). At 120-day REC, meniscal volume was reduced from FLIGHT mice (FLIGHT REC -8.3% vs GROUND REC) and preserved with BuOE treatment (FLIGHT+AOX REC +1.9% vs FLIGHT REC) (Fig 2b).

Transcriptomic analysis of menisci and proximal femoral cartilage after 35 days in orbit to characterize arthritic responses

Comparative analysis of 35-day FLIGHT vs GROUND menisci identified 2388 significantly altered genes ($p<0.05$) (Fig. 3a). Expression analysis showed enrichment of Pdk4, Imnt, Spock2, Gpmnb, Dpep1, Sgk1, and Galnt15 ($p<0.001$) which are associated with pro-osteoarthritic canonical pathway alterations in decreased collagen biosynthesis and modifying enzymes and cell cycle checkpoint regulation (Z-score>±2) (Fig. 3a). Comparative analysis of 35-day FLIGHT AOX vs GROUND menisci identified 3371 significantly altered genes ($p<0.05$) (Fig 3b). Expression analysis identified enrichment of Spock2, Cdo1, and Imnt ($p<0.001$) and further canonical pathway analysis indicated reduced oxidative stress via increased Hif1α and sirtuin signaling, and decreased cholesterol biosynthesis (Z-score>±2) (Fig 3b). Comparative analysis of 35-day FLIGHT vs GROUND proximal femoral cartilage identified 1287 significantly altered genes ($p<0.05$) (Fig 3c). Expression analysis showed downregulation of Col15A1, SerpinH, Ifitm3, Crabp2, and C1qTnf1 ($p<0.001$), as well as enrichment of Spock2 ($p<0.001$) and further canonical pathway analysis indicated pro-osteoarthritic pathway alterations in decreased collagen biosynthesis and modifying enzymes, assembly of collagen fibrils, and collagen chain trimerization and increased IL-7 (Z-score>±2) (Fig. 3c).

Bone histochemical alterations after 35 days in orbit and/or recovery

Histochemical assessment of TRAP concentration 500 µM below the tibial growth plate identified lower osteoclast surface/bone surface ratio in FLIGHT (-13.4% vs GROUND) and FLIGHT+AOX (-18.9% vs GROUND) compared to animals on the ground (Fig 4b). Post recovery, the osteoclast surface/bone surface ratio was lower in FLIGHT REC (-38.4% vs GROUND REC) and FLIGHT+AOX REC (-28.5% vs GROUND REC) vs GROUND (Fig 4b). A marginal difference in osteoclast number/bone surface was seen in FLIGHT (-8.5% vs GROUND) and a further decrease was observed with treatment with FLIGHT+AOX (-30.5% vs GROUND) (Fig 4a). Osteoclast number to bone surface was greater in all groups after 120 days of recovery with a lower number observed in both FLIGHT REC (-50.3% vs GROUND REC) and FLIGHT+AOX REC(-52.3& vs GROUND REC) compared to GROUND (Fig 4a). Assessment of TRAP5b concentration in the serum with ELISA revealed that TRAP5b concentration increased with FLIGHT (+0.9% vs GROUND) compared to ground and is mitigated with BuOE treatment with FLIGHT+AOX (-1.3%) (Fig 4c). Post-recovery FLIGHT+AOX REC treated animals had lower TRAP5b serum levels compared to FLIGHT REC (-2% vs FLIGHT REC) (Fig 4c).

Morphometric bone alterations after 35 days in orbit

Analysis of trabecular bone utilizing microCT revealed a loss in tibial trabecular volume vs GROUND after 35 days in FLIGHT (-22.3% vs GROUND), and a preservation with BuOE treatment in FLIGHT+AOX (-7.8% vs GROUND) (Fig 5a). After 35 days in orbit and 120 days on Earth recovery a larger disparity was observed in

FLIGHT REC (-30.7% vs GROUND REC) and FLIGHT+AOX REC (-44% vs GROUND REC) vs controls (Fig 5a). Trabecular bone volume fraction was lower in FLIGHT (-6.6% vs GROUND) and FLIGHT+AOX (-7%) vs controls, with no preservation seen with BuOE treatment (Fig 5b). After recovery a significant decrease was observed with FLIGHT REC (-38.8% vs GROUND REC, $p<0.05$) and FLIGHT+AOX REC (-46.2% vs GROUND REC, $p<0.01$) (Fig 5b). Cortical bone volume also was lower after 35 days of spaceflight with FLIGHT (-8% vs GROUND) and FLIGHT+AOX (-7.7% vs GROUND) vs control (Fig 5c). After 120 days of recovery a similar pattern was observed for both FLIGHT REC (-9.7% vs GROUND REC) and FLIGHT+AOX REC (-10.9% vs GROUND REC, $p<0.05$) (Fig 5c). Connectivity density lower after 35 days in FLIGHT (-22% vs GROUND) and was preserved with BuOE treatment in FLIGHT+AOX compared to GROUND (Fig 5d). After 35 days in orbit and 120 days on Earth recovery connectivity density decreased in FLIGHT REC (-34.9% vs GROUND REC) and FLIGHT+BuOE REC (-48.6% vs GROUND REC) compared to the GROUND (Fig 5d).

Finite element modeling of 35 day spaceflight and/or recovery bone

Finite element modeling identified that tibial bone stiffness was significantly lower vs GROUND after 35 days spaceflight regardless of BuOE treatment in FLIGHT (-14.4% vs GROUND) and FLIGHT+AOX (-13.5% vs GROUND) (Fig 6a). After 120 days recovery, the disparity in stiffness continued to significantly decline in flight groups with FLIGHT REC (-19.5% vs GROUND REC, $p<0.001$) and FLIGHT+AOX REC (-14% vs GROUND REC, $p<0.001$) respectively (Fig 6a). A marginal difference was observed in structural efficiency after 35 days of spaceflight and no preservative effect

was seen in BuOE treatment in FLIGHT+AOX (-3% vs FLIGHT) (Fig 6b). After 120 days of recovery, a decrease in structural efficiency was observed in flight groups regardless of treatment in comparison to GROUND in FLIGHT REC (-3.3% vs GROUND REC) and FLIGHT+AOX REC (-3% vs GROUND REC) (Fig 6b). Analysis of 90% von misses stress did not show a difference with BuOE treatment or with 35 days spaceflight compared to GROUND (Fig 6c). A decrease was seen in 90% von misses stress in recovery groups compared to 35-day groups but no difference were observed between the recovery groups (Fig 6c).

Morphometric joint soft tissue alterations after 75 days in orbit

Analysis of the medial menisci using contrast enhanced microCT revealed that after 75 days of spaceflight meniscal volume was elevated (+5.6% in FLIGHT vs GROUND; and +0.8% in FLIGHT+AOX vs GROUND) (Fig 7a). However, long term spaceflight resulted in decreased medial tibial articular cartilage thickness in FLIGHT (-10.7% vs GROUND); treatment with BuOE failed to preserve the meniscus with FLIGHT+AOX (-19.4% vs GROUND) compared to GROUND (Fig 7b). Histochemical assessment of sulfated GAG concentration in the medial tibial articular cartilage showed an increase in GAG content after 75 days spaceflight with FLIGHT (+9.9 vs GROUND) and a decrease with BuOE treatment with FLIGHT+AOX (-2.9% vs GROUND) compared to ground animals (Fig 7c). 75 days spaceflight resulted in a decrease in sulfated GAG concentration in the medial meniscus with FLIGHT (-34.6% vs GROUND) and FLIGHT+AOX (-45.1% vs GROUND) compared to GROUND (Fig 7d). OARSI scoring of the medial tibial articular cartilage as a measure of osteoarthritic progression was increased in FLIGHT (+0.5 vs GROUND) and that BuOE helps to

mitigate this increase with FLIGHT+AOX (-0.33 vs FLIGHT) (Fig 7e).

Bone alterations after 75 days in orbit

Morphometric assessment of the tibia using microCT revealed lower tibial trabecular bone volume with FLIGHT (-9.3% vs GROUND) and that BuOE treatment fails to preserve this loss with FLIGHT+AOX (-32.9% vs GROUND) compared to GROUND (Fig 8a) after 75 days aboard the ISS. The same trend was seen with tibial bone volume fraction in the flight cohorts with FLIGHT (-8.5% vs GROUND) and FLIGHT+AOX (-26.4% vs GROUND) (Fig 8b). 75 days of spaceflight resulted in a loss of tibial cortical bone volume in FLIGHT (-4.2% vs GROUND) and FLIGHT+AOX (-9.6% vs GROUND) (Fig 8c). A decrease in tibial trabecular connectivity density was observed in FLIGHT (-8.2% vs GROUND) and FLIGHT+AOX (-36.2% vs GROUND) after long duration spaceflight compared to GROUND (Fig 8d).

Discussion

Identifying well-tolerated countermeasures against spaceflight-induced musculoskeletal complications is important as damage to skeletal tissues poses a clear risk to astronaut health and mission success [45]–[48]. In this study with limited sample size and thus many observations are noted as qualitative or trends, short and long duration spaceflight induced some catabolic responses in the articular cartilage lining the medial tibial plateau, stabilizing menisci within knees, and bone within the tibia. For this study, the SOD mimetic BuOE was delivered during spaceflight, but then treatment stopped upon a return to weight bearing on Earth. Overall, BuOE was an effective

countermeasure after 35 days of spaceflight, but not for 75 days. Moreover, ceasing BuOE and then permitting the mice to recover in a full weight-bearing state for 120 days resulted in some degradation of the tissues that had previously been preserved.

Treatment with an SOD mimetic did have some preservative effects on cartilage after 35 days of spaceflight, but importantly not after 75 days. Cartilage degradation, specifically thinning of the articular cartilage in the medial tibial plateau and/or loss of GAGs was observed after 35 days of spaceflight both with/without on Earth recovery, and at 75 days. Treatment with BuOE did preserve the articular cartilage thickness and GAG concentration during 35 days of spaceflight. However, upon a return to weight bearing for 120 days, the GAG concentration in the knees of FLIGHT mice that had been preserved with BuOE then decreased compared with control, indicating progressive cartilage damage in the absence of the antioxidant but in the presence of Earth's gravity.

Trabecular and cortical bone volume declined after both 35 days of spaceflight with/without on Earth recovery and 75 days of spaceflight; BuOE preserved trabecular bone volume only in the 35-day spaceflight group. In the context of these findings, it is important to note that treatment with BuOE was ceased after a return to Earth for all groups, which may have impacted the results for the recovery group. These findings are aligned with a study analyzing extent of bone recovery in astronauts after spaceflight aboard ISS, which found that cortical, and trabecular bone mineral density declined in short term flight (6 months) and long term flight (12 months) [49]. The incomplete recovery of bone strength, density, and trabecular microarchitecture at the weight-bearing tibia, was equivalent to decade or more of terrestrial age-related bone loss, and astronauts that underwent long duration spaceflight saw poorer recovery in all of these metrics [49].

These findings taken in the context of future manned long duration missions planned for the moon and mars, indicate a critical need for the further investigation of countermeasures to mitigate spaceflight-induced musculoskeletal damage.

Meniscal volume declined after 35 days of spaceflight; BuOE treatment preserved meniscal volume. In contrast to the response from articular cartilage, degradation of the menisci was not observed out to 120 days recovery in full weight bearing. The limited studies that have investigated the effect of microgravity on the menisci have also observed a decline in meniscal volume coincident with a molecular pro-arthritic phenotype [10]. For this RR18 mission, pro-arthritic decreased Pdk4, Imnt, Gpmnb, Dpep1, Sgk1, and Galnt15 and increased Spock2 expression after 35 days spaceflight (Fig. 31). Decreased Imnt and Dpep1 expression indicate a disruption in inflammatory responses and cellular stress pathways during spaceflight [50]–[53]. Reduced Sgk1 and PDK4 expression has been found to stimulate cartilage degradation via the β-catenin pathway, IL1β-induced chondrocyte hypertrophy, and FOXO1-medicated chondrocyte autophagy[54]–[59]. A disruption in extracellular matrix remodeling markers, Spock2, Gpmnb, and Glant15 was seen in flight menisci, indicating pro-osteoarthritic disruption in cartilage homeostasis and degradation and synovial inflammation [60], [61]. Comparative analysis of FLIGHT AOX vs GROUND menisci showed that treatment with BuOE mitigated many of these marker alterations, but still Spock2, Imnt, and Cdo1 expression was elevated indicating that BuOE did not alter osteoarthritic progression in totality (Fig. 3b). Furthermore, canonical pathway analysis treatment with BuOE indicated reduced oxidative stress via increased Hif1α and sirtuin signaling and enrichment of these pathways may exert a protective effect against osteoarthritis

promoting chondrocyte survival, maintaining cartilage homeostasis, and suppressing inflammation [62]–[70].

Gene expression profiles after 35d in space from cartilage and menisci identified prearthritic responses to microgravity. Gene expression analysis revealed the downregulation of Col15A1, SerpinH, Ifitm3, Crabp2, and C1qTnf1, along with the enrichment of Spock2, in FLIGHT proximal femoral articular cartilage compared to GROUND indicated pro-osteoarthritic inflammation and disrupted collagen production during 35 days spaceflight (Fig. 3c). A dysregulation Col15A1 (a collagen protein involved in maintaining cartilage integrity) and SerpinH1 (a collagen-specific chaperone protein) indicated a disruption in chondrocyte differentiation, collagen synthesis and extracellular matrix remodeling which has been implicated in osteoarthritic progression [71]–[79]. Furthermore, reduced Crabp2 expression may lead to reduced retinoic acid signaling activity, impacting downstream pathways involved in cartilage homeostasis, ECM maintenance, and chondrocyte function contributing to cartilage degeneration, inflammation, and impaired cartilage repair mechanisms[80]–[84]. Ifitm3 and C1qTnf1 have been found to have chondroprotective effects via suppression of synovial cell inflammation, and a downregulation in these markers has been implicated in synovial joint inflammation and osteoarthritic pathogenesis [85]–[91]. Finally, elevated levels of Spock2, a gene involved in extracellular matrix modulation, has been shown to play a role in cartilage degradation and joint tissue remodeling, suggesting that increased Spock2 expression may disrupt the matrix turnover in joints during 35 days spaceflight [50].

This study is not without limitations. As noted, the primary science mission for

this study was to investigate the effects of BuOE on limiting retinal complications [44], thus it was not adequately powered to investigate musculoskeletal alterations. The authors acknowledge that many of the findings, including identification of the effects being different within or between groups, are therefore subjective. However, many findings were identified statistically as being acceptably noted as different from other groups, and that BuOE shows some promise as to protect some skeletal tissues after 35 days in orbit. The authors are comfortable but remain cautious with interpretations. Additionally, treatment with BuOE was provided to all animals a week prior to launch and weekly during flight and ceased upon a return to Earth. The cessation of treatment for the recovery cohort could have impacted the findings from that group; preservation may be dependent on continued use upon a return to weight bearing. Furthermore, the 75-day long term FLIGHT cohort was sacrificed in orbit and tissues were harvested from frozen carcasses. The freeze/thaw process may have impacted tissue and protein integrity for downstream analysis.

Elevated oxidative stress, production of reactive oxygen species (ROS) and a disruption of antioxidant defenses have been well characterized to contribute to osteoarthritic progression in the joint [45]–[48]. Furthermore, the combined hazards of reduced weightbearing and space radiation during spaceflight disrupt the normal redox biology and antioxidant defenses of the musculoskeletal system leading to degenerative disease. [92]–[97]. This degenerative progression can risk mission success and impact astronaut quality of life upon a return to Earth. Treatment with BuOE preserved articular cartilage, menisci, and trabecular bone volume during short duration spaceflight and downregulated pro-arthritic pathways in the joint soft tissue. Further investigation is still

needed to validate the protective effects of BuOE on the musculoskeletal system during spaceflight.

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Author contributions

The following authors served as science team leaders and contributed to the scientific design of the RR18 study: J.S.W., M.D.D., X.W.M. J.S.W. and C.M.P. contributed to the conception and design of the knee joint analyses. C.M.P., J.S.W., S.V.W., L.K., E.P., M.D.D., X.W.M. contributed to the performance of the studies and the collection of tissues. C.M.P., J.S.W., S.V.W., and L.K. contributed to the analysis and interpretation of the data. C.M.P., J.S.W., and S.V.W. wrote the main manuscript text. C.M.P and S.V.W. prepared Figs. 1-8. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

References

[1] C. P. Pal, P. Singh, S. Chaturvedi, K. K. Pruthi, and A. Vij, "Epidemiology of knee osteoarthritis in India and related factors," *Indian J. Orthop.*, vol. 50, no. 5, p. 518, Sep. 2016, doi: 10.4103/0019-5413.189608.

[2] N. Vaghela, D. Mishra, J. Patel, and V. Dani, "Promoting health and quality of life of patients with osteoarthritis of knee joint through non-pharmacological treatment strategies: A randomized controlled trial," *J. Educ. Health Promot.*, vol. 9, no. 1, Jun. 2020, doi: 10.4103/JEHP.JEHP_39_20.

[3] F. Guilak, "Biomechanical factors in osteoarthritis," *Best Pract. Res. Clin. Rheumatol.*, vol. 25, no. 6, p. 815, 2011, doi: 10.1016/J.BERH.2011.11.013.

[4] R. J. Reynolds, P. S. Walker, and J. Buza, "Mechanisms of anterior-posterior stability of the knee joint under load-bearing," *J. Biomech.*, vol. 57, pp. 39–45, May 2017, doi: 10.1016/J.JBIOMECH.2017.03.016.

[5] S. Hinterwimmer *et al.*, "Cartilage atrophy in the knees of patients after seven weeks of partial load bearing," *Arthritis Rheum.*, vol. 50, no. 8, pp. 2516–2520, Aug. 2004, doi: 10.1002/ART.20378.

[6] A. T. Kwok *et al.*, "Knee and Hip Joint Cartilage Damage from Combined

Spaceflight Hazards of Low-Dose Radiation Less than 1 Gy and Prolonged Hindlimb Unloading," *Radiat. Res.*, vol. 191, no. 6, p. 497, Jun. 2019, doi: 10.1667/RR15216.1.

[7] J. Fitzgerald, "Cartilage breakdown in microgravity-a problem for long-term spaceflight?," *NPJ Regen. Med.*, vol. 2, no. 1, Apr. 2017, doi: 10.1038/S41536-017-0016-1.

[8] J. Fitzgerald, J. Endicott, U. Hansen, and C. Janowitz, "Articular cartilage and sternal fibrocartilage respond differently to extended microgravity," *NPJ microgravity*, vol. 5, no. 1, Dec. 2019, doi: 10.1038/S41526-019-0063-6.

[9] B. Ganse, M. Cucchiarini, and H. Madry, "Joint Cartilage in Long-Duration Spaceflight," *Biomedicines*, vol. 10, no. 6, Jun. 2022, doi: 10.3390/BIOMEDICINES10061356.

[10] A. T. Kwok *et al.*, "Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents," *Sci. Rep.*, vol. 11, no. 1, p. 10469, Dec. 2021, doi: 10.1038/S41598-021-90010-2.

[11] A. M. Liphardt *et al.*, "Immobilization by 21 days of bed rest results in type II collagen degradation in healthy individuals," *Osteoarthr. Cartil.*, 2023, doi: 10.1016/J.JOCA.2023.11.007.

[12] A. M. Liphardt *et al.*, "Changes in mechanical loading affect arthritis-induced bone loss in mice," *Bone*, vol. 131, Feb. 2020, doi: 10.1016/J.BONE.2019.115149.

[13] M. Dreiner *et al.*, "Short-term Response of Serum Cartilage Oligomeric Matrix

Protein to Different Types of Impact Loading Under Normal and Artificial Gravity," *Front. Physiol.*, vol. 11, Aug. 2020, doi: 10.3389/FPHYS.2020.01032.

[14] A. M. Liphardt, R. Fernandez-Gonzalo, K. Albracht, J. Rittweger, and L. Vico, "Musculoskeletal research in human space flight - unmet needs for the success of crewed deep space exploration," *NPJ microgravity*, vol. 9, no. 1, Dec. 2023, doi: 10.1038/S41526-023-00258-3.

[15] D. J. Leong *et al.*, "Matrix Metalloproteinase-3 In Articular Cartilage Is Upregulated By Joint Immobilization And Suppressed By Passive Joint Motion," *Matrix Biol.*, vol. 29, no. 5, p. 420, Jun. 2010, doi: 10.1016/J.MATBIO.2010.02.004.

[16] J. S. Willey *et al.*, "Spaceflight-Relevant Challenges of Radiation and/or Reduced Weight Bearing Cause Arthritic Responses in Knee Articular Cartilage," *Radiat. Res.*, vol. 186, no. 4, pp. 333–344, Oct. 2016, doi: 10.1667/RR14400.1.

[17] E. Mesci, A. Icagasioglu, N. Mesci, and S. T. Turgut, "Relation of physical activity level with quality of life, sleep and depression in patients with knee osteoarthritis," *North. Clin. Istanbul*, vol. 2, no. 3, p. 215, 2015, doi: 10.14744/NCI.2015.95867.

[18] M. Y. Ansari, N. Ahmad, and T. M. Haqqi, "Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols," *Biomed. Pharmacother.*, vol. 129, p. 110452, Sep. 2020, doi: 10.1016/J.BIOPHA.2020.110452.

[19] T. M. Griffin and F. Guilak, "The role of mechanical loading in the onset and

progression of osteoarthritis," *Exerc. Sport Sci. Rev.*, vol. 33, no. 4, pp. 195–200, Oct. 2005, doi: 10.1097/00003677-200510000-00008.

[20] S. R. Goldring and M. B. Goldring, "The role of cytokines in cartilage matrix degeneration in osteoarthritis," *Clin. Orthop. Relat. Res.*, vol. 427, no. SUPPL., 2004, doi: 10.1097/01.BLO.0000144854.66565.8F.

[21] H. P. Nguyen, P. H. Tran, K. S. Kim, and S. G. Yang, "The effects of real and simulated microgravity on cellular mitochondrial function," *npj Microgravity 2021 71*, vol. 7, no. 1, pp. 1–11, Nov. 2021, doi: 10.1038/s41526-021-00171-7.

[22] Y. Henrotin, B. Kurz, and T. Aigner, "Oxygen and reactive oxygen species in cartilage degradation: friends or foes?," *Osteoarthr. Cartil.*, vol. 13, no. 8, pp. 643–654, Aug. 2005, doi: 10.1016/J.JOCA.2005.04.002.

[23] P. Lepetsos and A. G. Papavassiliou, "ROS/oxidative stress signaling in osteoarthritis," *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1862, no. 4, pp. 576–591, Apr. 2016, doi: 10.1016/J.BBADIS.2016.01.003.

[24] Y. Shi *et al.*, "A small molecule promotes cartilage extracellular matrix generation and inhibits osteoarthritis development," *Nat. Commun. 2019 101*, vol. 10, no. 1, pp. 1–14, Apr. 2019, doi: 10.1038/s41467-019-09839-x.

[25] E. A. Regan, R. P. Bowler, and J. D. Crapo, "Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury," *Osteoarthr. Cartil.*, vol. 16, no. 4, pp. 515–521, Apr. 2008, doi: 10.1016/J.JOCA.2007.09.001.

[26] M. Koike *et al.*, “Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage,” *PLoS One*, vol. 13, no. 9, p. e0203944, Sep. 2018, doi: 10.1371/JOURNAL.PONE.0203944.

[27] J. A. Bolduc, J. A. Collins, and R. F. Loeser, “Reactive oxygen species, aging and articular cartilage homeostasis,” *Free Radic. Biol. Med.*, vol. 132, pp. 73–82, Feb. 2019, doi: 10.1016/J.FREERADBIOMED.2018.08.038.

[28] Y. Liu, Z. Zhang, T. Li, H. Xu, and H. Zhang, “Senescence in osteoarthritis: from mechanism to potential treatment,” *Arthritis Res. Ther. 2022 241*, vol. 24, no. 1, pp. 1–15, Jul. 2022, doi: 10.1186/S13075-022-02859-X.

[29] E. Regan *et al.*, “Extracellular superoxide dismutase and oxidant damage in osteoarthritis,” *Arthritis Rheum.*, vol. 52, no. 11, pp. 3479–3491, Nov. 2005, doi: 10.1002/art.21387.

[30] J. L. Scott *et al.*, “Superoxide dismutase downregulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, pp. 1502–1510, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[31] C. Gavriilidis, S. Miwa, T. Von Zglinicki, R. W. Taylor, and D. A. Young, “Mitochondrial dysfunction in osteoarthritis is associated with down-regulation of superoxide dismutase 2,” *Arthritis Rheum.*, vol. 65, no. 2, pp. 378–387, Feb. 2013, doi: 10.1002/art.37782.

[32] J. L. Scott *et al.*, “Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug.

2010, doi: 10.1136/ARD.2009.119966.

[33] B. J. Day, “Catalytic antioxidants: A radical approach to new therapeutics,” *Drug Discov. Today*, vol. 9, no. 13, pp. 557–566, Jul. 2004, doi: 10.1016/S1359-6446(04)03139-3.

[34] J. D. Crapo, B. J. Day, and I. Fridovich, “Development of Manganic Porphyrin Mimetics of Superoxide Dismutase Activity,” 2013, Accessed: Dec. 14, 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK6050/.

[35] K. R. Olson *et al.*, “Manganese Porphyrin-Based SOD Mimetics Produce Polysulfides from Hydrogen Sulfide,” *Antioxidants 2019, Vol. 8, Page 639*, vol. 8, no. 12, p. 639, Dec. 2019, doi: 10.3390/ANTIOX8120639.

[36] X. Mao, S. Stanbouly, J. Holley, M. Pecaut, and J. Crapo, “Evidence of Spaceflight-Induced Adverse Effects on Photoreceptors and Retinal Function in the Mouse Eye,” *Int. J. Mol. Sci.*, vol. 24, no. 8, Apr. 2023, doi: 10.3390/IJMS24087362.

[37] S. T. Sonis, “Superoxide Dismutase as an Intervention for Radiation Therapy-Associated Toxicities: Review and Profile of Avasopasem Manganese as a Treatment Option for Radiation-Induced Mucositis,” *Drug Des. Devel. Ther.*, vol. 15, p. 1021, 2021, doi: 10.2147/DDDT.S267400.

[38] G. E. O. Borgstahl and R. E. Oberley-Deegan, “Superoxide Dismutases (SODs) and SOD Mimetics,” *Antioxidants*, vol. 7, no. 11, Nov. 2018, doi: 10.3390/ANTIOX7110156.

[39] M. Paździor, M. Kiełczykowska, J. Kurzepa, D. Luchowska-kocot, J. Kocot, and I. Musik, “The Oxidative Stress in Knee Osteoarthritis Patients. An Attempt of Evaluation of Possible Compensatory Effects Occurring in the Disease Development,” *Medicina (Kaunas).*, vol. 55, no. 5, May 2019, doi: 10.3390/MEDICINA55050150.

[40] A. Capozzi, C. Saucier, C. Bisbal, and K. Lambert, “Grape Polyphenols in the Treatment of Human Skeletal Muscle Damage Due to Inflammation and Oxidative Stress during Obesity and Aging: Early Outcomes and Promises,” *Molecules*, vol. 27, no. 19, Oct. 2022, doi: 10.3390/MOLECULES27196594.

[41] M. L. Bouxsein, S. K. Boyd, B. A. Christiansen, R. E. Guldberg, K. J. Jepsen, and R. Müller, “Guidelines for assessment of bone microstructure in rodents using micro–computed tomography,” *J. Bone Miner. Res.*, vol. 25, no. 7, pp. 1468–1486, Jul. 2010, doi: 10.1002/JBMR.141.

[42] C. M. Heveran *et al.*, “Chronic kidney disease and aging differentially diminish bone material and microarchitecture in C57Bl/6 mice,” *Bone*, vol. 127, pp. 91–103, Oct. 2019, doi: 10.1016/J.BONE.2019.04.019.

[43] W. Waldstein, G. Perino, S. L. Gilbert, S. A. Maher, R. Windhager, and F. Boettner, “OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee,” *J. Orthop. Res.*, vol. 34, no. 1, pp. 135–140, Jan. 2016, doi: 10.1002/JOR.23010.

[44] X. Mao, S. Stanbouly, J. Holley, M. Pecaut, and J. Crapo, “Evidence of Spaceflight-Induced Adverse Effects on Photoreceptors and Retinal Function in

the Mouse Eye," *Int. J. Mol. Sci.*, vol. 24, no. 8, Apr. 2023, doi: 10.3390/IJMS24087362.

[45] J. Stepanek, R. S. Blue, and S. Parazynski, "Space Medicine in the Era of Civilian Spaceflight," *N. Engl. J. Med.*, vol. 380, no. 11, pp. 1053–1060, Mar. 2019, doi: 10.1056/NEJMRA1609012.

[46] T. F. Lang, A. D. Leblanc, H. J. Evans, and Y. Lu, "Adaptation of the proximal femur to skeletal reloading after long-duration spaceflight," *J. Bone Miner. Res.*, vol. 21, no. 8, pp. 1224–1230, Aug. 2006, doi: 10.1359/JBMR.060509.

[47] T. Lang *et al.*, "Towards human exploration of space: the THESEUS review series on muscle and bone research priorities," *NPJ Microgravity*, vol. 3, no. 1, Dec. 2017, doi: 10.1038/S41526-017-0013-0.

[48] B. Vanwanseele, F. Eckstein, H. Knecht, A. Spaepen, and E. Stüssis, "Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord injury," *Arthritis Rheum.*, vol. 48, no. 12, pp. 3377–3381, 2003, doi: 10.1002/ART.11367.

[49] L. Gabel *et al.*, "Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia 1 year after return from long duration spaceflight," *Sci. Rep.*, vol. 12, no. 1, Dec. 2022, doi: 10.1038/S41598-022-13461-1.

[50] K. Panoutsopoulou *et al.*, "Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study," *Ann. Rheum. Dis.*, vol. 70, no. 5, pp. 864–

867, 2011, doi: 10.1136/ard.2010.141473.

[51] I. Tachmazidou *et al.*, “Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank.”

[52] M. Kim, A. Rubab, W. C. W. Chan, and D. Chan, “Osteoarthritis year in review 2022: Genetics, genomics and epigenetics,” *Osteoarthr. Cartil.*, vol. 31, no. 7, pp. 865–875, Jul. 2023, doi: 10.1016/J.JOCA.2023.03.003.

[53] Z. Zhang, Y. Mei, M. Feng, C. Wang, P. Yang, and R. Tian, “The relationship between common variants in the DPEP1 gene and the susceptibility and clinical severity of osteoarthritis,” *Int. J. Rheum. Dis.*, vol. 24, no. 9, pp. 1192–1199, Sep. 2021, doi: 10.1111/1756-185X.14182.

[54] Z. Wang, N. Zhou, W. Wang, Y. Yu, L. Xia, and N. Li, “HDAC2 interacts with microRNA-503-5p to regulate SGK1 in osteoarthritis,” *Arthritis Res. Ther.*, vol. 23, no. 1, pp. 1–11, Dec. 2021, doi: 10.1186/S13075-020-02373-Y/FIGURES/7.

[55] W. Huang *et al.*, “Knockdown of SGK1 alleviates the IL-1β-induced chondrocyte anabolic and catabolic imbalance by activating FoxO1-mediated autophagy in human chondrocytes,” *FEBS J.*, vol. 287, no. 1, pp. 94–107, Jan. 2020, doi: 10.1111/FEBS.15009.

[56] Z. Wang, S. Ni, H. Zhang, Y. Fan, L. Xia, and N. Li, “Silencing SGK1 alleviates osteoarthritis through epigenetic regulation of CREB1 and ABCA1 expression,” *Life Sci.*, vol. 268, Mar. 2021, doi: 10.1016/J.LFS.2020.118733.

[57] I. S. Sinam *et al.*, “Pyruvate dehydrogenase kinase 4 promotes ubiquitin–

proteasome system-dependent muscle atrophy," *J. Cachexia. Sarcopenia Muscle*, vol. 13, no. 6, pp. 3122–3136, 2022, doi: 10.1002/jcsm.13100.

[58] Z. Li, L. Xie, H. Zeng, and Y. Wu, "PDK4 inhibits osteoarthritis progression by activating the PPAR pathway," *J. Orthop. Surg. Res.*, vol. 19, no. 1, Dec. 2024, doi: 10.1186/S13018-024-04583-5.

[59] T. Kamo *et al.*, "Epigenetic mechanism controls PDK4 gene activation before and after exercise therapy following artificial knee arthroplasty," *Clin. Interv. Aging*, vol. 14, p. 1433, 2019, doi: 10.2147/CIA.S213154.

[60] S. C. Warner *et al.*, "IL-15 and IL15RA in Osteoarthritis: Association With Symptoms and Protease Production, but Not Structural Severity," *Front. Immunol.*, vol. 11, p. 1385, Jul. 2020, doi: 10.3389/FIMMU.2020.01385/FULL.

[61] A. Al Adlaan, N. Hussein, F. Jaber, and F. Safadi, "AB0103 A NOVEL PROTECTIVE ROLE OF GPNMB/OSTEOACTIVIN IN POST-TRAUMATIC OSTEOARTHRITIS," *Ann. Rheum. Dis.*, vol. 78, no. Suppl 2, pp. 1513–1513, Jun. 2019, doi: 10.1136/ANNRHEUMDIS-2019-EULAR.8175.

[62] Q. Lu, P. Liu, Z. Miao, D. Luo, S. Li, and M. Lu, "SIRT1 restoration enhances chondrocyte autophagy in osteoarthritis through PTEN-mediated EGFR ubiquitination," *Cell Death Discov. 2022 81*, vol. 8, no. 1, pp. 1–10, Apr. 2022, doi: 10.1038/s41420-022-00896-8.

[63] T. Matsuzaki *et al.*, "Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice,"

Ann. Rheum. Dis., vol. 73, no. 7, pp. 1397–1404, Jul. 2014, doi: 10.1136/ANNRHEUMDIS-2012-202620.

[64] K. Sun *et al.*, "The role of the sirtuin family in cartilage and osteoarthritis: molecular mechanisms and therapeutic targets," *Arthritis Res. Ther.*, vol. 24, no. 1, Dec. 2022, doi: 10.1186/S13075-022-02983-8.

[65] Y. Liu, Z. Zhang, C. Liu, and H. Zhang, "Sirtuins in osteoarthritis: current understanding," *Front. Immunol.*, vol. 14, p. 1140653, Apr. 2023, doi: 10.3389/FIMMU.2023.1140653/BIBTEX.

[66] F. J. Zhang, W. Luo, and G. H. Lei, "Role of HIF-1α and HIF-2α in osteoarthritis," *Jt. Bone Spine*, vol. 82, no. 3, pp. 144–147, May 2015, doi: 10.1016/J.JBSPIN.2014.10.003.

[67] H. Guo, J. Huang, Y. Liang, D. Wang, and H. Zhang, "Focusing on the hypoxia-inducible factor pathway: role, regulation, and therapy for osteoarthritis," *Eur. J. Med. Res. 2022 271*, vol. 27, no. 1, pp. 1–11, Dec. 2022, doi: 10.1186/S40001-022-00926-2.

[68] F. J. Zhang, W. Luo, and G. H. Lei, "Role of HIF-1α and HIF-2α in osteoarthritis," *Jt. bone spine*, vol. 82, no. 3, pp. 144–147, May 2015, doi: 10.1016/J.JBSPIN.2014.10.003.

[69] S. Hu *et al.*, "Stabilization of HIF-1α alleviates osteoarthritis via enhancing mitophagy," *Cell Death Dis. 2020 116*, vol. 11, no. 6, pp. 1–16, Jun. 2020, doi: 10.1038/s41419-020-2680-0.

[70] C. Y. Zeng, X. F. Wang, and F. Z. Hua, “HIF-1α in Osteoarthritis: From Pathogenesis to Therapeutic Implications,” *Front. Pharmacol.*, vol. 13, Jul. 2022, doi: 10.3389/FPHAR.2022.927126.

[71] C. Widmera *et al.*, “Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109, no. 33, pp. 13243–13247, Aug. 2012, doi: 10.1073/PNAS.1208072109/SUPPL_FILE/PNAS.201208072SI.PDF.

[72] W. Bin Xu *et al.*, “Identification of the circRNA–miRNA–mRNA regulatory network in osteoarthritis using bioinformatics analysis,” *Front. Genet.*, vol. 13, p. 994163, Sep. 2022, doi: 10.3389/FGENE.2022.994163/BIBTEX.

[73] K. C. Tsolis *et al.*, “Comparative proteomic analysis of hypertrophic chondrocytes in osteoarthritis,” *Clin. Proteomics*, vol. 12, no. 1, pp. 1–16, Apr. 2015, doi: 10.1186/S12014-015-9085-6/FIGURES/6.

[74] C. Marshall, J. Lopez, L. Crookes, R. C. Pollitt, and M. Balasubramanian, “A novel homozygous variant in SERPINH1 associated with a severe, lethal presentation of osteogenesis imperfecta with hydranencephaly,” *Gene*, vol. 595, no. 1, pp. 49–52, Dec. 2016, doi: 10.1016/J.GENE.2016.09.035.

[75] D. J. Wilkinson, “Serpins in cartilage and osteoarthritis: what do we know?,” *Biochem. Soc. Trans.*, vol. 49, no. 2, p. 1013, Apr. 2021, doi: 10.1042/BST20201231.

[76] J. Sun, B. Yan, W. Yin, and X. Zhang, “Identification of genes associated with

osteoarthritis by microarray analysis," *Mol. Med. Rep.*, vol. 12, no. 4, pp. 5211–5216, 2015, doi: 10.3892/mmr.2015.4048.

[77] R. Zhang *et al.*, "Potential candidate biomarkers associated with osteoarthritis: Evidence from a comprehensive network and pathway analysis," *J. Cell. Physiol.*, vol. 234, no. 10, pp. 17433–17443, Oct. 2019, doi: 10.1002/JCP.28365.

[78] C. Karlsson *et al.*, "Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis," *Osteoarthr. Cartil.*, vol. 18, no. 4, pp. 581–592, Apr. 2010, doi: 10.1016/J.JOCA.2009.12.002.

[79] K. M. Fisch *et al.*, "Identification of transcription factors responsible for dysregulated networks in human osteoarthritis cartilage by global gene expression analysis," *Osteoarthr. Cartil.*, vol. 26, no. 11, p. 1531, Nov. 2018, doi: 10.1016/J.JOCA.2018.07.012.

[80] I. D. Welch, M. F. Cowan, F. Beier, and T. M. Underhill, "The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease," *Arthritis Res. Ther.*, vol. 11, no. 1, pp. 1–9, 2009, doi: 10.1186/ar2604.

[81] R. Wang, Q. Yang, W. Xiao, R. Si, F. Sun, and Q. Pan, "Cellular retinoic acid binding protein 2 inhibits osteogenic differentiation by modulating LIMK1 in C2C12 cells," *Dev. Growth Differ.*, vol. 57, no. 8, pp. 581–589, Oct. 2015, doi: 10.1111/DGD.12240.

[82] I. Takazawa, A. Larange, and H. Cheroutre, "Cellular retinoic acid binding protein 2 (CRABP2) serves as a critical regulator in immune regulation and

inflammation," *J. Immunol.*, vol. 202, no. 1_Supplement, pp. 124.3-124.3, May 2019, doi: 10.4049/JIMMUNOL.202.SUPP.124.3.

[83] N. Mosquera, A. Rodriguez-Trillo, A. Mera-Varela, A. Gonzalez, and C. Conde, "Uncovering Cellular retinoic acid-binding protein 2 as a potential target for rheumatoid arthritis synovial hyperplasia," *Sci. Reports 2018 81*, vol. 8, no. 1, pp. 1–9, Jun. 2018, doi: 10.1038/s41598-018-26027-x.

[84] I. D. Welch, M. F. Cowan, F. Beier, and T. M. Underhill, "The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease," *Arthritis Res. Ther.*, vol. 11, no. 1, Jan. 2009, doi: 10.1186/AR2604.

