Effect of Spaceflight on Kidney-related Immune System Dysregulatory Diseases in Older *Mus musculus*

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OSD-771 Team 3

Abstract:

The combined effects of spaceflight have different physiological effects in all organisms. Spaceflight is known to affect immune system function, decrease bone strength, and affect almost every part of organisms' organ systems. In mice, these changes are no different, as studies of mice in space show similar effects on the organ systems of mice. The immune system itself shows downregulation in important genes after spaceflight, and even human astronauts become more vulnerable to both infectious and chronic diseases after time in space.

One less studied portion of the immune system in space, though, are **kidney-related immune conditions**. After analysing OSD-771, which included data from the kidneys of mice exposed to spaceflight, we discovered some immune changes in older female mice related to inflammation, namely upregulation of genes found in the **IL-17 signaling pathway**. Our proposed study involves testing mice for these conditions in order to analyze if stimulated spaceflight conditions, namely **microgravity**, increases the risk of developing kidney-related immune conditions such as autoimmune glomerulonephritis.

We propose to assess whether spaceflight factors induce inflammatory and autoimmune conditions in mice. By simulating microgravity on Earth with **hindlimb unloading**, then testing for increased interleukin and cytokine activation and other molecules related to the **immune and inflammatory response**, we can detect changes to the immune system related to autoimmune conditions in space. As most mice genes are **homologous** to human genes, the results of our study, if they support our hypothesis, could be used to develop **preventive measures or corrective action** to protect human astronauts and other organisms in space from autoimmune diseases and inflammation, thus furthering our understanding of necessary protocols for spaceflight.

Specific Aims:

After analyzing OSD-771, a database containing kidney samples from mice exposed to spaceflight, there appeared to be upregulation of genes found in the IL-17 pathway. As an **upregulation of genes in the IL-17 pathway** has been linked to autoimmune conditions [1], our experiments focus on identifying how exposure to simulated microgravity through hindlimb unloading could lead to an increase in autoimmune conditions in mice.

Hypothesis: If older (~29 week old) female mice (*Mus musculus*) are exposed to microgravity, then they will be more susceptible to **kidney-related autoimmune** and i**nflammatory diseases** due to **upregulation of the IL-17 inflammatory response pathway**.

Aim 1: Determine whether older female mice exposed to microgravity have an **overactive inflammatory response system**, as measured by changes in levels of **inflammatory cytokines** connected to the IL-17 pathway.

We propose to measure the levels of inflammatory cytokines by exposing the mice to a simulated microgravity environment on Earth and then running **qPCR** on the cell lysate to quantify the amount of cytokines produced within and downstream of the IL-17 pathway. We then would use a multiplex immunoassay to detect the downstream chemokines, which are all important in immune system activation and inflammation. By measuring the levels of these transcripts and proteins and comparing them to the ground control mice, this aim would show whether or not **IL-17 expression** is truly **increased** in mice exposed to spaceflight conditions.

Aim 2: Determine whether older female mice exposed to simulated microgravity have changes in **levels of key blood proteins present** in **urine** and **retention of waste products** in the blood indicative of kidney-related autoimmune conditions.

We propose to test the urine of mice exposed to simulated microgravity for 2 months for **abnormal protein content**, as proteinuria is a common indicator of kidney disease caused by inflammatory conditions. Other tests such as the blood urea nitrogen test as well as urine tests for proteins normally found in the blood and waste products that should be filtered out will be conducted. We will calculate creatinine clearance, albumin-to-creatinine ratio, and urea blood levels to **determine alterations to renal function**. By running these tests on the mice, we will determine whether older mice who have been exposed to simulated microgravity are **more likely** to develop kidney-related autoimmune conditions, such as **glomerulonephritis**.

Aim 3: Assess whether exposure to microgravity in older female mice has **chronic impacts** on the inflammatory response and the kidney by observing changes in levels of inflammation marker, **serum amyloid** P, and determining **how long** deviant levels may persist after returning to Earth-gravity levels.

We propose to analyze blood from mice exposed to microgravity for serum amyloid P during simulated microgravity exposure, 3 days, 2 weeks, 1 month, 3 months, and 5 months after simulated microgravity exposure. By measuring these proteins, which are produced in response to inflammation, we will determine whether the **risks** of developing inflammation and autoimmune diseases are **increased** due to spaceflight, and **how long** these changes **persist** following return to Earth.

Research Strategy:

Introduction:

Space is known to have adverse impacts on the immune system of living organisms, as continuously proven through past studies with model organisms such as mice and through analysis of the health of human astronauts from over twenty years in research [3]. Our current understanding regarding this matter is that the stressors of space (microgravity, ionizing radiation, high vacuum, intense temperatures, magnetic fields, and pressure) [12] contribute to impaired immune function that persists postflight [3]. Spaceflight-related immune dysfunction involves **persistent inflammation**, with **increased** levels of **pro-inflammatory** proteins and **decreased** levels of those which are **anti-inflammatory**, in addition to **reduced function** of T and NK cells and **dysregulation** of plasma **cytokine** concentrations [8,9]. The evidence garnered from over twenty years of research are strongly indicative of the relationship between exposure to spaceflight and immune system dysregulation as not merely correlation but **causal** [9].

One aspect of the space exposome, microgravity, has been especially researched on. The way the body of any organism functions is reliant on the gravitational force [4]. The lack of these forces have adverse effects on the physiology of living organisms, the greatest being a **significantly dysfunctional immune system** [4]. A study involving Human T-lymphoblastoid cells, a particular type of immune cells, showed increased rates of **apoptosis** when these cells were exposed to microgravity [12], connecting back to the idea that the body is in a **pro-inflammatory** state with exposure to space, but now specifically to microgravity.

