Characterizing GSK3β in murine skeletal muscles after 30 days of spaceflight

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Abstract

Background: Prolonged microgravity exposure causes muscle atrophy and fiber type transformations. The most affected muscle is the postural soleus due to the large proportion of slow-oxidative fibers that, on Earth, fights the downward pull of gravity. During prolonged spaceflight, the soleus undergoes atrophy and slow-oxidative to fast-glycolytic fiber type shift. Glycogen synthase kinase-3 (GSK3β) is a serine/threonine kinase and known negative regulator of NFAT and Wnt/β-catenin signaling. Together, NFAT and Wnt/β-catenin signaling pathways can activate the slow-oxidative myogenic program and muscle regeneration to potentially help combat the atrophic effects of spaceflight on muscle health.

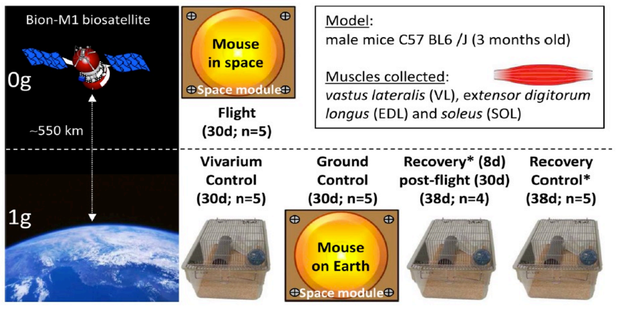
Methods: We obtained soleus muscles from male and female C57BL/6 mice from three separate missions (NASA RR9, NASA RR1, BION-M1; 30-37 days of spaceflight) to examine GSK3 activation via Western blotting. Specifically, we examined total and inhibitory serine9 phosphorylated GSK3 and total β-catenin content in Flight, Ground Control (GC) and Vivarium control (VIV) soleus muscles.

Results: In the RR9 mission, we found significant reductions in total and serine phosphorylated GSK3 content, which corresponded with significant increases in MHC IIb content. Interestingly we also observed a significant reduction in β-catenin, which could suggest enhanced GSK3 activity. Similar trends were observed in the BION-M1 and RR1 missions and combining the fold-change data from all 3 missions, show that spaceflight leads to a significant reduction in total and phosphorylated GSK3 and β-catenin compared with combined GC and VIV controls. This data could reveal GSK3 as a potential target for mitigating the decrements in muscle size and performance observed with spaceflight.

Introduction

1. Prolonged microgravity exposure causes muscle atrophy and fiber type transformations. The most affected muscle is the postural soleus due to the large proportion of slow-oxidative fibers that, on Earth, fights the downward pull of gravity.
2. Muscle disuse or unloading, such as microgravity, leads to a slow-to-fast fibre type shift, and calcineurin and Wnt activation has the potential to oppose this remodelling.
3. Calcineurin (CaN) is a calcium (Ca^{2+})/ calmodulin (CaM)- dependant serine/threonine phosphatase that promotes the slow oxidative myogenic program in skeletal muscle as well as myoblast fusion (1).
4. The canonical Wnt/β-catenin signaling pathway is a well-established anabolic cascade that promotes muscle health and bone formation (2).
5. Glycogen synthase kinase 3 beta (GSK3β) is a constitutively active serine/threonine kinase that serves as a potent negative regulator of both Calcineurin via NFAT prephosphorylation, and Wnt signalling via tagging β-catenin for proteasomal degradation (2). Furthermore, In conditions of disuse and muscle atrophy, GSK3β activity is elevated (3).
6. We wanted to characterize GSK3β activity and its effects on muscle health in an unloaded condition, such as prolonged spaceflight (30-37 days) across 3 separate missions.

Methods



Mice
- Soleus muscles from male and female C57BL/6 mice from three separate missions (NASA RR9, NASA RR1, BION-M1; 30-37 days of spaceflight

Western Blotting
- Soleus muscles were collected and homogenized 2 weeks post-spaceflight
- Blotting analysis for MHCI, MHCIIb, GSK3β, pGSK3β, PGC-1a, β-Catenin

Statistics
- Most comparisons were made using a one-way mixed plot and two-tailed t-test
- Vivarium (VIV) and ground control (GC) data were combined and compared to flight groups across all 3 missions
- Statistical significance was set to $p<0.05$.

Results

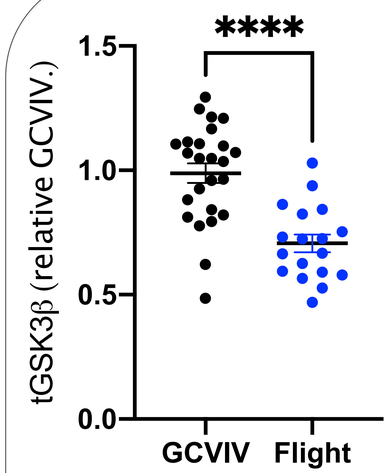


Figure 1. GSK3β content in ground control/vivarium (GCVIV) and flight groups (n = 17-18 per group). ****p = <0.0001