[85] Y. Huang, G. Wan, and J. Tao, "C1q/TNF-related protein-3 exerts the chondroprotective effects in IL-1β-treated SW1353 cells by regulating the FGFR1 signaling," *Biomed. Pharmacother.*, vol. 85, pp. 41–46, Jan. 2017, doi: 10.1016/J.BIOPHA.2016.11.128.

[86] F. Dong, M. Jiang, Z. Zhang, F. Li, and Y. Lian, "C1q/TNF-related protein-9 suppresses inflammation in synovial cells from patients with osteoarthritis," *Scand. J. Rheumatol.*, Sep. 2022, doi: 10.1080/03009742.2021.1946996.

[87] H. Raghu *et al.*, "Complement component C1q limits osteoarthritis pathology by regulating macrophage activation," *J. Immunol.*, vol. 196, no. 1_Supplement, pp. 124.7-124.7, May 2016, doi: 10.4049/JIMMUNOL.196.SUPP.124.7.

[88] F. Dong, M. Jiang, Z. Zhang, F. Li, and Y. Lian, "C1q/TNF-related protein-9 suppresses inflammation in synovial cells from patients with osteoarthritis,"

Scand. J. Rheumatol., vol. 51, no. 5, 2022, doi: 10.1080/03009742.2021.1946996.

[89] R. Lubbers *et al.*, "Complement component C1q is produced by isolated articular chondrocytes," *Osteoarthr. Cartil.*, vol. 28, no. 5, pp. 675–684, May 2020, doi: 10.1016/J.JOCA.2019.09.007.

[90] F. Comblain *et al.*, "Identification of Targets of a New Nutritional Mixture for Osteoarthritis Management Composed by Curcuminoids Extract, Hydrolyzed Collagen and Green Tea Extract," *PLoS One*, vol. 11, no. 6, Jun. 2016, doi: 10.1371/JOURNAL.PONE.0156902.

[91] S. Y. Lee, J. I. Yu, and S. C. Chae, "Associations of ifitm3 haplotypes with rheumatoid arthritis in a korean population," *Genes and Genomics*, vol. 34, no. 5, pp. 493–498, Oct. 2012, doi: 10.1007/S13258-011-0241-8/METRICS.

[92] K. Y. Kamal and J. M. Lawler, "Cellular and Molecular Signaling Meet the Space Environment," *Int. J. Mol. Sci.*, vol. 24, no. 6, Mar. 2023, doi: 10.3390/IJMS24065955.

[93] C. G. T. Tahimic and R. K. Globus, "Redox Signaling and Its Impact on Skeletal and Vascular Responses to Spaceflight," *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102153.

[94] M. A. Bucaro *et al.*, "Bone cell survival in microgravity: evidence that modeled microgravity increases osteoblast sensitivity to apoptogens," *Ann. N. Y. Acad. Sci.*, vol. 1027, pp. 64–73, 2004, doi: 10.1196/ANNALS.1324.007.

[95] J. M. Lawler, W. Song, and S. R. Demaree, "Hindlimb unloading increases

oxidative stress and disrupts antioxidant capacity in skeletal muscle," *Free Radic. Biol. Med.*, vol. 35, no. 1, pp. 9–16, Jul. 2003, doi: 10.1016/S0891-5849(03)00186-2.

[96] J. S. Alwood *et al.*, "Dose- and Ion-Dependent Effects in the Oxidative Stress Response to Space-Like Radiation Exposure in the Skeletal System," *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102117.

[97] G. J. Bell, T. P. Martin, E. I. Ilyina-Kakueva, V. S. Oganov, and V. R. Edgerton, "Altered distribution of mitochondria in rat soleus muscle fibers after spaceflight," *J. Appl. Physiol.*, vol. 73, no. 2, pp. 493–497, 1992, doi: 10.1152/JAPPL.1992.73.2.493.

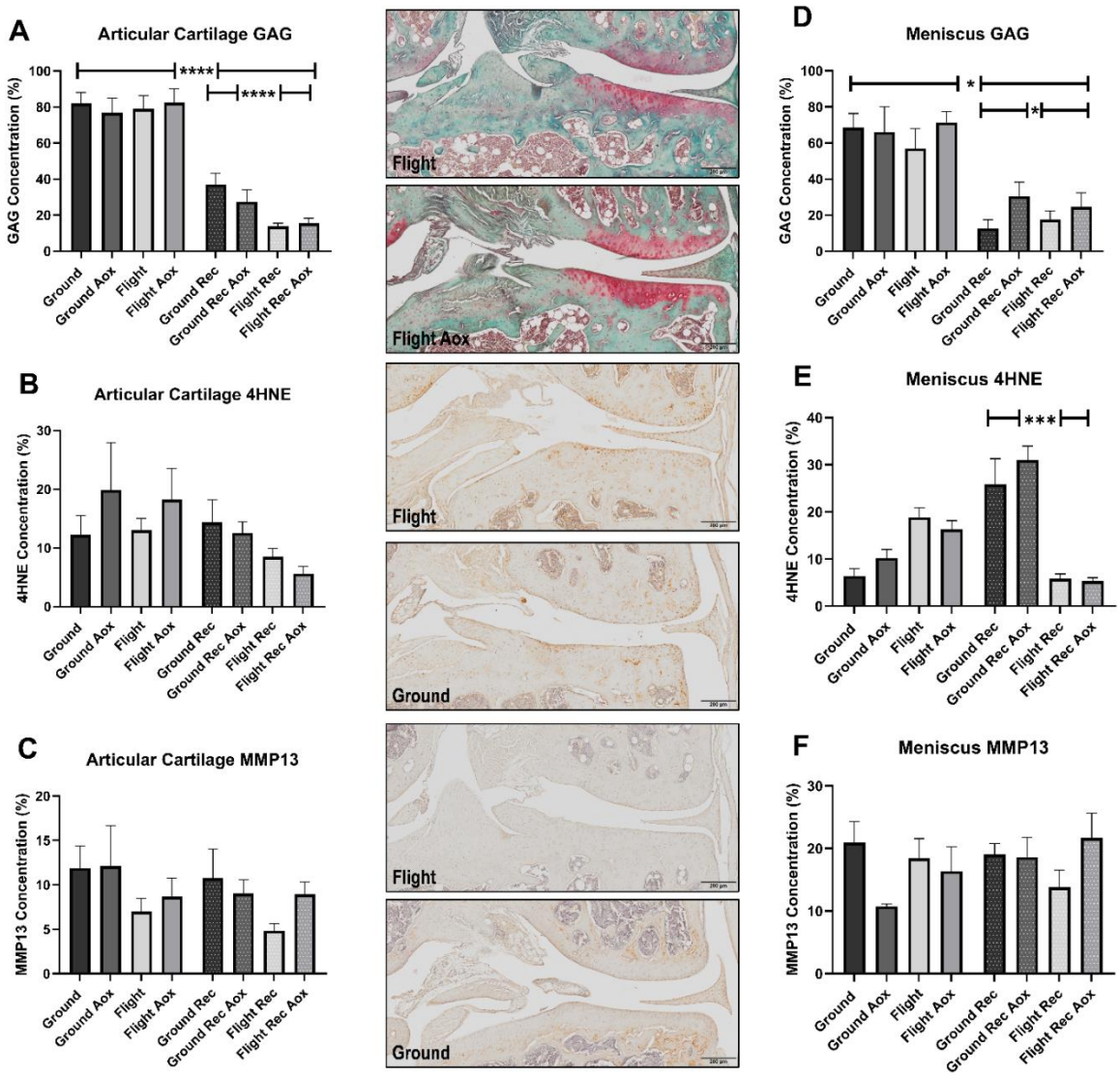

Figure 1. (A) Sulfated GAG in the medial tibial articular cartilage exhibited no significant differences between 35-day FLIGHT cohorts and GROUND controls, but a marked reduction was observed after 35 days in orbit and 120 days of Earth recovery, particularly in the FLIGHT REC groups as indicated by the red stain in representative images. ($p<0.001$). (D) Medial meniscal sulfated GAG concentration was lower after FLIGHT but preserved with AOX treatment ($p<0.05$). (B and E) Redox stress in the cartilage, assessed through 4HNE concentration, increased with AOX treatment during flight but decreased after 120 days of Earth recovery in the articular cartilage and menisci as indicated by the brown stain in repsentative images ($p<0.005$). (C and F) MMP13 concentration in the medial tibial articular cartilage was lower in all FLIGHT groups, and AOX treatment increased concentration in the FLIGHT+AOX recovery group as indicated by the brown stain in the representative images. Meniscal MMP13 concentration marginally decreased in 35-day cohorts with treatment. (*$p<0.05$,

***p<0.005, ****p<0.001)

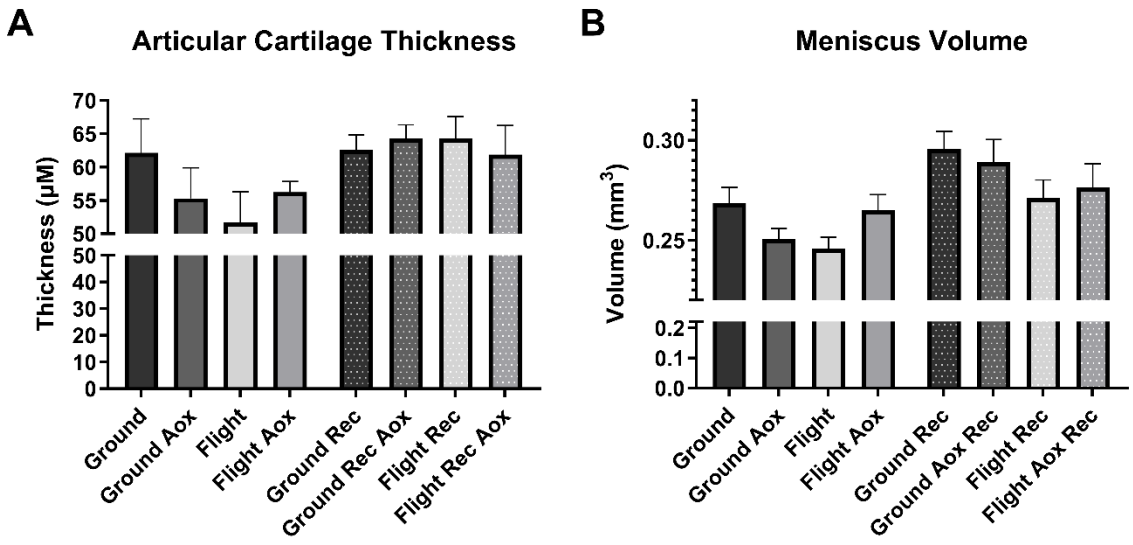

Figure 2. (A) Non-significant thinning of the cartilage at the tibial-femoral contact point was observed after 35 days in orbit, with subsequent preservation with Aox treatment. Articular cartilage thickness increased in all of the 120-day recovery cohorts. (B) Medial meniscal volume decreased after 35 days in orbit, and volume was preserved with AOX treatment. Meniscal volume increased for all 120-day recovery groups with a preservative effect seen with AOX treatment in the FLIGHT cohort.

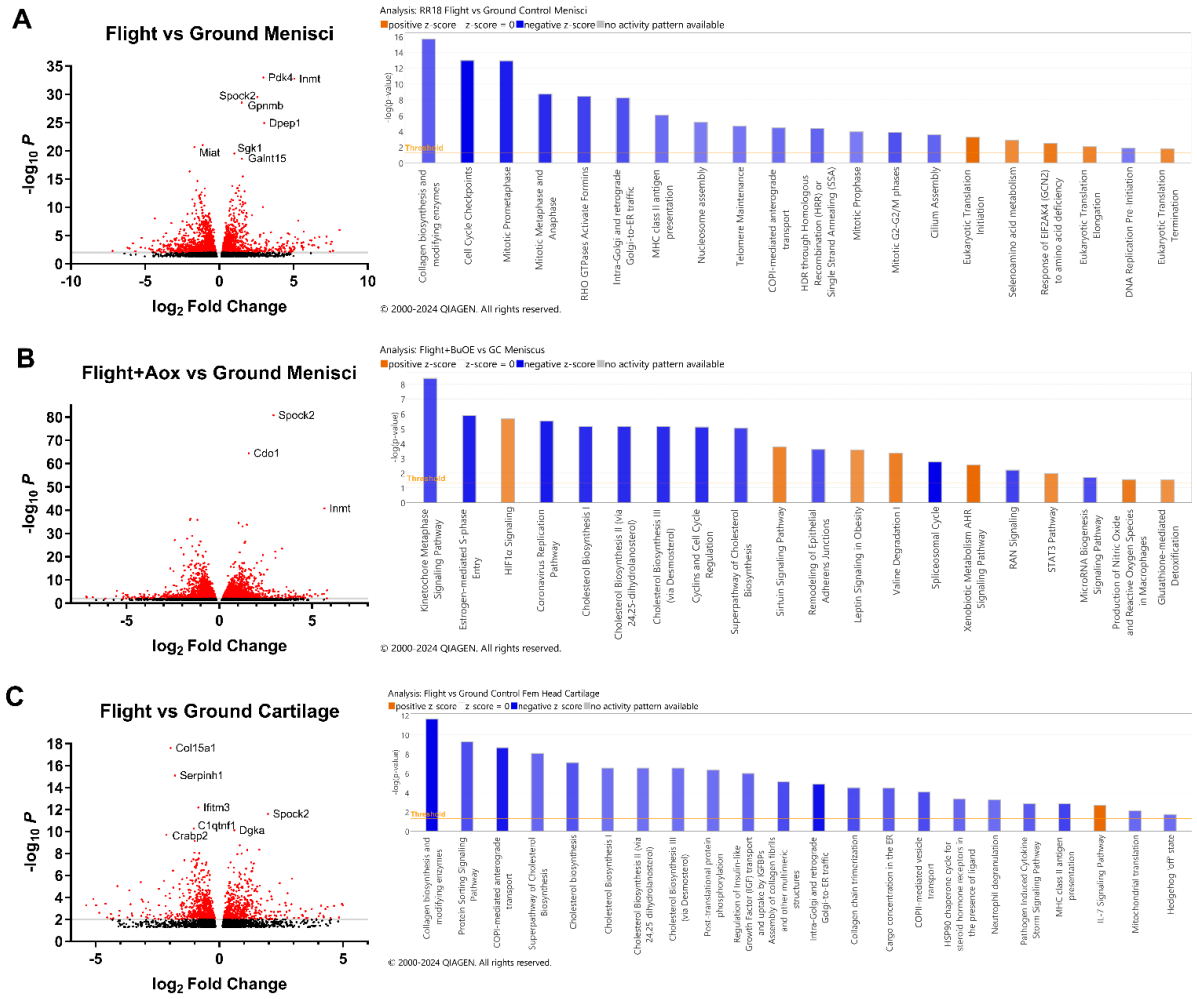

Figure 3. (A) Comparative analysis of 35-day FLIGHT vs GROUND menisci identified 2388 significantly altered genes ($p<0.05$). Canonical pathway comparisons of FLIGHT vs GROUND menisci indicate pro-osteoarthritic pathway alterations in decreased collagen biosynthesis and modifying enzymes and cell cycle checkpoints (Z-score>±2). (B) Comparative analysis of 35-day FLIGHT AOX vs GROUND menisci identified 3371 significantly altered genes ($p<0.05$). Canonical pathway comparisons of FLIGHT+AOX vs GROUND indicate reduced oxidative stress via increased HIF1α and sirtuin signaling, and decreased cholesterol biosynthesis (Z-score>±2). (C) Comparative analysis of 35-day FLIGHT vs GROUND cartilage identified 1287 significantly altered genes ($p<0.05$). Canonical pathway comparisons of FLIGHT vs GROUND proximal femoral articular cartilage indicate pro-osteoarthritic pathway alterations in decreased collagen biosynthesis and modifying enzymes, assembly of collagen fibrils, and collagen chain trimerization and increased IL-7 (Z-score>±2).

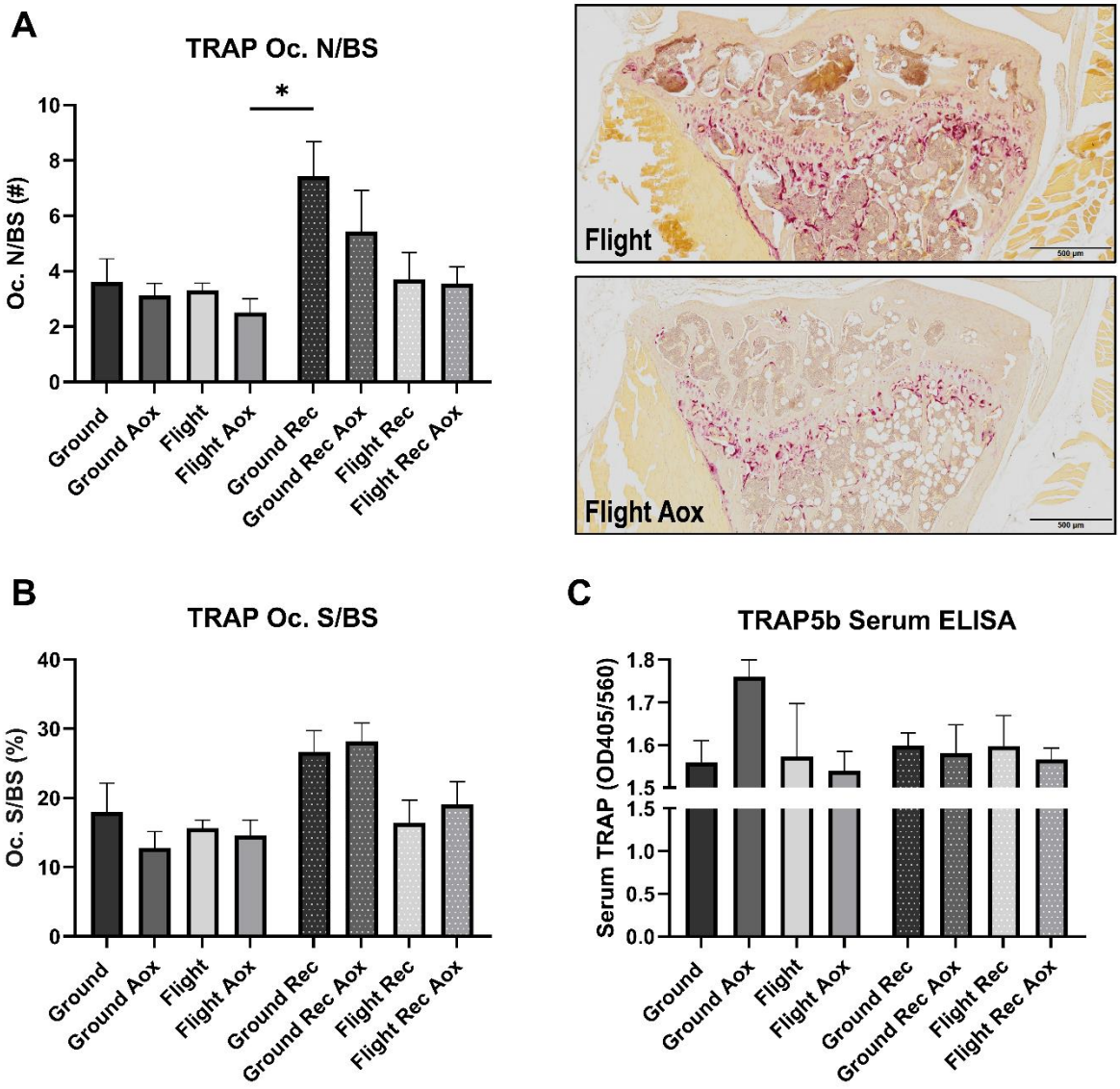

Figure 4. (A) No significant difference was seen in osteoclast number to bone surface (Oc. N/BS) after 35 days of FLIGHT compared to GROUND, and treatment with AOX marginally decreased Oc. N/BS as indicated by the red stain in representative images. An increase in Oc. N/BS after 120 days recovery was seen in all groups compared to the 35-day cohort, with a lower osteoclast number for FLIGHT REC groups compared to the GROUND REC. (B) No differences were observed in osteoclast surface to bone surface (Oc. S/BS) between all groups after 35 days of spaceflight. After 120 days of recovery Oc. S/BS increased in all groups compared to the 35-day cohorts and a lower Oc. S/BS was observed in the FLIGHT REC groups compared to the GROUND REC. (C) No change was seen in TRAP5b serum concentration in the 35-day or recovery groups. (*$p<0.05$)

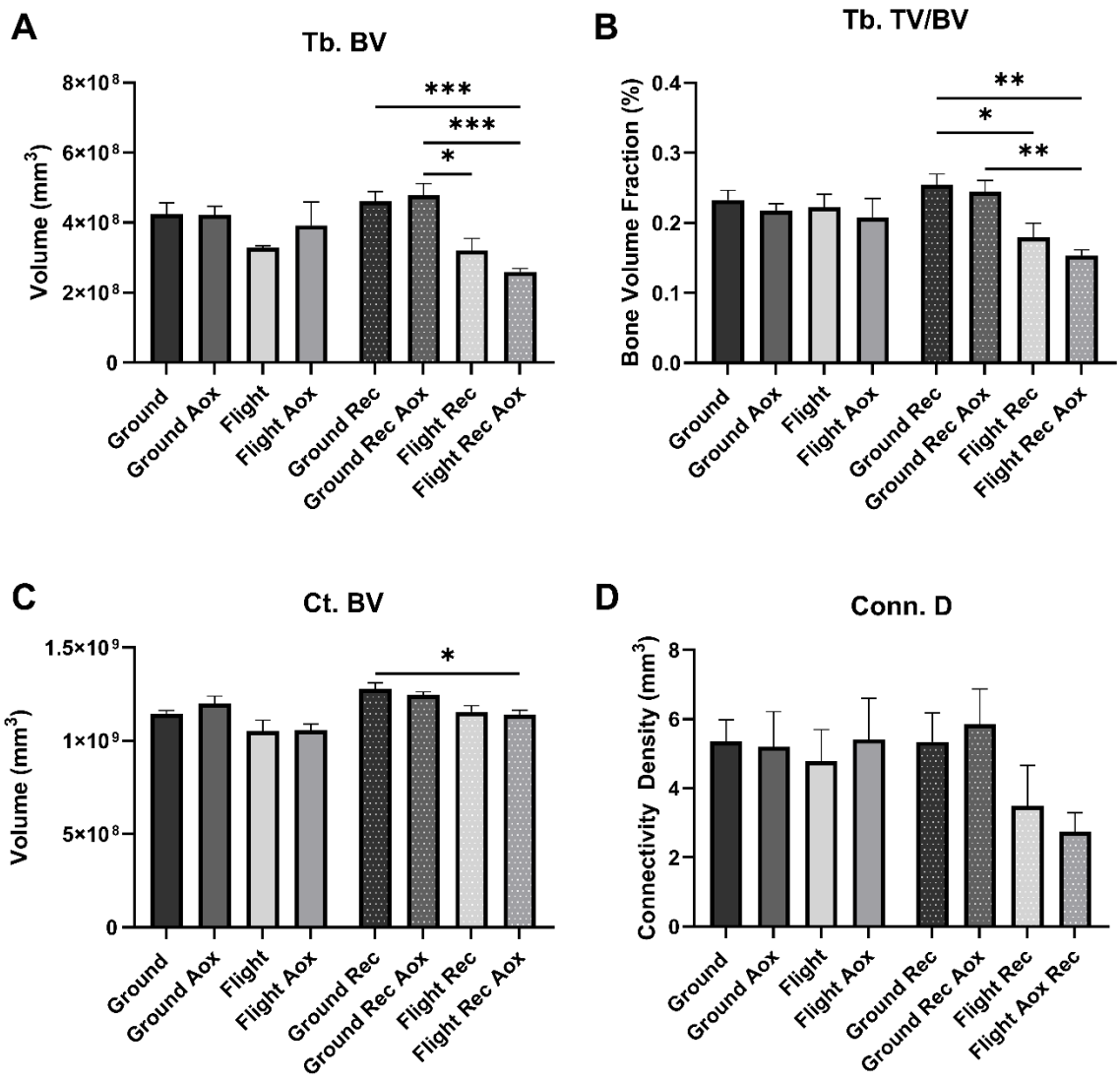

Figure 5. (A) A decrease in trabecular volume was seen after 35 days of spaceflight and mitigated by treatment with BuOE. After 120 days of recovery a significant decrease was observed in FLIGHT groups vs GROUND groups ($p<0.5$). (B) No differences were observed in trabecular volume/bone volume (Tb. TV/BV) after 35 days of FLIGHT compared to GROUND groups. After 120 days of recovery a significant decrease in Tb. TV/BV was observed in FLIGHT REC regardless of treatment compared to GROUND REC groups ($p<0.5$). (C) A decrease in cortical bone volume was observed after 35 days of FLIGHT regardless of treatment, and this same pattern was observed after 120 days of recovery. (D) A marginal decrease in connectivity density was seen after 35 days of FLIGHT, and this was mitigated with AOX treatment. After 120 days of recovery a decrease in connectivity density was observed in the FLIGHT REC groups compared to GROUND REC groups. (*$p<0.05$, **$p<0.01$, ***$p<0.005$)

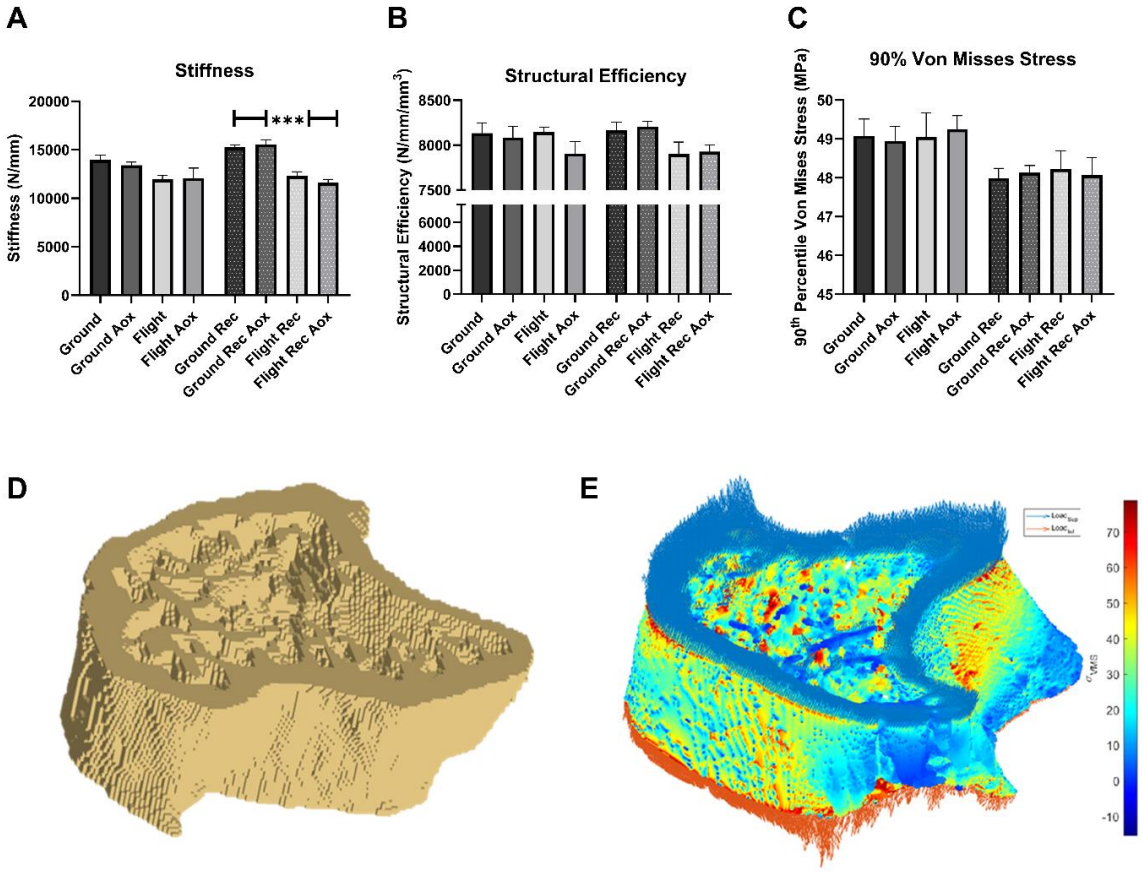

Figure 6. (A) Finite element modeling identified that tibial bone stiffness significantly decreases after 35 days spaceflight regardless of AOX treatment and recovery, the disparity in stiffness continued to significantly decline in FLIGHT groups ($p<0.001$). (B) A marginal difference was observed in structural efficiency after FLIGHT, and after recovery, a decrease in structural efficiency was observed in FLIGHT REC groups regardless of treatment. (C) 90% von misses stress did not show a difference with AOX treatment or after spaceflight. A decrease was seen in 90% von misses stress in recovery groups compared to 35-day groups. (D) A reconstruction demonstrating the 8 voxel, 1 mm tibia mesh used for finite element analysis. (E) A representative image of the color-coded reaction force analysis in the z-direction. (***$p<0.001$)

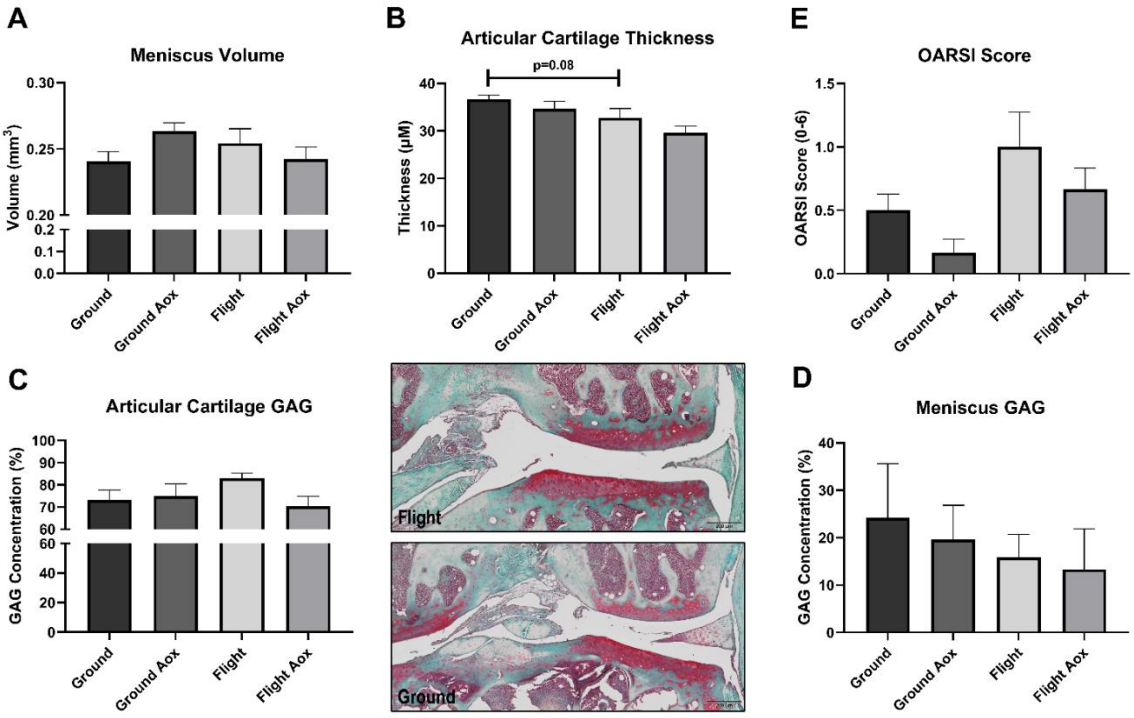

Figure 7. (A) Medial meniscal volume increased in FLIGHT animals after 35 days in orbit, but not in those treated with AOX as indicated by red stain in representative images. (B) 75 days FLIGHT decreased medial tibial articular cartilage thickness and AOX treatment failed to preserve the volume loss. (C) No change was observed in sulfated GAG concentration in the tibial articular cartilage after 75 days in FLIGHT. (D) No change was observed in sulfated GAG concentration in the medial meniscus after 75 days FLIGHT. (E) 75 days spaceflight resulted in osteoarthritic degradation of joint soft tissue structures and this damage was marginally mitigated with AOX treatment.

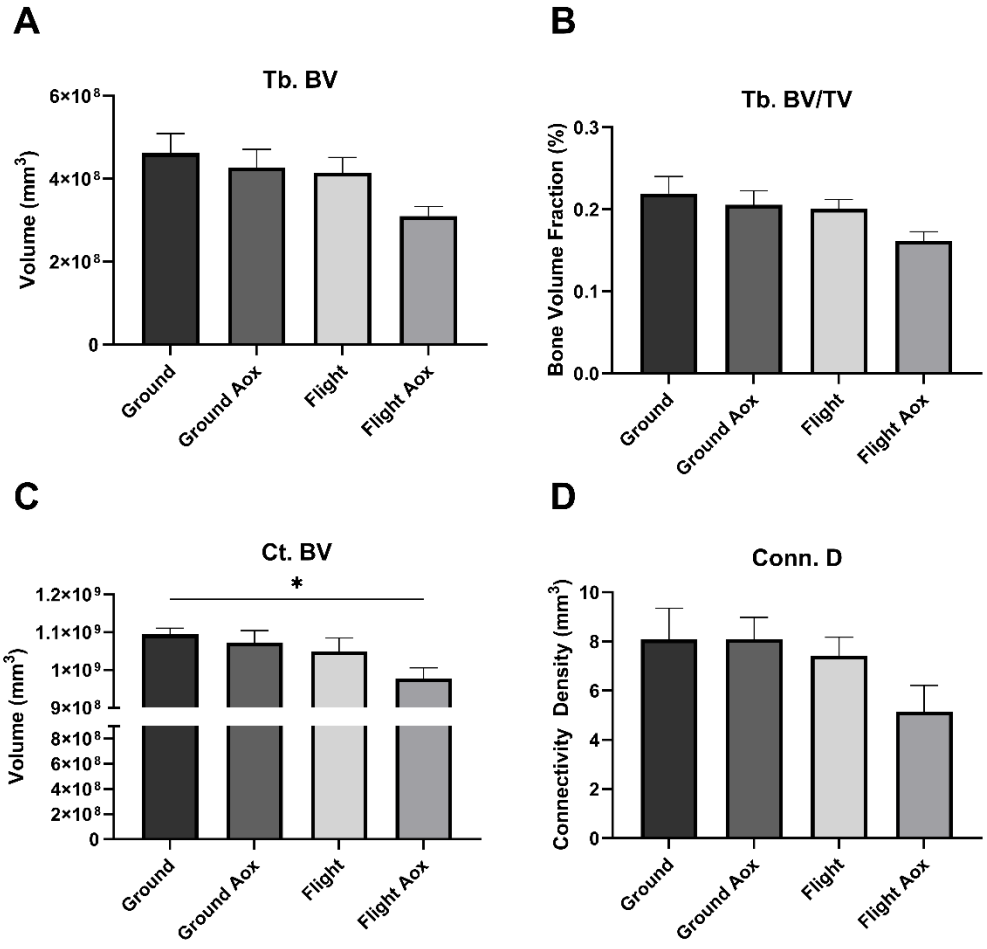

Figure 8. (A) After 75 days of spaceflight reduced tibial trabecular bone volume was observed with AOX treatment. (B) Reduced tibial bone volume fraction was observed after 75 days spaceflight with AOX treatment. (C) 75 days spaceflight resulted in a loss of tibial cortical bone volume with AOX treatment ($p<0.05$). (D) A decrease in tibial trabecular connectivity density was observed after 75 days of spaceflight with AOX treatment. (*$p<0.05$)

CHAPTER II

Artificial gravity during spaceflight prevents gait and performance deficits in a gravity-dose dependent manner

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Key words: International Space Station, Rodent Spaceflight, Gait, Performance, Artificial Gravity

Artificial gravity during spaceflight prevents gait and performance deficits in a gravity-dose dependent manner

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Abstract

Long-term adaptations to spaceflight outside of low earth orbit (LEO), during deep space transit, and readaptations to partial gravity environments upon reaching a destination, are unclear. The combined effect of these adaptations to the LEO environment can result in declines in mobility and balance in astronauts during and after spaceflight This study assessed gait and performance alterations in rodents exposed to one of 4 gravitational loading conditions (μg; 0.33g; 0.67g; and 1g) relative to preflight measurements after a ~35-day mission to the ISS in order to assess if a g-dose threshold exists with regard to induction of performance deficits. Pre-flight and post-flight gait measurements were conducted utilizing the portable gait analysis system (DigiGait, Mouse Specifics, Inc). Patterns of longitudinal gait changes were observed in the hind limbs and the forelimbs of the FLIGHT mice after ~35 days in orbit; few differences were observed in gait characteristics within the GC and VIV controls from the initial to the final gait assessment, and between groups. Continuous exposure to 1g of gravitational acceleration via centrifugation preserved gait patterns relative to both preflight and controls and gait patterns were preserved in a gravity-dose dependent manner; with major differences observed after 0.33g that were then attenuated / normal at 0.67g and normal after 1g. Notably, mice exposed to μg could not perform (locomote) at live animal return, with only 50% able to perform in the 0.33g-exposed group. As we plan for missions to the Moon and Mars, there is a critical need to characterize these neuromotor deficits in the microgravity and partial gravity environments to better mitigate the risks associated with long-duration spaceflight both in transit and upon reaching a destination.

Introduction

Multiple toxicity profiles result from exposure to microgravity during spaceflight that pose challenges to astronaut health and performance (Krittanawong et al. 2023; Orbit, Ball, and Charles H. Evans 2001; Patel et al. 2020; Tomsia et al. 2024; Yin et al. 2023), including neurovestibular complications (Carriot, Mackrous, and Cullen 2021; Clark 2019; G. Clément et al. 2022; Reschke and Clément 2018; Reschke, Good, and Clément 2017), musculoskeletal deficits, (Comfort et al. 2021; Liphardt et al. 2023a) and others. Alterations in normal gait patterns reflect many of the physiological alterations during space flight (Bloomberg and Mulavara 2003; Kim et al. 2018; A. Kwok et al. 2020; Saveko et al. 2022), including the aberrant vestibular and musculoskeletal responses. Gait pattern analysis in rodents is a useful performance assay to characterize the functional response to various injuries or challenges, with outcomes identifying several deficits such as instability or elevated fall risk with neuromotor complications (Karmakar et al. 2023; Santana Rizzi et al. n.d.; Wenger et al. 2022). Normal gait patterns will change with pathology in a relatively consistent manner reflecting the underlying dysfunction. Pre- and post-flight rodent gait analysis thus provides a non-invasive assay to measure the functional response of body systems to spaceflight and ground-based spaceflight hazards.

The Japan Aerospace Exploration Agency (JAXA) Mouse Artificial Gravity System (MARS) aboard the Kibo module on the ISS provides a useful platform to assess the cause, extent, and test countermeasures against the deleterious effects of various gravitational loading accelerations on rodent health (Shiba et al. 2017). Specifically, the 4 rotors of the centrifuge permit the assessment of up to 4 gravitational loading regimes on

the biologic and functional responses. Importantly, this thus permits the study of whether a gravitational threshold exists for incurring deficits across rodent tissues. In terms of gait assessment, only one has measured functional alterations due to microgravity as part of the Rodent Research 9 mission (A. Kwok et al. 2020), and none have assessed effects of partial gravity on gait and related performance in rodents. The Rodent Research 9 study (RR9) identified exposure to ~34d of microgravity resulted in gait pattern changes reflecting neuromotor deficits as well as metrics indicating instability, however the rodents could locomote in a linear fashion upon live animal return. It is unclear if or how continuous exposure to partial gravitational loads could mitigate these effects, and if a threshold exists for these responses. Therefore, the aim of this study was to assess gait and performance alterations in rodents exposed to one of 4 gravitational loading conditions (µg; 0.33g; 0.67g; and 1g) relative to preflight measurements after a ~35-day mission to the ISS in order to assess if a g-dose threshold exists with regard to induction of performance deficits.