The immune system has well-recognized relations with some of the other body systems, like the lymphatic system, but the relation between the kidneys and the immune system is, too, important. Studies done on the impact of a dysfunctional kidney on the immune system and vice versa reveals each are **adversely affected** in a significant way and how interconnected they are to each other's functions. For example, if the kidneys are unable to filter waste properly, and so incapable of maintaining **immune homeostasis**, the **accumulation** of **waste** will cause an immune reaction, contributing to **inflammation** and **immunodeficiency**. In a similar aspect, breakdowns in the immune system can contribute to **renal conditions** involving the inflammation and physical damage of kidneys, such as glomerulonephritis [5].

Researchers have analyzed the effect of space conditions on the kidney as well. Exposure to spaceflight-like conditions caused an increased risk of kidney stones, scarring, and a loss of function [11]. Beyond this, however, there still remains many questions, especially circulating **kidney-related immune conditions**. Despite the noticing of a correlation and possible causation relationship between space factors and the kidneys, there has not been much insight into renal diseases with relation to spaceflight factors, let alone autoimmune or inflammatory renal conditions, even though kidneys of astronauts developing issues is an ever-present problem. NASA has identified this issue as a clear obstacle to safe long-duration spaceflight. The Human Research Program (HRP) has explicitly stated the necessity for determining how spaceflight stressors contribute to dysfunction of the immune system and for validating **reliable biomarkers** to be able to monitor immune function status [8].

Despite extensive work on immune dysregulation as a whole, the way in which these mechanisms interact with specific organ systems, such as the kidneys, and how these interactions possibly influence the health of astronauts is far less understood. Addressing this underexplored connection would help to advance scientific understanding and also contribute to a recognized HRP research gap.

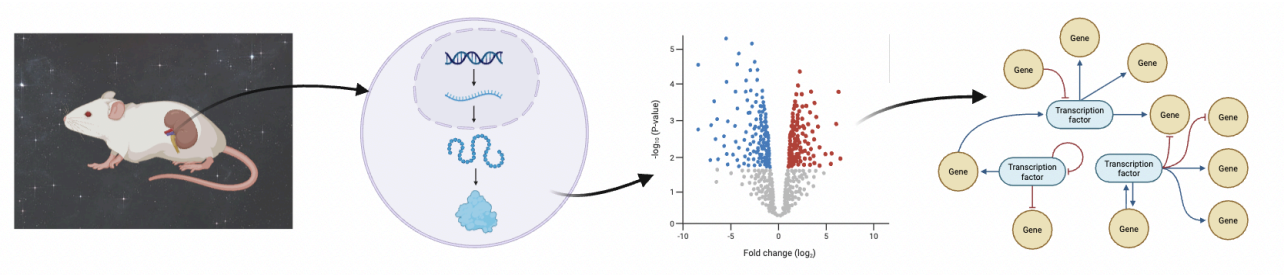


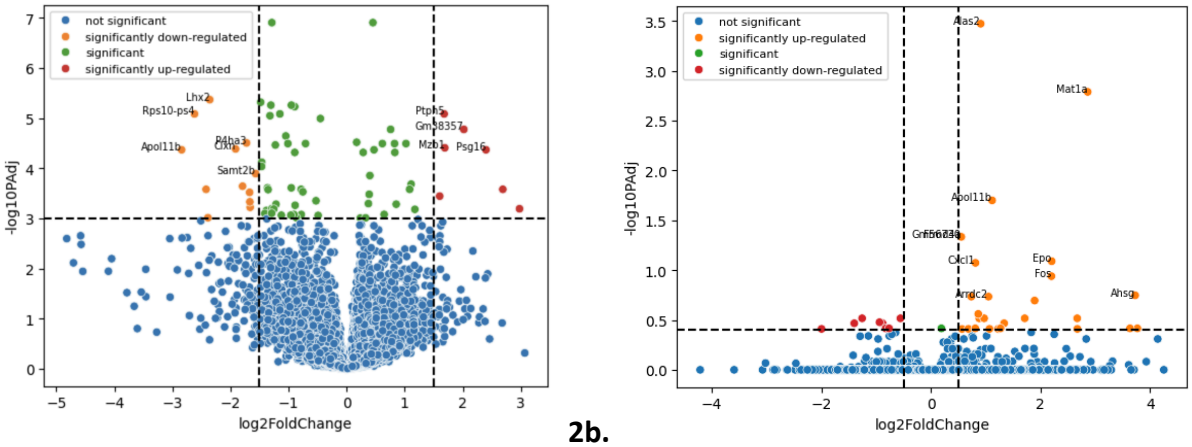
Figure 1. ***Flow diagram for OSD-771 and our analysis of the dataset.*** *Mice are exposed to simulated spaceflight conditions (hindlimb unloading to mimic microgravity), and kidney tissue is collected for further transcriptomic analysis. Gene expression fold changes are examined from RNA-seq, followed by differential gene expression analysis to identify up- and down-regulated genes plotted in volcano plots for younger and older mice. Based on the genes highlighted as having a significant change in expression, changes in whole pathways of genes and proteins could be identified, suggesting impacts related to their functions.*

Preliminary Data:

To address this question, we used the publicly available data from the NASA Open Science Data Repository (**OSD-771**). The dataset examined the effect of spaceflight factors on kidney gene expression and on aspects of female *Mus Musculus*'s physiology, including circadian rhythms, metabolism, and immune system. The samples were collected by euthanizing mice either on the International Space Station (FLT_ISS) or after returning to Earth (FLT_LAR). Researchers dissected the kidney tissue and then they snap-froze it at −80 °C for RNA extraction and sequencing. Ground control animals were kept at Kennedy Space Center in similar conditions. RNA was extracted from frozen kidney tissue using standard RNA-Seq preparation methods. While specific RIN scores and extraction methods were not mentioned, the dataset produced high-quality RNA-Seq data, which shows that the RNA integrity was consistently high, and no major differences in RNA quality were noted across experimental conditions [10].

