



# Heat Shock Protein 40 and Immune Function in Altered Gravity

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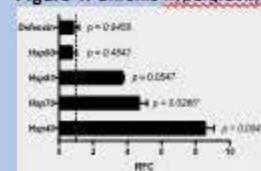
## Abstract

During spaceflight, astronauts are more susceptible to immunosuppression, which poses limits to their health and the mission. During space flight, stress-inducible heat shock proteins (HSPs) are robustly induced, and the overexpression of HSPs have been implicated in immune dysregulation. Therefore, HSPs may be critically involved in regulating immune homeostasis. HSP40 plays a major role in proper protein translation and folding. To determine the role of HSP40 during stress-induced altered gravity conditions, wild type and Hsp40 mutant *Drosophila melanogaster* were exposed to ground-based chronic hypergravity conditions, followed by quantitative PCR analysis of immune gene expression. This data indicates a role of Hsp40 in strengthening immune function during stress-induced spaceflight in flies. A critical need to evaluate the relationship between HSPs and immune suppression during space flight is necessary.

## Introduction

- Astronauts are immunocompromised during spaceflight. Understanding the mechanisms of this is vital for successful long-term spaceflight. (1)
- Heat shock proteins (HSPs) are a major class of proteins activated by stress of altered gravity. HSPs respond to oxidative stress induced by hypergravity. (2)
- Preliminary data shows that Hsp40 is upregulated in hypergravity conditions.

Figure 1. Chronic hypergravity (3g) flies, wild type male.



A.M. Paul, unpublished. qPCR results of various genes normalized to 1g conditions (dotted line). Hsp40 is particularly induced under chronic hypergravity conditions.

- So, further characterizing Hsp40 in hypergravity can prove useful to the stress response and immune response during hypergravity.
- Drosophila melanogaster* is a useful model organism for this study because of its ease of genetic modification, large sample size, and genetic similarity to humans.

## Hypothesis

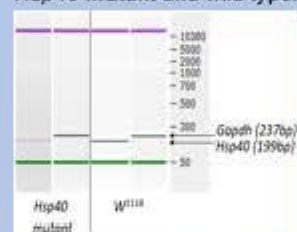
Loss of Hsp40 in stressful conditions of hypergravity will result in increased expression of innate immunity genes.

## Methods

Figure 2. Methods.

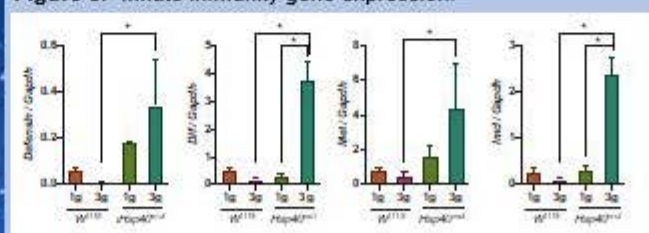


Figure 3. Gel electrophoresis of Hsp40 mutant and wild type.



Comparison of Hsp40 mutant to wild type. Fainter band on Hsp40 mutant confirms the reduced Hsp40 expression in mutants. Equally dark Gapdh bands on both samples indicates constitutive expression.

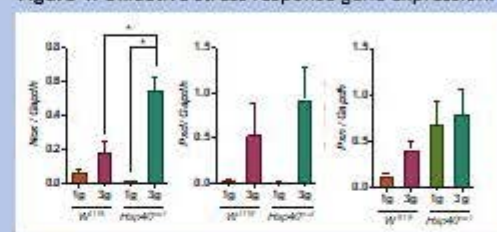
Figure 5. Innate immunity gene expression.



qPCR results for innate immunity genes. Significantly increased expression of all four genes. Data analysis performed by one-way ANOVA with post hoc Sidak's multiple comparisons test. Data represented as standard error of the mean (SEM).  $p < 0.05$  SEM. DmP=Dorsal-related immunity factor, Met=Methicillin, Imd=Drosophila immune deficiency gene.

## Results

Figure 4. Oxidative stress response gene expression.



qPCR results for oxidative stress response genes. Nox expression significantly increased in Hsp40 mutants compared to wild type flies, with similar trends in Pxd and Fxn. Data analysis performed by one-way ANOVA with post hoc Sidak's multiple comparisons test. Data represented as standard error of the mean (SEM).  $p < 0.05$  SEM. Nox=NADPH oxidase, Pxd=Drosophila peroxidase, Fxn=Peroxidase precursor.

Figure 6. Sample sizes.

Hsp40 1g	n=40 flies, 4 samples
Hsp40 3g	n=25 flies, 4 samples
WT 1g	n=25 flies, 3 samples
WT 3g	n=25 flies, 7 samples

Sample sizes for qPCR of oxidative stress genes and innate immunity genes. ~10 flies per sample.

## Conclusions

- Defensin, DmP, Met, and Imd are involved in the antibacterial response, antifungal response, and neonatal immunity. Because of their increased expression in Hsp40 mutant samples, Hsp40 may have a regulatory effect on these immune response genes.
- Hsp40 could also be involved in regulating the activity of oxidative stress response genes like Nox because of its increased expression in 3g Hsp40 knockdowns.

## References

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- Feger, B. J. et al. Microgravity induces proteomic changes involved in endoplasmic reticulum stress and mitochondrial protection. *Sci Rep* 6, 34091, doi:10.1038/srep34091 (2016).

## Acknowledgements

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