Methods and Materials

Joint Partial-gravity Rodent Research/ Mouse Habitat Unit-8 Spaceflight Mission:

Analyses were performed on male C57BL/6J mice (Jackson Labs) that spent ~35 days at varying g-levels in the JAXA Multiple Artificial-Gravity Research System (MARS) onboard the ISS's Kibo module as part of the Joint Partial-gravity Rodent Research (RR)/ Mouse Habitat Unit-8 (MHU) mission, which launched March 14, 2023 and returned April 16, 2023. A total of 24 mice were singly housed at the following G-levels: µg

(n=6), 0.33g (n=6), 0.67g (n=6), and 1g (n=6) in JAXA's mouse habitats. The experiment was launched as part of the SpaceX Commercial Resupply Services (CRS)-27 mission from the Kennedy Space Center (KSC) at Cape Canaveral, FL to the ISS. Six weeks prior to launch (L) or enrollment in the study (L-42), mice arrived at the KSC to begin their acclimation process. Upon arrival animals were singly housed and implanted with RFID tag for identification, and body mass, food, and water consumption were measured bi-weekly. At L-1, mice were placed into the three primary groupings based on weight gain over nine weeks and their body mass to ensure minimal pre-flight differences between groups. Twelve-week-old male mice were grouped as follows: mice launched to the ISS (FLIGHT, µg, 0.33g, 0.67g 1g; n=6/group) housed in habitat cage units (HCU), including rodent food bars (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan), ad libitum access to water containing (0.2/mg/L); Ground-based habitat controls (Ground Control, GC; n=12) were housed identically as flight mice exposed to artificial gravity, and maintained on a two-day delay to match environmental conditions (ambient temperature of 26-28 °C, humidity, and CO_2 partial pressure, and 12/12-hour light/dark cycle) on the ISS. One group of vivarium ground controls (VIV, n=12) were also selected, singly housed in standard cages in the animal care facility (ACF). At launch, all 12-week-old FLIGHT mice were loaded into a transporter and then loaded into the Dragon SpaceX Capsule. Mice were transferred to individual HCU and loaded in the MARS system aboard the ISS. Unberthing from ISS and splashdown of live mice occurred on L+32, and the live mice were collected by the science teams for functional testing and tissue collection at KSC.

Gait Analysis: Gait analysis was performed longitudinally on left limbs using the portable

DigiGait System (Mouse Specifics, Quincy, MA). The DigiGait system performs automated gait analysis by examining the ventral plane of the mouse as it walks on a transparent treadmill belt utilizing artificial intelligence to determine paw positions relative to the treadmill belt to generate gait signals for each limb. Continuous high-speed digital video capture at 176 frames/s was captured for 6-8 consecutive walking strides at 17 cm/s per animal to characterize motor deficits (Dorman et al. 2014; A. Kwok et al. 2020; Vincelette et al. 2007). Recordings of the footfall patterns were assessed for quality and truncated to a minimum criterion of 6 consecutive, non-interrupted strides. After this, the data was imported into the DigiGait Analysis v.14 software where spatial and temporal measurements were extracted from the video. The parameters that were selected for analysis were those that have been reported by other manuscripts utilizing the DigiGait to measure gait changes, e.g. (Dorman et al. 2014; A. Kwok et al. 2020; Vincelette et al. 2007) so that comparisons could be made. Percent change preflight and postflight in gait parameters for each animal was assessed in experimental and control groups.

Statistical Analysis: The comparisons of preflight and postflight changes in gait parameters either a one-way ANOVA with a Tukey post-hoc test for multiple comparisons or an unpaired t-test utilizing Graphpad Prism v10.2.

Results

One of the flight animals was lost due to an injection issue in orbit; the experiments, data capture and processing was available for all other animals from all groups. At launch, the

following average body masses were reported: FLIGHT: 26.26g (SD 1.42); GC 27.26g (0.82), and VIV 29.20g (0.97). Mice were placed into the three primary groupings based on weight gain over nine weeks at KSC and their body mass to ensure minimal pre-flight differences between groups. At recovery, rodents were deemed active and healthy by the lead veterinarian. Of note, all rodents in the µg FLIGHT group failed to perform and n=3 of the 0.33g group performed in contrast to the 0.67g, 1g, VIV, and GC where all animals were able to perform (n=5/6).

Longitudinal changes in left hindlimb within groups after 35 days spaceflight

No differences were seen for hindlimb gait patterns tested within the GC or the VIV groups longitudinally post-flight relative to pre-flight (Fig. 1). Likewise, no post- to pre-flight differences were observed within 1g FLIGHT groups (Fig. 1). In contrast, significant differences were observed longitudinally in the 0.33g and 0.67g FLIGHT groups for all measures (Fig. 1). These included: swing time (s) (0.33g, +242.2%, $p<0.001$; 0.67g, +82.3%, not significant (ns)), swing/stance (%) (0.33g, -81.2%, $p<0.001$; 0.67g, -46.6%, ns), swing stride (%) (0.33g, +136%, $p<0.001$; 0.67g, +54.8%, $p<0.01$), stance time (s) (0.33g, -55%, $p<0.01$; 0.67g, -13.4%, ns), stride width (cm) (0.33g, +23.9%, $p<0.05$; 0.67g, -5.4%, ns), stride width variability (cm) (0.33g, +134.9%, ns; 0.67g, +82.9%, ns), and paw angle variability (°) (0.33g, +150.9%, ns; 0.67g, +111.9%, ns).

Longitudinal changes in left hindlimb between groups after 35 days spaceflight

The magnitude of the changes in gait patterns measured post-flight relative to pre-flight were similar between GC, VIV, and 1g FLIGHT for all measured outcomes (Fig. 1). In contrast, hindlimb gait pattern changes for most metrics measured after 0.33g were substantially different than GC, VIV, and 1g (Fig. 1), and to a lesser degree for 0.67g vs GC, VIV, and 1g. FLIGHT vs GC/VIV.

Longitudinal changes in left forelimb within groups after 35 days spaceflight

No differences were seen for forelimb gait patterns tested within the GC or the VIV groups longitudinally (Fig. 2) post-flight relative to pre-flight. Likewise, no post- to pre-flight differences were observed within 1g FLIGHT groups (Fig 2). Similar to findings from the hind limbs, significant differences in the forelimbs were observed longitudinally in the 0.33g and 0.67g FLIGHT groups for all measures (Fig. 2). These included: swing stride percent (%) (0.33g, +75.9%, ns; 0.67g, +12.5%, ns), brake stride percent (%) (0.33g, -42%, ns; 0.67g, -27.4%, ns), stride length (cm) (0.33g, +100.6, $p<0.05$; 0.67g, +35.4%, ns) stride time (s) (0.33g, +100.5%, $p<0.05$; 0.67g, +35.7%, ns), stride frequency (steps/s) (0.33g, -23.5%, ns; 0.67g, -25.1%, ns), stride length variability (cm) (0.33g, +35.5%, ns; 0.67g, +6%, ns), and stride width (cm) (0.33g, -24.8%, ns; 0.67g, -19.2%, ns).

Longitudinal changes in left forelimb between groups after 35 days spaceflight

The magnitude of the changes in gait patterns measured post-flight relative to pre-flight

were similar between GC, VIV, and 1g FLIGHT for all measured outcomes (Fig. 2). As per the hind limbs, forelimb gait pattern changes for most metrics measured after 0.33g were substantially different than GC, VIV, and 1g (Fig. 2), and attenuated at 0.67g vs GC, VIV, and 1g. FLIGHT vs GC/VIV.

Discussion

Exposure to prolonged microgravity aboard the ISS, or partial gravitational loading, affected locomotor performance and/or altered mouse gait patterns. Importantly, this study identified that: 1] Continuous exposure to 1g of gravitational acceleration via centrifugation preserved gait patterns relative to both preflight and controls; 2] Gait patterns were preserved in a gravity-dose dependent manner, with major differences observed after 0.33g that were then attenuated / normal at 0.67g and, as noted, normal after 1g, and; 3] Mice exposed to µg could not perform (locomote) at live animal return, with only 50% able to perform in the 0.33g-exposed group. Aligned with the aim of the study, a g-dose threshold for gait and locomotor performance could be identified as being lower than 0.67g, as exposure to the Martian gravitational loads for ~30 days resulted in profound deficits relative to 0.67g or 1g.

For the RR9 rodent spaceflight mission, gait pattern deficits for mice exposed to microgravity in the Rodent Habitats aboard ISS were altered relative to preflight in a manner that reflected neuromotor deficits, as well as provided indication of reduced stability. But at live animal return, the mice in the RR9 study could locomote in a linear manner on the DigiGait rodent treadmill belt at 17.5cm/s. However, for this MHU8

mission, mice exposed to continuous microgravity within the MARS centrifuge (static condition) could not perform; only 2 of the mice had several strides that propelled the mouse forward linearly, and ultimately the mice were pushed to the back of the treadmill and/or rolled over onto their dorsal surface. Moreover, 50% of the mice exposed to 0.33g (simulating Marian gravity) were likewise unable to locomote linearly. Thus, these findings of performance capability (as defined by ability for linear forward motion) are in contrast to the RR9 findings (A. T. Kwok et al. 2021). However, all mice experiencing continuous exposure to 0.67g or 1g artificial gravity could locomote in a linear fashion, which then also permitted the gait assessment on both cohorts. As per RR9, gait pattern deficits for the MHU8 study largely reflected neuromotor deficits, and increased instability; these patterns were attenuated with increasing gravitational loading. Overall, these findings and the results regarding gait and performance of the MHU8 and RR9 study highlight that gravitational loading conditions can have major impact on functional and physiologic responses, but that also the experimental conditions (e.g., housing, return time for experiments after live animal return, etc), likely affect these physiologic responses.

Notably, there were some key environmental and housing differences between the MHU8 and RR9 missions that may have affected the differential responses regarding performance capabilities observed after continuous exposure to μg. Mice for RR9 rodents were group housed (10 rodents/habitat, with the divider removed (A. T. Kwok et al. 2021)). Moreover, the mice had access to enrichment, which was a “hut” in which the mice could huddle. For MHU8, the mice were single housed in the smaller MARS unit, void of enrichment. The larger volume of the rodent habitats for RR9, coupled with

housing conditions that permitted inter-individual interactions and possible generation of skeletal loading (e.g., potential for pushing against one another) could have affected physiologic responses to µg that affected the ability to perform upon live animal return. Furthermore, the time from splashdown to function testing varied between the two missions with an 18h return time for the RR9 mission and 4h return time for the MHU8 mission. Varied physiologic responses associated with time from exposure to 1g upon live animal return and function testing could, again, affect physiologic responses related to differential effects on performance between the µg groups between RR9 and MHU8. However, it is important to note that mice from each group were tested randomly but in succession postflight for MHU8, and given the normalization of gait patterns and the ability to perform in the 1g group (as well as the 0.67g group in terms of many gait patterns but also the ability to locomote) which were exposed to the same single housing and timing conditions as µg, it is unclear how either housing or timing issues impacted the results, most importantly that exposure to lower gravitational levels, especially µg and 0.33g, greatly impaired performance and gait patterns for the mice of the MHU8 mission. The impact of different housing units, single vs group housing, added enrichment, and difference in animal retrieval post-splashdown on partial limb loading and stress is difficult to characterize but should be considered for future rodent studies aboard the ISS.

Gait analysis provides a powerful diagnostic tool to assess functional alterations and physiological deficits (N. G. Lee, Kang, and Park 2020; Teixeira-Leite and Manhães 2012). Well-established induced injury rodent model studies have identified changes in limb specific alterations in parameters correlate with specific disease states, physiological

and neuromotor changes (Dienes et al. 2022; Lambert et al. 2014; Peter-Szabo et al. 2007). For instance, compensatory gait alterations associated with osteoarthritis have been established utilizing the destabilized medial menisci rodent model (Fang et al. 2018; Hu et al. 2022; Lakes et al. 2018). Furthermore, gait analysis from the RR9 study identified musculoskeletal deficits resulting from reduced weight-bearing in microgravity (A. Kwok et al. 2020). Importantly, this study identified that the patterns of gait abnormalities (particularly at 0.33g and attenuated at 0.67g) reflected a variety of different correlated conditions (Table 1), but primarily arthritis, traumatic brain injury, neurological dysfunction, and ataxia. However, it is important to note that typically these rodent studies in which we compare findings in order to speculate on a cause typically induce an injury, or use a genetically modified model for a pathologic condition in which gait patterns change in a regular manner (e.g., neurological dysfunction (Hung et al. 2016) and arthritis (Dorman et al. 2014; Vincelette et al. 2007)). This was not the case for this study – rather mice were launched into orbit, exposed to one of 4 gravitational loading conditions, and then returned live. As such, we can only speculate as to the cause of the gait abnormalities. Further collaboration regarding pathologic responses in the central nervous, peripheral nervous, and musculoskeletal systems are needed to better understand the true cause for these abnormalities.

While some partial recovery of inflight and postflight sensorimotor and musculoskeletal reconditioning does occur upon a return to weight bearing after flight to the ISS, the period and extent of recovery is varied (G. R. Clément et al. 2020; Liphardt et al. 2023b; Orwoll et al. 2013; Wood, Loehr, and Guilliams 2011). However, it is unclear how deep space travel outside LEO and prolonged exposure to sub 1g levels on

the Moon (0.17g) and Mars (0.33g) will impact astronaut health and performance. In this study we saw that for Martian gravity only 50% of the animals could function and none of the animals at µg could perform. Concerns should be considered as to whether astronauts' performance will be detrimentally affected during transit, upon reaching Mars, and/or after mission return, as these missions are expected to last ~2 years. Furthermore, current countermeasure that NASA employs to preserve musculoskeletal health is the advanced resistive exercise device (ARED) but we will not have ARED for missions outside of LEO like future deep space missions. Therefore, there is a critical need to investigate how sub 1g environments will affect astronaut health and performance, and more specifically identify if there is a gravitational threshold at which deficits are prevented.

Long duration spaceflight has deleterious effects on astronauts, from alterations in brain structure, musculoskeletal and joint soft tissue deficits, and impaired sensorimotor and vestibular function, to novel physiological alterations in space flight-associated neuro-ocular syndrome (SANS) (Deshpande et al. 2020; Hupfeld et al. 2022; A. G. Lee et al. 2018; Seidler et al. 2015; Yates and Kerman 1998). The combined effect of these adaptations to the LEO environment can result in declines in mobility and balance in astronauts during and after spaceflight (Tays et al. 2021; Weber and Stelzer 2022). As we plan for missions to the Moon and Mars, there is a critical need to characterize these neuromotor deficits (among others) in the microgravity and partial gravity environments to better mitigate the risks associated with long-duration spaceflight both in transit and upon reaching a destination, and thus continued assessment of gait pattern changes in rodents during spaceflight and ground-based analogue studies are essential to meet those

needs.

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Author contributions

The following authors served as science team leaders and contributed to the scientific design of the MHU8 study: J.S.W., M.L.B., S.T., C.F., J.C., and M.V. J.S.W. and C.M.P. contributed to the conception and design of the rodent gait analyses. C.M.P., J.S.W., and K.R. contributed to the performance of the studies. C.M.P., J.S.W. and L.K. contributed to the analysis and interpretation of the data. C.M.P. and J.S.W. wrote the main manuscript text. C.M.P., L.K. and J.S.W. prepared Figs. 1-3. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

References

Akula, Shyam K. et al. 2020. “The Trajectory of Gait Development in Mice.” *Brain and Behavior* 10(6): 1–12.

Amende, Ivo et al. 2005. “Gait Dynamics in Mouse Models of Parkinson’s Disease and Huntington’s Disease Gait VariabilityGaitMouse ModelsNeurodegenerationMovement DisordersAmyotrophic Lateral SclerosisSOD1.” http://www.jneuroengrehab.com/content/2/1/20 (May 8, 2024).

Bloomberg, Jacob J., and Ajitkumar P. Mulavara. 2003. “Changes in Walking Strategies after Spaceflight.” *IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society* 22(2): 58–62. https://pubmed.ncbi.nlm.nih.gov/12733460/ (April 23, 2024).

Carriot, Jérome, Isabelle Mackrous, and Kathleen E. Cullen. 2021. “Challenges to the Vestibular System in Space: How the Brain Responds and Adapts to Microgravity.” *Frontiers in Neural Circuits* 15: 760313.

Clark, Torin K. 2019. “Effects of Spaceflight on the Vestibular System.” *Handbook of Space Pharmaceuticals*: 1–39. https://link.springer.com/referenceworkentry/10.1007/978-3-319-50909-9_2-1 (April 22, 2024).

Clément, Gilles et al. 2022. “Mission-Critical Tasks for Assessing Risks from Vestibular

and Sensorimotor Adaptation during Space Exploration." *Frontiers in Physiology* 13: 1029161.

Clément, Gilles R. et al. 2020. "Challenges to the Central Nervous System during Human Spaceflight Missions to Mars." *Journal of Neurophysiology* 123(5): 2037–63. https://journals.physiology.org/doi/10.1152/jn.00476.2019 (April 23, 2024).

Comfort, Paul et al. 2021. "Effects of Spaceflight on Musculoskeletal Health: A Systematic Review and Meta-Analysis, Considerations for Interplanetary Travel." *Sports Medicine (Auckland, N.z.)* 51(10): 2097. /pmc/articles/PMC8449769/ (April 22, 2024).

Connell, James W., Rachel Allison, and Evan Reid. 2016. "Quantitative Gait Analysis Using a Motorized Treadmill System Sensitively Detects Motor Abnormalities in Mice Expressing ATPase Defective Spastin." *PLOS ONE* 11(3): e0152413. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0152413 (May 8, 2024).

Cops, Elisa J. et al. 2013. "Tissue-Type Plasminogen Activator Is an Extracellular Mediator of Purkinje Cell Damage and Altered Gait." *Experimental Neurology* 249: 8–19.

Deshpande, Nandini et al. 2020. "Vestibular and Cardiovascular Responses After Long-Duration Spaceflight." *Aerospace medicine and human performance* 91(8): 621–27. https://pubmed.ncbi.nlm.nih.gov/32693869/ (April 28, 2024).

Dienes, Jack et al. 2022. "Comprehensive Dynamic and Kinematic Analysis of the

Rodent Hindlimb during over Ground Walking." *Scientific Reports* 12(1).

Dorman, Christopher W. et al. 2014. "A Comparison of DigiGait™ and TreadScan™ Imaging Systems: Assessment of Pain Using Gait Analysis in Murine Monoarthritis." *Journal of Pain Research* 7: 25. /pmc/articles/PMC3883276/ (May 8, 2024).

Fang, Hang et al. 2018. "Early Changes of Articular Cartilage and Subchondral Bone in The DMM Mouse Model of Osteoarthritis." *Scientific reports* 8(1). https://pubmed.ncbi.nlm.nih.gov/29434267/ (April 23, 2024).

Glajch, Kelly E., Sheila M. Fleming, D. James Surmeier, and Pavel Osten. 2012. "Sensorimotor Assessment of the Unilateral 6-Hydroxydopamine Mouse Model of Parkinson's Disease." *Behavioural Brain Research* 230(2): 309. /pmc/articles/PMC3324279/ (May 8, 2024).

Hansen, Stephen T., and Stefan M. Pulst. 2013. "Response to Ethanol Induced Ataxia between C57BL/6J and 129X1/SvJ Mouse Strains Using a Treadmill Based Assay." *Pharmacology Biochemistry and Behavior* 103(3): 582–88.

Hu, W. et al. 2022. "Modelling Osteoarthritis in Mice via Surgical Destabilization of the Medial Meniscus with or without a Stereomicroscope." *Bone & Joint Research* 11(8): 518. /pmc/articles/PMC9396921/ (April 23, 2024).

Hulleck, Abdul Aziz et al. 2022. "Present and Future of Gait Assessment in Clinical Practice: Towards the Application of Novel Trends and Technologies." *Frontiers in Medical Technology* 4. /pmc/articles/PMC9800936/ (April 29, 2024).

Hung, Ya Hui et al. 2016. “Neurological Dysfunction in Early Maturity of a Model for Niemann–Pick C1 Carrier Status.” *Neurotherapeutics* 13(3): 614–22. https://link.springer.com/article/10.1007/s13311-016-0427-5 (May 8, 2024).

Hupfeld, K. E. et al. 2022. “Brain and Behavioral Evidence for Reweighting of Vestibular Inputs with Long-Duration Spaceflight.” *Cerebral cortex (New York, N.Y. : 1991)* 32(4): 755–69. https://pubmed.ncbi.nlm.nih.gov/34416764/ (April 28, 2024).

Kale, Ajit et al. 2004. “Ethanol’s Effects on Gait Dynamics in Mice Investigated by Ventral Plane Videography.” *Alcoholism: Clinical and Experimental Research* 28(12): 1839–48.

Karmakar, Moumita et al. 2023. “Baseline Gait and Motor Function Predict Long-Term Severity of Neurological Outcomes of Viral Infection.” *International Journal of Molecular Sciences* 24(3). /pmc/articles/PMC9917409/ (April 23, 2024).

Kim, Kyoung Jae et al. 2018. “Exposure to an Extreme Environment Comes at a Sensorimotor Cost.” *npj Microgravity 2018 4:1* 4(1): 1–8. https://www.nature.com/articles/s41526-018-0051-2 (April 23, 2024).

Krittanawong, Chayakrit et al. 2023. “Human Health during Space Travel: State-of-the-Art Review.” *Cells* 12(1). /pmc/articles/PMC9818606/ (April 23, 2024).

Kwok, Andy et al. 2020. “Altered Rodent Gait Characteristics after ~35 Days in Orbit Aboard the International Space Station.” *Life sciences in space research* 24: 9–17. https://pubmed.ncbi.nlm.nih.gov/31987483/ (April 23, 2024).

Kwok, Andy T. et al. 2021. “Spaceflight and Hind Limb Unloading Induces an Arthritic Phenotype in Knee Articular Cartilage and Menisci of Rodents.” *Scientific Reports* 11(1): 10469. /pmc/articles/PMC8131644/ (December 4, 2023).

Lakes, E.H., S.H. Haus, Y.C. Pacheco, and K.D. Allen. 2018. “Male and Female Gait Compensations in a Mouse Model of Medial Meniscus Destabilization.” *Osteoarthritis and Cartilage* 26: S366–67. http://www.oarsijournal.com/article/S1063458418308252/fulltext (April 23, 2024).

Lambert, C. S. et al. 2014. “Gait Analysis and the Cumulative Gait Index (CGI): Translational Tools to Assess Impairments Exhibited by Rats with Olivocerebellar Ataxia.” *Behavioural brain research* 274: 334–43. https://pubmed.ncbi.nlm.nih.gov/25116252/ (April 23, 2024).

Lee, Andrew G. et al. 2018. “Space Flight-Associated Neuro-Ocular Syndrome (SANS).” *Eye (London, England)* 32(7): 1164–67. https://pubmed.ncbi.nlm.nih.gov/29527011/ (April 28, 2024).

Lee, Nam Gi, Tae Woo Kang, and Hyun Ju Park. 2020. “Relationship Between Balance, Gait, and Activities of Daily Living in Older Adults With Dementia.” *Geriatric Orthopaedic Surgery & Rehabilitation* 11. /pmc/articles/PMC7262984/ (April 23, 2024).

Lin, Chin-Hsing et al. 2001. “Neurological Abnormalities in a Knock-in Mouse Model of Huntington’s Disease.” *Human Molecular Genetics* 10(2): 137–44.

Liphardt, Anna Maria et al. 2023a. “Musculoskeletal Research in Human Space Flight –

Unmet Needs for the Success of Crewed Deep Space Exploration." *npj Microgravity 2023 9:1* 9(1): 1–7. https://www.nature.com/articles/s41526-023-00258-3 (April 22, 2024).

———. 2023b. "Musculoskeletal Research in Human Space Flight – Unmet Needs for the Success of Crewed Deep Space Exploration." *NPJ Microgravity* 9(1). /pmc/articles/PMC9883469/ (April 23, 2024).

Orbit, Institute of Medicine (US) Committee on Creating a Vision for Space Medicine During Travel Beyond Earth, John R. Ball, and Jr. Charles H. Evans. 2001. "Risks to Astronaut Health During Space Travel." https://www.ncbi.nlm.nih.gov/books/NBK223785/ (April 23, 2024).

Orwoll, Eric S. et al. 2013. "Skeletal Health in Long-Duration Astronauts: Nature, Assessment, and Management Recommendations from the NASA Bone Summit." *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 28(6): 1243–55. https://pubmed.ncbi.nlm.nih.gov/23553962/ (April 23, 2024).

Patel, Zarana S. et al. 2020. "Red Risks for a Journey to the Red Planet: The Highest Priority Human Health Risks for a Mission to Mars." *npj Microgravity 2020 6:1* 6(1): 1–13. https://www.nature.com/articles/s41526-020-00124-6 (April 23, 2024).

Peter-Szabo, Mihaly et al. 2007. "Quantitative Characterization of a Repeated Acute Joint Inflammation Model in Rats." *Clinical and Experimental Pharmacology and Physiology* 34(5–6): 520–26.

Reed, John et al. 2021. “Gait Analysis in a Rat Model of Traumatic Brain Injury.” *Behavioural Brain Research* 405: 113210.

Reschke, Millard F., and Gilles Clément. 2018. “Vestibular and Sensorimotor Dysfunction During Space Flight.” *Current Pathobiology Reports* 6(3): 177–83. https://link.springer.com/article/10.1007/s40139-018-0173-y (April 22, 2024).

Reschke, Millard F., Edward F. Good, and Gilles R. Clément. 2017. “Neurovestibular Symptoms in Astronauts Immediately afterSpace Shuttle and International Space Missions.” *OTO Open* 1(4). /pmc/articles/PMC6239149/ (April 22, 2024).

Santana Rizzi, Joyce et al. “Characterization of Walking Locomotion in Aged C57BL/10 Mice: A Comprehensive 1 Gait Analysis 2 3.” https://doi.org/10.1101/2023.04.04.535545 (April 23, 2024).

Sashindranath, M., M. Daglas, and R. L. Medcalf. 2015. “Evaluation of Gait Impairment in Mice Subjected to Craniotomy and Traumatic Brain Injury.” *Behavioural Brain Research* 286: 33–38.

Saveko, Alina et al. 2022. “Adaptation in Gait to Lunar and Martian Gravity Unloading During Long-Term Isolation in the Ground-Based Space Station Model.” *Frontiers in Human Neuroscience* 15: 742664.

Seidler, Rachael D., Ajitkumar P. Mulavara, Jacob J. Bloomberg, and Brian T. Peters. 2015. “Individual Predictors of Sensorimotor Adaptability.” *Frontiers in systems neuroscience* 9(July). https://pubmed.ncbi.nlm.nih.gov/26217197/ (April 28, 2024).

Shiba, Dai et al. 2017. “Development of New Experimental Platform ‘MARS’—Multiple

Artificial-Gravity Research System—to Elucidate the Impacts of Micro/Partial Gravity on Mice." *Scientific Reports 2017 7:1* 7(1): 1–10. https://www.nature.com/articles/s41598-017-10998-4 (April 23, 2024).

Tays, Grant D. et al. 2021. "The Effects of Long Duration Spaceflight on Sensorimotor Control and Cognition." *Frontiers in Neural Circuits* 15: 723504. /pmc/articles/PMC8577506/ (April 28, 2024).

Teixeira-Leite, Homero, and Alex C. Manhães. 2012. "Association between Functional Alterations of Senescence and Senility and Disorders of Gait and Balance." *Clinics* 67(7): 719. /pmc/articles/PMC3400160/ (April 23, 2024).

Tomsia, Marcin et al. 2024. "Long-Term Space Missions' Effects on the Human Organism: What We Do Know and What Requires Further Research." *Frontiers in Physiology* 15: 1284644.

Vincelette, Jon et al. 2007. "Gait Analysis in a Murine Model of Collagen-Induced Arthritis." *Arthritis Research & Therapy 2007 9:6* 9(6): 1–7. https://arthritis-research.biomedcentral.com/articles/10.1186/ar2331 (May 8, 2024).

Vrinten, Dorien H., and Frank F.T. Hamers. 2003. "'CatWalk' Automated Quantitative Gait Analysis as a Novel Method to Assess Mechanical Allodynia in the Rat; a Comparison with von Frey Testing." *Pain* 102(1–2): 203–9.

Weber, Bernhard, and Martin Stelzer. 2022. "Sensorimotor Impairments during Spaceflight: Trigger Mechanisms and Haptic Assistance." *Frontiers in Neuroergonomics* 3: 959894.

Wenger, Nikolaus et al. 2022. “Rodent Models for Gait Network Disorders in Parkinson’s Disease – a Translational Perspective.” *Experimental Neurology* 352: 114011.

Wood, S. J., J. A. Loehr, and M. E. Guilliams. 2011. “Sensorimotor Reconditioning during and after Spaceflight.” *NeuroRehabilitation* 29(2): 185–95. https://pubmed.ncbi.nlm.nih.gov/22027081/ (April 23, 2024).

Yates, B. J., and I. A. Kerman. 1998. “Post-Spaceflight Orthostatic Intolerance: Possible Relationship to Microgravity-Induced Plasticity in the Vestibular System.” *Brain research. Brain research reviews* 28(1–2): 73–82. https://pubmed.ncbi.nlm.nih.gov/9795146/ (April 28, 2024).

Yin, Yishu et al. 2023. “Long-Term Spaceflight Composite Stress Induces Depression and Cognitive Impairment in Astronauts—Insights from Neuroplasticity.” *Translational Psychiatry 2023 13:1* 13(1): 1–7. https://www.nature.com/articles/s41398-023-02638-5 (April 23, 2024).

Figures and Tables

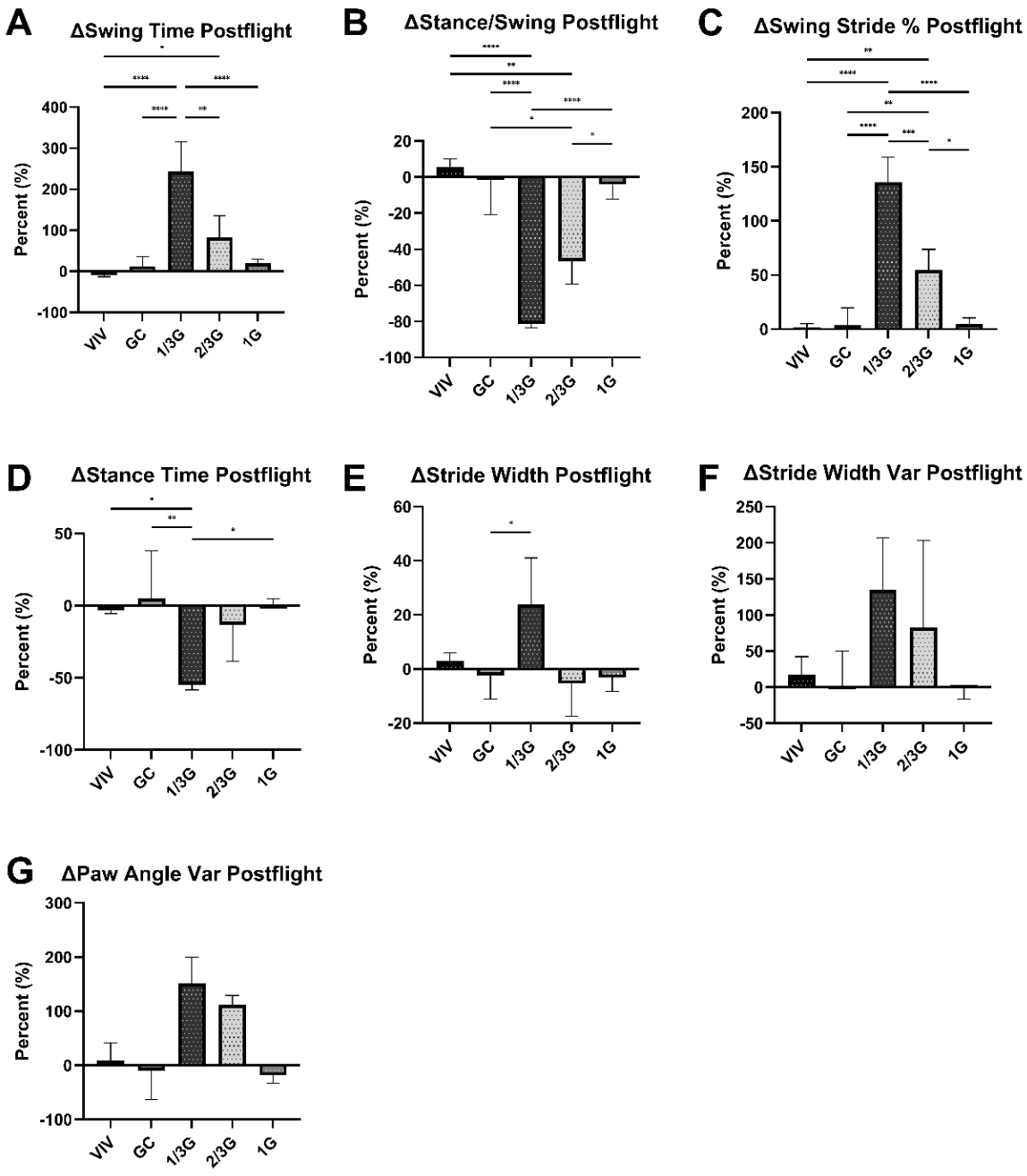

Figure 1. Longitudinal effects of spaceflight on gait kinematics of the left hindlimbs of mice exposed to artificial gravity. Within and between groups were tested using repeated measures ANOVA and Holm-Šídák post hoc (a=0.05, *p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

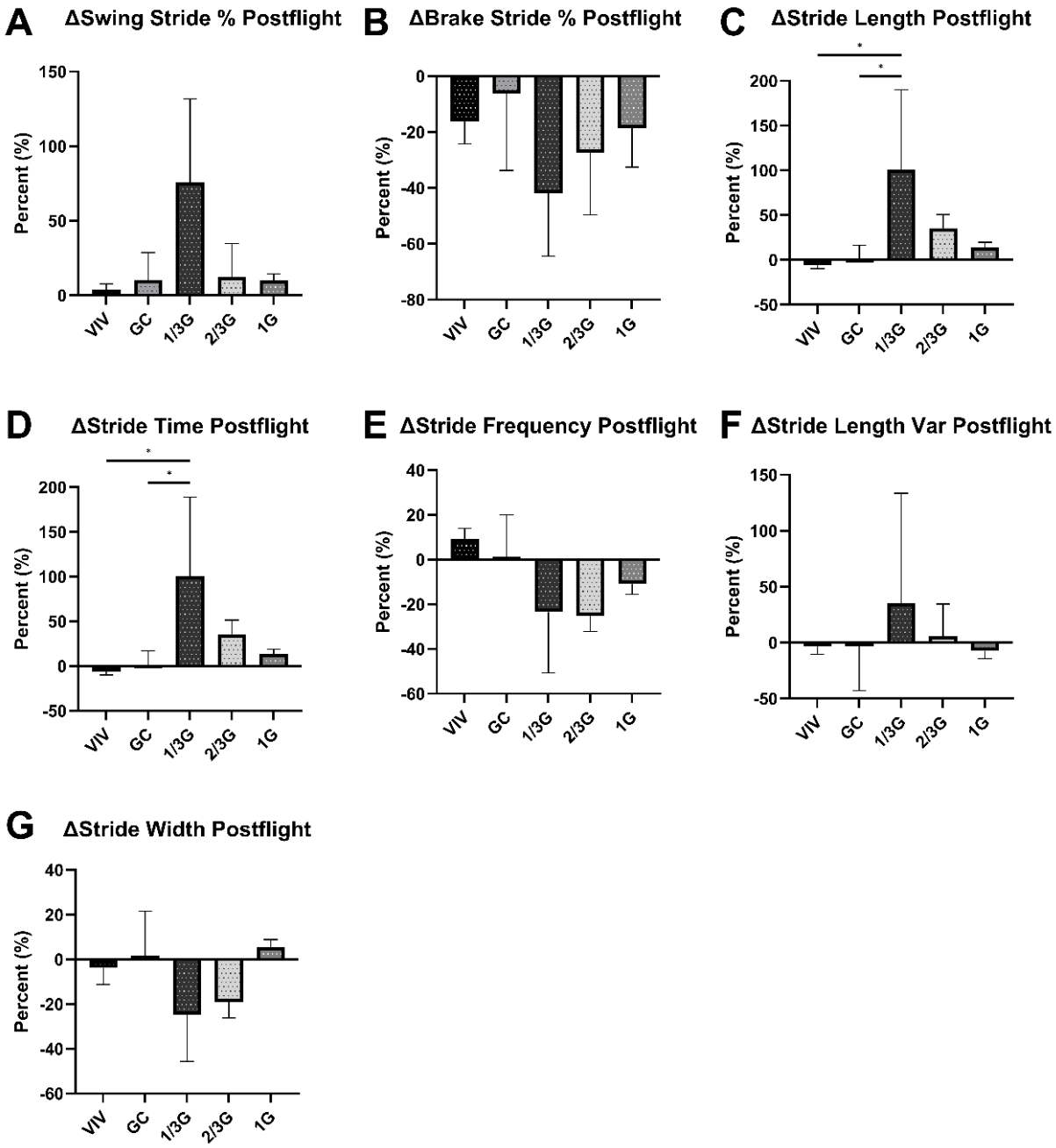

Figure 2. Longitudinal effects of spaceflight on gait kinematics of the left forelimbs of mice exposed to artificial gravity. Within and between groups were tested using repeated measures ANOVA and Holm-Šídák post hoc (a=0.05, *p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

Table 1. Altered gait patterns observed from the MHU8 mice that are associated with similar gait changes and related pathologic conditions in published rodent studies that utilized DigiGait.

Altered Gait parameters	Observed gait parameter trend	Reported correlated condition
Swing time (s)	Increase	Arthritis, Craniotomy and Traumatic Brain Injury, Inflammation, Pain, Ethanol Induced Ataxia (1,2,3,4,5)
Swing/stance (%)	Decrease	Neurodevelopmental disorders (6)
Swing/stride (%)	Increase	Traumatic Brain Injury, Neurodevelopmental Disorders (2,6)
Stance time (s)	Decrease	Arthritis, Ataxia, Ethanol Effects, Axonal Degeneration (7,8,9,10)
Stride width (cm)	Increase	Parkinson's Disease, Purkinje Cell Damage, Higher Fall Association (11,12,13)
Stride width variability (cm)	Increase	Purkinje Cell Damage, Higher Fall Association (12,13)
Paw angle variability (°)	Increase	Ataxia (14)
Brake/stride (%)	Decrease	Arthritis (7)
Stride length (cm)	Increase	Arthritis, Neurological Dysfunction, Axonal degeneration, Ataxia (1,5,8,10)
Stride time (s)	Increase	Arthritis, Axonal degeneration, Ataxia (1,5,10)
Stride frequency (steps/s)	Decrease	Arthritis, Neurological Dysfunction, Huntington's Disease, Ethanol Effects (1,8,9,16)
Stride length variability (cm)	Increase	Arthritis, Neurological Dysfunction (1,8)

References: (Dorman et al. 2014)[1], (Reed et al. 2021)[2], (Vrinten and Hamers 2003)[3], (Sashindranath, Daglas, and Medcalf 2015)[4], (Hansen and Pulst 2013)[5], (Akula et al. 2020)[6], (Vincelette et al. 2007)[7], (Hung et al. 2016)[8], (Kale et al. 2004)[9], (Connell, Allison, and Reid 2016)[10], (Glajch et al. 2012)[11], (Cops et al. 2013)[12], (Lin et al. 2001)[13], (Lambert et al. 2014)[14], (Amende et al. 2005)[16]

CHAPTER II

Arthritic responses of mouse articular cartilage and menisci to artificial gravity aboard the international space station

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Key words: International Space Station, Rodent Spaceflight, Meniscus, Knee Joint, Cartilage, Artificial Gravity

Arthritic responses of mouse articular cartilage and menisci to artificial gravity aboard the international space station

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Abstract

The impact of spaceflight outside of low earth orbit (LEO), during deep space transit, and readaptations to partial gravity environments upon reaching a destination, are unclear. Reduced loadbearing in sub 1g environments may have deleterious effects of joint health risking mission success and astronaut health. This study assessed the impact of artificial gravity via centrifugation on the preservation of knee and hip articular cartilage, and the menisci in rodents. Cartilage and meniscal degradation in mice were measured via microCT, histology, proteomics and transcriptomics in rodents exposed to one of 4 gravitational loading conditions (μg; 0.33g; 0.67g; and 1g) after a ~35-day mission to the ISS in order to assess if a g-dose threshold exists with regard to prevention of cartilage degradation. Continuous exposure to 1g of gravitational acceleration via centrifugation failed to preserve meniscal volume and articular cartilage thickness. However, proteomic and transcriptomic changes of the joint soft tissue were mitigated in a gravity-dose dependent manner compared to controls; with major differences observed after μg, 0.33g, and 0.67g that were then attenuated at 1g. Mice exposed to artificial 1g maintained meniscal ECM synthesis and sulfated GAG expression compared to controls. As we plan manned spaceflight missions to destinations outside of LEO there is a critical need to characterize cartilage degradation in the microgravity and partial gravity environments to better mitigate the risks associated with long-duration spaceflight both in transit and upon reaching a destination.