Using the metadata from OSD-771, we first did quality control and normalization on the raw count data. Genes with low expression were filtered out to avoid obscuring the results of the analysis (reducing noise), and counts were normalized using techniques like the **median-of-ratios** in DESeq2 to account for library size variations. Differential expression analysis was conducted with well-known R/Bioconductor packages **DESeq2** and **edgeR**, which model count data while taking biological variability and sample size into account. To manage multiple hypothesis testing, p-values were adjusted for false discovery rate (FDR). If any batch effects were present, they were modeled or corrected to avoid confusion. Significantly upregulated and downregulated genes were set as those with an **absolute $\log_2$ fold change greater than 1** and an **adjusted p-value of less than 0.05**.

With this, we were able to identify sets of genes that were differentially expressed under spaceflight conditions, which we then visualized for comparison and to notice anything interesting. After running the pyDeseq2 tool on the two different age groups of mice in the dataset, the following two volcano plots were produced. One group was **young female *Mus Musculus* (~ 12 weeks old)** and the other was **old female *Mus Musculus* (29 weeks old)**.



2a. **2b.**

Figure 2. ***(a) Volcano plot for young mice.*** *Analysis of gene expression in young mice kidney tissue revealed a quantity of genes which was significantly affected.* ***(b) Volcano plot for old mice****. Analysis of gene expression in old mice kidney tissue returned results for fewer genes in comparison to the young mice, but interestingly a quantity of genes is significantly upregulated.*

Examining closer, **27.6%** of the differentially expressed genes (across both age groups) were **immune-related** (the high percentage aligns with known information of interconnectedness between the kidneys and the immune system). A significant portion of those genes are involved in the stress and inflammatory response pathways. All of this points to an **overactive inflammatory state** in kidneys under spaceflight conditions. As shown in Figure 2b, comparison of old mice exposed to spaceflight compared to old mice at 1g resulted in 16 genes significantly upregulated. Using tools like StringDB (Fig. 3) and ShinyGO (Fig. 4), which we employed for functional analysis and visualizations, we observed a pathway that frequently showed up in our a cluster of those genes ***cxcl1, fos, and fosb***, are involved in the IL-17 pathway, which activates further inflammatory cytokines such as IL-6 and TNF-α.

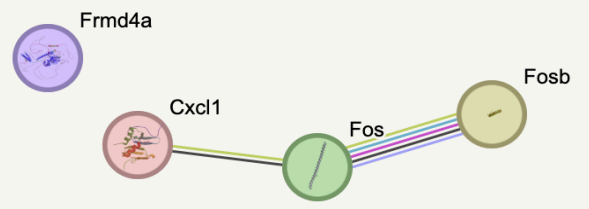


Figure 3. StringDB's Visualization of Common Pathways for Old Mice DEGs. *Cxcl1, fos, and fosb all share one common pathway, which upon further analysis reveals to be the IL-17 pathway.*

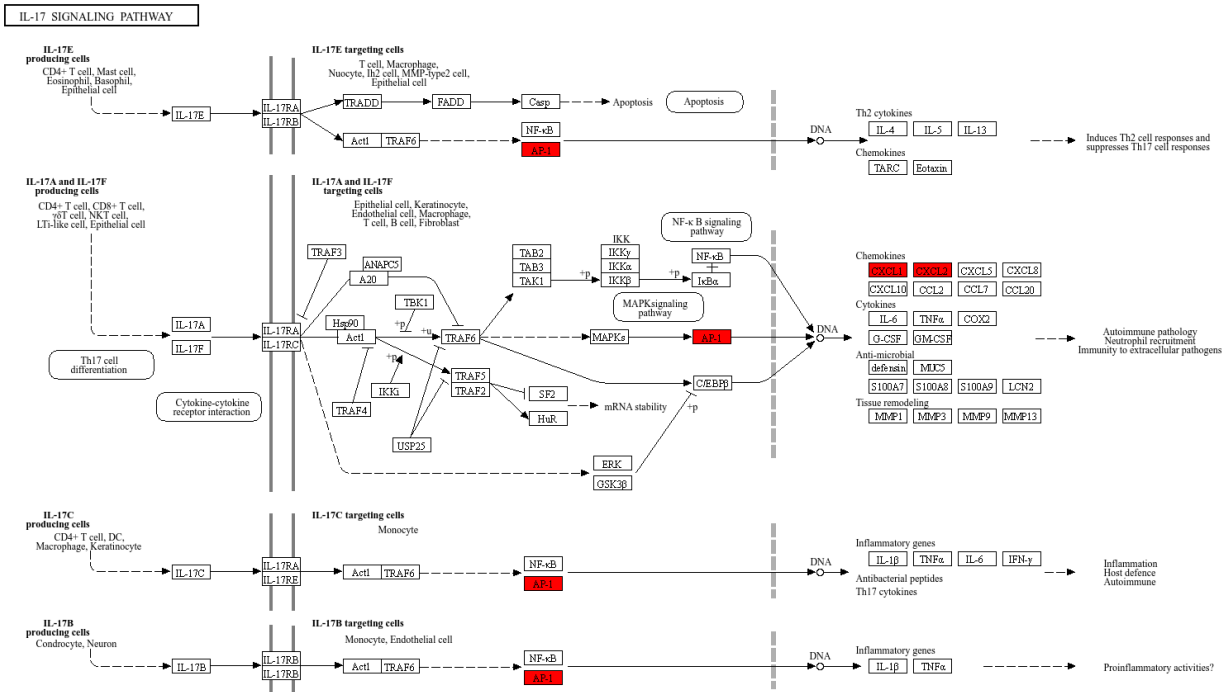


Figure 4. ShinyGO's Visualization of the IL-17 Pathway. *After the old mice DEGs (Differentially Expressed Genes) were run through ShinyGO, it listed common pathways between those genes. The genes cxcl1, fos, and fosb share this pathway in common – while fos and fosb are not directly shown in the above diagram, AP-1 is a complex with those two [1].*

