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Gravity as a Continuum: Effects of Altered Gravity on Drosophila Melanogaster Immunity

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Gravity as a continuum: Effects of Altered Gravity on *Drosophila melanogaster* Immunity

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Introduction

Monitoring and maintaining the health of astronauts is essential to ensure space exploration missions are successful. As we continue to push the boundaries of space exploration and set our sights on landing the first humans on Mars, astronauts will be exposed to unprecedented amounts of stressors during spaceflight, for example, social confinement, ionizing radiation, closed/dangerous environments and variable gravity. Therefore, further research into the effects of these stressors on immune function is paramount. This study will focus on the effects of altered gravity, more specifically hypergravity (elevated g-force), on *Drosophila melanogaster* immunity.

Why Fruit Flies?

- ~75% of human disease genes are found in the fruit fly[1]
- Innate immune genes first discovered using the fruit fly (i.e. Toll pathway)[2]
- Excellent tool for genetic manipulations
- Short life cycle and exhibit multiple generations to study
- Few resources required for their development

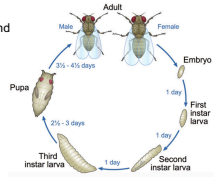


Image from Ong et al., 2014

Spaceflight dysregulates astronaut immunity by:

- Increasing the number of granulocytes (granulopoesis)
- Impairs granulocyte and leukocyte function, i.e. reduced phagocytosis and T cell/NK function

Astronauts spend the majority of missions under the influence of microgravity. Unsurprisingly, the effect of microgravity on astronaut health is an ongoing, hot topic of research, yet the impact of hypergravity is not well understood. Questions to this subject include: (1) can exposure to launch (elevated g-force) *initiate* immune dysfunction? (2) Is there a certain g-force that triggers immune dysfunction? And (3) Are there sex-specific responses generated? These questions we pose, in addition to the g-forces chosen for our experimental protocol, are especially important in terms of applicability and the translational implications. In order to address these questions, our study exposed flies to simulated hypergravity (1.2g, 3g, and 5g) via centrifugation at both acute (1-hour) and chronic (7-day) durations.

Previous studies in our lab showed that acute hypergravity induced the oxidative stress response and cellular survival signals via heat shock proteins (Hsp) (unpublished). In line with this, flies that developed in and were subjected to spaceflight (12-day) also showed elevated Hsp expression (Taylor, K et al., 2014). Since Hsp70 is well-documented, we wanted to determine if the expression of this cellular survival gene could regulate immunity. *Therefore, collectively we hypothesize that acute hypergravity at increased g-force will dysregulate immunity and a loss of Hsp70 will impair immune function, under the stress-inducing model of chronic hypergravity.*

Figure 1. Transcriptional Profile (Ground vs. Space Flies)

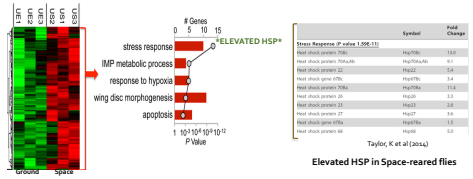
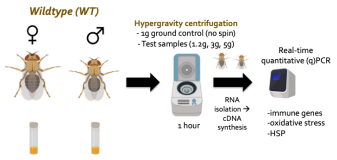


Figure 1. Summary

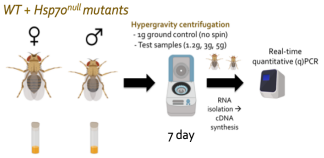
Comparison of transcriptional profiles for ground and space-reared flies. The graph above represents differential gene expression for flies that spent their entire development in space (12 days).

Methodology

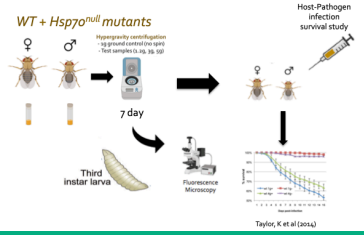
Acute Conditions (1 hour exposure)



Chronic Conditions (7 day exposure)



Chronic Conditions (7 day exposure)



Results

Figure 2. Acute hypergravity gene expression (WT Male vs. WT Female flies)