Introduction

Maintaining musculoskeletal health and performance is vital for mission success during a long-duration spaceflight, as well as for preserving quality of life post-mission. Prolonged exposure to reduced weight bearing in microgravity during spaceflight is well-documented to cause muscle atrophy [1–3] and bone loss [4–6], with more recent studies identifying degradation of soft-tissue structures in both the knee (e.g., cartilage, menisci) and hip (e.g., cartilage) joints [7, 8]. It is estimated that with a mission to Mars, astronauts will be exposed to approximately 8 to 10 months of weightlessness total during transit to and from Mars, and approximately 12 to 18 months of partial gravity exposure while on surface [9]. Recent studies have shown that neither bisphosphonate treatment [10–12] or the use of the advanced resistive exercise device (ARED) prevent spaceflight-induced BMD decline and bone turnover [13, 14], with limited studies having been conducted on the load-sensitive soft tissues of the knee. There is a critical need to investigate novel countermeasures to preserve astronaut joint health during and after deep space travel such as artificial gravity to counteract the deleterious effects of reduced gravity, as well as, pharmaceutical interventions to counteract spaceflight-induced osteoarthritis (OA).

Intermittent or continuous exposure to elevated gravitational loading is a possible countermeasure to prevent spaceflight-induced decline in musculoskeletal function and performance [15], [16]. Prior work has established that the deficits in bone and muscle following exposure to partial gravity, simulated by partial weight bearing in mice, are directly proportional to the degree of unloading [17]. A previous study utilizing the mouse habitat unit (MHU) has demonstrated significant decreases in femur bone density and the soleus/gastrocnemius muscle weights of μG mice, whereas artificial 1G mice maintained the same bone density and muscle weight as the ground control animals after

35 days aboard the ISS, indicating that artificial G preserves musculoskeletal health during spaceflight [18-20]. Yet, the ability of partial gravity applied to rodent limbs while in orbit using the existing Kibo module as a means to prevent osteoarthritic changes are unstudied [21].

Mechanical loading is required for the maintenance of the musculoskeletal tissues, including joint soft tissues. Cartilage and meniscal degradation in mice have been documented with reduced weight bearing during spaceflight aboard both the International Space Station (ISS) and space shuttle, and from hind limb unloading (HLU). Cartilage and joint degradation could increase the risk of joint failure during dynamic loading conditions, and if painful, could lead to performance deficits. Molecular mechanisms leading to the observed arthritic responses in rodents are unknown. The Joint Partial Gravity (Rodent Research)-Mouse Habitat Unit 8 mission (MHU8) exposed mice to various gravitational (g) exposures via centrifugation aboard the ISS. We tested if varied levels of artificial gravity (AG) via centrifugation aboard the ISS can prevent/attenuate gross and transcriptomic arthritic responses in mice.

Methods and Materials

Joint Partial-gravity Rodent Research/ Mouse Habitat Unit-8 Spaceflight Mission (MHU8): Male C57BL/6J mice (Jackson Labs) underwent approximately 35 days of exposure to various gravitational levels within the JAXA Multiple Artificial-Gravity Research System (MARS) aboard the International Space Station (ISS). The MHU8 mission launched aboard SpaceX CRS-27, March 15, 2023, from the Kennedy Space

Center (KSC) to the ISS to the ISS and returned April 16, 2023. A total of 24 mice were individually housed at gravity levels of 0g (n=6), 0.33g (n=6), 0.67g (n=6), and 1g (n=6) within JAXA's mouse habitats. The mice arrived to KSC, 6 weeks prior to study enrollment for an acclimation period at the animal control facility (ACF). Mice were singly housed and fitted with RFID tags for identification, with bi-weekly measurements of body mass, food, and water consumption. At L-1, twelve-week-old male mice were categorized into three main groups based on weight gain over nine weeks and their body mass to ensure minimal pre-flight differences between groups. Mice launched to the ISS were subdivided into the following g-level groupings: 0g, 0.33g, 0.67g, 1g; n=6/group. FLIGHT mice were housed in habitat cage units (HCU) aboard the ISS, receiving rodent food bars (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan), and ad libitum access to water containing 0.2/mg/L; Ground-based habitat controls (Ground Control, HGC; n=12) were maintained identically to flight mice exposed to artificial gravity, with a two-day delay to match environmental conditions (ambient temperature of 26-28°C, humidity, CO2 partial pressure, and 12/12-hour light/dark cycle) on the ISS. Another group of vivarium ground controls (VGC, n=12) were selected and individually housed in standard cages within ACF. At launch, all 12-week-old FLIGHT mice were transported and loaded into the Dragon SpaceX Capsule, then transferred to individual HCU and loaded into the MARS system aboard the ISS. Unberthing from the ISS and splashdown of live mice occurred on L+32, and the live mice were collected by the science teams for functional testing and tissue collection at KSC.

Tissue Harvest: The right intact knees of the mice were harvested and fixed in 10% Neutral-buffered formalin for preservation for 3 days prior to being placed into 70%

ethanol for storage. The right proximal femur was isolated, placed in RNAlater, flash frozen in liquid nitrogen, and stored at -80 °C. The femur and tibia from the left hind limb were disarticulated, and the meniscus was micro-dissected, placed in RNAlater, flash frozen in liquid nitrogen, and stored at -80 °C.

Microcomputed Tomography (MicroCT): To assess articular cartilage and meniscal structural changes, the intact right knees was were immersed in a 1% solution of phosphotungstic acid, a contrast-enhancing agent [22], [23], and imaged using the Bruker Skyscan 1275 instrument at the following settings: X-Ray emission parameters of 65 kV and 153 µA, and at a 10µm resolution. Two-dimensional X-ray data were gathered by averaging two images at each of 1400 positions, spaced 0.3° apart. Image reconstruction was conducted using NRecon v1.7.4.2. Subsequently, Mimics 23.0 software was employed for segmentation of the meniscus and cartilage to assess meniscal volume and cartilage thickness at the medial tibial plateau.

Bulk RNAseq Analysis: Total RNA from the menisci was isolated using the Bead Ruptor 24 (Omni International) and RNA integrity was verified using an Agilent TapeStation and the High Sensitivity RNA ScreenTape Kit® (Agilent Technologies). RNA-seq libraries was constructed from ~5 ng of total RNA using the SMARTer Stranded Total RNA-Seq Kit v2 – Pico Input Mammalian (Takara Bio USA, Inc.). The libraries were normalized, pooled and then sequenced on the Illumina NovaSeq 6000. Trimmomatic v0.33 was used to trim and clean the reads and read alignment was conducted using the STAR v2.5.3. Differentially expressed genes (DEGs) were verified using edgeR v4.2.0 and statistically significant results defined as $p \leq 0.05$ after adjusting for false discovery (FDR) were analyzed using Ingenuity Pathway Analysis (IPA).

Proteomic Analysis: Protein was isolated from proximal femoral cartilage from the right hindlimb and were analyzed on a LC-MS/MS system consisted of an Orbitrap Eclipse Mass Spectrometer (Thermo Scientific, Waltham, MA) and a Vanquish Neo nano-UPLC system (Thermo Scientific, Waltham, MA). Peptides were separated on a DNV PepMap Neo (1500 bar, 75 μm x 500 mm) column for 120min employing linear gradient elution consisted of water (A) and 80% acetonitrile (B) both of which contained 0.1% formic acid. Data were acquired by top speed data dependent mode where maximum MS/MS scans were acquired per cycle time of 1 seconds between adjacent survey spectra. Dynamic exclusion option was enabled which duration was set to 120 seconds. To identify proteins, spectra were searched against the UniProt mouse protein FASTA database (17,082 annotated entries, Oct 2021) using the Sequest HT search engine with the Proteome Discoverer v2.5 (Thermo Scientific, Waltham, MA). Search parameters were as follows: FT-trap instrument; parent mass error tolerance, 10 ppm; fragment mass error tolerance, 0.6 Da (monoisotopic); enzyme, trypsin (full); # maximum missed cleavages, 2; variable modification, +15.995 Da (oxidation) on methionine; static modification, +57.021 Da (carbamidomethyl) on cysteine.

Histology: Histology was performed on the intact right knee joint following contrast-enhanced microCT imaging to characterize degradation of soft tissue structures. Tissues were decalcified in EDTA for 14 days with a solution change at day 7 following previously published protocol [24], [25]. The hindlimbs were paraffin embedded and 5 μm-thick coronal sections at the cartilage-cartilage contact point were harvested. Safranin-O histochemical staining was conducted to quantify the proteoglycan content.

Stained sections were scanned at 20x using the Olympus vs120 and analyzed utilizing the Viziopharm v2023.01 image analysis software.

Statistical Analysis: The comparisons for cartilage and meniscal health were calculated with either a one-way ANOVA with a Tukey post-hoc test for multiple comparisons or an unpaired t-test. For transcriptomic and proteomic analyses, differentially expressed genes/proteins that were statistically significant results defined as $p \leq 0.05$ after adjusting for false discovery (FDR) were analyzed using Ingenuity Pathway Analysis (IPA).

Results

Joint soft tissue morphometric alterations after 35 days in orbit at µg, 0,33g, 0.67g, and 1g

Morphometric analyses via contrast enhanced microCT identified no significant differences in any FLIGHT groups compared to HGC or VGC (Fig 1a). Medial meniscal volume was lower after 35 days of spaceflight compared to HGC and VIV across groups (Fig 1b); (-16.8% for 1g vs HGC, $p<0.001$; -14.6% for 0.66g vs HGC, $p<0.005$; -11.9% for 0.33g vs HGC, $p<0.05$; -15.4% for µg vs HGC, $p<0.005$)

Differential gene expression analysis of menisci after 35 days in orbit at µg, 0,33g, 0.67g, and 1g compared to HGC to characterize arthritic responses

Comparative analysis of µg FLIGHT vs HGC menisci identified 1260 significantly altered genes ($p<0.05$) (Fig. 2a). Pro-osteoarthritic differential gene expression

enrichment of Tppp3 and Slc6a2, and decreased expression of Gabarapl1, Pip3ip1, Spock2, Adamts5, and Dio3 was observed (Fig. 2a). Comparative analysis of 0.33g FLIGHT vs HGC menisci identified 2159 significantly altered genes ($p<0.05$) (Fig. 2b). Pro-osteoarthritic differential gene expression enrichment of Slc6a2 and decreased expression of Gabarapl1, Pip3ip1, Pdk4, and Spock2 was observed (Fig. 2b). Comparative analysis of 0.67g FLIGHT vs HGC menisci identified 1075 significantly altered genes ($p<0.05$) (Fig. 2c). Pro-osteoarthritic differential gene expression enrichment of Slc6a2, Tppp, and Tppp3, and decreased expression of Gabarapl1, Pip3ip1, Pdk4, and Dio3 was observed (Fig 2c). Comparative analysis of 1g FLIGHT vs HGC menisci identified 101 significantly altered genes ($p<0.05$) (Fig. 2d). Pro-osteoarthritic differential gene expression enrichment of Mpo and decreased expression of Penk and Dio3 was observed (Fig. 2d).

Comparative transcriptomic analysis of menisci after 35 days in orbit at µg, 0,33g, 0.67g, and 1g compared to HGC to characterize arthritic responses

Comparative analysis of µg vs HGC menisci indicated enrichment of markers associated with osteoarthritis (ADAMTS5, MMP9, MMP13, FOXO3) (Z-score>2, $p<0.001$) (Fig. 3a). Additionally, a downregulation of GP6 signaling pathway, cholesterol biosynthesis pathways, and osteoclastic signaling characterized by a reduction in collagen and increased matrix degrading enzymes was observed after 35 days in orbit. (Z-score>-2, $p<0.001$) (Fig. 3a). Comparative analysis of 0.33g FLIGHT vs HGC menisci indicated enrichment of pro-arthritic pathways associated with oxidative phosphorylation, reduced

collage production, inflammation, cellular autophagy, and cellular senescence, and a downregulation of matrix protein synthesis (chondroitin sulfate, dermatan sulfate) and antioxidant defenses characterized by mitochondrial dysfunction (Z-score>±2, p<0.001) (Fig. 3b). Comparative analysis of 0.67g vs HGC menisci indicated enrichment of pro-arthritic pathways associated cytokine signaling, cell cycle progression and matrix degradation, and decreased expression of cholesterol biosynthesis (Z-score>±2, p<0.001) (Fig 3c). Comparative analysis of 1g vs HGC menisci indicated lower magnitude of changes indicating pro-arthritic pathways compared to µg, 0.33g and 0.67g, with a partial enrichment in pathways associated with cell cycle progression, DNA damage and inflammation (Z-score>2, p<0.001) (Fig. 4d).

Comparative transcriptomic conical pathway analysis of menisci after 35 days in orbit at µg, 0,33g, 0.67g, and 1g compared to HGC to characterize arthritic responses

Comparative analysis of canonical pathway alterations in the menisci of mice exposed to µg, 0.33g, and 0.67g vs HGC indicated differentially enriched cellular stress and injury pathways (TRAPP, HIF1α, p53, PI3K/AKT), cell cycle progression pathways, lipid metabolism (fatty acid α-oxidation, pyridoxal 5'-phophate salvage pathway), pro-inflammatory pathways (MAPK, USP18, KL, DYSF), pro-arthritic pathways (S100, SIRT1/AMPK, ER-α, ET-1), and pro-apoptotic pathways (AMPK, PTEN, DDB1, AHR, CASP8) (p<0.001, z-score >2) (Fig. 4). Furthermore, comparative analysis indicated downregulation of cellular stress response pathways (UPR, RHOA, 14-3-3α, NCF1, IL5), extracellular matrix (ECM) synthesis (CS, DS, PDXN, trigylcerol, cholesterol), and

antioxidant defenses (CCR2, VitC) in µg, 0.33g, and 0.67g vs GC ($p<0.001$, z-score >-2) (Fig. 4). However, pathway expression was similar between 1g and HGC especially for cartilage ECM synthesis (CS, DS, PDXN, trigylcerol, cholesterol) indicating diminished pre-arthritic pathway alterations at 1g compared to partial gravity and µg groups. ($p<0.001$, z-score >±2) (Fig. 4).

Comparative proteomic analysis of proximal femoral cartilage after 35 days in orbit at µg, 0,33g, 0.67g, and 1g compared to HGC to characterize arthritic responses

Comparative analysis of µg FLIGHT vs HGC proximal femoral cartilage identified 80 significantly altered proteins ($p<0.05$) (Fig. 5a). Pro-osteoarthritic enriched differential protein expression of Ki67, RSP27a, and SNRPA1, and decreased expression of GSTP1-1 and R-Ras was observed (Fig. 5a). Comparative analysis of 0.33g FLIGHT vs HGC proximal femoral cartilage identified 56 significantly altered proteins ($p<0.05$) (Fig. 5b). Pro-osteoarthritic enriched differential protein expression of RSP27a and SNRPA1, and decreased expression of GSTP1-1, RPL32, and R-Ras was observed (Fig. 5b). Comparative analysis of 0.67g FLIGHT vs HGC proximal femoral cartilage identified 132 significantly altered proteins ($p<0.05$) (Fig. 5c). Pro-osteoarthritic enriched differential protein expression of RSP27a and SNRPA1 and decreased expression of GSTP1-1 was observed (Fig. 5c). Comparative analysis of 1g FLIGHT vs HGC proximal femoral cartilage identified 20 significantly altered proteins ($p<0.05$) (Fig. 5d). Pro-osteoarthritic enriched differential protein expression of Ki67 and RSP27a, and decreased expression of GSTP1-1, RPL32, and R-Ras was observed (Fig. 5d).

Comparative proteomic analysis of proximal femoral cartilage after 35 days in orbit at µg, 0,33g, 0.67g, and 1g compared to HGC to characterize arthritic responses

Comparative analysis of µg vs HGC proximal femoral cartilage indicated pro-osteoarthritic enrichment of markers associated with mitochondrial dysfunction, cell cycle progression and a decrease in markers associated with oxidative phosphorylation (Z-score>±2, $p<0.001$) (Fig. 6a). Comparative analysis of 0.33g vs HGC proximal femoral cartilage indicated pro-osteoarthritic decrease in markers associated with EIF2 signaling and neutrophil degranulation (Z-score>-2, $p<0.001$) (Fig. 6b). Comparative analysis of 0.67g vs HGC proximal femoral cartilage indicated pro-osteoarthritic enrichment of markers associated with mitochondrial dysfunction, cell cycle progression, RUNX3 transcriptional regulation, and a decrease in markers associated with oxidative phosphorylation (Z-score>±2, $p<0.001$) (Fig. 6c). Comparative analysis of 1g vs HGC proximal femoral cartilage indicated pro-osteoarthritic enrichment of markers associated with mitochondrial dysfunction, cell cycle progression, RUNX3 transcriptional regulation, and a decrease in markers associated with oxidative phosphorylation and neutrophil degranulation (Z-score>±2, $p<0.001$) (Fig. 6d).

Joint soft tissue histological and immunohistochemical alterations after 35 days in orbit at µg, 0,33g, 0.67g, and 1g.

Sulfated glycosaminoglycans (GAG) in the medial tibial articular cartilage were similar in all FLIGHT cohorts (Fig. 7a). All FLIGHT cohorts had reduced sulfated GAG

concentration compared to HGC (-17.0% µg vs HGC, -20.4% 0.33g vs HGC, 6.3% 0.67g vs HGC, -20.1% vs HGC) (Fig. 7a). Sulfated GAG concentration in the medial menisci was lower after in µg (13%) and 0.33g (11.1%) compared to 0.67g (17.9%) and 1g (18.7%) FLIGHT cohorts (Fig. 7b). All flight cohorts had a lower sulfated GAG concentration in the medical menisci compared to HGC and VGC (Fig. 7b).

Discussion

Identifying countermeasures against spaceflight-induced musculoskeletal complications and characterizing how partial gravity environments will affect musculoskeletal health is important for the preservation of astronaut health and mission success for missions outside of LEO. Importantly, this study identified that: 1] Continuous exposure to 1g of gravitational acceleration via centrifugation failed to preserve meniscal volume and articular cartilage thickness; 2] Proteomic and transcriptomic changes of the joint soft tissue were mitigated in a gravity-dose dependent manner compared to controls, with major differences observed after µg, 0.33g, and 0.67g that were then attenuated at 1g, and; 3] Mice exposed to artificial 1g maintained meniscal ECM synthesis and sulfated GAG expression compared to controls, and ECM synthesis was significantly downregulated in µg, 0.33g, and 0.67g groups.

Rodents exposed to artificial gravity at µg, 0.33g, 0.67g, and 1g via centrifugation for 35 days aboard the ISS had the similar articular cartilage thickness at the medial tibial plateau as HGC and VGC (Fig. 1a). However, sulfated GAG expression in the articular cartilage decreased in all FLIGHT groups compared to HGC (Fig. 7a). These findings

differ from the rodent research 9 (RR9 mission) which found that articular cartilage thickness at the medial tibial plateau decreased in FLIGHT animals compared to HGC and VGC [25]. It is important to note that the housing differences between the MHU8 and RR9 missions may have affected the differential findings regarding cartilage thickness observed after continuous exposure to µg. Mice for RR9 rodents were group housed (10 rodents/habitat, with the divider removed [25]) whereas for MHU8, the mice were single housed in the smaller MARS unit.

Exposure to prolonged artificial gravity via centrifugation for 35 days aboard the ISS failed to preserve medial meniscal volume; µg, 0.33g, 0.67g, and 1g FLIGHT groups exhibited a similar decrease in volume compared to HGC and VGC (Fig 1b). While meniscal sulfated GAG content was lower in all FLIGHT groups compared to ground, there was some preservation seen at the 0.67g and 1g groups compared to HGC and VGC (Fig 7b). Differential gene expression analysis vs HGC revealed pro-arthritic increased Tppp3, Tppp, and Slc6a2, and decreased Pdk4, Gabarapl1, Spock2, Pik3ip1, Dio3, and Adamts5 expression after 35 days spaceflight in the µg, 0.33g and 0.67g FLIGHT groups (Fig. 2). Studies have indicated that increased Tppp and Tppp3 contributes to heterotopic ossification resulting from aberrant osteochondral differentiation after trauma resulting in osteoarthritis and gait impairment in rodents [26]–[28]. Furthermore, studies performing transcriptomic analysis of cartilage have indicated an increased expression of Tppp3 and Tppp genes in osteoarthritic tissue [29], [30]. A decrease in meniscal Pdk4, Gabarapl1, and Pik3ip1 expression indicate pre-arthritic increased chondrocyte autophagy and synovial inflammation [31]–[36]. Additionally, a decrease in extracellular matrix remodeling markers, Spock2 and Adamts5 was seen in flight menisci, indicating pro-

osteoarthritic disruption in cartilage homeostasis and joint degradation [37]–[39]. Notably, the 1g FLIGHT cohort did not see any significant alteration in the gene expression profiles of these markers and moreover, there were only 101 significantly altered genes vs HGC, compared to 1260, 2159, and 1075 significantly altered genes in the µg, 0.33g, and 0.67g flight groups, respectively. These findings indicate that there may be a threshold between 0.33g and 0.67g where meniscal volume can be preserved under artificial gravity. The large decrease in meniscal volume at µg and 0.33g, Martian gravity, may indicate a need to develop countermeasures to preserve joint soft tissue in transit and in partial gravity environments at destination.

Protein expression profiles of cartilage lining the proximal femur after 35d in artificial gravity revealed a similar pattern with a reduced number of significant differentially expressed proteins in the 1g FLIGHT group (20), vs µg (80), 0.33g (56), and 0.67g (120). A reduced expression of GTSP1-1, R-Ras, and RPL32 and an increased expression of RSP27a, Ki67, and SNRPA1 was seen across all FLIGHT cohorts. Reduced expression of R-Ras and RPL32 indicate synovial inflammation and chondrocyte hypertrophy [40]–[43]. Furthermore, treatment GTSP-1 has demonstrated efficacy in reducing cartilage degradation and inflammation markers as well as significant improvements in pain and functional outcomes indicating its protective role against osteoarthritis [44]. Elevated protein expression of RSP27a, Ki67, and SNRPA1 in the articular cartilage further support that spaceflight regardless of artificial gravity induces a pre-arthritic phenotype in the knee joint [45]–[49].

Chondrocyte autophagy, synovial inflammation, and chondrocyte hypertrophy have been well characterized to contribute to the development of osteoarthritis in the

knee joint [50]–[52]. Reduced weight-bearing due to a lack of gravity can have deleterious effects on the musculoskeletal system, and this degeneration can risk mission success and impact astronaut performance and health [53]. The study supports the findings of the RR9 mission in spaceflight damages the joint soft tissue and induces arthritic responses [24]. Artificial gravity via centrifugation failed to preserve the menisci of articular cartilage, however at the transcriptomic and proteomic levels, artificial 1g did prevent significant differential expression compared to HGC. Further investigation is still needed to validate the protective effects of artificial gravity on the knee joint during spaceflight.

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Author contributions

The following authors served as science team leaders and contributed to the scientific design of the MHU8 study: J.S.W., M.L.B., J.C., S.T., C.F., and M.V. J.S.W. and C.M.P. contributed to the conception and design of the knee joint analysis. C.M.P., J.S.W., K.R. and A.C. contributed to the performance of the studies. C.M.P., J.S.W., S.V.W., L.K. and A.C. contributed to the analysis and interpretation of the data. C.M.P. and J.S.W. wrote the main manuscript text. C.M.P. and J.S.W. prepared Figs. 1-7. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

References

[1] R. O. Nielsen, P. Schjerling, P. Tesch, P. Stål, and H. Langberg, "Collagen content in the vastus lateralis and the soleus muscle following a 90-day bed rest period with or without resistance exercises," *Muscles. Ligaments Tendons J.*, vol. 5, no. 4, p. 305, Oct. 2015, doi: 10.11138/MLTJ/2015.5.4.305.

[2] B. J. Parmenter, J. Raymond, P. J. Dinnen, R. J. Lusby, and M. A. Fiatarone Singh, "Preliminary evidence that low ankle-brachial index is associated with reduced bilateral hip extensor strength and functional mobility in peripheral arterial disease," *J. Vasc. Surg.*, vol. 57, no. 4, pp. 963-973.e1, 2013, doi: 10.1016/J.JVS.2012.08.103.

[3] A. Leblanc *et al.*, "Muscle volume, MRI relaxation times (T2), and body

composition after spaceflight," 2000, Accessed: Dec. 13, 2022. [Online]. Available: http://www.jap.org.

[4] J. S. Willey, S. A. J. Lloyd, G. A. Nelson, and T. A. Bateman, "Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications," *Clin Rev Bone Min. Metab*, vol. 9, no. 1, pp. 54–62, 2011, doi: 10.1007/s12018-011-9092-8.

[5] J. S. Willey, S. A. J. Lloyd, G. A. Nelson, and T. A. Bateman, "Space Radiation and Bone Loss."

[6] T. Tominari *et al.*, "Hypergravity and microgravity exhibited reversal effects on the bone and muscle mass in mice," *Sci. Reports 2019 91*, vol. 9, no. 1, pp. 1–10, Apr. 2019, doi: 10.1038/s41598-019-42829-z.

[7] A. T. Kwok *et al.*, "Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents," *Sci. Rep.*, vol. 11, no. 1, Dec. 2021, doi: 10.1038/S41598-021-90010-2.

[8] A. Kwok *et al.*, "Altered rodent gait characteristics after ~35 days in orbit aboard the International Space Station," *Life Sci. Sp. Res.*, vol. 24, pp. 9–17, Feb. 2020, doi: 10.1016/J.LSSR.2019.10.010.

[9] J. C. Buckey, "Preparing for Mars: the physiologic and medical challenges.," *European journal of medical research*, 1999. https://pubmed.ncbi.nlm.nih.gov/10477498/ (accessed Dec. 13, 2022).

[10] J. Sibonga *et al.*, "Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss," *Bone*, vol. 128, Nov.

2019, doi: 10.1016/j.bone.2019.07.013.

[11] A. LeBlanc *et al.*, “Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight,” *Osteoporos. Int.*, vol. 24, no. 7, pp. 2105–2114, Jul. 2013, doi: 10.1007/S00198-012-2243-Z.

[12] A. Okada *et al.*, “Bisphosphonate Use May Reduce the Risk of Urolithiasis in Astronauts on Long-Term Spaceflights,” *JBMR Plus*, vol. 6, no. 1, Jan. 2022, doi: 10.1002/JBM4.10550.

[13] J. Sibonga *et al.*, “Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss,” *Bone*, vol. 128, Nov. 2019, doi: 10.1016/J.BONE.2019.07.013.

[14] K. A. Greene, J. A. Tooze, L. Lenchik, and A. A. Weaver, “Change in Lumbar Muscle Size and Composition on MRI with Long-Duration Spaceflight,” *Ann. Biomed. Eng.*, vol. 50, no. 7, pp. 816–824, Jul. 2022, doi: 10.1007/S10439-022-02968-3.

[15] J. M. Evans, C. F. Knapp, and N. Goswami, “Artificial gravity as a countermeasure to the cardiovascular deconditioning of spaceflight: Gender perspectives,” *Front. Physiol.*, vol. 9, no. JUL, p. 716, Jul. 2018, doi: 10.3389/FPHYS.2018.00716/BIBTEX.

[16] G. R. Clément, A. P. Bukley, W. H. Paloski, A. Mulavara, M. M. Cohen, and B. Mcgrath, “Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions,” 1961, doi: 10.3389/fnsys.2015.00092.

[17] R. Ellman, J. Spatz, A. Cloutier, R. Palme, B. A. Christiansen, and M. L. Bouxsein, “Partial reductions in mechanical loading yield proportional changes in bone density, bone architecture, and muscle mass,” *J. Bone Miner. Res.*, vol. 28, no. 4, pp. 875–885, Apr. 2013, doi: 10.1002/JBMR.1814.

[18] M. Shimbo *et al.*, “Ground-based assessment of JAXA mouse habitat cage unit by mouse phenotypic studies,” *Exp. Anim.*, vol. 65, no. 2, pp. 175–187, 2016, doi: 10.1538/EXPANIM.15-0077.

[19] D. Shiba *et al.*, “Development of new experimental platform ‘MARS’—Multiple Artificial-gravity Research System—to elucidate the impacts of micro/partial gravity on mice,” *Sci. Reports 2017 71*, vol. 7, no. 1, pp. 1–10, Sep. 2017, doi: 10.1038/S41598-017-10998-4.

[20] T. Tominari *et al.*, “Hypergravity and microgravity exhibited reversal effects on the bone and muscle mass in mice,” *Sci. Rep.*, vol. 9, no. 1, Dec. 2019, doi: 10.1038/S41598-019-42829-Z.

[21] G. Clément, “International roadmap for artificial gravity research,” *NPJ microgravity*, vol. 3, no. 1, Dec. 2017, doi: 10.1038/S41526-017-0034-8.

[22] A. Capozzi, C. Saucier, C. Bisbal, and K. Lambert, “Grape Polyphenols in the Treatment of Human Skeletal Muscle Damage Due to Inflammation and Oxidative Stress during Obesity and Aging: Early Outcomes and Promises,” *Molecules*, vol. 27, no. 19, Oct. 2022, doi: 10.3390/MOLECULES27196594.

[23] M. Paździor, M. Kiełczykowska, J. Kurzepa, D. Luchowska-kocot, J. Kocot, and

I. Musik, “The Oxidative Stress in Knee Osteoarthritis Patients. An Attempt of Evaluation of Possible Compensatory Effects Occurring in the Disease Development,” *Medicina (Kaunas).*, vol. 55, no. 5, May 2019, doi: 10.3390/MEDICINA55050150.

[24] A. T. Kwok *et al.*, “Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents,” *Sci. Rep.*, vol. 11, no. 1, p. 10469, Dec. 2021, doi: 10.1038/S41598-021-90010-2.

[25] J. S. Willey *et al.*, “Spaceflight-Relevant Challenges of Radiation and/or Reduced Weight Bearing Cause Arthritic Responses in Knee Articular Cartilage,” *Radiat. Res.*, vol. 186, no. 4, pp. 333–344, Oct. 2016, doi: 10.1667/RR14400.1.

[26] J. H. Yea *et al.*, “Tppp3+ synovial/tendon sheath progenitor cells contribute to heterotopic bone after trauma,” *Bone Res. 2023 111*, vol. 11, no. 1, pp. 1–12, Jul. 2023, doi: 10.1038/s41413-023-00272-x.

[27] J. A. Staverosky, B. A. Pryce, S. S. Watson, and R. Schweitzer, “Tubulin polymerization-promoting protein family member 3, Tppp3, is a specific marker of the differentiating tendon sheath and synovial joints,” *Dev. Dyn.*, vol. 238, no. 3, pp. 685–692, Mar. 2009, doi: 10.1002/DVDY.21865.

[28] L. Ameye, D. Aria, K. Jepsen, A. Oldberg, T. Xu, and M. F. Young, “Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis,” *FASEB J.*, vol. 16, no. 7, pp. 673–680, May 2002, doi: 10.1096/FJ.01-0848COM.

[29] M. F. Rai, E. D. Tycksen, L. Cai, J. Yu, R. W. Wright, and R. H. Brophy,

"Distinct Degenerative Phenotype of Articular Cartilage from Knees with Meniscus Tear Compared to Knees with Osteoarthritis," *Osteoarthr. Cartil.*, vol. 27, no. 6, p. 945, Jun. 2019, doi: 10.1016/J.JOCA.2019.02.792.

[30] Y. Hu *et al.*, "Transcriptomic analyses of joint tissues during osteoarthritis development in a rat model reveal dysregulated mechanotransduction and extracellular matrix pathways," *Osteoarthr. Cartil.*, vol. 31, no. 2, pp. 199–212, Feb. 2023, doi: 10.1016/J.JOCA.2022.10.003.

[31] B. Brandstetter *et al.*, "FOXO3 is involved in the tumor necrosis factor-driven inflammatory response in fibroblast-like synoviocytes," *Lab. Investig.*, vol. 99, no. 5, pp. 648–658, May 2019, doi: 10.1038/S41374-018-0184-7.

[32] J. H. Park *et al.*, "Anti-Ischemic Effects of PIK3IP1 Are Mediated through Its Interactions with the ETA-PI3Kγ-AKT Axis," *Cells*, vol. 11, no. 14, Jul. 2022, doi: 10.3390/CELLS11142162/S1.

[33] J. Wang *et al.*, "Screening crucial lncRNAs and genes in osteoarthritis by integrated analysis," *Adv. Rheumatol.*, vol. 63, no. 1, pp. 1–10, Dec. 2023, doi: 10.1186/S42358-023-00288-1/FIGURES/6.

[34] J. Liu, Q. Fu, and S. Liu, "Transcriptional Regulation Based on Network of Autophagy Identifies Key Genes and Potential Mechanisms in Human Osteoarthritis," *Cartilage*, vol. 13, no. 2, pp. 1431S-1441S, Dec. 2021, doi: 10.1177/1947603520951632/ASSET/IMAGES/LARGE/10.1177_1947603520951632-FIG6.JPEG.

[35] H. Cao *et al.*, “Advances in the study of mitophagy in osteoarthritis,” *J. Zhejiang Univ. Sci. B*, vol. 25, no. 3, p. 197, Mar. 2024, doi: 10.1631/JZUS.B2300402.

[36] Z. Li, L. Xie, H. Zeng, and Y. Wu, “PDK4 inhibits osteoarthritis progression by activating the PPAR pathway,” *J. Orthop. Surg. Res.*, vol. 19, no. 1, p. 109, Dec. 2024, doi: 10.1186/S13018-024-04583-5.

[37] S. S. Zhao, V. Karhunen, A. P. Morris, and D. Gill, “ADAMTS5 as a therapeutic target for osteoarthritis: Mendelian randomisation study,” *Ann. Rheum. Dis.*, vol. 81, no. 6, pp. 903–904, Jun. 2022, doi: 10.1136/ANNRHEUMDIS-2021-222007.

[38] A. Latourte and P. Richette, “Inhibition of ADAMTS-5: the right target for osteoarthritis?,” *Osteoarthr. Cartil.*, vol. 30, no. 2, pp. 175–177, Feb. 2022, doi: 10.1016/j.joca.2021.09.012.

[39] I. C. M. Pelsma *et al.*, “Variants of FOXO3 and RPA3 genes affecting IGF-1 levels alter the risk of development of primary osteoarthritis,” *Eur. J. Endocrinol.*, vol. 184, no. 1, pp. 29–39, Jan. 2021, doi: 10.1530/EJE-20-0904.

[40] S. Fauteux-Daniel, L. M. Merlo Pich, C. Girard-Guyonvarc’h, A. Caruso, E. Rodriguez, and C. Gabay, “The role of interleukin-18 and interleukin-18 binding protein in K/BxN serum transfer-induced arthritis,” *Front. Immunol.*, vol. 14, p. 1215364, Jun. 2023, doi: 10.3389/FIMMU.2023.1215364/BIBTEX.

[41] F. Rousset *et al.*, “IL-1beta mediates MMP secretion and IL-1beta neosynthesis via upregulation of p22phox and NOX4 activity in human articular chondrocytes,” *Osteoarthr. Cartil.*, vol. 23, no. 11, pp. 1972–1980, Nov. 2015, doi:

10.1016/j.joca.2015.02.167.

[42] Y. Wu *et al.*, “Renin-angiotensin system in osteoarthritis: A new potential therapy,” *Int. Immunopharmacol.*, vol. 75, p. 105796, Oct. 2019, doi: 10.1016/J.INTIMP.2019.105796.

[43] M. Zayoud, E. Vax, G. Elad-Sfadia, I. Barshack, R. Pinkas-Kramarski, and I. Goldstein, “Inhibition of Ras GTPases prevents Collagen-Induced Arthritis by Reducing the Generation of Pathogenic CD4+ T Cells and the Hyposialylation of Autoantibodies,” *ACR Open Rheumatol.*, vol. 2, no. 9, p. 512, Sep. 2020, doi: 10.1002/ACR2.11169.

[44] T. Setti, M. G. L. Arab, G. S. Santos, N. Alkass, M. A. P. Andrade, and J. F. S. D. Lana, “The protective role of glutathione in osteoarthritis,” *J. Clin. Orthop. Trauma*, vol. 15, p. 145, Apr. 2021, doi: 10.1016/J.JCOT.2020.09.006.

[45] G. G. H. Van Den Akker, M. M. J. Caron, M. J. Peffers, and T. J. M. Welting, “Ribosome dysfunction in osteoarthritis,” *Curr. Opin. Rheumatol.*, vol. 34, no. 1, p. 61, Jan. 2022, doi: 10.1097/BOR.0000000000000858.

[46] W. Madej, P. Buma, and P. van der Kraan, “Inflammatory conditions partly impair the mechanically mediated activation of Smad2/3 signaling in articular cartilage,” *Arthritis Res. Ther.*, vol. 18, no. 1, Jun. 2016, doi: 10.1186/S13075-016-1038-6.

[47] “Integrative meta-analysis of differentially expressed genes in osteoarthritis using microarray technology.” https://www.spandidos-publications.com/10.3892/mmr.2015.3790 (accessed Jun. 06, 2024).

[48] Y. Ge *et al.*, “Identification and validation of hub genes of synovial tissue for

patients with osteoarthritis and rheumatoid arthritis," *Hereditas*, vol. 158, no. 1, pp. 1–16, Dec. 2021, doi: 10.1186/S41065-021-00201-0/FIGURES/7.

[49] F. Pessler *et al.*, "Subintimal Ki-67 as a synovial tissue biomarker for inflammatory arthropathies," *Ann. Rheum. Dis.*, vol. 67, no. 2, pp. 162–167, Feb. 2008, doi: 10.1136/ARD.2007.071670.

[50] E. Sanchez-Lopez, R. Coras, A. Torres, N. E. Lane, and M. Guma, "Synovial inflammation in osteoarthritis progression," *Nat. Rev. Rheumatol.*, vol. 18, no. 5, p. 258, May 2022, doi: 10.1038/S41584-022-00749-9.

[51] R. Duan, H. Xie, and Z. Z. Liu, "The Role of Autophagy in Osteoarthritis," *Front. Cell Dev. Biol.*, vol. 8, p. 608388, Nov. 2020, doi: 10.3389/FCELL.2020.608388.

[52] S. Chawla *et al.*, "Chondrocyte Hypertrophy in Osteoarthritis: Mechanistic Studies and Models for the Identification of New Therapeutic Strategies," *Cells*, vol. 11, no. 24, Dec. 2022, doi: 10.3390/CELLS11244034.

[53] B. Ganse, M. Cucchiarini, and H. Madry, "Joint Cartilage in Long-Duration Spaceflight," *Biomedicines*, vol. 10, no. 6, Jun. 2022, doi: 10.3390/BIOMEDICINES10061356.