An upregulation of the IL-17 pathway has previously been linked to the onset of autoimmune glomerulonephritis in the kidney [2] - suggesting there may be an increased risk for **autoimmune** and **inflammatory kidney** diseases when organisms are exposed to space. Thus in this proposal, we aim to evaluate the impact of stimulated microgravity on the IL-17 pathway.

Using stimulated microgravity will prevent exposure to a multitude of unaccounted spaceflight factors, like radiation and temperature [13], and allow us to determine what changes in the physiology result from microgravity [7].

Hypothesis and Aims:

Our data demonstrates that spaceflight conditions, including microgravity, can significantly alter kidney gene expression in older female mice, particularly upregulating immune and inflammatory pathways like IL-17, which are associated with kidney inflammation and autoimmune responses.

Thus, we hypothesize that if older females (~29 weeks old) *Mus musculus* are exposed to microgravity, then they will be **more susceptible** to kidney-related **inflammatory diseases** due to upregulation of the inflammatory response genes, resulting in tissue damage and impaired renal function.

Our three aims focus on a link between microgravity and the IL-17 pathway, the IL-17 pathway and the inflammatory disorders within mice kidneys, and the subsequent recovery or lack of recovery from the inflammatory state.

Successfully completing this work will enable a better understanding of how microgravity contributes to renal immune conditions that are autoimmune or involve inflammation, potentially assisting interventions to mitigate related health risks for astronauts, especially for long-duration spaceflight.

Research Design and Methods:

Specific Aim 1: To determine whether older female mice exposed to microgravity will have an overactive inflammatory response system as measured by changes in levels of inflammatory cytokines connected to the IL-17 pathway relative to Earth gravity mice of the same age.

Overview: Older age positively correlates with chronic low-grade inflammation [1], and microgravity has also been reported to further compromise immune regulation by musculoskeletal unloading and fluid redistribution [2]. IL-17 signaling is a key inducer of inflammatory and autoimmune events in the kidney [14]. Our preliminary data in OSD-771 established increased expression of IL-17 linked genes and preliminary evidence of increased downstream cytokines in kidneys following simulated microgravity [8], suggesting a possible correlation of microgravity exposure with renal inflammation in aged mice.

Hypothesis: Simulated microgravity will induce an exaggerated IL-17 mediated inflammatory reaction in aged mice, with elevated mRNA transcripts and protein production levels of IL-1β, IL-6, and TNF-α relative to age-matched Earth-gravity controls.

Approach:

Simulated Microgravity: Female mice, approximately **29 weeks of age**, will be exposed to simulated microgravity using the **hindlimb unloading (HU)** model for **2 months**. This model has been well-validated through Friedman (2025) which found it to accurately mimic key physiological characteristics of spaceflight, including musculoskeletal unloading and fluid shift distribution, which can influence immune function [14]. Normally loaded age- and sex-matched mice will be used as controls, **enabling direct comparison** of the impact of microgravity on inflammatory and immune pathways.

Sample Collection and Preservation: Following HU exposure, mice will be euthanized, the kidney tissue will be perfused with PBS in order to remove any contaminants from the blood, and then harvested from each mouse. **The left kidney** will be dissected, then flash frozen in liquid nitrogen and stored at -80.0℃ for transcriptomic and proteomic analyses (qPCR and multiplex immunoassay). The **right kidney will be divided in half.** One half will be flash frozen, used as a backup for molecular analysis. The other half will be fixed with formalin for potential histological evaluation of inflammation or structural alteration. The left kidney will be the **main tissue** used for molecular analysis whereas the right kidney will be preserved as a back up in case of experimental error. *(Fig 3.)* All of the frozen samples will then be homogenized into cell lysates for future assays.

Transcriptomic Analysis: Quantitative PCR (qPCR) will determine mRNA expression of IL17a and downstream pro-inflammatory cytokines **IL-1β, IL-6, and TNF-α** within kidney tissue of HU and control mice (*Fig. 5*). These genes were selected since IL-17 signaling is **implicated in autoimmune kidney disease and chronic inflammation**, and IL-1β, IL-6, and TNF-α are recognized downstream mediators of this pathway that go on to enhance **the inflammatory response** in body tissues. Quantifying their expression will allow us to determine if simulated microgravity enhances IL-17–mediated inflammatory signaling at the transcriptional level.

Cytokine Analysis: To complement the transcriptomic data, we will measure the corresponding protein levels of **IL-1β, IL-6, TNF-α, and IL-17** in kidney tissue and serum by multiplex immunoassay. These cytokines are immediate downstream effectors of the IL-17 pathway and the mediators of **inflammatory injury in kidney disease**. Multiplex immunoassays allow for the simultaneous measurement of multiple cytokines from small sample volumes, with increased sensitivity and efficiency over single-analyte methods such as ELISA. This will allow us to determine if **simulated microgravity induces greater cytokine production**, hence backing up transcriptional findings and provisioning functional evidence of inflammatory dysregulation.