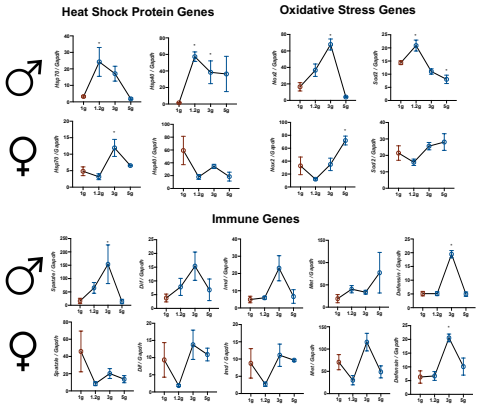


Figure 2. Summary

- Males displayed a robust oxidative stress response to lower g-forces, therefore more sensitive to hypergravity.
- Innate immune-related responses are similar between female and males at acute exposure → Immune-related changes may not appear until longer exposure to stress-related signals?
- Elevated Hsp in males compared to females → Potential sex-specific immune response during chronic hypergravity?

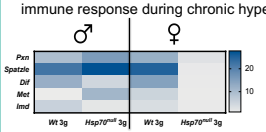


Figure 3. Chronic Hypergravity Immune Gene Expression The blue color gradient on the far right denotes that a darker blue equates to a higher gene expression level, while the lighter shade reveals a lower expression level.

Figure 3. Summary

- Differential immune profiles following chronic hypergravity → *Hsp70*^{null} females overall lower immune gene expression.
- Gene expression immunoprofiles are a simple measurement of immunity, but complementary functional assays are required

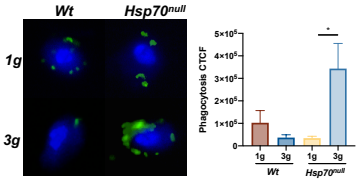


Figure 4. Functional Immune Assay (Larval Phagocytosis Assay) Post-chronic hypergravity

Phagocytosis CTCF values from the hemocytes of third instar larva (1g controls and 3g chronic spin; average of technical replicates (n=6-12)) are represented on the left. Images on the right depict the larval hemocytes in blue, while the *E. coli* bioparticles fluoresce green.

Figure 4. Summary

- Increased phagocytosis in *Hsp70*^{null} larva following chronic hypergravity exposure → Stronger immune response in *Hsp70*^{null} compared to *Wt* controls

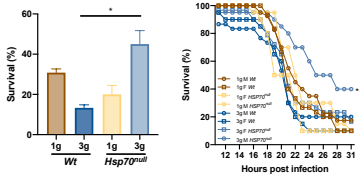


Figure 5. Host-Pathogen Survival Assay (*WT* vs *Hsp70*^{null} flies) *Wt* and *Hsp70*^{null} flies (average of technical replicates (n=2-3)) where exposed to chronic hypergravity for 7-days followed by abdomen injection.

Figure 5. Summary

- *Hsp70*^{null} male flies displayed higher survival → elevated immunity in males partially protects against pathogen challenge

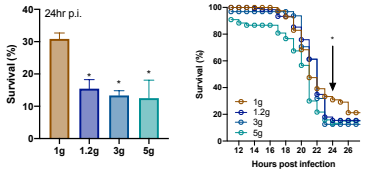
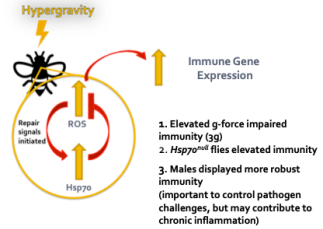


Figure 6. Host-Pathogen Survival Assay (WT flies)

Figure 6. Summary

- WT flies exposed to hypergravity exhibited greater susceptibility to death from infection, compared to our controls

Summary



A better understanding of the effects of increasing g-forces on the initiation of immune dysfunction and sex-specific outcomes were identified in this study. While immune markers were elevated with acute hypergravity, chronic elevation lead to differential immune pathways, therefore countermeasures tailored to reverse pathological effects triggered at launch/landing (acute) and those that persist during long-duration (chronic) missions, when medical intervention is limited should be considered. Furthermore, the sex-specific effects describe the need for more personalized medicine for astronauts. Furthermore, specific countermeasures that can inhibit Hsp70 may be necessary for short term effects, while its chronic inhibition may be detrimental to immune health.

As we delve deeper into space, an understanding of the stress of altered gravity on astronaut immune health is critical. Hence, our goal of landing the first woman on the moon means that analyzing and remaining cognizant of the differences in immune impact for males and females is especially important.

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