Figures and Tables

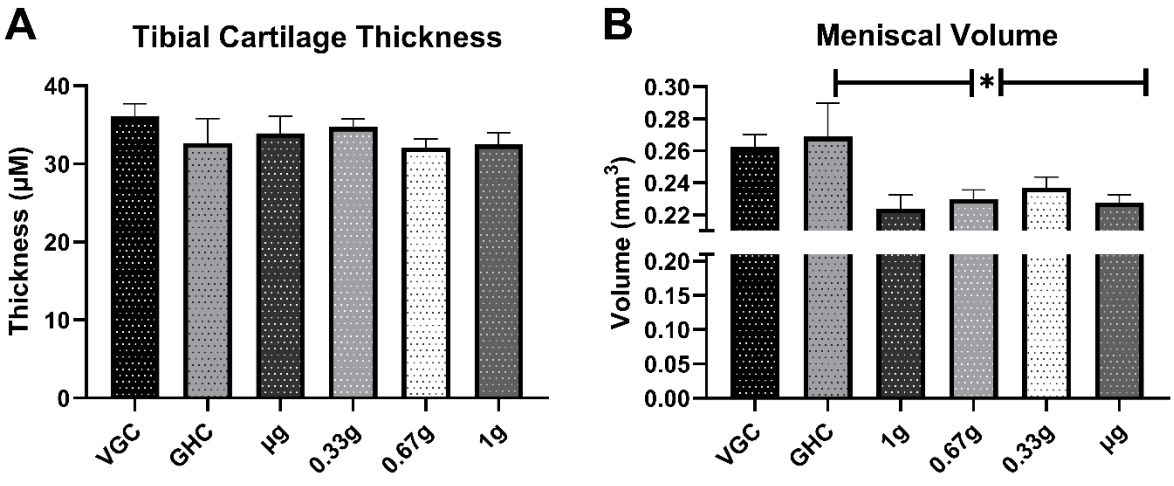


Figure 1. (A) Meniscal volume decreased after 35 days in orbit in all FLIGHT groups regardless of artificial gravity conditions. (*$p<0.05$) (B) No significant difference in thinning of the tibial articular cartilage at the medica tibial plateau was observed after 35 days in orbit.

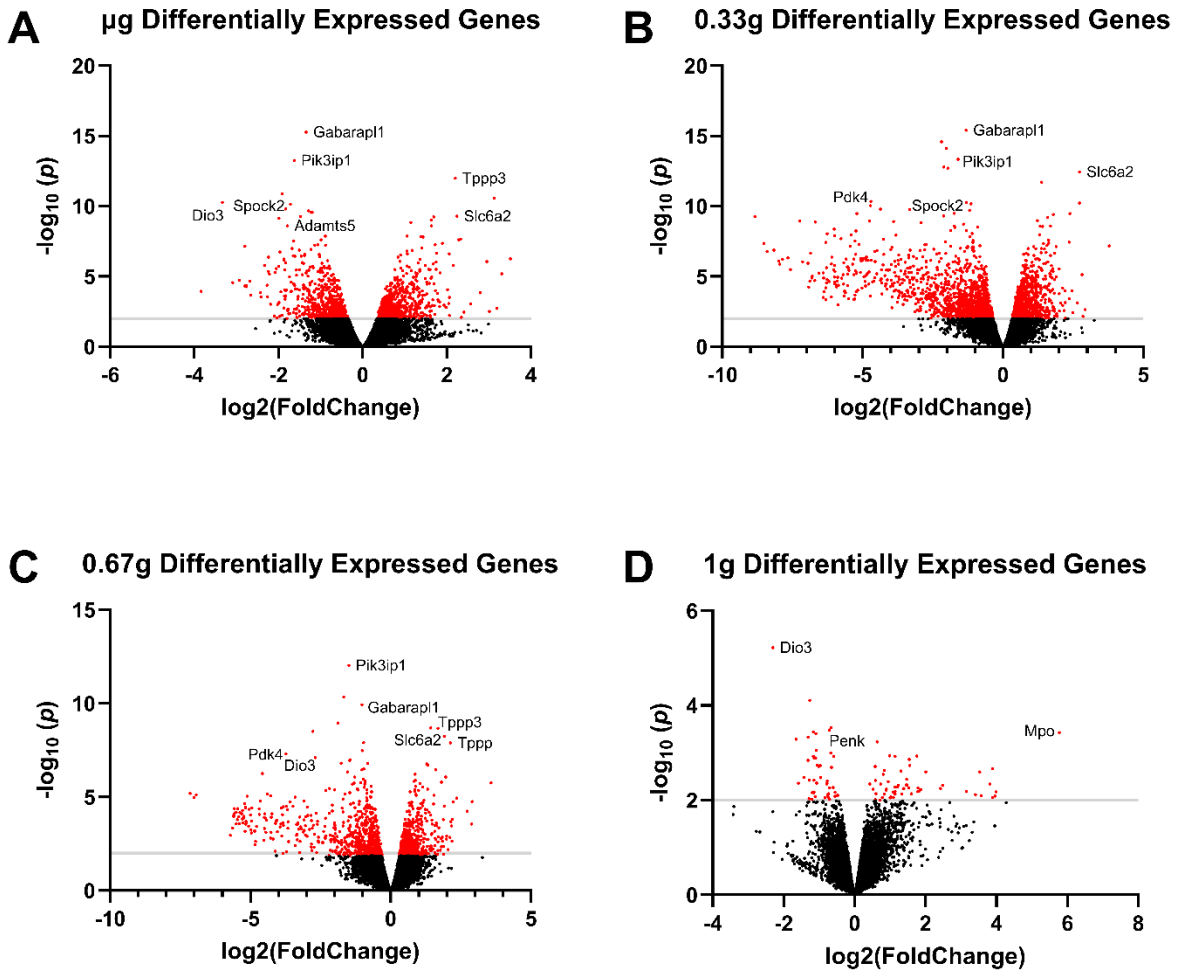
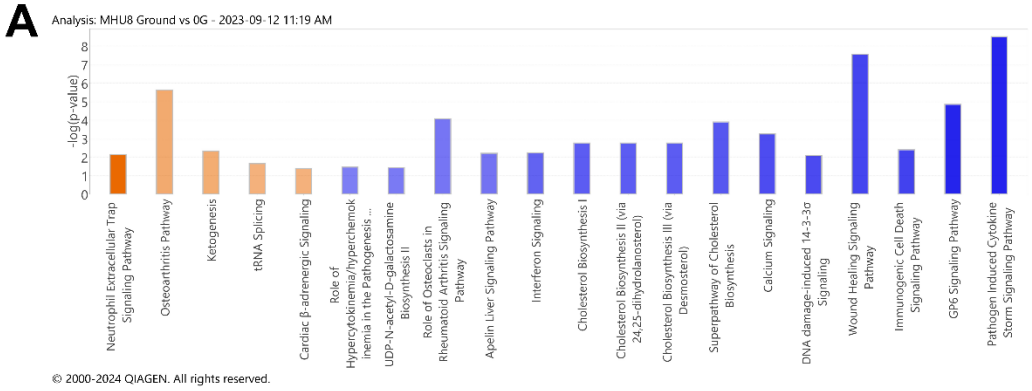


Figure 2. (A) Comparative analysis of µg FLIGHT vs HGC menisci identified 1260 significantly altered genes ($p<0.05$) (B) Comparative analysis of 0.33g FLIGHT vs HGC menisci identified 2159 significantly altered genes ($p<0.05$ (C) Comparative analysis of 0.67g FLIGHT vs HGC menisci identified 1075 significantly altered genes ($p<0.05$). (D) Comparative analysis of 1g FLIGHT vs HGC menisci identified 101 significantly altered genes ($p<0.05$).


A
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-log(p-value)
Neutrophil Extracellular Trap Signaling Pathway
Osteoarthritis Pathway
Ketogenesis
tRNA Splicing
Cardiac β-adrenergic Signaling
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis ...
UDP-N-acetyl-D-galactosamine Biosynthesis II
Role of Osteoclasts in Rheumatoid Arthritis Signaling Pathway
Apelin Liver Signaling Pathway
Interferon Signaling
Cholesterol Biosynthesis I
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)
Cholesterol Biosynthesis III (via Desmosterol)
Superpathway of Cholesterol Biosynthesis
Calcium Signaling
DNA damage-induced 14-3-3σ Signaling
Wound Healing Signaling Pathway
Immunogenic Cell Death Signaling Pathway
GP6 Signaling Pathway
Pathogen Induced Cytokine Storm Signaling Pathway
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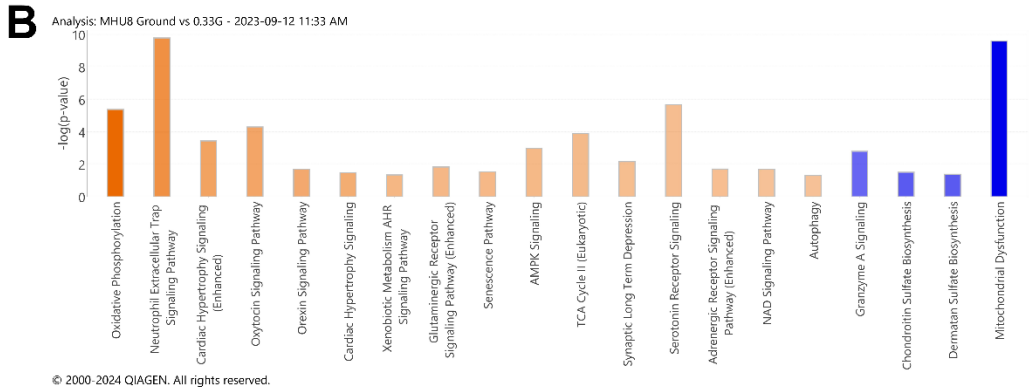
B
Analysis: MHU8 Ground vs 0.33G - 2023-09-12 11:33 AM
-log(p-value)
Oxidative Phosphorylation
Neutrophil Extracellular Trap Signaling Pathway
Cardiac Hypertrophy Signaling (Enhanced)
Oxytocin Signaling Pathway
Orexin Signaling Pathway
Cardiac Hypertrophy Signaling
Xenobiotic Metabolism AHR Signaling Pathway
Glutaminergic Receptor Signaling Pathway (Enhanced)
Senescence Pathway
AMPK Signaling
TCA Cycle II (Eukaryotic)
Synaptic Long Term Depression
Serotonin Receptor Signaling
Adrenergic Receptor Signaling Pathway (Enhanced)
NAD Signaling Pathway
Autophagy
Granzyme A Signaling
Chondroitin Sulfate Biosynthesis
Dermatan Sulfate Biosynthesis
Mitochondrial Dysfunction
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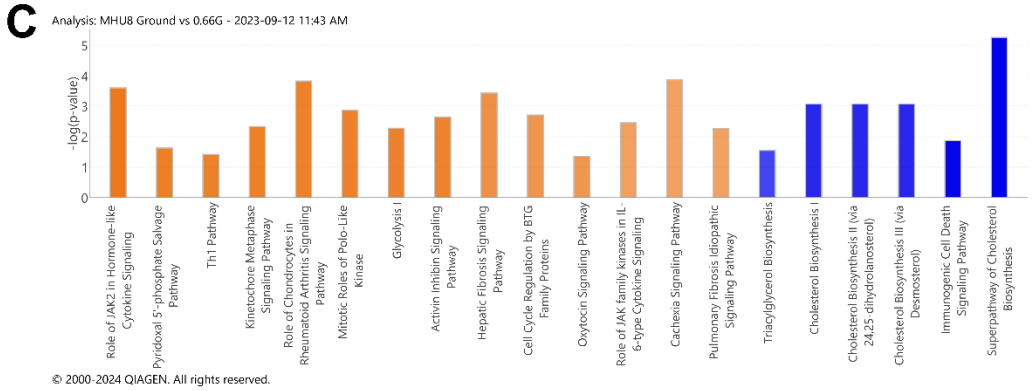
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Analysis: MHU8 Ground vs 0.66G - 2023-09-12 11:43 AM
-log(p-value)
Role of JAK2 in Hormone-like Cytokine Signaling
Pyridoxal 5'-phosphate Salvage Pathway
Th1 Pathway
Kinetochore Metaphase Signaling Pathway
Role of Chondrocytes in Rheumatoid Arthritis Signaling Pathway
Mitotic Roles of Polo-Like Kinase
Glycolysis I
Activin Inhibin Signaling Pathway
Hepatic Fibrosis Signaling Pathway
Cell Cycle Regulation by BTG Family Proteins
Oxytocin Signaling Pathway
Role of JAK family kinases in IL-6-type Cytokine Signaling
Cachexia Signaling Pathway
Pulmonary Fibrosis Idiopathic Signaling Pathway
Triacylglycerol Biosynthesis
Cholesterol Biosynthesis I
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)
Cholesterol Biosynthesis III (via Desmosterol)
Immunogenic Cell Death Signaling Pathway
Superpathway of Cholesterol Biosynthesis
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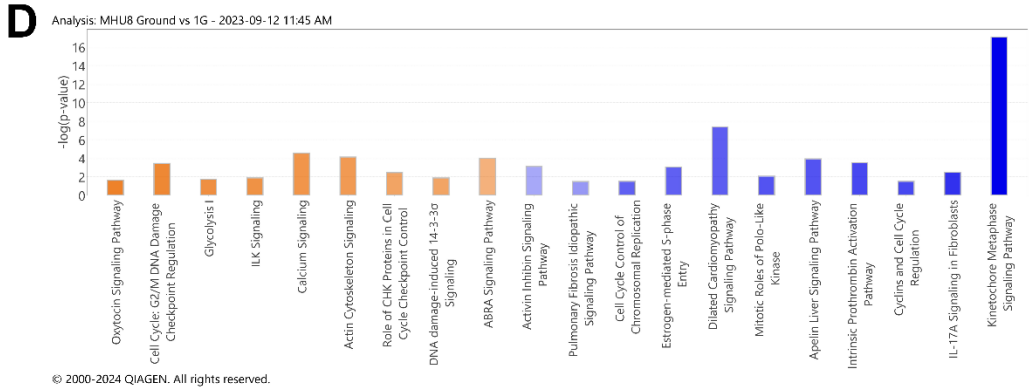
D
Analysis: MHU8 Ground vs 1G - 2023-09-12 11:45 AM
-log(p-value)
Oxytocin Signaling Pathway
Cell Cycle: G2/M DNA Damage Checkpoint Regulation
Glycolysis I
ILK Signaling
Calcium Signaling
Actin Cytoskeleton Signaling
Role of CHK Proteins in Cell Cycle Checkpoint Control
DNA damage-induced 14-3-3σ Signaling
ABRA Signaling Pathway
Activin Inhibin Signaling Pathway
Pulmonary Fibrosis Idiopathic Signaling Pathway
Cell Cycle Control of Chromosomal Replication
Estrogen-mediated S-phase Entry
Dilated Cardiomyopathy Signaling Pathway
Mitotic Roles of Polo-Like Kinase
Apelin Liver Signaling Pathway
Intrinsic Prothrombin Activation Pathway
Cyclins and Cell Cycle Regulation
IL-17A Signaling in Fibroblasts
Kinetochore Metaphase Signaling Pathway
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Figure 3. (A) Most significantly altered pathways from comparative analysis of μg vs HGC menisci (Z-score>±2, p<0.001). (B) Most significantly altered pathways from comparative analysis of 0.33g FLIGHT vs HGC menisci (Z-score>±2, p<0.001). (C) Most significantly altered pathways from comparative analysis of 0.67g vs HGC menisci (Z-score>±2, p<0.001). (D) Most significantly altered pathways from comparative analysis of 1g vs HGC menisci indicated decreased alterations in pro-arthritic pathways compared to μg, 0.33g. And 0.67g (Z-score>±2, p<0.001).


Canonical Pathway
0g 0.3g 0.6g 1g
RHOGDI Signaling
tRNA Splicing
Dilated Cardiomyopathy Signaling Pathway
Sertoli Cell Germ Cell Junction Signaling Pathway (Enhanced)
Neutrophil Extracellular Trap Signaling Pathway
AMPK Signaling
Cardiac β-adrenergic Signaling
Osteoarthritis Pathway
Nicotine Degradation II
Role of BRCA1 in DNA Damage Response
Pyridoxal 5'-phosphate Salvage Pathway
Kinetochore Metaphase Signaling Pathway
Cell Cycle Control of Chromosomal Replication
DNA Methylation and Transcriptional Repression Signaling
Adrenomedullin signaling pathway
Cell Cycle Regulation by BTG Family Proteins
Role of Chondrocytes in Rheumatoid Arthritis Signaling Pathway
Cyclins and Cell Cycle Regulation
NAD Signaling Pathway
HIF1α Signaling
IL-17A Signaling in Fibroblasts
Sirtuin Signaling Pathway
Sertoli Cell-Sertoli Cell Junction Signaling
Netrin Signaling
Gluconeogenesis I
Glutaminergic Receptor Signaling Pathway (Enhanced)
Oxytocin Signaling Pathway
Inhibition of Matrix Metalloproteases
Wound Healing Signaling Pathway
Pathogen Induced Cytokine Storm Signaling Pathway
GP6 Signaling Pathway
ILK Signaling
Opioid Signaling Pathway
SNARE Signaling Pathway
Calcium Signaling
Regulation of Actin-based Motility by Rho
DNA damage-induced 14-3-3σ Signaling
Semaphorin Neuronal Repulsive Signaling Pathway
RHOA Signaling
Actin Cytoskeleton Signaling
PFKFB4 Signaling Pathway
Triacylglycerol Biosynthesis
Ketogenesis
p53 Signaling
Xenobiotic Metabolism AHR Signaling Pathway
Nicotine Degradation III
Superpathway of Cholesterol Biosynthesis
Estrogen-mediated S-phase Entry
Intrinsic Prothrombin Activation Pathway
Immunogenic Cell Death Signaling Pathway
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)
Cholesterol Biosynthesis III (via Desmosterol)
Cholesterol Biosynthesis I
Dermatan Sulfate Biosynthesis
Chondroitin Sulfate Biosynthesis

Figure 4. Comparative analysis of canonical pathway alterations in the menisci of mice exposed to µg, 0.33g, and 0.67g vs HGC indicated differentially enriched cellular stress and injury pathways cell cycle progression pathways, lipid metabolism, pro-inflammatory pathways (MAPK, USP18, KL, DYSF), pro-arthritic pathways, and pro-apoptotic pathways ($p<0.001$, z-score $\geq\pm2$). Furthermore, comparative analysis indicated downregulation of cellular stress response pathways, ECM synthesis, and antioxidant defenses in µg, 0.33g, and 0.67g vs GC ($p<0.001$, z-score $\geq\pm2$). However, pathway expression was similar between 1g and HGC. ($p<0.001$, z-score $\geq\pm2$).

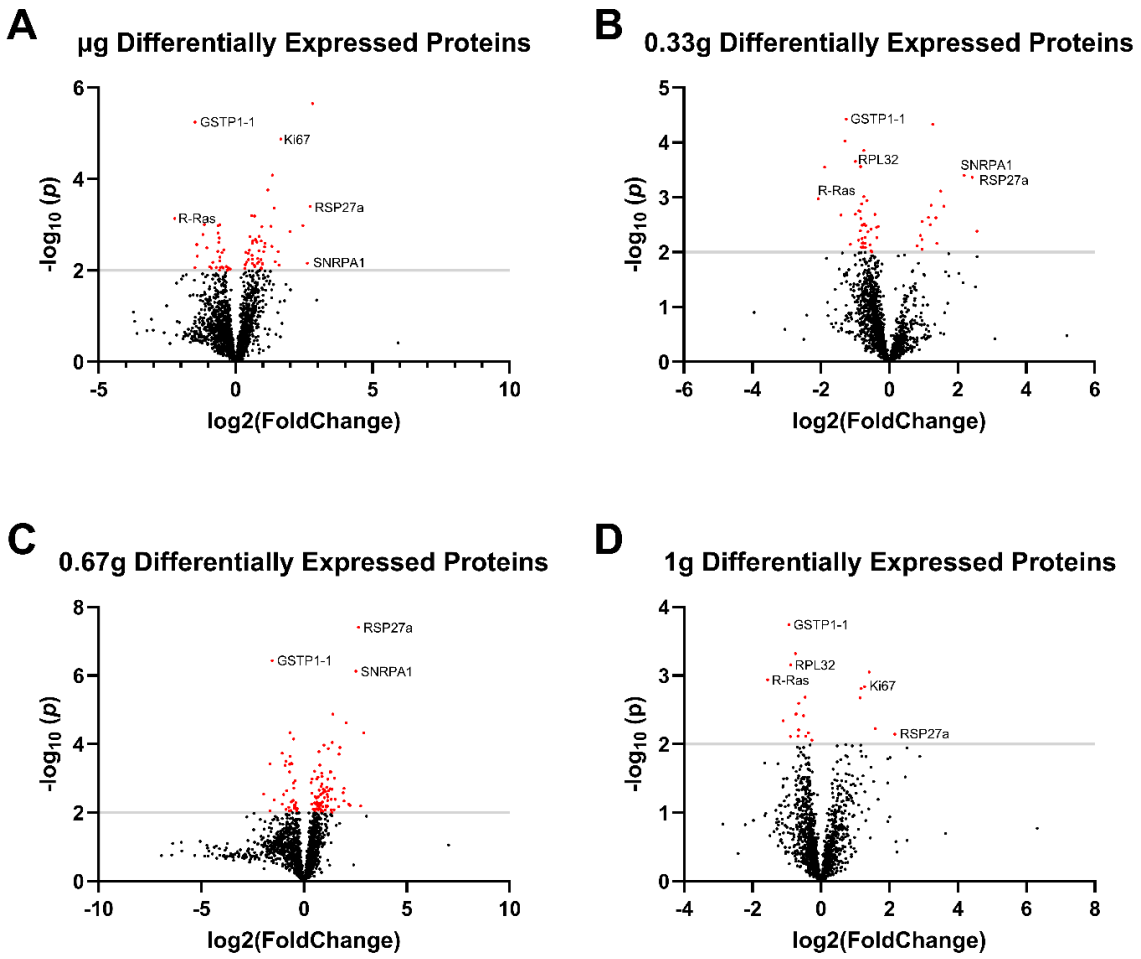
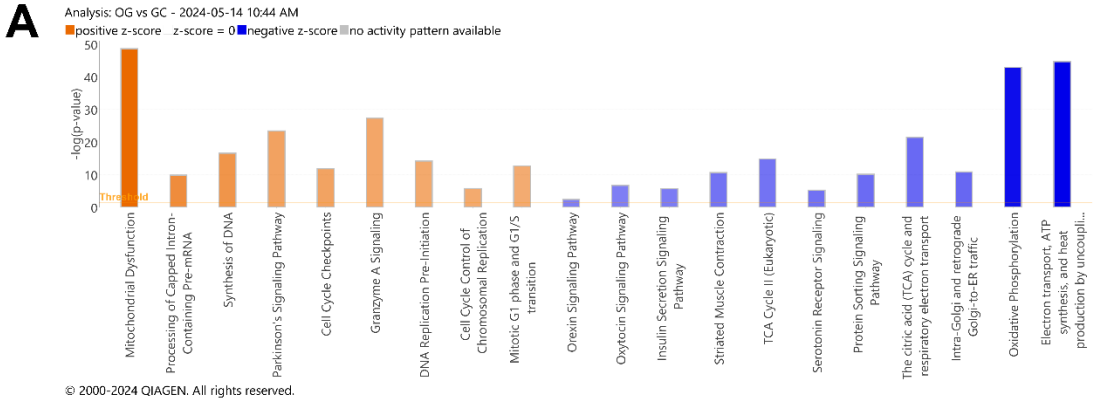


Figure 5. (A) Comparative analysis of µg FLIGHT vs HGC proximal femoral cartilage identified 80 significantly altered proteins ($p<0.05$). (B) Comparative analysis of 0.33g FLIGHT vs HGC proximal femoral cartilage identified 56 significantly altered proteins ($p<0.05$). (C) Comparative analysis of 0.67g FLIGHT vs HGC proximal femoral cartilage identified 132 significantly altered proteins ($p<0.05$). (D) Comparative analysis of 1g FLIGHT vs HGC proximal femoral cartilage identified 20 significantly altered proteins ($p<0.05$).


A
Analysis: OG vs GC - 2024-05-14 10:44 AM
positive z-score z-score = 0 negative z-score no activity pattern available
-log(p-value)
Threshold
Mitochondrial Dysfunction
Processing of Capped Intron-Containing Pre-mRNA
Synthesis of DNA
Parkinson's Signaling Pathway
Cell Cycle Checkpoints
Granzyme A Signaling
DNA Replication Pre-Initiation
Cell Cycle Control of Chromosomal Replication
Mitotic G1 phase and G1/S transition
Orexin Signaling Pathway
Oxytocin Signaling Pathway
Insulin Secretion Signaling Pathway
Striated Muscle Contraction
TCA Cycle II (Eukaryotic)
Serotonin Receptor Signaling
Protein Sorting Signaling Pathway
The citric acid (TCA) cycle and respiratory electron transport
Intra-Golgi and retrograde Golgi-to-ER traffic
Oxidative Phosphorylation
Electron transport, ATP synthesis, and heat production by uncoupli...
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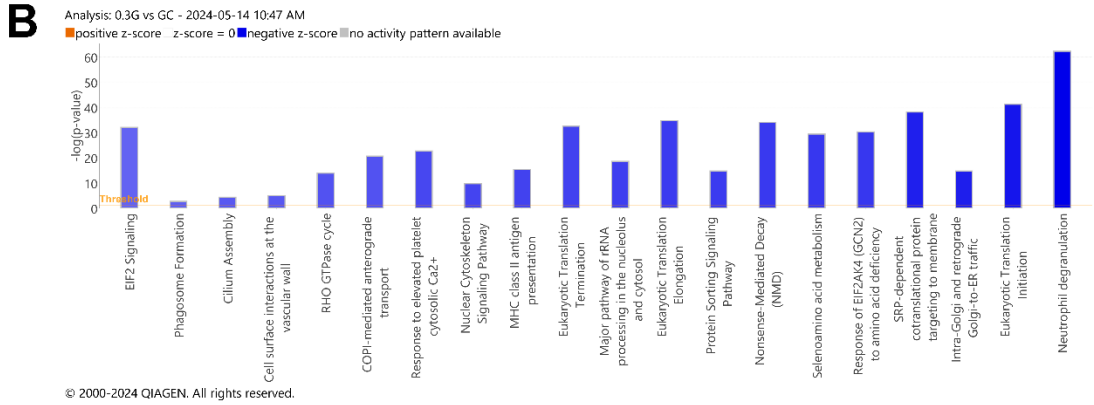
B
Analysis: 0.3G vs GC - 2024-05-14 10:47 AM
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-log(p-value)
Threshold
Eif2 Signaling
Phagosome Formation
Cilium Assembly
Cell surface interactions at the vascular wall
RHO GTPase cycle
COPI-mediated anterograde transport
Response to elevated platelet cytosolic Ca2+
Nuclear Cytoskeleton Signaling Pathway
MHC class II antigen presentation
Eukaryotic Translation Termination
Major pathway of rRNA processing in the nucleolus and cytosol
Eukaryotic Translation Elongation
Protein Sorting Signaling Pathway
Nonsense-Mediated Decay (NMD)
Selenoamino acid metabolism
Response of Eif2ak4 (GCN2) to amino acid deficiency
SRP-dependent cotranslational protein targeting to membrane
Intra-Golgi and retrograde Golgi-to-ER traffic
Eukaryotic Translation Initiation
Neutrophil degranulation
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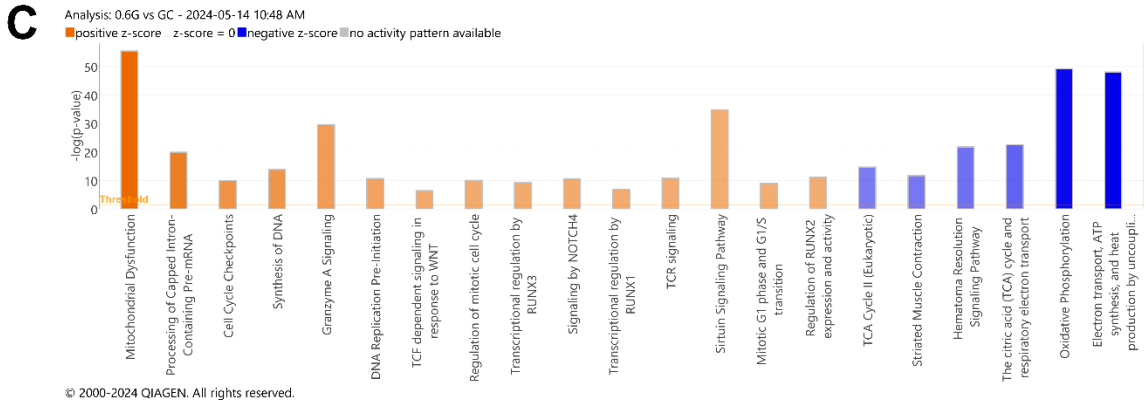
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Analysis: 0.6G vs GC - 2024-05-14 10:48 AM
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Threshold
Mitochondrial Dysfunction
Processing of Capped Intron-Containing Pre-mRNA
Cell Cycle Checkpoints
Synthesis of DNA
Granzyme A Signaling
DNA Replication Pre-Initiation
TCF dependent signaling in response to WNT
Regulation of mitotic cell cycle
Transcriptional regulation by RUNX3
Signaling by NOTCH4
Transcriptional regulation by RUNX1
TCR signaling
Sirtuin Signaling Pathway
Mitotic G1 phase and G1/S transition
Regulation of RUNX2 expression and activity
TCA Cycle II (Eukaryotic)
Striated Muscle Contraction
Hematoma Resolution Signaling Pathway
The citric acid (TCA) cycle and respiratory electron transport
Oxidative Phosphorylation
Electron transport, ATP synthesis, and heat production by uncoupli...
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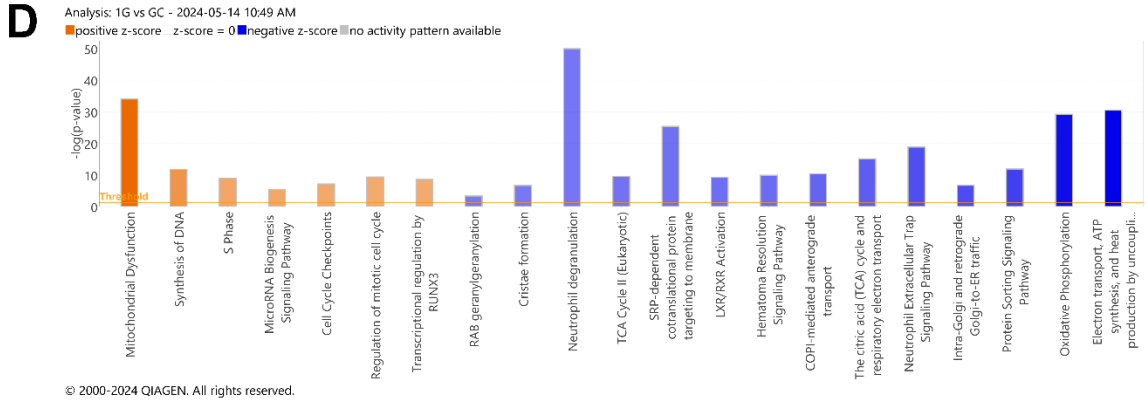
D
Analysis: 1G vs GC - 2024-05-14 10:49 AM
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-log(p-value)
Threshold
Mitochondrial Dysfunction
Synthesis of DNA
S Phase
MicroRNA Biogenesis Signaling Pathway
Cell Cycle Checkpoints
Regulation of mitotic cell cycle
Transcriptional regulation by RUNX3
RAB geranylgeranylation
Cristae formation
Neutrophil degranulation
TCA Cycle II (Eukaryotic)
SRP-dependent cotranslational protein targeting to membrane
LXR/RXR Activation
Hematoma Resolution Signaling Pathway
COPI-mediated anterograde transport
The citric acid (TCA) cycle and respiratory electron transport
Neutrophil Extracellular Trap Signaling Pathway
Intra-Golgi and retrograde Golgi-to-ER traffic
Protein Sorting Signaling Pathway
Oxidative Phosphorylation
Electron transport, ATP synthesis, and heat production by uncoupli...
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Figure 6. (A) Most significantly altered pathways from comparative analysis of µg vs HGC proximal femoral cartilage (Z-score>±2, p<0.001). (B) Most significantly altered pathways from comparative analysis of 0.33g FLIGHT vs HGC proximal femoral cartilage (Z-score>-2, p<0.001). (C) Most significantly altered pathways from comparative analysis of 0.67g vs HGC proximal femoral cartilage (Z-score>±2, p<0.001). (D) Most significantly altered pathways from comparative analysis of 1g vs HGC proximal femoral cartilage (Z-score>±2, p<0.001).

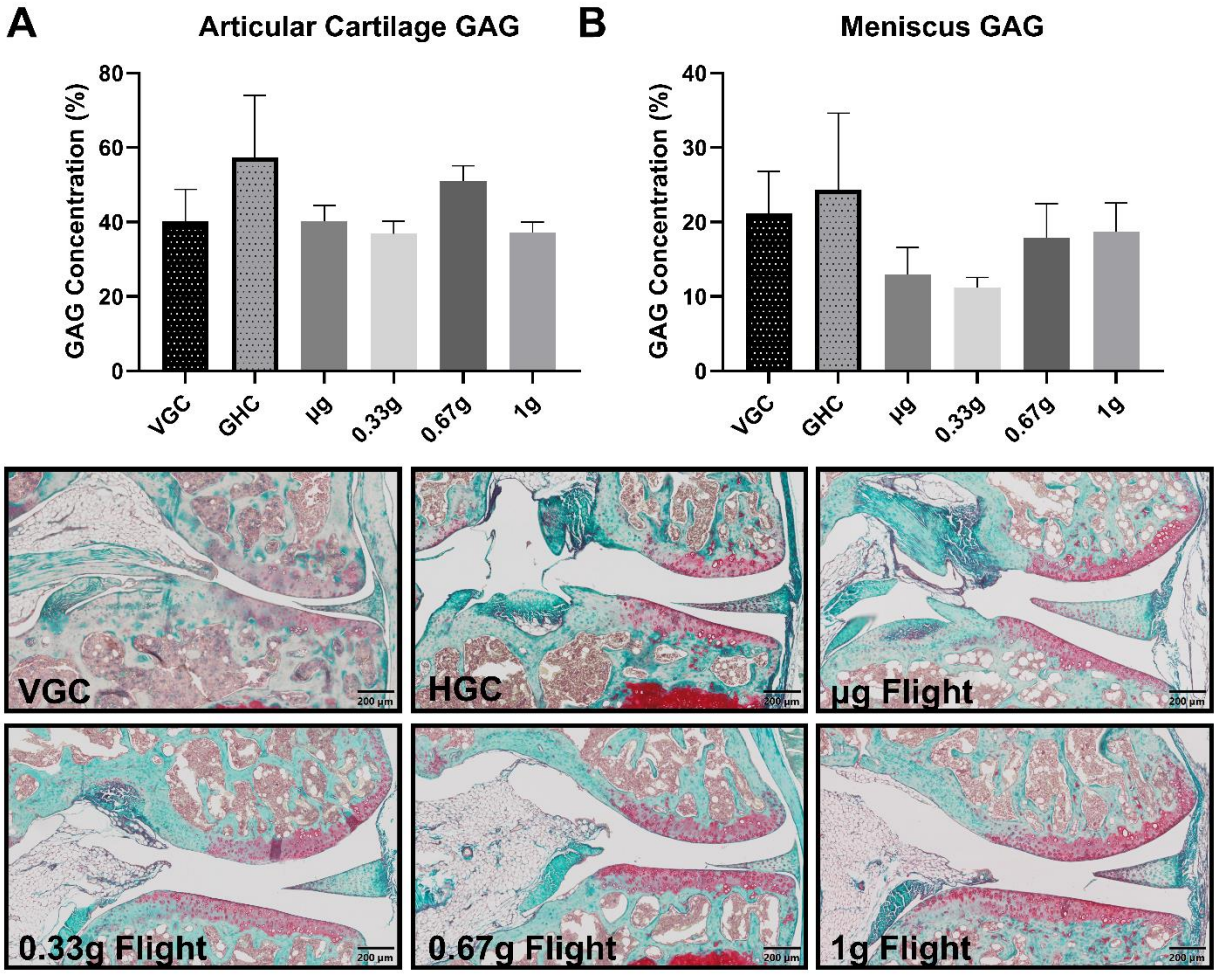

Figure 7. (A) Sulfated GAG in the medial tibial articular cartilage exhibited no significant differences between FLIGHT cohorts, but all FLIGHT groups had a lower sulfated GAG expression compared to HGC. (B) Medial meniscal sulfated GAG concentration was lower in all FLIGHT cohorts compared to HGC and VGC. Partial preservation of meniscal GAG concentration was observed in 0.67g and 1g FLIGHT compared to µg and 0.33g FLIGHT.

CHAPTER IV

An Introduction on the Musculoskeletal Complications During Spaceflight, Current Countermeasures, and Rationale for Study

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Summary of Doctoral Thesis

NASA has announced its Journey to Mars framework for deep space human exploration to Mars as early as 2030. Current Artemis missions aim to establish our long-term human presence on the Moon as a proving ground for future Mars missions. The progression of commercial technology and launch systems has increased feasibility of establishing a lunar and/or Martian colony and is becoming increasingly more realistic. However, before deep space travel can be considered, we must develop countermeasures to mitigate human health risks as outlined by NASA's Human Research Program, including the challenges: reduced weight bearing in partial gravity or microgravity, and radiation caused by solar flares or galactic cosmic events [1], [2]. Among these, risks of bone fracture due to spaceflight-induced changes to bone and risk of altered sensorimotor/vestibular function impacting critical mission tasks have been identified [3], [4]. While degradation of bone and muscle are well-documented during spaceflight [5]–[9], it is unclear how spaceflight affects mechanically sensitive menisci within the knees and synovial joints. Degraded joints during and after spaceflight can risk mission success, as pain, failure, and lowered stability can impact performance of astronauts during dynamic loading conditions, or as necessary, rapid egress maneuver. Because it is critical to identify these issues in joint health and investigate countermeasures for spaceflight-induced musculoskeletal complications, we performed a combination of spaceflight studies to the ISS assessing the efficacy of antioxidant treatment, partial gravity, and 1g artificial gravity on the preservation of knee joint health.

We measured the arthritic responses of the menisci and articular cartilage of mice during and after spaceflight as part of the Rodent Research-18 mission in 3 conditions:

spaceflight aboard the ISS for ~35 days, spaceflight aboard the ISS for ~35 days with 4-months on Earth recovery, and spaceflight aboard the ISS for ~60 days with animal sacrifice on orbit. Our structural and histological analysis identified cartilage degradation, specifically thinning of the articular cartilage in the medial tibial plateau and/or loss of GAGs, was observed after 35 days of spaceflight both with/without on Earth recovery, and at 75 days. Additionally, meniscal volume declined after 35 days of spaceflight; BuOE treatment preserved meniscal volume. In contrast to the response from articular cartilage, degradation of the menisci was not observed out to 120 days recovery in full weight bearing. Further examination of gene expression profiles of menisci found that spaceflight disrupts inflammatory responses and cellular stress pathways and increases IL1β-induced chondrocyte hypertrophy and FOXO1-medicated chondrocyte autophagy, inducing an osteoarthritis phenotype. Transcriptomic alterations within the proximal femoral cartilage after spaceflight indicated a disruption in chondrocyte differentiation, collagen synthesis, and extracellular matrix remodeling and a downregulation in these markers has been implicated in synovial joint inflammation and osteoarthritic pathogenesis. Notably, canonical pathway analysis treatment with BuOE indicated reduced oxidative stress via increased Hif1α and sirtuin signaling and enrichment of these pathways may exert a protective effect against osteoarthritis promoting chondrocyte survival, maintaining cartilage homeostasis, and suppressing inflammation. Our findings demonstrated treatment with BuOE preserved articular cartilage and menisci during short duration spaceflight by downregulating pro-arthritic pathways in the joint soft tissue through the reduction of oxidative stress.