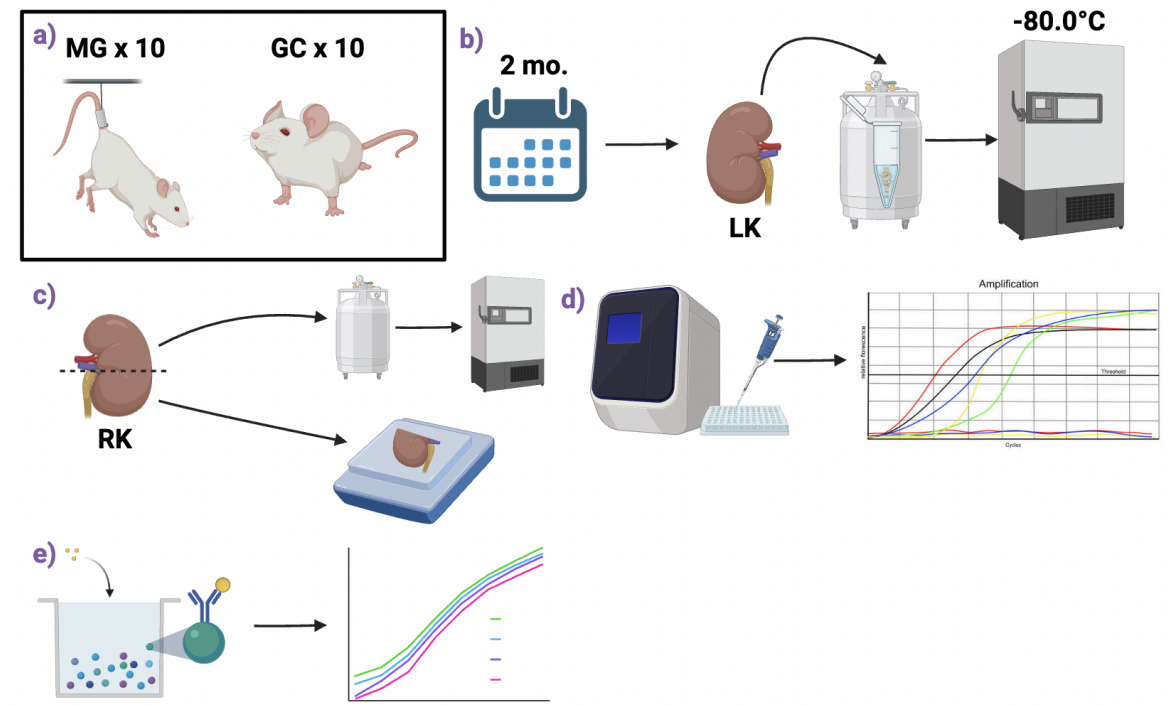


Figure 5. ***Procedure for Aim 1 (analyzing changes in protein production and gene expression of interleukins and cytokines connected to IL-17 pathway).*** *a. Ten 29-weeks old mice exposed to simulated microgravity and ten remaining at Earth gravity levels. b. The left kidney will be collected, homogenized into cell lysates, and frozen at -80.0°C after the two months of exposure until analysed. c. The right kidney will go on to be split in half with the one half undergoing the same procedure as the left and the other being fixed in formalin and embedded in paraffin for future immunohistological analysis. d. Cells will undergo qPCR to determine changes in mRNA transcript production of IL-17-related genes. e. Corresponding proteins will be isolated and tagged in a multiplex immunoassay with fluorescence intensity measured.*

Statistical Analysis: To determine the impact of simulated microgravity on IL-17-induced inflammatory responses, biological replicates will be determined through power analysis, and pilot studies will consist of **at least 6–8 mice** per group in experiments for variance estimation. Quantitative PCR (qPCR) will be normalized to any housekeeping genes, and the calculated **ΔΔCt values** (indicating relative change in gene expression) between microgravity and Earth-gravity groups will be with two-tailed t-tests or ANOVA for more than two groups or time points. For experiments with multiple timepoints, **repeated-measures ANOVA or mixed-effects models will be utilized** to identify trends over time, with strict statistical testing of gene and protein expression alterations induced by microgravity exposure.

Expected Outcomes: Because of the **increased expression we observed in IL-17 signaling genes** in OSD-771, we expect that our results substantiate this and that there will be an increase in the production of mRNA and subsequently, the associated proteins downstream of that pathway, **IL-1β, IL-6, and TNF-α**, relative to Earth-gravity controls. These findings would confirm that simulated microgravity **enhances inflammatory response** in aged mice and provide a mechanistic link between immune dysregulation during spaceflight and the potential for kidney injury. We further expect that primary kidney cell cultures stimulated with IL-17 will **demonstrate increased expression** of these same **downstream cytokines**, providing functional validation of the pathway in vitro.

Potential Pitfalls & Alternative Approaches: The hindlimb unloading model will not replicate all physiological effects of actual spaceflight and may lead to **smaller than expected changes** in IL-17 signaling or cytokine production. We will be monitoring for this by assessing whether expected transcriptional and protein level changes in IL-17 pathway members and downstream cytokines are statistically different from controls. If **observed effects are less significant than anticipated,** we will enhance our approach by prioritizing sequencing of RNA from IL-17 pathway genes, incorporating **additional biological replicates**, or extending the duration of microgravity exposure to enhance the detection of inflammatory responses.

Specific Aim 2: To determine whether older female mice exposed to simulated microgravity have **increased levels of key blood proteins** present in **urine** and **retention of waste products** in the blood indicative of kidney-related autoimmune conditions.

Overview: IL-17 signaling has been shown to be involved in the pathogenesis of **autoimmune glomerulonephritis** [12], an inflammatory kidney disease characterized by injury to glomerular structures. IL-17 blockade in mice reduces inflammatory cell infiltrate in the kidney and downregulates **downstream cytokine expression**, demonstrating a fundamental role for this pathway in autoimmune renal disease. Building on our preliminary evidence of increased IL-17 pathway activity in kidneys of aged mice (OSD-771) [8], we hypothesize to test whether simulated microgravity exposure activates functional and biochemical markers of autoimmune kidney damage. Assessment of **renal function by urine and blood markers** will allow us to determine whether immune dysregulation caused by microgravity leads to measurable renal injury in aged mice.