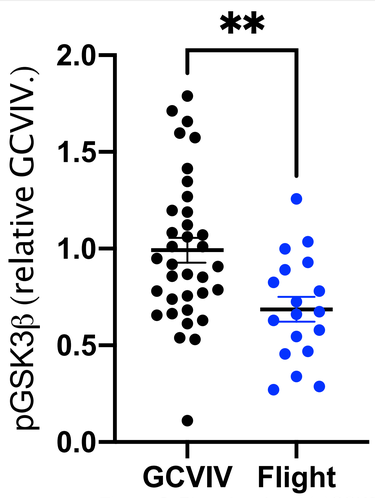


Figure 2. Phosphorylated (p)GSK3β content in ground control/vivarium (GCVIV) and flight groups (n = 17-18 per group). **p = 0.004

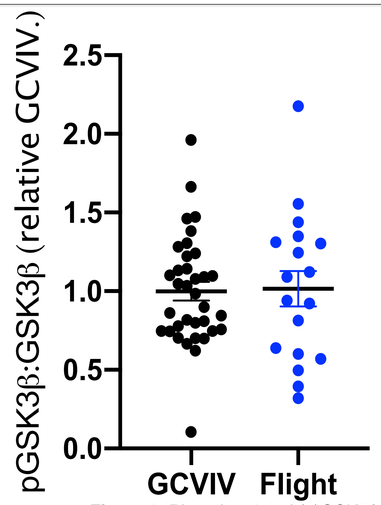


Figure 3. Phosphorylated (p)GSK3β normalized to GSK3β content in ground control/vivarium (GCVIV) and flight groups (n = 17-18 per group).

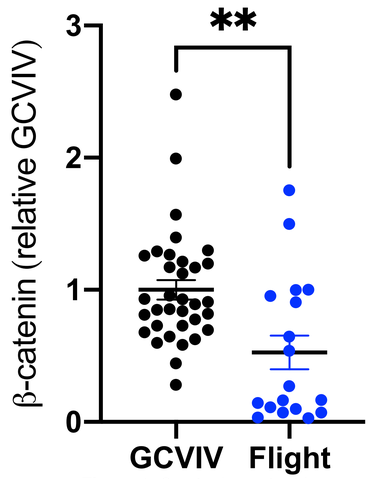
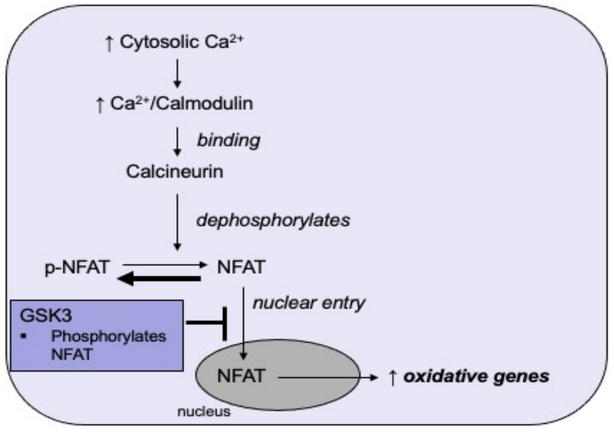
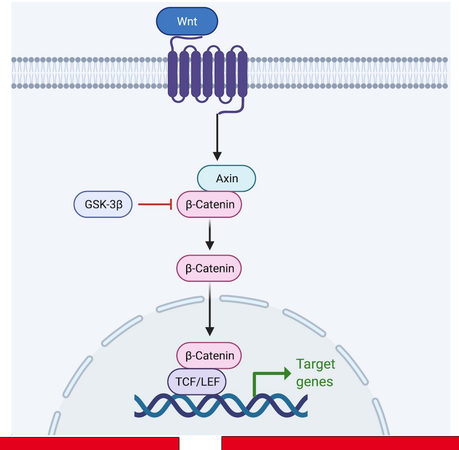
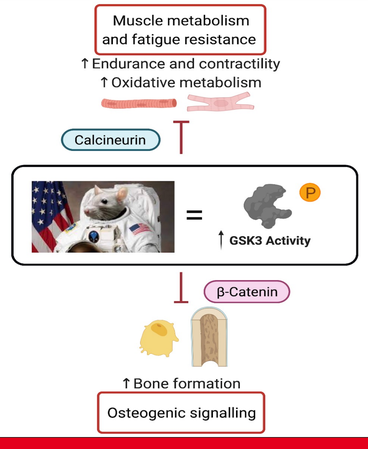


Figure 4. β-catenin content in ground control/vivarium (GCVIV) and flight groups (n = 17-18 per group). **p = 0.001

Supplemental Figures







Conclusions

Total GSK3β
- GSK3 content is significantly lowered in the flight group compared to the control groups which is consistent with muscle atrophy and protein loss.

Phosphorylated (p)GSK3β
- Phosphorylation levels of GSK3 are significantly lowered in the flight group relative to GCVIV

(p)GSK3β: GSK3β ratio

B-catenin
- Total β-catenin levels are significantly lowered in the flight group
- Potentially indicative of higher GSK3β activity therefore leading to its phosphorylation and subsequently proteasomal degradation (3)

Future Directions

Hindlimb suspension in GSK3 knockdown mice
- Well-known model of muscle unloading commonly used by NASA for its accuracy in mimicking spaceflight conditions
- Knocking down GSK3 will potentially help mitigate the atrophic effects seen in skeletal muscle.

References & Funding

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