In addition to the joint soft tissue responses, we also evaluated the trabecular and cortical bone alterations as part of the RR18 mission. Trabecular and cortical bone volume declined after both 35 days of spaceflight with/without on Earth recovery and 75 days of spaceflight; BuOE preserved trabecular bone volume only in the 35-day spaceflight group. In the context of these findings, it is important to note that treatment with BuOE was ceased after a return to Earth for all groups, which may have impacted the results for the recovery group. These findings are aligned with a study analyzing extent of bone recovery in astronauts after spaceflight aboard ISS, which found that cortical, and trabecular bone mineral density declined in short-term flight (6 months) and long-term flight (12 months) [7]. Overall, BuOE showed promising results as a potential countermeasure for whole joint preservation during long duration spaceflight.

The impact of spaceflight outside of low earth orbit, during deep space transit, and readaptations to partial gravity environments upon reaching a destination, are unclear. Reduced loadbearing in sub 1g environments may have deleterious effects on joint health, risking mission success and astronaut health. The Joint Partial Gravity (Rodent Research)-Mouse Habitat Unit 8 mission exposed mice to various gravitational exposures via centrifugation aboard the ISS. We tested if varied levels of artificial gravity (AG) via centrifugation aboard the ISS can prevent/attenuate gross and transcriptomic arthritic responses in mice. Spaceflight-induced meniscal degradation occurred in all flight groups and artificial gravity failed to preserve the tissue. However, continuous exposure to 1g prevented the pro-arthritic, pro-inflammatory, pro-apoptotic, anti-cellular stress response, anti-proteoglycan synthesis, anti-lipid synthesis, antioxidant defense pathways that were enriched or downregulated in μg, 0.33g (Mars gravity), and 0.67g

mice. In conclusion, 1g of AG aboard the ISS prevented pro-arthritic pathway enrichment and chondrocyte apoptosis, but exposure to lower g-levels resulted in pro-arthritic transcriptomic changes similar to those seen in the μg group. These findings were supported by proteomic analysis which indicated synovial inflammation and chondrocyte hypertrophy in μg, 0.33g, and 0.67g proximal femoral cartilage. These findings further support the findings from the RR18 study in that spaceflight results in a pre-arthritic phenotype of the knee joint. While artificial gravity via centrifugation failed to preserve the menisci of articular cartilage tissue, artificial 1g did prevent significant differential expression compared to ground animals at the transcriptomic and proteomic levels indicating that further investigation is necessary on AG's efficacy for the prevention of spaceflight-induced joint degradation.

Neurovestibular and musculoskeletal deficits resulting from exposure to microgravity during spaceflight can pose challenges to astronaut health and performance [1], [2], [10]–[19]. Pre- and post-flight gait analysis provides a non-invasive assay to measure the anatomic and functional consequences of spaceflight hazards in rodents, as gait pattern changes in mice reflect pathologies. Gait pattern changes in mice after ~35 days aboard the ISS as part of the Rodent Research 9 (RR9) mission reflected deficits observed in astronauts post-flight, including skeletal and neuromotor deficits, among others [20]. In addition to assessing joint soft tissue alterations as part of the MHU8 mission, additional investigation was conducted to test if artificial gravity can prevent gait and performance deficits, and if gait and performance deficits/prevention occur in a gravity-dose dependent manner. Continuous exposure to artificial gravity at 1g prevented gait abnormalities, while 0.67g attenuated these deficits. Importantly, exposure to 0.33g

(Mars gravity) resulted in pronounced gait pattern deficits, with 50% of mice unable to locomote linearly, while exposure to μg largely yielded mice non-functional with 0% of the mice able to locomote. This study identified that the patterns of gait abnormalities (particularly at 0.33g and attenuated at 0.67g) reflected a variety of different correlated conditions, but primarily arthritis, traumatic brain injury, neurological dysfunction, and ataxia. These findings highlight that gravitational loading conditions can have a major impact on functional and physiologic responses.

Importance of Findings

We determined that potential joint degradation does occur after exposure to spaceflight conditions. Structural analysis via contrast enhanced microCT identified a decrease in volume at the tibial-femoral cartilage-cartilage contact point, decreased meniscal volume, and a decrease in cortical and trabecular bone volume after 35 days aboard the ISS. These findings were further supported via proteomic and/or transcriptomic analyses which identified an increase in pro-osteoarthritic signaling in the joint soft tissue after exposure to spaceflight conditions in both the RR18 and MHU8 missions. These findings are in agreement with RR9, further establishing an evidence base of spaceflight-induced osteoarthritis of the knee and/or hip cartilage. Furthermore, the results from the MHU8 study indicate that there is no gravitational threshold for spaceflight-induced joint degradation, as the meniscal volume loss and decrease in articular cartilage was similar across all flight cohorts. This study addresses the overall importance of understanding joint health deficiencies caused by the spaceflight environment that will need to be addressed for long duration spaceflight outside of LEO.

Our study examined two potential methods of countermeasures against spaceflight-induced joint soft tissue damage in treatment with the SOD mimetic BuOE (RR18), and exposure to in-flight artificial gravity at 0.33g, 0.67g, and 1g (MHU8). Treatment with BuOE reduced meniscal, articular cartilage, trabecular and cortical bone degradation in the 35 day flight cohort. Treatment with BuOE did not elicit the same protective properties in the 75-day flight and 120-day recovery cohorts. It is important to note that BuOE treatment was provided 1 week prior to launch and then weekly aboard the ISS, and this may have impacted the results for the 120-day cohort. Importantly, this study also identified that AG does not protect against joint soft tissue damage during spaceflight but does reduce pro-osteoarthritic transcriptomic and proteomic changes in a gravitational dose dependent manner. If joint health in astronauts exemplifies similar degradation effects, the damages caused by extended spaceflight missions could potentially jeopardize a mission requiring long-term inhabitance and/or decreased quality of life. Furthermore, this study identified that the partial gravity that astronauts will encounter on missions to the Moon and Mars may not be sufficient to prevent the deleterious effects of spaceflight seen at μg on joint health.

Our study addressed a proposed methodology for mitigating potential function and performance deficiencies resulting from spaceflight by utilizing rodent gait analysis. Gait analysis is already performed in astronauts [21], [22], but implementation into spaceflight murine models has only been performed once previously as part of the RR9 mission [20]. Our findings were in agreement with RR9, as gait pattern deficits for the MHU8 study largely reflected neuromotor deficits and increased instability; these patterns were attenuated with increasing gravitational loading. Animals exposed to

continuous 1g AG maintained gait patterns similar to controls, however, significant performance deficits were observed with only 50% of the 0.33g and 0% of the μg groups able to locomote linearly. With spaceflight providing many challenges to the musculoskeletal, neuromotor, and vestibular systems in astronauts, rodent gait analysis could provide a rapid non-invasive method to investigate potential preventative treatments against performance deficits as a result of spaceflight.

Conclusion

We have identified that spaceflight conditions have deleterious effects on joint health which will need to be addressed prior to future manned missions outside of LEO. Treatment with BuOE reduced meniscal, articular cartilage, trabecular and cortical bone degradation during 35 days of spaceflight. The efficacy of BuOE treatment could be further evaluated with continued treatment after a return to Earth. Artificial gravity does not protect against joint soft tissue degradation during spaceflight but does reduce pro-osteoarthritic signaling in the articular cartilage and joint-stabilizing menisci in a gravitational dose dependent manner. Gait analysis identified that spaceflight results in neuromotor deficits and increased instability, which were prevented in a gravitational dose dependent manner. Further investigation of these countermeasures against spaceflight-induced joint degradation and performance deficits would help eliminate potential risk to future spaceflight endeavors and preserve quality of life in astronauts.

Future Directions for Research

These results provide opportunities to continue the studies assessing BuOE treatment and artificial gravity for joint preservation and prevention of performance deficits during spaceflight. It also provides further direction towards potential targeted therapeutics on mechanisms and pathways for preservative effects. Major questions include: 1) would continued treatment with antioxidants continue to preserve joint health after spaceflight; 2) would continuous exposure to artificial gravity for longer duration prevent further joint degradation; 3) what role do housing conditions play in spaceflight experiments (i.e. JAXA habitats vs NASA habitats); 4) are there additional countermeasures that preserve gait after spaceflight; and 5) would a combination countermeasure with AG protect joint health in space.

Our canonical pathway analysis indicated a disruption in SPARC mediated IGF-1 expression, and reduced PDK4 mediated PPAR pathway activation in the joint soft tissue across both flight studies, indicating that a disruption in these pathways may contribute to spaceflight-induced joint degradation. Developing treatments that may help counteract these alterations may provide a novel countermeasures for the preservation of musculoskeletal health during spaceflight. While our study has demonstrated the efficacy of SOD mimetic treatment for joint preservation, the next step would be to perform a second spaceflight study with continued treatment after a return to Earth. Our findings also suggest that artificial gravity may have some preservative effect, however a second longer duration study may be needed to further validate its efficacy. Our study further demonstrated the utility of gait analysis for the characterization of functional deficiencies in rodents after spaceflight, tracking the animals' gait could prove useful for understanding how astronauts can recover from neuromotor and balance deficiencies

caused by the mission. Cartilage and meniscal degradation in mice have been documented with reduced weight bearing during spaceflight aboard the ISS. Cartilage and joint degradation could increase the risk of joint failure during dynamic loading conditions, and potential performance deficits in mission and health complications post return in astronauts. Adverse biologic adaptations to microgravity during spaceflight challenge astronaut health and performance, and further characterization and investigation is still needed to better prepare for upcoming long duration missions outside of LEO.

References

[1] Z. S. Patel *et al.*, "Red risks for a journey to the red planet: The highest priority human health risks for a mission to Mars," *npj Microgravity 2020 61*, vol. 6, no. 1, pp. 1–13, Nov. 2020, doi: 10.1038/s41526-020-00124-6.

[2] C. Krittanawong *et al.*, "Human Health during Space Travel: State-of-the-Art Review," *Cells*, vol. 12, no. 1, Jan. 2023, doi: 10.3390/CELLS12010040/S1.

[3] R. D. Seidler, A. P. Mulavara, J. J. Bloomberg, and B. T. Peters, "Individual predictors of sensorimotor adaptability.," *Front. Syst. Neurosci.*, vol. 9, no. July, p. 100, Jul. 2015, doi: 10.3389/fnsys.2015.00100.

[4] "HRR - Risk - Risk of Bone Fracture due to Spaceflight-induced Changes to Bone." https://humanresearchroadmap.nasa.gov/Risks/risk.aspx?i=77 (accessed Jun. 08, 2024).

[5] M. A. Bucaro *et al.*, "Bone cell survival in microgravity: evidence that modeled microgravity increases osteoblast sensitivity to apoptogens," *Ann. N. Y. Acad. Sci.*, vol. 1027, pp. 64–73, 2004, doi: 10.1196/ANNALS.1324.007.

[6] T. F. Lang, A. D. Leblanc, H. J. Evans, and Y. Lu, "Adaptation of the proximal femur to skeletal reloading after long-duration spaceflight," *J. Bone Miner. Res.*, vol. 21, no. 8, pp. 1224–1230, Aug. 2006, doi: 10.1359/JBMR.060509.

[7] L. Gabel *et al.*, "Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia 1 year after return from long duration spaceflight," *Sci. Rep.*, vol. 12, no. 1, Dec. 2022, doi: 10.1038/S41598-022-13461-1.

[8] P. Comfort *et al.*, “Effects of Spaceflight on Musculoskeletal Health: A Systematic Review and Meta-analysis, Considerations for Interplanetary Travel,” *Sports Med.*, vol. 51, no. 10, p. 2097, Oct. 2021, doi: 10.1007/S40279-021-01496-9.

[9] C. G. T. Tahimic and R. K. Globus, “Redox Signaling and Its Impact on Skeletal and Vascular Responses to Spaceflight,” *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102153.

[10] A. M. Liphardt, R. Fernandez-Gonzalo, K. Albracht, J. Rittweger, and L. Vico, “Musculoskeletal research in human space flight – unmet needs for the success of crewed deep space exploration,” *npj Microgravity 2023 91*, vol. 9, no. 1, pp. 1–7, Jan. 2023, doi: 10.1038/s41526-023-00258-3.

[11] P. Comfort *et al.*, “Effects of Spaceflight on Musculoskeletal Health: A Systematic Review and Meta-analysis, Considerations for Interplanetary Travel,” *Sports Med.*, vol. 51, no. 10, p. 2097, Oct. 2021, doi: 10.1007/S40279-021-01496-9.

[12] M. Tomsia *et al.*, “Long-term space missions’ effects on the human organism: what we do know and what requires further research,” *Front. Physiol.*, vol. 15, p. 1284644, Feb. 2024, doi: 10.3389/FPHYS.2024.1284644.

[13] Y. Yin *et al.*, “Long-term spaceflight composite stress induces depression and cognitive impairment in astronauts—insights from neuroplasticity,” *Transl. Psychiatry 2023 131*, vol. 13, no. 1, pp. 1–7, Nov. 2023, doi: 10.1038/s41398-023-02638-5.

[14] I. of M. (US) C. on C. a V. for S. M. D. T. B. E. Orbit, J. R. Ball, and J. Charles H. Evans, “Risks to Astronaut Health During Space Travel,” 2001, Accessed: Apr. 23, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK223785/.

[15] G. Clément, S. C. Moudy, T. R. Macaulay, M. O. Bishop, and S. J. Wood, “Mission-critical tasks for assessing risks from vestibular and sensorimotor adaptation during space exploration,” *Front. Physiol.*, vol. 13, p. 1029161, Nov. 2022, doi: 10.3389/FPHYS.2022.1029161/BIBTEX.

[16] T. K. Clark, “Effects of Spaceflight on the Vestibular System,” *Handb. Sp. Pharm.*, pp. 1–39, 2019, doi: 10.1007/978-3-319-50909-9_2-1.

[17] M. F. Reschke and G. Clément, “Vestibular and Sensorimotor Dysfunction During Space Flight,” *Curr. Pathobiol. Rep.*, vol. 6, no. 3, pp. 177–183, Sep. 2018, doi: 10.1007/S40139-018-0173-Y/METRICS.

[18] M. F. Reschke, E. F. Good, and G. R. Clément, “Neurovestibular Symptoms in Astronauts Immediately afterSpace Shuttle and International SpaceStation Missions,” *OTO Open*, vol. 1, no. 4, 2017, doi: 10.1177/2473974X17738767.

[19] J. Carriot, I. Mackrous, and K. E. Cullen, “Challenges to the Vestibular System in Space: How the Brain Responds and Adapts to Microgravity,” *Front. Neural Circuits*, vol. 15, p. 760313, Nov. 2021, doi: 10.3389/FNCIR.2021.760313/BIBTEX.

[20] A. Kwok *et al.*, “Altered rodent gait characteristics after ~35 days in orbit aboard

the International Space Station," *Life Sci. Sp. Res.*, vol. 24, pp. 9–17, Feb. 2020, doi: 10.1016/J.LSSR.2019.10.010.

[21] A. Saveko *et al.*, "Foot-ground reaction force during long-term space flight and after it: walking in active treadmill mode," *Gait Posture*, vol. 76, pp. 382–388, Feb. 2020, doi: 10.1016/J.GAITPOST.2019.12.033.

[22] A. Saveko, V. Brykov, V. Kitov, A. Shpakov, and E. Tomilovskaya, "Adaptation in Gait to Lunar and Martian Gravity Unloading During Long-Term Isolation in the Ground-Based Space Station Model," *Front. Hum. Neurosci.*, vol. 15, Jan. 2021, doi: 10.3389/FNHUM.2021.742664.

CURRICULUM VITAE

Chirayu Patel

EDUCATION

Doctor of Philosophy in Molecular Medicine & Translational Science 2024
Wake Forest Graduate School, Winston-Salem, NC
Concentration: Radiation Biology
Dissertation: *The Investigation of Countermeasures Against Spaceflight-Induced Joint Degeneration*

Master of Science in Molecular and Cellular Biosciences December 2019
Wake Forest Graduate School, Winston-Salem, NC
Concentration: Cancer Biology
Thesis: *Characterization of the Bone Microenvironment Cell Composition in Ovarian Cancer*

Bachelor of Science in Cell/Molecular Biology May 2014
Appalachian State University, Boone, NC
Minors: Chemistry, Medical Humanities
Honors: University, Departmental, Latin Honors
Thesis: *The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer.*

PROFESSIONAL EXPERIENCE

2024-2024 **Research Scientist**: Seward Rutkove, Ph.D., National Aeronautics and Space Administration Space Radiation Lab, Brookhaven National Laboratory

2023-2024 **MHU8 Dissection Team Member:** Mary Bouxsein, Ph.D., National Aeronautics and Space Administration, Kennedy Space Center

2023-2024 **MHU8 Behavioral Testing Team Member:** Mary Bouxsein, Ph.D., National Aeronautics and Space Administration, Kennedy Space Center

2022-2023 **Research Scientist**: Jeff Chancellor, Ph.D., National Aeronautics and Space Administration Space Radiation Lab, Brookhaven National Laboratory

2022-2023 **Musculoskeletal RR18 Dissector:** Xiao Mao, Ph.D., National Aeronautics and Space Administration, Rodent Research 18 Mission

2021-2022 **Research Scientist**: John Baker, Ph.D., National Aeronautics and Space Administration Space Radiation Lab, Brookhaven National Laboratory

2020-2021 **Research Laboratory Technician III**: Yusuke Shiozawa, M.D., Ph.D., Cancer Biology, Wake Forest University, School of Medicine

2019-2020 **Graduate Research Assistant: Thesis Project.** Bethany Kerr, Ph.D., Department of Cancer Biology, Wake Forest University, School of Medicine.

2016-2019 **Research Laboratory Technician II:** Neveen Said, M.D., Ph.D., Department of Cancer Biology, Wake Forest University, School of Medicine.

2012-2014 **Research Assistant: Thesis Project**. Maryam Ahmed, Ph.D., Department of Biology, Appalachian State University.

INTERNSHIPS

2013 **Zambia Clinical Shadowing Internship**
Ndola Central Hospital (Surgery, Medicine, OBGYN)
Arthur Davidson Children's Hospital (Pediatrics)

2012 **South Africa Clinical Shadowing Internship**
Riverlea Clinic (AIDS/TB)
Chris Hani Baragwanath hospital (Endocrinology, Maternity)
DFC Clinic at Univ. of Johannesburg (Chiropractic, Biokinetics)
Johannesburg General Hospital (Podiatry, Spine Specialist)
Soweto Children's Clinic (Homeopathic Medicine)

PUBLICATIONS

1. **Patel CM**, Vander Wiele SS, Reno K, Kim L, Costa-Teryll A, Coulombe J, Viaterna M, Fuller C, Takahasi S, Bouxsein ML, Willey JS. (2024). Arthritic responses of mouse articular cartilage and menisci to artificial gravity aboard the international space station. *Nature Microgravity*. (in preparation).

2. **Patel CM**, Kim L, Reno K, Coulombe J, Viaterna M, Fuller C, Takahasi S, Bouxsein ML, Willey JS. (2024). Artificial gravity during spaceflight prevents gait and performance deficits in a gravity-dose dependent manner. *Nature Microgravity*. (in submission).

3. **Patel CM**, Vander Wiele SS, Kim L, Payne E, Bruno-Garcia M, Kaganov DE, Devorak A, Guthold M, Lau A, Guthold M, Delp MD, Crapo J, Mao XW, Willey JS. (2024). Treatment with a superoxide dismutase mimetic for joint preservation

during 35 and 75 days in orbit aboard the International Space Station, and after 120 days recovery on Earth. *Life Sciences in Space Research*. (in submission)

4. Nemec-Bakk AS, Sridharan V, Willey JS, Koturebash I, Williams DK, **Patel CM**, Reno K, Gifford G, Newhauser W, Williams J, Chancellor JC, Boerma M. (2024). Sex-specific effects on the heart from combined exposure to simulated galactic cosmic radiation and microgravity. *Life Sciences and Space Research*. (in preparation)

5. Tan L, Armstrong AR, Rosas S, **Patel CM**, Vander Wiele SS, Willey JS, Carlson CS, Yammani RR. (2023). Nuclear protein-1 is the common link for pathways activated by aging and obesity in chondrocytes: A potential therapeutic target for osteoarthritis. *FASEB Journal*.

6. Foster BM, Shi L, Harris KS, **Patel CM**, Surratt VE, Langsten KL, Kerr BA. (2022). Bone marrow-derived stem cell factor regulates prostate cancer-induced shifts in pre-metastatic niche composition. *Front Oncol.*

7. **Patel CM**, Shi L, Whitesides JF, Foster BM, Fajardo RJ, Quillen EE, Kerr BA. (2022). A new method of bone stromal cell characterization by flow cytometry. *Current Protocols*.

8. **Patel CM**, Wadas TJ, Shiozawa Y. (2021). Progress in Targeted Alpha-Particle-Emitting Radiopharmaceuticals as Treatments for Prostate Cancer Patients with Bone Metastases. *Molecules.*

9. Park SH, Eber MR, Fonseca MM, **Patel CM**, Cunnane KA, Ding H, Hsu FC, Peters CM, Ko MC, Strowd RE, Wilson JA, Hsu W, Romero-Sandoval EA, Shiozawa Y. (2021). Usefulness of the measurement of neurite outgrowth of primary sensory neurons to study cancer-related painful complications. *Biochem Pharmacol*.
10. Eber MR, Park SH, Contino KF, **Patel CM**, Hsu FC, Shiozawa Y. (2020). Osteoblasts derived from mouse mandible enhance tumor growth of prostate cancer more than osteoblasts derived from long bone. *J Bone Oncol.*

11. John B, Naczki C, **Patel C**, Ghoneum A, Qasem S, Salih Z, Said N. (2019). Regulation of the bi-directional cross-talk between ovarian cancer cells and adipocytes by SPARC. *Oncogene.*

12. Naczki C, John B, **Patel C**, Lafferty A, Ghoneum A, Affify H, White M, Davis A, Jin G, Kridel S, Said N. (2019) SPARC Inhibits Metabolic Plasticity in Ovarian Cancer. *Cancers (Basel).*

13. Ahmed M, **Patel CM**, Fehl, DJ. (2015). The use of oncolytic vesicular stomatitis virus in conjunction with natural products for the treatment of cervical cancers. *Adv. Tech. Biol. Med.*

PRESENTATIONS (* indicates mentored student presentation)

2024 Ashley Nemec-Bakk, Marjan Boerma, Jeffrey Willey, Igor Koturbash, Vijakalakshmi Sidharan, **Chirayu Patel**, Alex Borg, Kaitlyn Reno, Wayne Newhauser, Abdelraham Fouda, Jackie Williams, Jeffery Chancellor. Degenerative Effects of Long-Distance Space Travel on the Mouse Retina. Committee on Space Research Scientific Assembly, July 2024. (poster presentation).

2024 Kaitlyn Reno, Sun Park, Micheal Farris, Ryan Hughes, Joseph Moore, **Chirayu Patel**, Jeffrey Willey. Cartilage oligomeric matrix protein as a potential biomarker and therapeutic target of radiation resistance in NSCLC. American Association for Cancer Research Annual Meeting 2024. (abstract 2889, poster presentation)

2024 Daniel Kaganov, **Chirayu Patel***, Sabrina Vander Wiele, Leslie Kim, Kaitlyn Reno, Michelle Bruno-Garcia, Micheal Delp, Xiao Mao, and Jeffrey Willey. A Use of a Superoxide Dismutase Mimetic to Protect Joint Soft-Tissue During Short and Long Duration Spaceflight. Aerospace Medical Association Annual Meeting, May 2024. (poster presentation).

2024 Jeffrey S. Willey, **Chirayu M. Patel**, Kaitlyn Reno, Rae Young Leslie Kim, Jennifer Coulombe, Charles Fuller, Satoru Takahashi, Martha Hotz Vitaterna, Mary L. Bouxsein, Artificial Gravity During Spaceflight Prevents Gait and Performance Deficits in a Gravity-Dose Dependent Manner. Human Research Program Investigators' Workshop, February 2024. (oral presentation).

2024 **Chirayu Patel**, Kaitlyn Reno, Alex Borg, Deborah Snyder, Paula Bennett, Ashley Nemec-Bakk, Charles Pearson, Micheal Sivertz, Adam Rusek, Peter Guida, Igor Koturbash, Maryan Boerma, Jeffery Chancellor, Jeffrey. Willey, Prolonged Low-Dose Rate Irradiation of Both Tail Suspended and Full Weight Bearing Mice Over 24H Using Simulated Galactic Cosmic Rays. Human Research Program Investigators' Workshop, February 2024. (poster presentation).

2024 **Chirayu Patel**, Sabrina Vander Wiele, Kaitlyn Reno, Leslie Kim, Jennifer Coulombe, Charles Fuller, Satoru Takahashi, Martha Hotz Vitaterna, Mary L. Bouxsein, Jeffrey S. Willey, Arthritic Responses of Mouse Knee Menisci to Artificial Gravity Aboard the International Space Station. Human Research Program Investigators' Workshop, February 2024. (poster presentation).

2024 Kirsten Clement, Ashley Nemec-Bakk, Vijakalakshmi Sidharan, **Chirayu Patel**, Wayne Newhauser, Abdelraham Fouda, Jeffrey Willey, Jackie Williams, Marjan Boerma, Jeffery Chancellor, Igor Koturbash. Combination of Galactic Cosmic Radiation Exposure and Simulated Microgravity Result in Late Pro-Inflammatory Responses and Epigentic Reprogramming in the Mouse Lung. Human Research Program Investigators' Workshop, February 2024. (oral presentation).

2024 Ashley Nemec-Bakk, Marjan Boerma, Jeffrey Willey, Igor Koturbash, **Chirayu Patel**, Alex Borg, Vijakalakshmi Sidharan, Gabriel Gifford, Wayne Newhauser, Abdelraham Fouda, Jackie Williams, Jeffery Chancellor. Effects of Galactic Cosmic Radiation and Simulated Microgravity on the Cardiovascular System and Retina. Human Research Program Investigators' Workshop, February 2024. (poster presentation).

2023 **Chirayu Patel**, Jeff Willey, Kaitlyn Reno, Debroah Snyder, Paula Bennett, Ashley Nemec-Bakk, Charles Pearson, Micheal Sivertz, Adam Rusek, Peter Guida, Igor Koturbash, Maryan Boerma, Jeff Chancellor. Prolonged Low-Dose Rate Irradiation of Both Tail Suspended and Full Weight Bearing Mice over 24h using Simulated Galactic Cosmic Rays. American Society for Gravitational and Space Research, November 2023. (oral presentation).

2023 **Chirayu Patel**, Sabrina Vander Wiele, Kaitlyn Reno, Leslie Kim, Michelle Bruno-Garcia, Daniel Kaganov, Micheal Delp, Xiao Mao, and Jeffrey Willey. A Superoxide Dismutase Mimetic is a Countermeasure against Knee and Hip Joint Soft Tissue Arthritic Responses during Short and Long Duration Spaceflight. American Society for Gravitational and Space Research, November 2023. (poster presentation).

2023 Daniel Kaganov, **Chirayu Patel***, Sabrina Vander Wiele, Leslie Kim, Kaitlyn Reno, Michelle Bruno-Garcia, Micheal Delp, Xiao Mao, and Jeffrey Willey. A Use of a Superoxide Dismutase Mimetic to Protect Joint Soft-Tissue During Short and Long Duration Spaceflight. Medical Student Research Day (MSRD) – Wake Forest School of Medicine, October 2023. (poster presentation).

2023 Leslie Kim, **Chirayu Patel***, Kaitlyn Reno, Mary Bouxsein, and Jeffrey Willey. Increased Gravitational Loading Prevents Performance Deficits

During Spaceflight. Biomedical Engineering Society Annual Meeting, October 2023. (poster presentation).

2023 Leslie Kim, **Chirayu Patel***, Kaitlyn Reno, Mary Bouxsein, and Jeff Willey. Increased Gravitational Loading Prevents Performance Deficits During Spaceflight. NSF Research Experience for Undergraduates-Wake Forest Symposium, August 2023. (oral presentation).

2023 Michelle Bruno, **Chirayu Patel***, Sabrina Vander Wiele, Xiao Mao, Jeffrey Willey. An Antioxidant Countermeasure to Protect Joint Soft Tissue during Long Duration Spaceflight Aboard the International Space Station. Program to Enhance Diversity in Cancer Research-Wake Forest Symposium, July 2023. (poster presentation).

2023 Sabrina Vander Wiele, **Chirayu Patel***, Jeffrey Willey, Anthony Lau. Antioxidant countermeasure effects on bone properties during and after spaceflight. New Jersey Space Grant Consortium Internship Poster Session, April 2022. (poster presentation).

2022 **Chirayu Patel**, Andy Kwok, Sam Rosas, Ted Bateman, Eric Livingston, Joseph Moore, Ragunatha Yammani, Xiao Mao, Michael Delp, Jeffrey Willey. Spaceflight and reduced weight-bearing on earth damaged menisci. Orthopedic Research Society Meniscus Seminar Series, December 2022. (invited oral presentation).

2022 Anne Devorak, **Chirayu Patel***, Ethan Payne, Sabrina Vander Wiele, Xiao Mao, Jeffrey Willey (2022). Use of a superoxide dismutase mimetic to prevent spaceflight-induced bone and joint degradation in mice. The American College of Veterinary Pathology, Boston, MA, November 2022. (poster presentation).

2022 Sabrina Vander Wiele, **Chirayu Patel***, James Crapo, Xiao Mao, Jeffrey Willey. An antioxidant countermeasure protects joint health during spaceflight. Biomedical Engineering Society Annual Meeting, October 2022. (poster presentation).

2022 Anne Devorak, **Chirayu Patel***, Ethan Payne, Xiao Mao, Jeffrey Willey. Use of a superoxide dismutase mimetic to prevent spaceflight-induced bone and joint degradation in mice. National Veterinary Scholars Symposium, Minneapolis, MN, August 2022. (poster presentation).

2022 Ethan Payne, **Chirayu Patel***, Xiao Mao, Jeffrey Willey. Treatment with a superoxide dismutase mimetic and/or return to full weight-bearing to prevent bone and joint degradation from spaceflight. Wake Forest University Undergraduate Student Research Day, October 2022. (poster presentation).

2022 **Chirayu Patel**, Sabrina Vander Wiele, Nequesha Mohammed, Michael Delp, Xiao Mao, Jeffrey Willey. A Superoxide Dismutase Mimetic and/or Return to Full Weight-Bearing to Prevent Bone and Joint Degradation from Spaceflight. American Society for Gravitational and Space Research, October 2022. (oral presentation).

2022 Ethan Payne, **Chirayu Patel***, Sabrina Vander Wiele, Xiao Mao, Jeffrey Willey. An antioxidant Countermeasure Protects Joint Health during Spaceflight. The Wake Forest Department of Physics Colloquium, August 2022. (oral presentation).

2022 Sabrina Vander Wiele, **Chirayu Patel***, Xiao Mao, Jeffrey Willey. An antioxidant countermeasure protects joint health during spaceflight. NSF Research Experience for Undergraduates-Wake Forest Symposium, August 2022. (oral presentation).

2022 Nequesha Mohamed, John Olson, Johannes Plate, **Chirayu Patel**, Mark Cline, Jeffrey Willey. Diabetes is associated with an increased osteoarthritis incidence in non-human primates. International Cartilage Regeneration and Joint Preservation Society World Congress Meeting, April 2022. (oral presentation).

2021 **Chirayu Patel**, Andy Kwok, Joseph Moore, Jeffrey Willey. Suppressing IRE-1α to reduce ER stress may not effectively prevent radiation-induced bone loss. Radiation Research Society Annual Meeting 2021. (poster presentation).

2019 **Chirayu Patel**, Lihong Shi, Sam Rosas, Katherine Cook, Jeffery Willey, Ellen Quillen, Bethany Kerr. Bone Microenvironment Alterations with Ovarian Cancer and Comorbidities. Gordon Research Conference-Hormone Dependent Cancer 2019. (poster presentation).

2018 **Chirayu Patel**, Cristina Ivan, Anthony Atala, Neveen Said. Identification of novel therapeutics of bladder cancer cells through upregulation of SPARC expression using 2D cell cultures and 3D bladder organoids. American Association for Cancer Research Annual Meeting 2018. (abstract 5035, poster presentation).

2018 Christine Naczki, Bincy John, **Chirayu Patel**, Ashlyn Lafferty, Alia Ghoneum, Hesham Afify, Amanda Davis, Guangxu Jin, Neveen Said. Integrated Molecular Analysis Reveals Novel SPARC-Regulated Metabolic Programming in Ovarian Cancer. American Association for Cancer Research Annual Meeting 2018. (abstract 1448, poster presentation).

2017 **Chirayu Patel**. Development of Fluorescent-Cell Based High Throughput Assay to Identify Novel Therapeutics of Bladder Cancer Cells Through Upregulation of SPARC Expression. American Association for Cancer Research Annual Meeting 2018. (abstract 967, poster presentation).

2014 **Chirayu Patel**. The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer. Student Research and Creative Endeavors 2014, Appalachian State University. (oral presentation).

2013 **Chirayu Patel**. The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer. Student Research and Creative Endeavors 2013, Appalachian State University. (poster presentation).

2013 **Chirayu Patel**. The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer. 2013 Meeting of the North Carolina Branch of the American Society for Microbiology. (abstract 7, poster presentation).

2013 **Chirayu Patel**. Response of Cervical Cancer Cells to Infection with Vesicular Stomatitis Virus. 2013 Science in the Mountains Meeting, Appalachian State University. (poster presentation).

2012 **Chirayu Patel**. Response of Cervical Cancer Cells to Infection with Vesicular Stomatitis Virus. Student Research and Creative Endeavors 2012, Appalachian State University. (poster presentation).

2012 **Chirayu Patel**. Response of Cervical Cancer Cells to Infection with Vesicular Stomatitis Virus. The State of North Carolina Undergraduate Research and Creativity Symposium 2012, Duke University. (poster presentation).

2012 **Chirayu Patel**. Response of Cervical Cancer Cells to Infection with Vesicular Stomatitis Virus. 2012 Meeting of the North Carolina Branch of the American Society for Microbiology. (abstract 28, poster presentation).

LEADERSHIP ACTIVITIES

2023-present **President**, American Society for Gravitational and Space Research

2022-2023 **President-Elect**, American Society for Gravitational and Space Research

2011-2104 **President of BBB Honor Society**, Appalachian State University

2012-2103 **South African Exchange Student Welcome Committee Leader**, Appalachian State University

2011 **Health Professions Club Homecoming Committee Leader**, Appalachian State University

2011 **Camp Open Airways Camp Counselor**, Jeff Gordon Children's Hospital

ACADEMIC AND COMMITTEE SERVICE

ALSDA Analysis Working Group (2023-present)
ASGSR Inclusion, Diversity, Equity Committee (2024-present)
ASGSR Award Committee (2022-present)
ASGSR Education and Outreach Committee (2022-present)
ASGSR Programs Committee (2022-present)
ASGSR Inspiration Scholar Selection Committee (2022-present)

PROFESSIONAL AND ACADEMIC MEMBERSHIPS

American Society for Gravitational and Space Research
Orthopedic Research Society
Radiation Research Society
The American Association for Cancer Research
Phi Kappa Phi Honor Society
Beta Beta Beta, Biological Science Honor Society
Pi Gamma Mu, Social Science Honor Society
Omicron Delta Kappa, Leadership Honor Society

AWARDS AND HONORS

2024 Student Leadership Congressional Advocate for the Advanced Funding for Spaceflight Research and ISS Utilization, ASGSR.
2023 Molecular Medicine and Translational Science Travel Award
2023 Student Leadership Congressional Advocate for the Advanced Funding for Spaceflight Research and ISS Utilization, ASGSR.
2023 Student Leadership Travel Award ASGSR
2023 MHU8 Research featured by Wake Forest School of Medicine
2023 MHU8 Research featured by NC-PBS highlighting research conducted in North Carolina
2023 Excellence Award, MSRD 2023, Wake Forest School of Medicine
2023 First Place, Research Experience for Undergraduates-Wake Forest Symposium
2023 Invited to Congress to Advocate for the Advanced Funding for Spaceflight Research and ISS Utilization, ASGSR.
2022 NASA BSP Honored Guest, ASGSR 2022 Awards Banquet
2022 Platform Presentation, The American College of Veterinary Pathology Meeting

2022	First Place, Research Experience for Undergraduates-Wake Forest Symposium
2022	Molecules Volume 26 Issue 8 Journal Cover Recipient, Wake Forest Baptist Health
2020	Gordon A. Melson Outstanding MS Student Nominee, Wake Forest Graduate School
2014	Derrick Biology Scholarship, Appalachian State University
2014	Biology Alumni/Faculty Scholarship, Appalachian State University
2013	Office of Student Research Travel Grant, Appalachian State University
2013	FND-Study Abroad Scholarship, Appalachian State University
2013	Peggy Harmon Healthcare Scholarship, Appalachian State University
2013	Biology Alumni/Faculty Scholarship, Appalachian State University
2013	Lloyd L. Hobbs Memorial for the Physical Sciences Scholarship, Appalachian State University
2012	Honors Study Abroad Scholarship, Appalachian State University
2012	Williamsen Study Abroad Scholarship, Appalachian State University
2012	Ida Bell Ledbetter Scholarship, Appalachian State University
2012	Lloyd L. Hobbs Memorial for the Physical Sciences Scholarship, Appalachian State University
2010	Biology Alumni/Faculty Scholarship, Appalachian State University

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MENTORING RELATIONSHIPS

Medical Students and Veterinary Students:

2023 Daniel Kaganov: Wake Forest University School of Medicine Medical Student. Special Opportunity Research (MS2024). Project – "Cartilage preservation during spaceflight" **Winner: Excellence Award, Outstanding Student of Space Medicine**

2022 Anne Devorak
Doctor of Veterinary Medicine Student
T35 Summer Program
Project: Bone and kidney toxicity after spaceflight: pathologic assessment
Winner: Distinguished Poster Podium Award

Undergraduate Students:

2023 Michelle Bruno-Garcia: Training Program to Promote Diversity in Cancer Research (PIs, Watabe and Kridel). Winston-Salem State University Undergraduate. "An antioxidant countermeasure for joint damage during very long duration spaceflight". **Winner: First Place Poster Award**

2023 Rae Young L. Kim: Biomedical Engineering Department Summer Research Experience for Undergraduates, University of Virginia.

	"Increased Gravitational Loading Mitigates Performance Deficits During Spaceflight" **Winner: First Place Injury Biomechanics Section.**
2022-2023	Ethan Payne. Wake Forest University Physics Major Honors Thesis: Histological Analysis of Spaceflight Tissue to Analyze Effectiveness of Antioxidant Treatment to Prevent Bone and Joint Degradation, and A Method to Analyze Mechanical Effects of Spaceflight on Joint Tissue.
2022	Sabrina Vander Weihl, Biomedical Engineering Dept. Research Experience for Undergraduates. The College of New Jersey Rising Senior. "Image-based assessment of cartilage and bone damage during spaceflight" **Winner: First Place Injury Biomechanics Section**

REFERENCES

[1] A. Saveko *et al.*, "Foot-ground reaction force during long-term space flight and after it: walking in active treadmill mode," *Gait Posture*, vol. 76, pp. 382–388, Feb. 2020, doi: 10.1016/J.GAITPOST.2019.12.033.

[2] A. Saveko, V. Brykov, V. Kitov, A. Shpakov, and E. Tomilovskaya, "Adaptation in Gait to Lunar and Martian Gravity Unloading During Long-Term Isolation in the Ground-Based Space Station Model," *Front. Hum. Neurosci.*, vol. 15, Jan. 2021, doi: 10.3389/FNHUM.2021.742664.

[3] R. D. Seidler, A. P. Mulavara, J. J. Bloomberg, and B. T. Peters, "Individual predictors of sensorimotor adaptability.," *Front. Syst. Neurosci.*, vol. 9, no. July, p. 100, Jul. 2015, doi: 10.3389/fnsys.2015.00100.