Hypothesis: Simulated microgravity will **impair kidney filtration** in older mice, as evidenced by greater proteinuria, blood urea nitrogen and creatinine, altered albumin levels, and reduced creatinine clearance compared to **age-matched Earth-gravity controls**, consistent with autoimmune renal injury at an early stage.

Approach:

Simulated Microgravity: Old female *Mus musculus* mice, approximately 29 weeks old, that were previously defined in Aim 1 will be the study group for Aim 2. Mice will be subjected to the same 2-month hindlimb unloading (HU) protocol for mimicking microgravity, with age- and sex-matched Earth-gravity controls. Urine from these animals also will be collected at baseline (prior to HU), during the exposure period when feasible, at completion of exposure of 2 months, and at post-return recovery points. Blood also will be collected for serum and plasma analysis to quantify renal biomarkers such as BUN, creatinine, and albumin, and all samples will be kept on −80 °C until analysis time.

Sample Collection and Preservation: Urine will be collected at baseline (prior to exposure), during microgravity exposure as feasible, at the end of the 2-month exposure, and at post-return recovery time intervals. Samples of all urine will be aliquoted and stored at **−80°C** for subsequent biochemical and proteomic analysis (*Fig. 6*). Blood will be sampled for serum and plasma to evaluate markers such as **blood urea nitrogen (BUN)**, **creatinine**, and **albumin,** and it will be stored at −80°C. This systematic acquisition assures the availability of good-quality specimens for the assessment of renal impairment and autoimmune-mediated changes.

Proteomic Analysis: Our main focus will be on the **blood urea nitrogen tests** to test for elevated urea as well as albumin and creatinine in the urine, measuring these concentrations twice during the two-month period. **Urea and creatinine are both waste products** normally filtered out of the blood by the kidneys and excreted via the urine, whereas albumin is a key protein normally found in the blood. Blood urea nitrogen tests would be conducted on blood samples collected from both groups of mice and treated with DAMO, which reacts with urea and produces a colored product in an acid solution after heating that can be quantified by a spectrophotometer. We will then utilize a mouse urine **ELISA**, or **Enzyme-Linked Immunosorbent Assay**, to measure albumin and creatinine levels. Antibodies for albumin and creatinine will be tagged with fluorescent tags, and the intensity of the fluorescence will be determined through a plate reader. *(Fig. 6)*

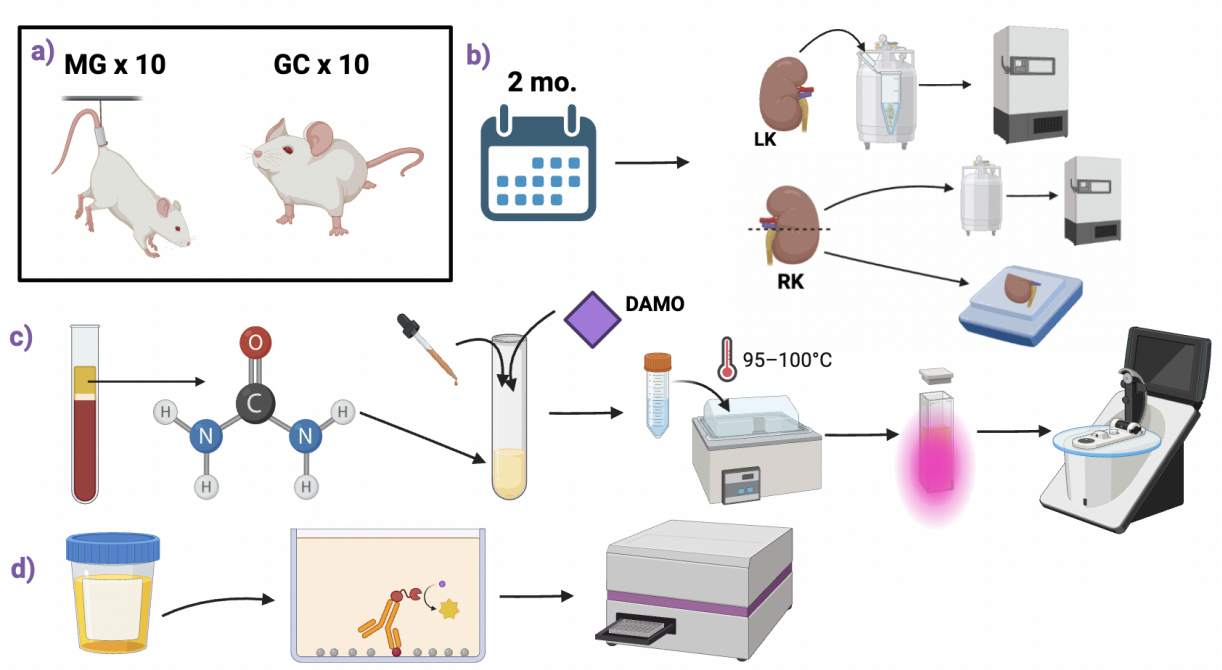


Figure 6. ***Procedure for Aim 2.*** *a, b. Same procedure for experimental setup followed as Aim 1 above. c. Blood drawn from the submandibular vein will be spun down and serum with urea is isolated. DAMO will produce a magenta product after reacting with urea (structure pictured on left) in an acidic solution (addition of acidic ferric solution) and being heated near boiling temperature. Using a spectrophotometer, the absorbance of each solution can be measured and blood urea concentrations determined. d. Urine will be collected from each mice and a urine direct ELISA will be conducted with both albumin and creatinine and their respective antibodies with fluorescent tags, intensity determined by plate reader.*

Statistical Analysis: All of these analyses combined will give an **exhaustive assessment** of functional, biochemical, and pathological presentations of renal injury upon **exposure to microgravity**. By utilizing various tests to determine levels of these molecules in the blood and urine in control versus microgravity-exposed mice, we can **infer kidney efficiency and function**, which would be hindered by inflammation in the tissue. Specifically, an increase in the ratio of the concentration of albumin and creatinine is a well-known indicator of kidney damage, as there should be very little to no albumin present in the urine. We will also calculate the creatinine clearance using the **Cockcroft-Gault equation**, which is a metric that indicates how effectively the kidneys filter such waste products.