[4] "HRR - Risk - Risk of Bone Fracture due to Spaceflight-induced Changes to Bone." https://humanresearchroadmap.nasa.gov/Risks/risk.aspx?i=77 (accessed Jun. 08, 2024).

[5] P. Comfort *et al.*, "Effects of Spaceflight on Musculoskeletal Health: A Systematic Review and Meta-analysis, Considerations for Interplanetary Travel," *Sports Med.*, vol. 51, no. 10, p. 2097, Oct. 2021, doi: 10.1007/S40279-021-01496-9.

[6] E. Sanchez-Lopez, R. Coras, A. Torres, N. E. Lane, and M. Guma, "Synovial inflammation in osteoarthritis progression," *Nat. Rev. Rheumatol.*, vol. 18, no. 5, p. 258, May 2022, doi: 10.1038/S41584-022-00749-9.

[7] R. Duan, H. Xie, and Z. Z. Liu, "The Role of Autophagy in Osteoarthritis," *Front. Cell Dev. Biol.*, vol. 8, p. 608388, Nov. 2020, doi: 10.3389/FCELL.2020.608388.

[8] S. Chawla *et al.*, "Chondrocyte Hypertrophy in Osteoarthritis: Mechanistic Studies and Models for the Identification of New Therapeutic Strategies," *Cells*, vol. 11, no. 24, Dec. 2022, doi: 10.3390/CELLS11244034.

[9] G. G. H. Van Den Akker, M. M. J. Caron, M. J. Peffers, and T. J. M. Welting, "Ribosome dysfunction in osteoarthritis," *Curr. Opin. Rheumatol.*, vol. 34, no. 1, p. 61, Jan. 2022, doi: 10.1097/BOR.0000000000000858.

[10] W. Madej, P. Buma, and P. van der Kraan, "Inflammatory conditions partly impair the mechanically mediated activation of Smad2/3 signaling in articular cartilage," *Arthritis Res. Ther.*, vol. 18, no. 1, Jun. 2016, doi: 10.1186/S13075-016-1038-6.

[11] "Integrative meta-analysis of differentially expressed genes in osteoarthritis using microarray technology." https://www.spandidos-publications.com/10.3892/mmr.2015.3790 (accessed Jun. 06, 2024).

[12] Y. Ge *et al.*, “Identification and validation of hub genes of synovial tissue for patients with osteoarthritis and rheumatoid arthritis,” *Hereditas*, vol. 158, no. 1, pp. 1–16, Dec. 2021, doi: 10.1186/S41065-021-00201-0/FIGURES/7.

[13] F. Pessler *et al.*, “Subintimal Ki-67 as a synovial tissue biomarker for inflammatory arthropathies,” *Ann. Rheum. Dis.*, vol. 67, no. 2, pp. 162–167, Feb. 2008, doi: 10.1136/ARD.2007.071670.

[14] S. Fauteux-Daniel, L. M. Merlo Pich, C. Girard-Guyonvarc’h, A. Caruso, E. Rodriguez, and C. Gabay, “The role of interleukin-18 and interleukin-18 binding protein in K/BxN serum transfer-induced arthritis,” *Front. Immunol.*, vol. 14, p. 1215364, Jun. 2023, doi: 10.3389/FIMMU.2023.1215364/BIBTEX.

[15] F. Rousset *et al.*, “IL-1beta mediates MMP secretion and IL-1beta neosynthesis via upregulation of p22phox and NOX4 activity in human articular chondrocytes,” *Osteoarthr. Cartil.*, vol. 23, no. 11, pp. 1972–1980, Nov. 2015, doi: 10.1016/j.joca.2015.02.167.

[16] Y. Wu *et al.*, “Renin-angiotensin system in osteoarthritis: A new potential therapy,” *Int. Immunopharmacol.*, vol. 75, p. 105796, Oct. 2019, doi: 10.1016/J.INTIMP.2019.105796.

[17] M. Zayoud, E. Vax, G. Elad-Sfadia, I. Barshack, R. Pinkas-Kramarski, and I. Goldstein, “Inhibition of Ras GTPases prevents Collagen-Induced Arthritis by Reducing the Generation of Pathogenic CD4+ T Cells and the Hyposialylation of Autoantibodies,” *ACR Open Rheumatol.*, vol. 2, no. 9, p. 512, Sep. 2020, doi: 10.1002/ACR2.11169.

[18] T. Setti, M. G. L. Arab, G. S. Santos, N. Alkass, M. A. P. Andrade, and J. F. S. D. Lana, “The protective role of glutathione in osteoarthritis,” *J. Clin. Orthop. Trauma*, vol. 15, p. 145, Apr. 2021, doi: 10.1016/J.JCOT.2020.09.006.

[19] L. Ameye, D. Aria, K. Jepsen, A. Oldberg, T. Xu, and M. F. Young, “Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis,” *FASEB J.*, vol. 16, no. 7, pp. 673–680, May 2002, doi: 10.1096/FJ.01-0848COM.

[20] M. F. Rai, E. D. Tycksen, L. Cai, J. Yu, R. W. Wright, and R. H. Brophy, “Distinct Degenerative Phenotype of Articular Cartilage from Knees with Meniscus Tear Compared to Knees with Osteoarthritis,” *Osteoarthr. Cartil.*, vol. 27, no. 6, p. 945, Jun. 2019, doi: 10.1016/J.JOCA.2019.02.792.

[21] Y. Hu *et al.*, “Transcriptomic analyses of joint tissues during osteoarthritis development in a rat model reveal dysregulated mechanotransduction and extracellular matrix pathways,” *Osteoarthr. Cartil.*, vol. 31, no. 2, pp. 199–212, Feb. 2023, doi: 10.1016/J.JOCA.2022.10.003.

[22] J. H. Yea *et al.*, “Tppp3+ synovial/tendon sheath progenitor cells contribute to heterotopic bone after trauma,” *Bone Res. 2023 111*, vol. 11, no. 1, pp. 1–12, Jul. 2023, doi: 10.1038/s41413-023-00272-x.

[23] J. A. Staverosky, B. A. Pryce, S. S. Watson, and R. Schweitzer, “Tubulin polymerization-promoting protein family member 3, Tppp3, is a specific marker of the differentiating tendon sheath and synovial joints,” *Dev. Dyn.*, vol. 238, no. 3, pp. 685–692, Mar. 2009, doi: 10.1002/DVDY.21865.

[24] S. S. Zhao, V. Karhunen, A. P. Morris, and D. Gill, “ADAMTS5 as a therapeutic target for osteoarthritis: Mendelian randomisation study,” *Ann. Rheum. Dis.*, vol. 81, no. 6, pp. 903–904, Jun. 2022, doi: 10.1136/ANNRHEUMDIS-2021-222007.

[25] A. Latourte and P. Richette, “Inhibition of ADAMTS-5: the right target for osteoarthritis?,” *Osteoarthr. Cartil.*, vol. 30, no. 2, pp. 175–177, Feb. 2022, doi: 10.1016/j.joca.2021.09.012.

[26] I. C. M. Pelsma *et al.*, “Variants of FOXO3 and RPA3 genes affecting IGF-1 levels alter the risk of development of primary osteoarthritis,” *Eur. J. Endocrinol.*, vol. 184, no. 1, pp. 29–39, Jan. 2021, doi: 10.1530/EJE-20-0904.

[27] B. Brandstetter *et al.*, “FOXO3 is involved in the tumor necrosis factor-driven inflammatory response in fibroblast-like synoviocytes,” *Lab. Investig.*, vol. 99, no. 5, pp. 648–658, May 2019, doi: 10.1038/S41374-018-0184-7.

[28] J. H. Park *et al.*, “Anti-Ischemic Effects of PIK3IP1 Are Mediated through Its Interactions with the ETA-PI3Kγ-AKT Axis,” *Cells*, vol. 11, no. 14, Jul. 2022, doi: 10.3390/CELLS11142162/S1.

[29] J. Wang *et al.*, “Screening crucial lncRNAs and genes in osteoarthritis by integrated analysis,” *Adv. Rheumatol.*, vol. 63, no. 1, pp. 1–10, Dec. 2023, doi: 10.1186/S42358-023-00288-1/FIGURES/6.

[30] J. Liu, Q. Fu, and S. Liu, “Transcriptional Regulation Based on Network of Autophagy Identifies Key Genes and Potential Mechanisms in Human Osteoarthritis,” *Cartilage*, vol. 13, no. 2, pp. 1431S-1441S, Dec. 2021, doi: 10.1177/1947603520951632/ASSET/IMAGES/LARGE/10.1177_1947603520951632-FIG6.JPEG.

[31] H. Cao *et al.*, “Advances in the study of mitophagy in osteoarthritis,” *J. Zhejiang Univ. Sci. B*, vol. 25, no. 3, p. 197, Mar. 2024, doi: 10.1631/JZUS.B2300402.

[32] Z. Li, L. Xie, H. Zeng, and Y. Wu, “PDK4 inhibits osteoarthritis progression by activating the PPAR pathway,” *J. Orthop. Surg. Res.*, vol. 19, no. 1, p. 109, Dec. 2024, doi: 10.1186/S13018-024-04583-5.

[33] H. Cao *et al.*, “Advances in the study of mitophagy in osteoarthritis,” *J. Zhejiang Univ. Sci. B*, vol. 25, no. 3, p. 197, Mar. 2024, doi: 10.1631/JZUS.B2300402.

[34] C. M. Heveran *et al.*, “Chronic kidney disease and aging differentially diminish bone material and microarchitecture in C57Bl/6 mice,” *Bone*, vol. 127, pp. 91–103, Oct. 2019, doi: 10.1016/J.BONE.2019.04.019.

[35] Q. Lu, P. Liu, Z. Miao, D. Luo, S. Li, and M. Lu, “SIRT1 restoration enhances chondrocyte autophagy in osteoarthritis through PTEN-mediated EGFR ubiquitination,” *Cell Death Discov. 2022 81*, vol. 8, no. 1, pp. 1–10, Apr. 2022, doi: 10.1038/s41420-022-00896-8.

[36] T. Matsuzaki *et al.*, “Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice,” *Ann. Rheum. Dis.*, vol. 73, no. 7, pp. 1397–1404, Jul. 2014, doi: 10.1136/ANNRHEUMDIS-2012-202620.

[37] K. Sun *et al.*, “The role of the sirtuin family in cartilage and osteoarthritis: molecular mechanisms and therapeutic targets,” *Arthritis Res. Ther.*, vol. 24, no. 1, Dec. 2022, doi: 10.1186/S13075-022-02983-8.

[38] Y. Liu, Z. Zhang, C. Liu, and H. Zhang, “Sirtuins in osteoarthritis: current understanding,” *Front. Immunol.*, vol. 14, p. 1140653, Apr. 2023, doi: 10.3389/FIMMU.2023.1140653/BIBTEX.

[39] F. J. Zhang, W. Luo, and G. H. Lei, “Role of HIF-1α and HIF-2α in osteoarthritis,” *Jt. Bone Spine*, vol. 82, no. 3, pp. 144–147, May 2015, doi: 10.1016/J.JBSPIN.2014.10.003.

[40] H. Guo, J. Huang, Y. Liang, D. Wang, and H. Zhang, “Focusing on the hypoxia-inducible factor pathway: role, regulation, and therapy for osteoarthritis,” *Eur. J. Med. Res. 2022 271*, vol. 27, no. 1, pp. 1–11, Dec. 2022, doi: 10.1186/S40001-022-00926-2.

[41] F. J. Zhang, W. Luo, and G. H. Lei, “Role of HIF-1α and HIF-2α in osteoarthritis,” *Jt. bone spine*, vol. 82, no. 3, pp. 144–147, May 2015, doi: 10.1016/J.JBSPIN.2014.10.003.

[42] S. Hu *et al.*, “Stabilization of HIF-1α alleviates osteoarthritis via enhancing mitophagy,” *Cell Death Dis. 2020 116*, vol. 11, no. 6, pp. 1–16, Jun. 2020, doi: 10.1038/s41419-020-2680-0.

[43] C. Y. Zeng, X. F. Wang, and F. Z. Hua, “HIF-1α in Osteoarthritis: From Pathogenesis to Therapeutic Implications,” *Front. Pharmacol.*, vol. 13, Jul. 2022, doi: 10.3389/FPHAR.2022.927126.

[44] I. S. Sinam *et al.*, “Pyruvate dehydrogenase kinase 4 promotes ubiquitin–proteasome system-dependent muscle atrophy,” *J. Cachexia. Sarcopenia Muscle*, vol. 13, no. 6, pp. 3122–3136, 2022, doi: 10.1002/jcsm.13100.

[45] Z. Li, L. Xie, H. Zeng, and Y. Wu, “PDK4 inhibits osteoarthritis progression by activating the PPAR pathway,” *J. Orthop. Surg. Res.*, vol. 19, no. 1, Dec. 2024, doi: 10.1186/S13018-024-04583-5.

[46] T. Kamo *et al.*, “Epigenetic mechanism controls PDK4 gene activation before and after exercise therapy following artificial knee arthroplasty,” *Clin. Interv. Aging*, vol. 14, p. 1433, 2019, doi: 10.2147/CIA.S213154.

[47] S. C. Warner *et al.*, “IL-15 and IL15RA in Osteoarthritis: Association With Symptoms and Protease Production, but Not Structural Severity,” *Front. Immunol.*, vol. 11, p. 1385, Jul. 2020, doi: 10.3389/FIMMU.2020.01385/FULL.

[48] A. Al Adlaan, N. Hussein, F. Jaber, and F. Safadi, “AB0103 A NOVEL PROTECTIVE ROLE OF GPNMB/OSTEOACTIVIN IN POST-TRAUMATIC OSTEOARTHRITIS,” *Ann. Rheum. Dis.*, vol. 78, no. Suppl 2, pp. 1513–1513, Jun. 2019, doi: 10.1136/ANNRHEUMDIS-2019-EULAR.8175.

[49] Z. Wang, N. Zhou, W. Wang, Y. Yu, L. Xia, and N. Li, “HDAC2 interacts with microRNA-503-5p to regulate SGK1 in osteoarthritis,” *Arthritis Res. Ther.*, vol. 23, no. 1, pp. 1–11, Dec. 2021, doi: 10.1186/S13075-020-02373-Y/FIGURES/7.

[50] W. Huang *et al.*, “Knockdown of SGK1 alleviates the IL-1β-induced chondrocyte anabolic and catabolic imbalance by activating FoxO1-mediated autophagy in human chondrocytes,” *FEBS J.*, vol. 287, no. 1, pp. 94–107, Jan. 2020, doi: 10.1111/FEBS.15009.

[51] Z. Wang, S. Ni, H. Zhang, Y. Fan, L. Xia, and N. Li, “Silencing SGK1 alleviates osteoarthritis through epigenetic regulation of CREB1 and ABCA1 expression,” *Life Sci.*, vol. 268, Mar. 2021, doi: 10.1016/J.LFS.2020.118733.

[52] K. Panoutsopoulou *et al.*, “Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study,” *Ann. Rheum. Dis.*, vol. 70, no. 5, pp. 864–867, 2011, doi: 10.1136/ard.2010.141473.

[53] I. Tachmazidou *et al.*, “Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank.”

[54] M. Kim, A. Rubab, W. C. W. Chan, and D. Chan, “Osteoarthritis year in review 2022: Genetics, genomics and epigenetics,” *Osteoarthr. Cartil.*, vol. 31, no. 7, pp. 865–875, Jul. 2023, doi: 10.1016/J.JOCA.2023.03.003.

[55] Z. Zhang, Y. Mei, M. Feng, C. Wang, P. Yang, and R. Tian, “The relationship between common variants in the DPEP1 gene and the susceptibility and clinical severity of osteoarthritis,” *Int. J. Rheum. Dis.*, vol. 24, no. 9, pp. 1192–1199, Sep. 2021, doi: 10.1111/1756-185X.14182.

[56] I. C. M. Pelsma *et al.*, “Variants of FOXO3 and RPA3 genes affecting IGF-1 levels alter the risk of development of primary osteoarthritis,” *Eur. J. Endocrinol.*, vol. 184, no. 1, pp. 29–39, Jan. 2021, doi: 10.1530/EJE-20-0904.

[57] A. Sebastian and G. G. Loots, “Genomic Profiling in Bone,” *Genet. Bone Biol. Skelet. Dis. Second Ed.*, pp. 55–75, Jan. 2018, doi: 10.1016/B978-0-12-804182-6.00005-8.

[58] I. C. M. Pelsma *et al.*, “Variants of FOXO3 and RPA3 genes affecting IGF-1 levels alter the risk of development of primary osteoarthritis,” *Eur. J. Endocrinol.*, vol. 184, no. 1, pp. 29–39, Jan. 2021, doi: 10.1530/EJE-20-0904.

[59] Y. Huang, G. Wan, and J. Tao, “C1q/TNF-related protein-3 exerts the chondroprotective effects in IL-1β-treated SW1353 cells by regulating the FGFR1 signaling,” *Biomed. Pharmacother.*, vol. 85, pp. 41–46, Jan. 2017, doi: 10.1016/J.BIOPHA.2016.11.128.

[60] F. Dong, M. Jiang, Z. Zhang, F. Li, and Y. Lian, “C1q/TNF-related protein-9 suppresses inflammation in synovial cells from patients with osteoarthritis,” *Scand. J. Rheumatol.*, Sep. 2022, doi: 10.1080/03009742.2021.1946996.

[61] H. Raghu *et al.*, “Complement component C1q limits osteoarthritis pathology by regulating macrophage activation,” *J. Immunol.*, vol. 196, no. 1_Supplement, pp. 124.7-124.7, May 2016, doi: 10.4049/JIMMUNOL.196.SUPP.124.7.

[62] F. Dong, M. Jiang, Z. Zhang, F. Li, and Y. Lian, “C1q/TNF-related protein-9 suppresses inflammation in synovial cells from patients with osteoarthritis,” *Scand. J. Rheumatol.*, vol. 51, no. 5, 2022, doi: 10.1080/03009742.2021.1946996.

[63] R. Lubbers *et al.*, “Complement component C1q is produced by isolated articular chondrocytes,” *Osteoarthr. Cartil.*, vol. 28, no. 5, pp. 675–684, May 2020, doi: 10.1016/J.JOCA.2019.09.007.

[64] F. Comblain *et al.*, “Identification of Targets of a New Nutritional Mixture for Osteoarthritis Management Composed by Curcuminoids Extract, Hydrolyzed Collagen and Green Tea Extract,” *PLoS One*, vol. 11, no. 6, Jun. 2016, doi: 10.1371/JOURNAL.PONE.0156902.

[65] S. Y. Lee, J. I. Yu, and S. C. Chae, “Associations of ifitm3 haplotypes with rheumatoid arthritis in a korean population,” *Genes and Genomics*, vol. 34, no. 5, pp. 493–498, Oct. 2012, doi: 10.1007/S13258-011-0241-8/METRICS.

[66] R. Wang, Q. Yang, W. Xiao, R. Si, F. Sun, and Q. Pan, “Cellular retinoic acid binding protein 2 inhibits osteogenic differentiation by modulating LIMK1 in C2C12 cells,” *Dev. Growth Differ.*, vol. 57, no. 8, pp. 581–589, Oct. 2015, doi: 10.1111/DGD.12240.

[67] I. Takazawa, A. Larange, and H. Cheroutre, “Cellular retinoic acid binding protein 2 (CRABP2) serves as a critical regulator in immune regulation and inflammation,” *J. Immunol.*, vol. 202, no. 1_Supplement, pp. 124.3-124.3, May 2019, doi: 10.4049/JIMMUNOL.202.SUPP.124.3.

[68] N. Mosquera, A. Rodriguez-Trillo, A. Mera-Varela, A. Gonzalez, and C. Conde, “Uncovering Cellular retinoic acid-binding protein 2 as a potential target for rheumatoid arthritis synovial hyperplasia,” *Sci. Reports 2018 81*, vol. 8, no. 1, pp. 1–9, Jun. 2018, doi: 10.1038/s41598-018-26027-x.

[69] I. D. Welch, M. F. Cowan, F. Beier, and T. M. Underhill, “The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease,” *Arthritis Res. Ther.*, vol. 11, no. 1, Jan. 2009, doi: 10.1186/AR2604.

[70] C. Widmera *et al.*, “Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109, no. 33, pp. 13243–13247, Aug. 2012, doi: 10.1073/PNAS.1208072109/SUPPL_FILE/PNAS.201208072SI.PDF.

[71] W. Bin Xu *et al.*, “Identification of the circRNA–miRNA–mRNA regulatory network in osteoarthritis using bioinformatics analysis,” *Front. Genet.*, vol. 13, p. 994163, Sep. 2022, doi: 10.3389/FGENE.2022.994163/BIBTEX.

[72] K. C. Tsolis *et al.*, “Comparative proteomic analysis of hypertrophic chondrocytes in osteoarthritis,” *Clin. Proteomics*, vol. 12, no. 1, pp. 1–16, Apr. 2015, doi: 10.1186/S12014-015-9085-6/FIGURES/6.

[73] C. Marshall, J. Lopez, L. Crookes, R. C. Pollitt, and M. Balasubramanian, “A novel homozygous variant in SERPINH1 associated with a severe, lethal presentation of osteogenesis imperfecta with hydranencephaly,” *Gene*, vol. 595, no. 1, pp. 49–52, Dec. 2016, doi: 10.1016/J.GENE.2016.09.035.

[74] D. J. Wilkinson, “Serpins in cartilage and osteoarthritis: what do we know?,” *Biochem. Soc. Trans.*, vol. 49, no. 2, p. 1013, Apr. 2021, doi: 10.1042/BST20201231.

[75] R. Zhang *et al.*, “Potential candidate biomarkers associated with osteoarthritis: Evidence from a comprehensive network and pathway analysis,” *J. Cell. Physiol.*, vol. 234, no. 10, pp. 17433–17443, Oct. 2019, doi: 10.1002/JCP.28365.

[76] C. Karlsson *et al.*, “Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis,” *Osteoarthr. Cartil.*, vol. 18, no. 4, pp. 581–592, Apr. 2010, doi: 10.1016/J.JOCA.2009.12.002.

[77] K. M. Fisch *et al.*, “Identification of transcription factors responsible for dysregulated networks in human osteoarthritis cartilage by global gene expression analysis,” *Osteoarthr. Cartil.*, vol. 26, no. 11, p. 1531, Nov. 2018, doi: 10.1016/J.JOCA.2018.07.012.

[78] J. A. Bolduc, J. A. Collins, and R. F. Loeser, “Reactive oxygen species, aging and articular cartilage homeostasis,” *Free Radic. Biol. Med.*, vol. 132, pp. 73–82, Feb. 2019, doi: 10.1016/J.FREERADBIOMED.2018.08.038.

[79] Y. Liu, Z. Zhang, T. Li, H. Xu, and H. Zhang, “Senescence in osteoarthritis: from mechanism to potential treatment,” *Arthritis Res. Ther. 2022 241*, vol. 24, no. 1, pp. 1–15, Jul. 2022, doi: 10.1186/S13075-022-02859-X.

[80] E. Regan *et al.*, “Extracellular superoxide dismutase and oxidant damage in osteoarthritis,” *Arthritis Rheum.*, vol. 52, no. 11, pp. 3479–3491, Nov. 2005, doi: 10.1002/art.21387.

[81] J. L. Scott *et al.*, “Superoxide dismutase downregulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, pp. 1502–1510, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[82] C. Gavriilidis, S. Miwa, T. Von Zglinicki, R. W. Taylor, and D. A. Young, “Mitochondrial dysfunction in osteoarthritis is associated with down-regulation of superoxide dismutase 2,” *Arthritis Rheum.*, vol. 65, no. 2, pp. 378–387, Feb. 2013, doi: 10.1002/art.37782.

[83] J. L. Scott *et al.*, “Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[84] Y. Shi *et al.*, “A small molecule promotes cartilage extracellular matrix generation and inhibits osteoarthritis development,” *Nat. Commun. 2019 101*, vol. 10, no. 1, pp. 1–14, Apr. 2019, doi: 10.1038/s41467-019-09839-x.

[85] E. A. Regan, R. P. Bowler, and J. D. Crapo, “Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury,” *Osteoarthr. Cartil.*, vol. 16, no. 4, pp. 515–521, Apr. 2008, doi: 10.1016/J.JOCA.2007.09.001.

[86] M. Koike *et al.*, “Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage,” *PLoS One*, vol. 13, no. 9, p. e0203944, Sep. 2018, doi: 10.1371/JOURNAL.PONE.0203944.

[87] X. Mao, S. Stanbouly, J. Holley, M. Pecaut, and J. Crapo, “Evidence of Spaceflight-Induced Adverse Effects on Photoreceptors and Retinal Function in the Mouse Eye,” *Int. J. Mol. Sci.*, vol. 24, no. 8, Apr. 2023, doi: 10.3390/IJMS24087362.

[88] K. Y. Kamal and J. M. Lawler, “Cellular and Molecular Signaling Meet the Space Environment,” *Int. J. Mol. Sci.*, vol. 24, no. 6, Mar. 2023, doi: 10.3390/IJMS24065955.

[89] C. G. T. Tahimic and R. K. Globus, “Redox Signaling and Its Impact on Skeletal and Vascular Responses to Spaceflight,” *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102153.

[90] M. A. Bucaro *et al.*, “Bone cell survival in microgravity: evidence that modeled microgravity increases osteoblast sensitivity to apoptogens,” *Ann. N. Y. Acad. Sci.*, vol. 1027, pp. 64–73, 2004, doi: 10.1196/ANNALS.1324.007.

[91] J. M. Lawler, W. Song, and S. R. Demaree, “Hindlimb unloading increases oxidative stress and disrupts antioxidant capacity in skeletal muscle,” *Free Radic. Biol. Med.*, vol. 35, no. 1, pp. 9–16, Jul. 2003, doi: 10.1016/S0891-5849(03)00186-2.

[92] J. S. Alwood *et al.*, “Dose- and Ion-Dependent Effects in the Oxidative Stress Response to Space-Like Radiation Exposure in the Skeletal System,” *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102117.

[93] G. J. Bell, T. P. Martin, E. I. Ilyina-Kakueva, V. S. Oganov, and V. R. Edgerton, “Altered distribution of mitochondria in rat soleus muscle fibers after spaceflight,” *J. Appl. Physiol.*, vol. 73, no. 2, pp. 493–497, 1992, doi: 10.1152/JAPPL.1992.73.2.493.

[94] C. S. Thudium *et al.*, “A comparison of osteoclast-rich and osteoclast-poor osteopetrosis in adult mice sheds light on the role of the osteoclast in coupling bone resorption and bone formation,” *Calcif. Tissue Int.*, vol. 95, no. 1, pp. 83–93, 2014, doi: 10.1007/S00223-014-9865-4.

[95] R. R. Yammani, D. Long, and R. F. Loeser, “Interleukin-7 stimulates secretion of S100A4 by activating the JAK/STAT signaling pathway in human articular chondrocytes,” *Arthritis Rheum.*, vol. 60, no. 3, pp. 792–800, Mar. 2009, doi: 10.1002/ART.24295.

[96] J. Haywood and R. R. Yammani, “Free fatty acid palmitate activates unfolded protein response pathway and promotes apoptosis in meniscus cells,” *Osteoarthr. Cartil.*, vol. 24, no. 5, pp. 942–945, May 2016, doi: 10.1016/J.JOCA.2015.11.020.

[97] S. L. Dunn, J. Soul, S. Anand, J. M. Schwartz, R. P. Boot-Handford, and T. E. Hardingham, “Gene expression changes in damaged osteoarthritic cartilage identify a signature of non-chondrogenic and mechanical responses,” *Osteoarthr. Cartil.*, vol. 24, no. 8, pp. 1431–1440, Aug. 2016, doi: 10.1016/J.JOCA.2016.03.007.

[98] W. Jingbo, C. Aimin, W. Qi, L. Xin, and L. Huaining, “Betulinic acid inhibits IL-1β-induced inflammation by activating PPAR-γ in human osteoarthritis chondrocytes,” *Int. Immunopharmacol.*, vol. 29, no. 2, pp. 687–692, Dec. 2015, doi: 10.1016/J.INTIMP.2015.09.009.

[99] R. ming Zeng *et al.*, “Knockdown of FOXM1 attenuates inflammatory response in human osteoarthritis chondrocytes,” *Int. Immunopharmacol.*, vol. 68, pp. 74–80, Mar. 2019, doi: 10.1016/J.INTIMP.2018.12.057.

[100] S. Zhu, Y. Deng, H. Gao, K. Huang, and Z. Nie, “miR-877-5p alleviates chondrocyte dysfunction in osteoarthritis models via repressing FOXM1,” *J. Gene Med.*, vol. 22, no. 11, Nov. 2020, doi: 10.1002/JGM.3246.

[101] W. Wang, P. Guo, M. Chen, D. Chen, Y. Cheng, and L. He, “FOXM1/LINC00152 feedback loop regulates proliferation and apoptosis in rheumatoid arthritis fibroblast-like synoviocytes via Wnt/β-catenin signaling pathway,” *Biosci. Rep.*, vol. 40, no. 1, Jan. 2020, doi: 10.1042/BSR20191900.

[102] J. Song *et al.*, “Upregulated FOXM1 stimulates chondrocyte senescence in Acot12-/-Nudt7-/- double knockout mice,” *Theranostics*, vol. 13, no. 15, pp. 5207–5222, 2023, doi: 10.7150/THNO.89033.

[103] R. Zeng *et al.*, “FOXM1 activates JAK1/STAT3 pathway in human osteoarthritis cartilage cell inflammatory reaction,” *Exp. Biol. Med. (Maywood).*, vol. 246, no. 6, pp. 644–653, Mar. 2021, doi: 10.1177/1535370220974933.

[104] L. Gabel *et al.*, “Pre-flight exercise and bone metabolism predict unloading-induced bone loss due to spaceflight,” *Br. J. Sports Med.*, vol. 56, no. 4, pp. 196–203, Feb. 2022, doi: 10.1136/BJSPORTS-2020-103602.

[105] M. L. Bouxsein, S. K. Boyd, B. A. Christiansen, R. E. Guldberg, K. J. Jepsen, and R. Müller, “Guidelines for assessment of bone microstructure in rodents using micro–computed tomography,” *J. Bone Miner. Res.*, vol. 25, no. 7, pp. 1468–1486, Jul. 2010, doi: 10.1002/JBMR.141.

[106] L. Gabel *et al.*, “Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia 1 year after return from long duration spaceflight,” *Sci. Rep.*, vol. 12, no. 1, Dec. 2022, doi: 10.1038/S41598-022-13461-1.

[107] A. M. Liphardt *et al.*, “Immobilization by 21 days of bed rest results in type II collagen degradation in healthy individuals,” *Osteoarthr. Cartil.*, 2023, doi: 10.1016/J.JOCA.2023.11.007.

[108] A. M. Liphardt *et al.*, “Changes in mechanical loading affect arthritis-induced bone loss in mice,” *Bone*, vol. 131, Feb. 2020, doi: 10.1016/J.BONE.2019.115149.

[109] M. Dreiner *et al.*, “Short-term Response of Serum Cartilage Oligomeric Matrix Protein to Different Types of Impact Loading Under Normal and Artificial Gravity,” *Front. Physiol.*, vol. 11, Aug. 2020, doi: 10.3389/FPHYS.2020.01032.

[110] A. M. Liphardt, R. Fernandez-Gonzalo, K. Albracht, J. Rittweger, and L. Vico, “Musculoskeletal research in human space flight - unmet needs for the success of crewed deep space exploration,” *NPJ microgravity*, vol. 9, no. 1, Dec. 2023, doi: 10.1038/S41526-023-00258-3.

[111] J. Fitzgerald, J. Endicott, U. Hansen, and C. Janowitz, “Articular cartilage and sternal fibrocartilage respond differently to extended microgravity,” *NPJ microgravity*, vol. 5, no. 1, Dec. 2019, doi: 10.1038/S41526-019-0063-6.

[112] J. Fitzgerald, “Cartilage breakdown in microgravity-a problem for long-term spaceflight?,” *NPJ Regen. Med.*, vol. 2, no. 1, Apr. 2017, doi: 10.1038/S41536-017-0016-1.

[113] Y. Henrotin, B. Kurz, and T. Aigner, “Oxygen and reactive oxygen species in cartilage degradation: friends or foes?,” *Osteoarthr. Cartil.*, vol. 13, no. 8, pp. 643–654, Aug. 2005, doi: 10.1016/J.JOCA.2005.04.002.

[114] D. Li, G. Xie, and W. Wang, “Reactive oxygen species: the 2-edged sword of osteoarthritis,” *Am. J. Med. Sci.*, vol. 344, no. 6, pp. 486–490, 2012, doi: 10.1097/MAJ.0B013E3182579DC6.

[115] J. W. J. Bijlsma, F. Berenbaum, and F. P. J. G. Lafeber, “Osteoarthritis: an update with relevance for clinical practice,” *Lancet (London, England)*, vol. 377, no. 9783, pp. 2115–2126, 2011, doi: 10.1016/S0140-6736(11)60243-2.

[116] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J. P. Pelletier, and H. Fahmi, “Role of proinflammatory cytokines in the pathophysiology of osteoarthritis,” *Nat. Rev. Rheumatol.*, vol. 7, no. 1, pp. 33–42, Jan. 2011, doi: 10.1038/NRRHEUM.2010.196.

[117] J. Stepanek, R. S. Blue, and S. Parazynski, “Space Medicine in the Era of Civilian Spaceflight,” *N. Engl. J. Med.*, vol. 380, no. 11, pp. 1053–1060, Mar. 2019, doi: 10.1056/NEJMRA1609012.

[118] T. F. Lang, A. D. Leblanc, H. J. Evans, and Y. Lu, “Adaptation of the proximal femur to skeletal reloading after long-duration spaceflight,” *J. Bone Miner. Res.*, vol. 21, no. 8, pp. 1224–1230, Aug. 2006, doi: 10.1359/JBMR.060509.

[119] T. Lang *et al.*, “Towards human exploration of space: the THESEUS review series on muscle and bone research priorities,” *NPJ Microgravity*, vol. 3, no. 1, Dec. 2017, doi: 10.1038/S41526-017-0013-0.

[120] B. Vanwanseele, F. Eckstein, H. Knecht, A. Spaepen, and E. Stüssis, “Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord injury,” *Arthritis Rheum.*, vol. 48, no. 12, pp. 3377–3381, 2003, doi: 10.1002/ART.11367.

[121] W. Waldstein, G. Perino, S. L. Gilbert, S. A. Maher, R. Windhager, and F. Boettner, “OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee,” *J. Orthop. Res.*, vol. 34, no. 1, pp. 135–140, Jan. 2016, doi: 10.1002/JOR.23010.

[122] S. L. Schlichte *et al.*, “Nanoformulation of the superoxide dismutase mimic, MnTnBuOE-2-PyP5+, prevents its acute hypotensive response,” *Redox Biol.*, vol. 36, p. 101610, Sep. 2020, doi: 10.1016/J.REDOX.2020.101610.

[123] S. T. Sonis, “Superoxide Dismutase as an Intervention for Radiation Therapy-Associated Toxicities: Review and Profile of Avasopasem Manganese as a Treatment Option for Radiation-Induced Mucositis,” *Drug Des. Devel. Ther.*, vol. 15, p. 1021, 2021, doi: 10.2147/DDDT.S267400.

[124] G. E. O. Borgstahl and R. E. Oberley-Deegan, “Superoxide Dismutases (SODs) and SOD Mimetics,” *Antioxidants*, vol. 7, no. 11, Nov. 2018, doi: 10.3390/ANTIOX7110156.

[125] B. J. Day, “Catalytic antioxidants: A radical approach to new therapeutics,” *Drug Discov. Today*, vol. 9, no. 13, pp. 557–566, Jul. 2004, doi: 10.1016/S1359-6446(04)03139-3.

[126] X. Mao, S. Stanbouly, J. Holley, M. Pecaut, and J. Crapo, “Evidence of Spaceflight-Induced Adverse Effects on Photoreceptors and Retinal Function in the Mouse Eye,” *Int. J. Mol. Sci.*, vol. 24, no. 8, Apr. 2023, doi: 10.3390/IJMS24087362.

[127] J. D. Crapo, B. J. Day, and I. Fridovich, “Development of Manganic Porphyrin Mimetics of Superoxide Dismutase Activity,” 2013, Accessed: Dec. 14, 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK6050/.

[128] K. R. Olson *et al.*, “Manganese Porphyrin-Based SOD Mimetics Produce Polysulfides from Hydrogen Sulfide,” *Antioxidants 2019, Vol. 8, Page 639*, vol. 8, no. 12, p. 639, Dec. 2019, doi: 10.3390/ANTIOX8120639.

[129] L. Liu, P. Luo, M. Yang, J. Wang, W. Hou, and P. Xu, “The role of oxidative stress in the development of knee osteoarthritis: A comprehensive research review,” *Front. Mol. Biosci.*, vol. 9, p. 1001212, Sep. 2022, doi: 10.3389/FMOLB.2022.1001212/BIBTEX.

[130] P. Lepetsos and A. G. Papavassiliou, “ROS/oxidative stress signaling in osteoarthritis,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1862, no. 4, pp. 576–591, Apr. 2016, doi: 10.1016/J.BBADIS.2016.01.003.

[131] J. L. Scott *et al.*, “Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[132] Y. Henrotin, B. Kurz, and T. Aigner, “Oxygen and reactive oxygen species in cartilage degradation: friends or foes?,” *Osteoarthr. Cartil.*, vol. 13, no. 8, pp. 643–654, Aug. 2005, doi: 10.1016/J.JOCA.2005.04.002.

[133] H. P. Nguyen, P. H. Tran, K. S. Kim, and S. G. Yang, “The effects of real and simulated microgravity on cellular mitochondrial function,” *npj Microgravity 2021 71*, vol. 7, no. 1, pp. 1–11, Nov. 2021, doi: 10.1038/s41526-021-00171-7.

[134] M. Y. Ansari, N. Ahmad, and T. M. Haqqi, “Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols,” *Biomed. Pharmacother.*, vol. 129, p. 110452, Sep. 2020, doi: 10.1016/J.BIOPHA.2020.110452.

[135] D. J. Leong *et al.*, “Matrix Metalloproteinase-3 In Articular Cartilage Is Upregulated By Joint Immobilization And Suppressed By Passive Joint Motion,” *Matrix Biol.*, vol. 29, no. 5, p. 420, Jun. 2010, doi: 10.1016/J.MATBIO.2010.02.004.

[136] J. S. Willey *et al.*, “Spaceflight-Relevant Challenges of Radiation and/or Reduced Weight Bearing Cause Arthritic Responses in Knee Articular Cartilage,” *Radiat. Res.*, vol. 186, no. 4, pp. 333–344, Oct. 2016, doi: 10.1667/RR14400.1.

[137] A. T. Kwok *et al.*, “Knee and Hip Joint Cartilage Damage from Combined Spaceflight Hazards of Low-Dose Radiation Less than 1 Gy and Prolonged Hindlimb Unloading,” *Radiat. Res.*, vol. 191, no. 6, p. 497, Jun. 2019, doi: 10.1667/RR15216.1.

[138] T. M. Griffin and F. Guilak, “The role of mechanical loading in the onset and progression of osteoarthritis,” *Exerc. Sport Sci. Rev.*, vol. 33, no. 4, pp. 195–200, Oct. 2005, doi: 10.1097/00003677-200510000-00008.

[139] S. R. Goldring and M. B. Goldring, “The role of cytokines in cartilage matrix degeneration in osteoarthritis,” *Clin. Orthop. Relat. Res.*, vol. 427, no. SUPPL., 2004, doi: 10.1097/01.BLO.0000144854.66565.8F.

[140] A. T. Kwok *et al.*, “Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents,” *Sci. Rep.*, vol. 11, no. 1, p. 10469, Dec. 2021, doi: 10.1038/S41598-021-90010-2.

[141] B. Ganse, M. Cucchiarini, and H. Madry, “Joint Cartilage in Long-Duration Spaceflight,” *Biomedicines*, vol. 10, no. 6, Jun. 2022, doi: 10.3390/BIOMEDICINES10061356.