Expected Outcomes and Potential Pitfalls: Microgravity-aged mice will show increased proteinuria, elevated BUN and creatinine, reduced creatinine clearance, and potential albumin alterations compared to Earth-gravity controls, consistent with previous evidence of autoimmune kidney damage. A **limitation** is that **renal damage might be less than optimal** with the two-month exposure regimen. To account for this, we will quantify sensitive early indicators of damage such as NGAL and KIM-1 and, if necessary, prolong follow-up or exposure to detect delayed impacts. Another possible limitation is **variability in blood pressure measurement**; to mitigate against this, telemetry will be utilized in a group of mice to confirm trends and obtain genuine physiological information.

Specific Aim 2 is critical because it **connects immune dysregulation in space to real clinical consequences** in the kidney. Building on OSD-771 findings, we will identify whether aged mice exposed to microgravity develop proteinuria and renal impairment (symptoms of autoimmune kidney disease). Demonstrating kidney functional injury would provide the first evidence that spaceflight not only alters immune signaling but also leads to measurable health risks. **Such discoveries** can identify early biomarkers to **safeguard astronaut health** on longer missions.

Specific Aim 3: To determine whether exposure to microgravity in older female mice has chronic impacts on the inflammatory response and the kidney by observing changes in levels of inflammation marker, **serum amyloid** P, and determining **how long** deviant levels may persist after returning to Earth-gravity levels.

Overview: Spaceflight and simulated microgravity can induce systemic inflammation, particularly in aged organisms [2], which could increase susceptibility to autoimmune and renal disease. Serum amyloid P (SAP) is a clinically validated marker of systemic inflammation in mice and humans and rapidly responds to inflammatory challenges. Previous results in OSD-771 indicate that aged mice exposed to spaceflight upregulate pro-inflammatory genes linked with IL-17 signaling [8], which would signify chronic systemic inflammation [10]. Objective 3 will quantify longitudinal dynamics of systemic inflammation after microgravity exposure using serum amyloid P as a quantitative biomarker, and look for its correlation with renal function.

Hypothesis: Mice exposed to simulated microgravity will exhibit elevated serum amyloid P levels during and shortly after the exposure period, indicating elevated systemic inflammation, with resolution over several months. Elevation of SAP later in time will be accompanied by evidence of kidney dysfunction, implying that inflammation caused by microgravity contributes to long-term renal risk and damage.

Simulated Microgravity: Old female *Mus musculus* mice, approximately **29 weeks of age**, will be randomly assigned to a microgravity group, which is subjected to 2 months of simulated microgravity via **hindlimb unloading (HU)**, or an age- and sex-matched Earth-gravity control group. Random assignment decreases bias, and matched controls ensure measured differences in systemic inflammation and kidney function are the result of exposure to microgravity and not to natural differences. This model provides a controlled environment to examine the influence of spaceflight simulation on **inflammatory markers** and **renal function** in aged mice.

Sample Collection and Preservation: Blood will be drawn at baseline, one month into the exposure period, and at five post-return recovery time points: **3 days, 2 weeks, 1 month, 3 months, and 5 months.** The blood from each timepoint for each mouse will be spun down in a centrifuge so the serum can be isolated and frozen at −80°C to stabilize for eventual batch analysis. Longitudinal sampling in this way provides for accurate monitoring of both acute and chronic change in systemic inflammation, which can be used to evaluate the recovery course after returning to Earth gravity. *(Fig. 7)*

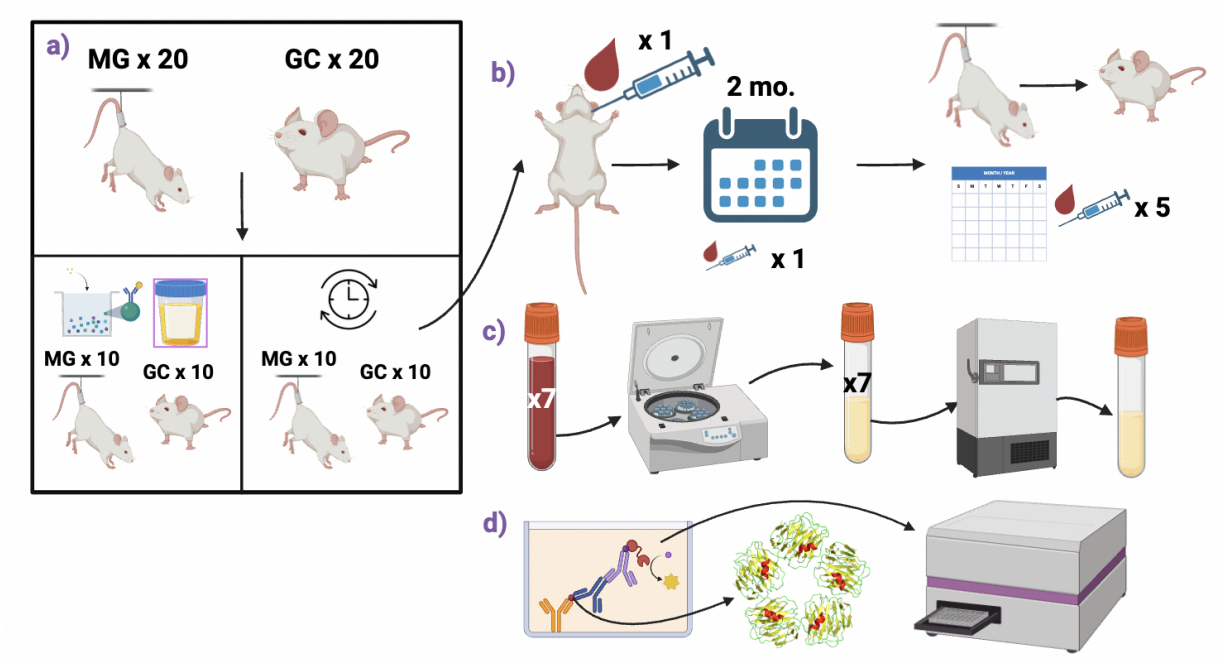


Figure 7. ***Procedure for Aim 3.*** *a. Twenty mice will be placed in each cohort for a total of 40 mice. Ten will randomly be chosen from each group to undergo longitudinal experimentation while the rest will proceed under Aims 1 and 2. b. The ten MG and ten GC mice will have 20 mL blood drawn from their submandibular vein before exposure to the environment, 1 month into exposure, immediately after, as well as 3 days, 2 weeks, 1 month, 3 months, and 5 months subsequent. c. The blood collected each time will be spun down in a centrifuge to isolate the serum and frozen at -80.0℃ until analyzed. d. Antibodies targeting serum amyloid P will be used in a sandwich ELISA to tag the protein with biotin which will then bind horseradish-peroxidase-conjugated streptavidin strongly. The fluorescent signal emitted can be measured with a plate reader.*

Statistical Analysis: Two-way ANOVA with factors of treatment and time will be utilized to compare serum amyloid P between the microgravity and control groups. Pairwise post-hoc comparisons will be conducted using **Tukey's HSD**. Pearson's or Spearman's correlation test, as appropriate for data distribution, will assess serum amyloid P concentration relationships with markers of renal disease. **Repeated measures tests** will account for within-animal **variability across timepoints**. Statistical significance at **$p < 0.05$** will be employed to ensure strict interpretation of longitudinal inflammatory and renal data.

Expected Outcomes and Potential Pitfalls: **Serum amyloid P** concentrations in the blood will be elevated in microgravity mice [1] which is a change that will persist during early recovery timepoints (~1 month) and gradually normalizes around 3-4 months after exposure. This would be indicated by increased fluorescence intensity in MG mice compared to GC mice. Serum amyloid P can be transient and **return to baseline quickly**. Then, the recovery rate is valuable information, pointing to the resilience of the immune response. An additional concern is that serum amyloid P is too heterogeneous to reliably distinguish between groups; if so, we will expand analysis to other systemic inflammation markers **(IL-1β, IL-6, TNF-α)** to validate findings.

This work will determine if **systemic inflammation**, quantified by serum amyloid P, persists following microgravity exposure in elderly mice and to what extent it is **associated with the risk of kidney disease**. Due to the fact that **serum amyloid P is a validated clinical biomarker**, these findings have direct clinical applicability for monitoring astronaut health. If space travel causes long-term inflammation, then astronauts might be at a greater risk for autoimmune and inflammatory disorders on long-duration missions. By **identifying serum amyloid P as a biomarker** and defining recovery schedules, this study will guide countermeasure and monitoring programs designed to protect crew health on long-duration spaceflight missions [8].

Significance and Conclusions:

Spaceflight exposes organisms to physiological stressors (e.g. microgravity, radiation, and altered circadian rhythms) that impair immune regulation and increase susceptibility to infection and inflammatory disease. While immune dysfunction in astronauts is well documented, its effect on specific organs , including the kidneys, remain less understood. The kidney plays a key role in **waste removal, fluid balance, and systemic homeostasis**, making it crucial to study how microgravity impacts its immune-related functions.

Our objective is to test whether **microgravity exposure** triggers an overactive inflammatory response through the **IL-17 signaling pathway** in *Mus musculus*, thereby increasing susceptibility to autoimmune and inflammatory kidney conditions. By combining gene expression and protein analysis, this research directly addresses NASA Human Research Program priorities to **understand immune dysregulation** in space and to **identify reliable biomarkers** of immune health**.**

Exploring whether IL-17-driven inflammation is amplified in microgravity through **Aim 1** would inform NASA about **early immune dysregulation** that could be targeted in **pre-flight prevention measures** to reduce possible kidney damage during missions. By identifying any measurable kidney function decline and impacts on filtration in **Aim 2**, NASA could establish space-specific **screening** and **intervention protocols** in-flight to detect renal damage **before** it compromises astronaut health. Lastly, the insight gained from the longitudinal study of **Aim 3** would guide **long-term timelines** and countermeasure planning for astronauts during **re-adaptation** to the Earth environment.

All together, these findings could guide new **countermeasures to protect astronaut health**, support development of reliable in-flight biomarkers, and advance understanding of the mechanisms for autoimmune and inflammatory kidney disease on Earth. This proposal builds on **preliminary data** (OSD-771), targets a well-characterized pathway and employs rigorous methods to provide deeper insight into the interaction between **aging, inflammation, and kidney function** in spaceflight. By focusing on the IL-17 pathway, we aim to provide **actionable insights** for astronaut health and **broader biomedical science.**

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