[142] S. Hinterwimmer *et al.*, “Cartilage atrophy in the knees of patients after seven weeks of partial load bearing,” *Arthritis Rheum.*, vol. 50, no. 8, pp. 2516–2520, Aug. 2004, doi: 10.1002/ART.20378.

[143] R. J. Reynolds, P. S. Walker, and J. Buza, “Mechanisms of anterior-posterior stability of the knee joint under load-bearing,” *J. Biomech.*, vol. 57, pp. 39–45, May 2017, doi: 10.1016/J.JBIOMECH.2017.03.016.

[144] F. Guilak, “Biomechanical factors in osteoarthritis,” *Best Pract. Res. Clin. Rheumatol.*, vol. 25, no. 6, p. 815, 2011, doi: 10.1016/J.BERH.2011.11.013.

[145] C. P. Pal, P. Singh, S. Chaturvedi, K. K. Pruthi, and A. Vij, “Epidemiology of knee osteoarthritis in India and related factors,” *Indian J. Orthop.*, vol. 50, no. 5, p. 518, Sep. 2016, doi: 10.4103/0019-5413.189608.

[146] N. Vaghela, D. Mishra, J. Patel, and V. Dani, “Promoting health and quality of life of patients with osteoarthritis of knee joint through non-pharmacological treatment strategies: A randomized controlled trial,” *J. Educ. Health Promot.*, vol. 9, no. 1, Jun. 2020, doi: 10.4103/JEHP.JEHP_39_20.

[147] E. Mesci, A. Icagasioglu, N. Mesci, and S. T. Turgut, “Relation of physical activity level with quality of life, sleep and depression in patients with knee osteoarthritis,” *North. Clin. Istanbul*, vol. 2, no. 3, p. 215, 2015, doi: 10.14744/NCI.2015.95867.

[148] E. R. Morey-Holton and R. K. Globus, “Hindlimb unloading of growing rats: a model for predicting skeletal changes during space flight,” *Bone*, vol. 22, no. 5 Suppl, pp. 83S-88S, 1998, doi: 10.1016/S8756-3282(98)00019-2.

[149] A. Zhuang *et al.*, “SOD2 in skeletal muscle: New insights from an inducible deletion model,” *Redox Biol.*, vol. 47, p. 102135, Nov. 2021, doi: 10.1016/J.REDOX.2021.102135.

[150] F. L. Muller *et al.*, “Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy,” *Free Radic. Biol. Med.*, vol. 40, no. 11, pp. 1993–2004, Jun. 2006, doi: 10.1016/J.FREERADBIOMED.2006.01.036.

[151] S. Arbogast *et al.*, “Bowman-Birk inhibitor concentrate prevents atrophy, weakness, and oxidative stress in soleus muscle of hindlimb-unloaded mice,” *J. Appl. Physiol.*, vol. 102, no. 3, pp. 956–964, Mar. 2007, doi: 10.1152/JAPPLPHYSIOL.00538.2006.

[152] J. Abrigo, J. C. Rivera, F. Simon, D. Cabrera, and C. Cabello-Verrugio, “Transforming growth factor type beta (TGF-β) requires reactive oxygen species to induce skeletal muscle atrophy,” *Cell. Signal.*, vol. 28, no. 5, pp. 366–376, May 2016, doi: 10.1016/J.CELLSIG.2016.01.010.

[153] S. K. Powers, A. N. Kavazis, and J. M. McClung, “Oxidative stress and disuse muscle atrophy,” *J. Appl. Physiol.*, vol. 102, no. 6, pp. 2389–2397, Jun. 2007, doi: 10.1152/JAPPLPHYSIOL.01202.2006/ASSET/IMAGES/LARGE/ZDG0050772520005.JPEG.

[154] S. K. Powers, A. J. Smuder, and A. R. Judge, “Oxidative stress and disuse muscle atrophy: cause or consequence?,” *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 15, no. 3, pp. 240–245, Jul. 2012, doi: 10.1097/MCO.0B013E328352B4C2.

[155] J. Ježek, K. F. Cooper, and R. Strich, “Reactive Oxygen Species and Mitochondrial Dynamics: The Yin and Yang of Mitochondrial Dysfunction and Cancer Progression,” *Antioxidants*, vol. 7, no. 1, Mar. 2018, doi: 10.3390/ANTIOX7010013.

[156] S. Bolisetty and E. A. Jaimes, “Mitochondria and Reactive Oxygen Species: Physiology and Pathophysiology,” *Int. J. Mol. Sci.*, vol. 14, no. 3, p. 6306, Mar. 2013, doi: 10.3390/IJMS14036306.

[157] J. S. Willey *et al.*, “Spaceflight-Relevant Challenges of Radiation and/or Reduced Weight Bearing Cause Arthritic Responses in Knee Articular Cartilage,” *Radiat. Res.*, vol. 186, no. 4, pp. 333–344, Oct. 2016, doi: 10.1667/RR14400.1.

[158] E. A. Regan, R. P. Bowler, and J. D. Crapo, “Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury,” *Osteoarthr. Cartil.*, vol. 16, no. 4, pp. 515–521, Apr. 2008, doi: 10.1016/J.JOCA.2007.09.001.

[159] A. Mobasheri, H. K. Biesalski, M. Shakibaei, and Y. Henrotin, “Antioxidants and osteoarthritis,” *Syst. Biol. Free Radicals Antioxidants*, vol. 9783642300189, pp. 2997–3026, May 2014, doi: 10.1007/978-3-642-30018-9_130/COVER.

[160] A. K. Grover and S. E. Samson, “Benefits of antioxidant supplements for knee osteoarthritis: rationale and reality,” *Nutr. J.*, vol. 15, no. 1, Jan. 2016, doi: 10.1186/S12937-015-0115-Z.

[161] M. Y. Ansari, N. Ahmad, and T. M. Haqqi, “Oxidative Stress and Inflammation in Osteoarthritis Pathogenesis: Role of Polyphenols,” *Biomed. Pharmacother.*, vol. 129, p. 110452, Sep. 2020, doi: 10.1016/J.BIOPHA.2020.110452.

[162] E. Regan *et al.*, “Extracellular superoxide dismutase and oxidant damage in osteoarthritis,” *Arthritis Rheum.*, vol. 52, no. 11, pp. 3479–3491, Nov. 2005, doi: 10.1002/ART.21387.

[163] J. L. Scott *et al.*, “Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[164] B. Poulet and F. Beier, “Targeting oxidative stress to reduce osteoarthritis,” *Arthritis Res. Ther.*, vol. 18, no. 1, pp. 1–2, Jan. 2016, doi: 10.1186/S13075-015-0908-7/METRICS.

[165] M. Y. Ansari, N. Ahmad, and T. M. Haqqi, “Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols,” *Biomed. Pharmacother.*, vol. 129, p. 110452, Sep. 2020, doi: 10.1016/J.BIOPHA.2020.110452.

[166] P. Lepetsos and A. G. Papavassiliou, “ROS/oxidative stress signaling in osteoarthritis,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1862, no. 4, pp. 576–591, Apr. 2016, doi: 10.1016/J.BBADIS.2016.01.003.

[167] O. M. Zahan, O. Serban, C. Gherman, and D. Fodor, “The evaluation of oxidative stress in osteoarthritis,” *Med. Pharm. Reports*, vol. 93, no. 1, p. 12, 2020, doi: 10.15386/MPR-1422.

[168] C. R. Chu, M. Szczodry, and S. Bruno, “Animal Models for Cartilage Regeneration and Repair,” *Tissue Eng. Part B. Rev.*, vol. 16, no. 1, p. 105, Feb. 2010, doi: 10.1089/TEN.TEB.2009.0452.

[169] D. M. Green, P. C. Noble, J. R. Bocell, J. S. Ahuero, B. A. Poteet, and H. H. Birdsall, “Effect of early full weight-bearing after joint injury on inflammation and cartilage degradation,” *J. Bone Joint Surg. Am.*, vol. 88, no. 10, pp. 2201–2209, 2006, doi: 10.2106/JBJS.E.00812.

[170] S. E. Bove *et al.*, “Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis,” *Osteoarthr. Cartil.*, vol. 11, no. 11, pp. 821–830, Nov. 2003, doi: 10.1016/S1063-4584(03)00163-8.

[171] K. Messner and W. Maletius, “The long-term prognosis for severe damage to weight-bearing cartilage in the knee: a 14-year clinical and radiographic follow-up in 28 young athletes,” *Acta Orthop. Scand.*, vol. 67, no. 2, pp. 165–168, 1996, doi: 10.3109/17453679608994664.

[172] I. Takahashi, T. Matsuzaki, S. Yoshida, I. Kitade, and M. Hoso, “Differences in Cartilage Repair between Loading and Unloading Environments in the Rat Knee,” *J. Japanese Phys. Ther. Assoc.*, vol. 17, no. 1, p. 22, 2014, doi: 10.1298/JJPTA.VOL17_004.

[173] H. B. Sun, “Mechanical loading, cartilage degradation, and arthritis,” *Ann. N. Y. Acad. Sci.*, vol. 1211, no. 1, pp. 37–50, Nov. 2010, doi: 10.1111/J.1749-6632.2010.05808.X.

[174] G. Musumeci, “The Effect of Mechanical Loading on Articular Cartilage,” *J. Funct. Morphol. Kinesiol. 2016, Vol. 1, Pages 154-161*, vol. 1, no. 2, pp. 154–161, Apr. 2016, doi: 10.3390/JFMK1020154.

[175] A. E. M. Jørgensen, M. Kjær, and K. M. Heinemeier, “The Effect of Aging and Mechanical Loading on the Metabolism of Articular Cartilage,” *J. Rheumatol.*, vol. 44, no. 4, pp. 410–417, Apr. 2017, doi: 10.3899/JRHEUM.160226.

[176] S. Fu *et al.*, “Mechanical loading inhibits cartilage inflammatory signalling via an HDAC6 and IFT-dependent mechanism regulating primary cilia elongation,” *Osteoarthr. Cartil.*, vol. 27, no. 7, pp. 1064–1074, Jul. 2019, doi: 10.1016/J.JOCA.2019.03.003.

[177] K. W. Aguilar-Agon, A. J. Capel, N. R. W. Martin, D. J. Player, and M. P. Lewis, “Mechanical loading stimulates hypertrophy in tissue-engineered skeletal muscle: Molecular and phenotypic responses,” *J. Cell. Physiol.*, vol. 234, no. 12, p. 23547, Dec. 2019, doi: 10.1002/JCP.28923.

[178] K. W. Aguilar-Agon, A. J. Capel, J. W. Fleming, D. J. Player, N. R. W. Martin, and M. P. Lewis, “Mechanical loading of tissue engineered skeletal muscle prevents dexamethasone induced myotube atrophy,” *J. Muscle Res. Cell Motil.*, vol. 42, no. 2, pp. 149–159, Jun. 2021, doi: 10.1007/S10974-020-09589-0/FIGURES/6.

[179] J. Klein-Nulend, R. G. Bacabac, and A. D. Bakker, “Mechanical loading and how it affects bone cells: the role of the osteocyte cytoskeleton in maintaining our skeleton,” *Eur. Cell. Mater.*, vol. 24, pp. 278–291, 2012, doi: 10.22203/ECM.V024A20.

[180] C. W. Dorman, H. E. Krug, S. P. Frizelle, S. Funkenbusch, and M. L. Mahowald, “A comparison of digigait™ and treadscan™ imaging systems: Assessment of

pain using gait analysis in murine monoarthritis," *J. Pain Res.*, vol. 7, pp. 25–35, 2013, doi: 10.2147/JPR.S52195.

[181] A. S. P. Lin, G. E. Salazar-noratto, and R. E. Guldberg, "EPIC-μCT imaging of articular cartilage," *Methods Mol. Biol.*, vol. 1226, pp. 131–140, 2015, doi: 10.1007/978-1-4939-1619-1_11.

[182] C. M. Bagi, D. E. Zakur, E. Berryman, C. J. Andresen, and D. Wilkie, "Correlation between μCT imaging, histology and functional capacity of the osteoarthritic knee in the rat model of osteoarthritis," *J. Transl. Med.*, vol. 13, no. 1, pp. 1–12, Aug. 2015, doi: 10.1186/S12967-015-0641-7/FIGURES/4.

[183] M. Y. Ansari *et al.*, "Mitochondrial dysfunction in osteoarthritis and aged cartilage triggers inflammatory response and matrix degradation via ros mediated activation of JNK-MAPK/cFos-AP1 axis in chondrocytes," *Osteoarthr. Cartil.*, vol. 28, p. S187, Apr. 2020, doi: 10.1016/j.joca.2020.02.304.

[184] F. J. Blanco, M. J. López-Armada, and E. Maneiro, "Mitochondrial dysfunction in osteoarthritis," *Mitochondrion*, vol. 4, no. 5–6, pp. 715–728, Sep. 2004, doi: 10.1016/J.MITO.2004.07.022.

[185] X. Mao, P. Fu, L. Wang, and C. Xiang, "Mitochondria: Potential Targets for Osteoarthritis," *Front. Med.*, vol. 7, p. 581402, Nov. 2020, doi: 10.3389/FMED.2020.581402.

[186] S. S. Gandhi, C. Muraresku, E. M. McCormick, M. J. Falk, and S. E. McCormack, "Risk factors for poor bone health in primary mitochondrial disease," *J. Inherit. Metab. Dis.*, vol. 40, no. 5, p. 673, Sep. 2017, doi: 10.1007/S10545-017-0046-2.

[187] W. A. Da Silveira *et al.*, "Comprehensive Multi-omics Analysis Reveals Mitochondrial Stress as a Central Biological Hub for Spaceflight Impact," *Cell*, vol. 183, pp. 1185-1201.e20, 2020, doi: 10.1016/j.cell.2020.11.002.

[188] R. Angireddy *et al.*, "Cytochrome c oxidase dysfunction enhances phagocytic function and osteoclast formation in macrophages," *FASEB J.*, vol. 33, no. 8, pp. 9167–9181, Aug. 2019, doi: 10.1096/FJ.201900010RR.

[189] P. F. Dobson *et al.*, "Mitochondrial dysfunction impairs osteogenesis, increases osteoclast activity, and accelerates age related bone loss," *Sci. Reports 2020 101*, vol. 10, no. 1, pp. 1–14, Jul. 2020, doi: 10.1038/s41598-020-68566-2.

[190] J. L. Scott *et al.*, "Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease," *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[191] L. Di Cesare Mannelli *et al.*, “Therapeutic Effects of the Superoxide Dismutase Mimetic Compound MnIIMe2DO2A on Experimental Articular Pain in Rats,” *Mediators Inflamm.*, vol. 2013, p. 11, 2013, doi: 10.1155/2013/905360.

[192] J. Y. LEE, S. U. LEE, T. LIM, and S. H. CHOI, “Healing Effects and Superoxide Dismutase Activity of Diode/Ga-As Lasers in a Rabbit Model of Osteoarthritis,” *In Vivo (Brooklyn).*, vol. 28, no. 6, 2014.

[193] A. Capozzi, C. Saucier, C. Bisbal, and K. Lambert, “Grape Polyphenols in the Treatment of Human Skeletal Muscle Damage Due to Inflammation and Oxidative Stress during Obesity and Aging: Early Outcomes and Promises,” *Molecules*, vol. 27, no. 19, Oct. 2022, doi: 10.3390/MOLECULES27196594.

[194] M. Paździor, M. Kiełczykowska, J. Kurzepa, D. Luchowska-kocot, J. Kocot, and I. Musik, “The Oxidative Stress in Knee Osteoarthritis Patients. An Attempt of Evaluation of Possible Compensatory Effects Occurring in the Disease Development,” *Medicina (Kaunas).*, vol. 55, no. 5, May 2019, doi: 10.3390/MEDICINA55050150.

[195] C. Ziskoven, M. Jäger, C. Zilkens, W. Bloch, K. Brixius, and R. Krauspe, “Oxidative stress in secondary osteoarthritis: from cartilage destruction to clinical presentation?,” *Orthop. Rev. (Pavia).*, vol. 2, no. 2, p. 23, Sep. 2010, doi: 10.4081/OR.2010.E23.

[196] V. Domazetovic, G. Marcucci, T. Iantomasi, M. L. Brandi, and M. T. Vincenzini, “Oxidative stress in bone remodeling: role of antioxidants,” *Clin. Cases Miner. Bone Metab.*, vol. 14, no. 2, p. 209, 2017, doi: 10.11138/CCMBM/2017.14.1.209.

[197] D. Lian, M. M. Chen, H. Wu, S. Deng, and X. Hu, “The Role of Oxidative Stress in Skeletal Muscle Myogenesis and Muscle Disease,” *Antioxidants*, vol. 11, no. 4, Apr. 2022, doi: 10.3390/ANTIOX11040755.

[198] M. Shimbo *et al.*, “Ground-based assessment of JAXA mouse habitat cage unit by mouse phenotypic studies,” *Exp. Anim.*, vol. 65, no. 2, pp. 175–187, 2016, doi: 10.1538/EXPANIM.15-0077.

[199] D. Shiba *et al.*, “Development of new experimental platform ’MARS’-Multiple Artificial-gravity Research System-to elucidate the impacts of micro/partial gravity on mice,” *Sci. Rep.*, vol. 7, no. 1, Dec. 2017, doi: 10.1038/S41598-017-10998-4.

[200] G. Clément, “International roadmap for artificial gravity research,” *npj Microgravity 2017 31*, vol. 3, no. 1, pp. 1–7, Nov. 2017, doi: 10.1038/s41526-017-0034-8.

[201] R. Ellman, J. Spatz, A. Cloutier, R. Palme, B. A. Christiansen, and M. L. Bouxsein, “Partial Reductions in Mechanical Loading Yield Proportional Changes

in Bone Density, Bone Architecture, and Muscle Mass," *J. Bone Miner. Res.*, vol. 28, no. 4, p. 875, Apr. 2013, doi: 10.1002/JBMR.1814.

[202] G. R. Clément, A. P. Bukley, and W. H. Paloski, "Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions," *Front. Syst. Neurosci.*, vol. 9, no. June, p. 92, Jun. 2015, doi: 10.3389/FNSYS.2015.00092/BIBTEX.

[203] S. Hinterwimmer *et al.*, "Cartilage atrophy in the knees of patients after seven weeks of partial load bearing," *Arthritis Rheum.*, vol. 50, no. 8, pp. 2516–2520, Aug. 2004, doi: 10.1002/ART.20378.

[204] H. Q. Luan *et al.*, "Use of micro-computed tomography to evaluate the effects of exercise on preventing the degeneration of articular cartilage in tail-suspended rats," *Life Sci. Sp. Res.*, vol. 6, pp. 15–20, Jul. 2015, doi: 10.1016/J.LSSR.2015.06.001.

[205] R. B. Souza *et al.*, "Effects of unloading on knee articular cartilage T1rho and T2 magnetic resonance imaging relaxation times: a case series," *J. Orthop. Sports Phys. Ther.*, vol. 42, no. 6, pp. 511–520, 2012, doi: 10.2519/JOSPT.2012.3975.

[206] A. M. Liphardt *et al.*, "Vibration training intervention to maintain cartilage thickness and serum concentrations of cartilage oligometric matrix protein (COMP) during immobilization.," *Osteoarthr. Cartil.*, vol. 17, no. 12, pp. 1598–1603, Dec. 2009, doi: 10.1016/J.JOCA.2009.07.007.

[207] A. V. Stone, R. F. Loeser, K. S. Vanderman, D. L. Long, S. C. Clark, and C. M. Ferguson, "Pro-inflammatory stimulation of meniscus cells increases production of matrix metalloproteinases and additional catabolic factors involved in osteoarthritis pathogenesis," *Osteoarthr. Cartil.*, vol. 22, no. 2, pp. 264–274, Feb. 2014, doi: 10.1016/J.JOCA.2013.11.002.

[208] D. T. Felson, "Osteoarthritis as a disease of mechanics," *Osteoarthr. Cartil.*, vol. 21, no. 1, pp. 10–15, Jan. 2013, doi: 10.1016/J.JOCA.2012.09.012.

[209] G. N. Williams, T. Chmielewski, K. S. Rudolph, T. S. Buchanan, and L. Snyder-Mackler, "Dynamic knee stability: current theory and implications for clinicians and scientists," *J. Orthop. Sports Phys. Ther.*, vol. 31, no. 10, pp. 546–566, 2001, doi: 10.2519/JOSPT.2001.31.10.546.

[210] D. L. Bader, D. M. Salter, and T. T. Chowdhury, "Biomechanical influence of cartilage homeostasis in health and disease," *Arthritis*, vol. 2011, p. 979032, Sep. 2011, doi: 10.1155/2011/979032.

[211] B. Vanwanseele, F. Eckstein, H. Knecht, A. Spaepen, and E. Stüssis, "Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord

injury," *Arthritis Rheum.*, vol. 48, no. 12, pp. 3377–3381, 2003, doi: 10.1002/ART.11367.

[212] H. S. Yercan, T. Ait Si Selmi, T. S. Sugun, and P. Neyret, "Tibiofemoral instability in primary total knee replacement: a review, Part 1: Basic principles and classification," *Knee*, vol. 12, no. 4, pp. 257–266, 2005, doi: 10.1016/J.KNEE.2005.01.004.

[213] H. B. Sun, "Mechanical loading, cartilage degradation, and arthritis," *Ann. N. Y. Acad. Sci.*, vol. 1211, pp. 37–50, 2010, doi: 10.1111/J.1749-6632.2010.05808.X.

[214] L. S. Lohmander, P. M. Englund, L. L. Dahl, and E. M. Roos, "The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis," *Am. J. Sports Med.*, vol. 35, no. 10, pp. 1756–1769, Oct. 2007, doi: 10.1177/0363546507307396.

[215] D. J. Leong *et al.*, "Matrix metalloproteinase-3 in articular cartilage is upregulated by joint immobilization and suppressed by passive joint motion," *Matrix Biol.*, vol. 29, no. 5, pp. 420–426, Jun. 2010, doi: 10.1016/J.MATBIO.2010.02.004.

[216] J. S. Willey, S. A. J. Lloyd, G. A. Nelson, and T. A. Bateman, "Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications," *Clin. Rev. Bone Miner. Metab.*, vol. 9, no. 1, pp. 54–62, 2011, doi: 10.1007/S12018-011-9092-8.

[217] S. A. Lloyd *et al.*, "Effect of Proton Irradiation Followed by Hindlimb Unloading on Bone in Mature Mice: A Model of Long-Duration Spaceflight," *Bone*, vol. 51, no. 4, p. 756, Oct. 2012, doi: 10.1016/J.BONE.2012.07.001.

[218] J. S. Alwood, A. Kumar, L. H. Tran, A. Wang, C. L. Limoli, and R. K. Globus, "Low-dose, ionizing radiation and age-related changes in skeletal microarchitecture," *J. Aging Res.*, vol. 2012, 2012, doi: 10.1155/2012/481983.

[219] L. Gabel *et al.*, "Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia 1 year after return from long duration spaceflight," *Sci. Reports 2022 121*, vol. 12, no. 1, pp. 1–13, Jun. 2022, doi: 10.1038/s41598-022-13461-1.

[220] R. Cappellesso, L. Nicole, A. Guido, and D. Pizzol, "Spaceflight osteoporosis: current state and future perspective," *Endocr. Regul.*, vol. 49, no. 4, pp. 231–239, 2015, doi: 10.4149/ENDO_2015_04_231.

[221] M. Stavnichuk, N. Mikolajewicz, T. Corlett, M. Morris, and S. V. Komarova, "A systematic review and meta-analysis of bone loss in space travelers," *npj Microgravity 2020 61*, vol. 6, no. 1, pp. 1–9, May 2020, doi: 10.1038/s41526-020-0103-2.

[222] J. D. Sibonga *et al.*, “Recovery of spaceflight-induced bone loss: bone mineral density after long-duration missions as fitted with an exponential function,” *Bone*, vol. 41, no. 6, pp. 973–978, Dec. 2007, doi: 10.1016/J.BONE.2007.08.022.

[223] S. A. Davis and B. L. Davis, “Exercise equipment used in microgravity: challenges and opportunities,” *Curr. Sports Med. Rep.*, vol. 11, no. 3, pp. 142–147, May 2012, doi: 10.1249/JSR.0B013E3182578FC3.

[224] S. M. Smith, M. A. Heer, L. C. Shackelford, J. D. Sibonga, L. Ploutz-Snyder, and S. R. Zwart, “Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry,” *J. Bone Miner. Res.*, vol. 27, no. 9, pp. 1896–1906, Sep. 2012, doi: 10.1002/JBMR.1647.

[225] S. Khosla *et al.*, “Benefits and risks of bisphosphonate therapy for osteoporosis,” *J. Clin. Endocrinol. Metab.*, vol. 97, no. 7, pp. 2272–2282, Jul. 2012, doi: 10.1210/JC.2012-1027.

[226] J. D. Sibonga, “Spaceflight-induced bone loss: is there an osteoporosis risk?,” *Curr. Osteoporos. Rep.*, vol. 11, no. 2, pp. 92–98, Jun. 2013, doi: 10.1007/S11914-013-0136-5.

[227] A. C. Looker *et al.*, “Updated data on proximal femur bone mineral levels of US adults,” *Osteoporos. Int.*, vol. 8, no. 5, pp. 468–489, 1998, doi: 10.1007/S001980050093.

[228] J. H. Keyak, A. K. Koyama, A. LeBlanc, Y. Lu, and T. F. Lang, “Reduction in proximal femoral strength due to long-duration spaceflight,” *Bone*, vol. 44, no. 3, pp. 449–453, Mar. 2009, doi: 10.1016/J.BONE.2008.11.014.

[229] B. PAt, L. Beery Mack, P. A. Lacuaxce, G. P. Vose, and F. B. Vogt, “AMERICAN JOURNAL OF ROENTGENOLOGY RADIUM THERAPY AND NUCLEAR MEDICINE BONE DEMINERALIZATION OF FOOT AND HAND OF GEMINI-TITAN IV, V AND VII ASTRO-NAUTS DURING ORBITAL FLIGHT*,” Accessed: Dec. 26, 2022. [Online]. Available: www.ajronline.org.

[230] E. S. Orwoll *et al.*, “Skeletal health in long-duration astronauts: Nature, assessment, and management recommendations from the NASA bone summit,” *J. Bone Miner. Res.*, vol. 28, no. 6, pp. 1243–1255, Jun. 2013, doi: 10.1002/JBMR.1948.

[231] J. Yang, G. Zhang, D. Dong, and P. Shang, “Effects of Iron Overload and Oxidative Damage on the Musculoskeletal System in the Space Environment: Data from Spaceflights and Ground-Based Simulation Models,” *Int. J. Mol. Sci. 2018, Vol. 19, Page 2608*, vol. 19, no. 9, p. 2608, Sep. 2018, doi: 10.3390/IJMS19092608.

[232] A. Kumar, C. G. T. Tahimic, E. A. C. Almeida, and R. K. Globus, “Spaceflight modulates the expression of key oxidative stress and cell cycle related genes in heart,” *Int. J. Mol. Sci.*, vol. 22, no. 16, Aug. 2021, doi: 10.3390/IJMS22169088/S1.

[233] T. P. Stein, “Space flight and oxidative stress,” *Nutrition*, vol. 18, no. 10, pp. 867–871, 2002, doi: 10.1016/S0899-9007(02)00938-3.

[234] C. G. T. Tahimic and R. K. Globus, “Redox Signaling and Its Impact on Skeletal and Vascular Responses to Spaceflight,” *Int. J. Mol. Sci.*, vol. 18, no. 10, Oct. 2017, doi: 10.3390/IJMS18102153.

[235] L. Vico *et al.*, “Cortical and Trabecular Bone Microstructure Did Not Recover at Weight-Bearing Skeletal Sites and Progressively Deteriorated at Non-Weight-Bearing Sites During the Year Following International Space Station Missions,” *J. Bone Miner. Res.*, vol. 32, no. 10, pp. 2010–2021, Oct. 2017, doi: 10.1002/JBMR.3188.

[236] H. S. Cohen, K. T. Kimball, A. P. Mulavara, J. J. Bloomberg, and W. H. Paloski, “Posturography and locomotor tests of dynamic balance after long-duration spaceflight,” *J. Vestib. Res.*, vol. 22, no. 4, pp. 191–196, 2012, doi: 10.3233/VES-2012-0456.

[237] K. L. English, S. M. C. Lee, J. A. Loehr, R. J. Ploutz-Snyder, L. L. Ploutz-Snyder, and J. M. Reeves, “Isokinetic Strength Changes Following Long-Duration Spaceflight on the ISS,” *Aerosp. Med. Hum. Perform.*, vol. 86, no. 12 Suppl, pp. A68–A77, Dec. 2015, doi: 10.3357/AMHP.EC09.2015.

[238] N. Petersen, G. Lambrecht, J. Scott, N. Hirsch, M. Stokes, and J. Mester, “Postflight reconditioning for European Astronauts - A case report of recovery after six months in space,” *Musculoskelet. Sci. Pract.*, vol. 27 Suppl 1, pp. S23–S31, Jan. 2017, doi: 10.1016/J.MSKSP.2016.12.010.

[239] G. D. Tays *et al.*, “The Effects of Long Duration Spaceflight on Sensorimotor Control and Cognition,” *Front. Neural Circuits*, vol. 15, p. 110, Oct. 2021, doi: 10.3389/FNCIR.2021.723504/BIBTEX.

[240] J. Fitzgerald, “Cartilage breakdown in microgravity—a problem for long-term spaceflight?,” *npj Regen. Med. 2017 21*, vol. 2, no. 1, pp. 1–2, Apr. 2017, doi: 10.1038/s41536-017-0016-1.

[241] V. Ramachandran, R. Wang, S. S. Ramachandran, A. S. Ahmed, K. Phan, and E. L. Antonsen, “Effects of spaceflight on cartilage: implications on spinal physiology,” *J. Spine Surg.*, vol. 4, no. 2, p. 433, Jun. 2018, doi: 10.21037/JSS.2018.04.07.

[242] O. J. Juhl, E. G. Buettmann, M. A. Friedman, R. C. DeNapoli, G. A. Hoppock, and H. J. Donahue, “Update on the effects of microgravity on the musculoskeletal system,” *npj Microgravity*, vol. 7, no. 1, Dec. 2021, doi: 10.1038/S41526-021-00158-4.

[243] B. Ganse, M. Cucchiarini, and H. Madry, “Joint Cartilage in Long-Duration Spaceflight,” *Biomedicines*, vol. 10, no. 6, Jun. 2022, doi: 10.3390/BIOMEDICINES10061356.

[244] P. H. U. Lee, M. Chung, Z. Ren, D. B. Mair, and D. H. Kim, “Factors mediating spaceflight-induced skeletal muscle atrophy,” *Am. J. Physiol. Cell Physiol.*, vol. 322, no. 3, pp. C567–C580, Mar. 2022, doi: 10.1152/AJPCELL.00203.2021.

[245] E. A. Radugina, E. A. C. Almeida, E. Blaber, V. A. Poplinskaya, Y. V. Markitantova, and E. N. Grigoryan, “Exposure to microgravity for 30 days onboard Bion M1 caused muscle atrophy and impaired regeneration in murine femoral Quadriceps,” *Life Sci. Sp. Res.*, vol. 16, pp. 18–25, Feb. 2018, doi: 10.1016/j.lssr.2017.08.005.

[246] H. Vandenburgh, J. Chromiak, J. Shansky, M. Del Tatto, and J. Lemaire, “Space travel directly induces skeletal muscle atrophy,” *FASEB J.*, vol. 13, no. 9, pp. 1031–1038, Jun. 1999, doi: 10.1096/FASEBJ.13.9.1031.

[247] A. D. Kokkinias and S.-O. D. Kokkinias, “Mechanical loading effect to the functional bone adaptation,” *J. Frailty, Sarcopenia Falls*, vol. 2, no. 3, p. 62, Sep. 2017, Accessed: Dec. 26, 2022. [Online]. Available: /pmc/articles/PMC7161930/.

[248] P. Bergmann *et al.*, “Loading and Skeletal Development and Maintenance,” *J. Osteoporos.*, vol. 2011, pp. 1–15, 2011, doi: 10.4061/2011/786752.

[249] J. C. Fritton, E. R. Myers, T. M. Wright, and M. C. H. Van Der Meulen, “Loading induces site-specific increases in mineral content assessed by microcomputed tomography of the mouse tibia,” *Bone*, vol. 36, no. 6, pp. 1030–1038, Jun. 2005, doi: 10.1016/J.BONE.2005.02.013.

[250] H. M. Frost, “From Wolff’s law to the Utah paradigm: Insights about bone physiology and its clinical applications,” *Anat. Rec.*, vol. 262, no. 4, pp. 398–419, Apr. 2001, doi: 10.1002/AR.1049.

[251] E. Isasi, M. E. Isasi, and J. J. W. A. van Loon, “The application of artificial gravity in medicine and space,” *Front. Physiol.*, vol. 13, Aug. 2022, doi: 10.3389/FPHYS.2022.952723.

[252] M. Shimbo *et al.*, “Ground-based assessment of JAXA mouse habitat cage unit by mouse phenotypic studies,” *Exp. Anim.*, vol. 65, no. 2, pp. 175–187, 2016, doi: 10.1538/EXPANIM.15-0077.

[253] D. Shiba *et al.*, “Development of new experimental platform ‘MARS’—Multiple Artificial-gravity Research System—to elucidate the impacts of micro/partial gravity on mice,” *Sci. Reports 2017 71*, vol. 7, no. 1, pp. 1–10, Sep. 2017, doi: 10.1038/S41598-017-10998-4.

[254] T. Tominari *et al.*, “Hypergravity and microgravity exhibited reversal effects on the bone and muscle mass in mice,” *Sci. Rep.*, vol. 9, no. 1, Dec. 2019, doi: 10.1038/S41598-019-42829-Z.

[255] A. Okada *et al.*, “Bisphosphonate Use May Reduce the Risk of Urolithiasis in Astronauts on Long-Term Spaceflights,” *JBMR Plus*, vol. 6, no. 1, Jan. 2022, doi: 10.1002/JBM4.10550.

[256] J. Sibonga *et al.*, “Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss,” *Bone*, vol. 128, Nov. 2019, doi: 10.1016/J.BONE.2019.07.013.

[257] K. A. Greene, J. A. Tooze, L. Lenchik, and A. A. Weaver, “Change in Lumbar Muscle Size and Composition on MRI with Long-Duration Spaceflight,” *Ann. Biomed. Eng.*, vol. 50, no. 7, pp. 816–824, Jul. 2022, doi: 10.1007/S10439-022-02968-3.

[258] T. Gui *et al.*, “Superoxide dismutase-loaded porous polymersomes as highly efficient antioxidant nanoparticles targeting synovium for osteoarthritis therapy,” *Biomaterials*, vol. 283, p. 121437, Apr. 2022, doi: 10.1016/J.BIOMATERIALS.2022.121437.

[259] L. Di Cesare Mannelli *et al.*, “Therapeutic Effects of the Superoxide Dismutase Mimetic Compound MnIIMe2DO2A on Experimental Articular Pain in Rats,” *Mediators Inflamm.*, vol. 2013, p. 11, 2013, doi: 10.1155/2013/905360.

[260] D. H. Yu *et al.*, “Over-expression of extracellular superoxide dismutase in mouse synovial tissue attenuates the inflammatory arthritis,” *Exp. Mol. Med. 2012 449*, vol. 44, no. 9, pp. 529–535, Jun. 2012, doi: 10.3858/emm.2012.44.9.060.

[261] M. Koike *et al.*, “Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage,” *PLoS One*, vol. 13, no. 9, p. e0203944, Sep. 2018, doi: 10.1371/JOURNAL.PONE.0203944.

[262] J. L. Scott *et al.*, “Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease,” *Ann. Rheum. Dis.*, vol. 69, no. 8, p. 1502, Aug. 2010, doi: 10.1136/ARD.2009.119966.

[263] J. M. Evans, C. F. Knapp, and N. Goswami, “Artificial gravity as a countermeasure to the cardiovascular deconditioning of spaceflight: Gender

perspectives," *Front. Physiol.*, vol. 9, no. JUL, p. 716, Jul. 2018, doi: 10.3389/FPHYS.2018.00716/BIBTEX.

[264] G. Clément, "International roadmap for artificial gravity research," *NPJ microgravity*, vol. 3, no. 1, Dec. 2017, doi: 10.1038/S41526-017-0034-8.

[265] R. Ellman, J. Spatz, A. Cloutier, R. Palme, B. A. Christiansen, and M. L. Bouxsein, "Partial reductions in mechanical loading yield proportional changes in bone density, bone architecture, and muscle mass," *J. Bone Miner. Res.*, vol. 28, no. 4, pp. 875–885, Apr. 2013, doi: 10.1002/JBMR.1814.

[266] G. R. Clément, A. P. Bukley, W. H. Paloski, A. Mulavara, M. M. Cohen, and B. Mcgrath, "Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions," 1961, doi: 10.3389/fnsys.2015.00092.

[267] J. Sibonga *et al.*, "Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss," *Bone*, vol. 128, Nov. 2019, doi: 10.1016/j.bone.2019.07.013.

[268] A. LeBlanc *et al.*, "Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight," *Osteoporos. Int.*, vol. 24, no. 7, pp. 2105–2114, Jul. 2013, doi: 10.1007/S00198-012-2243-Z.

[269] S. M. Smith, M. A. Heer, L. C. Shackelford, J. D. Sibonga, L. Ploutz-Snyder, and S. R. Zwart, "Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry," *J. Bone Miner. Res.*, vol. 27, no. 9, pp. 1896–1906, Sep. 2012, doi: 10.1002/jbmr.1647.

[270] J. C. Buckey, "Preparing for Mars: the physiologic and medical challenges.," *European journal of medical research*, 1999. https://pubmed.ncbi.nlm.nih.gov/10477498/ (accessed Dec. 13, 2022).

[271] J. S. Willey, S. A. J. Lloyd, G. A. Nelson, and T. A. Bateman, "Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications," *Clin Rev Bone Min. Metab*, vol. 9, no. 1, pp. 54–62, 2011, doi: 10.1007/s12018-011-9092-8.

[272] J. S. Willey, S. A. J. Lloyd, G. A. Nelson, and T. A. Bateman, "Space Radiation and Bone Loss."

[273] T. Tominari *et al.*, "Hypergravity and microgravity exhibited reversal effects on the bone and muscle mass in mice," *Sci. Reports 2019 91*, vol. 9, no. 1, pp. 1–10, Apr. 2019, doi: 10.1038/s41598-019-42829-z.

[274] R. O. Nielsen, P. Schjerling, P. Tesch, P. Stål, and H. Langberg, "Collagen content in the vastus lateralis and the soleus muscle following a 90-day bed rest period

with or without resistance exercises," *Muscles. Ligaments Tendons J.*, vol. 5, no. 4, p. 305, Oct. 2015, doi: 10.11138/MLTJ/2015.5.4.305.

[275] B. J. Parmenter, J. Raymond, P. J. Dinnen, R. J. Lusby, and M. A. Fiatarone Singh, "Preliminary evidence that low ankle-brachial index is associated with reduced bilateral hip extensor strength and functional mobility in peripheral arterial disease," *J. Vasc. Surg.*, vol. 57, no. 4, pp. 963-973.e1, 2013, doi: 10.1016/J.JVS.2012.08.103.

[276] A. Leblanc *et al.*, "Muscle volume, MRI relaxation times (T2), and body composition after spaceflight," 2000, Accessed: Dec. 13, 2022. [Online]. Available: http://www.jap.org.

[277] A. T. Kwok *et al.*, "Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents," *Sci. Rep.*, vol. 11, no. 1, Dec. 2021, doi: 10.1038/S41598-021-90010-2.

[278] A. Kwok *et al.*, "Altered rodent gait characteristics after ~35 days in orbit aboard the International Space Station," *Life Sci. Sp. Res.*, vol. 24, pp. 9–17, Feb. 2020, doi: 10.1016/J.LSSR.2019.10.010.