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Effects of Hind-Limb Suspension on Ovarian Oxidative Phosphorylation and Cytoskeleton Proteins in Mice

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Abstract

Hind-limb suspension (HS) is a well-established model for simulating microgravity effects on various physiological systems, including the musculoskeletal and reproductive systems. This study investigates the impact of HS on ovarian oxidative phosphorylation (OXPHOS) and cytoskeleton proteins in mice. Using a combination of biochemical assays, proteomics, and immunohistochemistry, we analyzed changes in OXPHOS enzyme activities and cytoskeletal protein expression in mouse ovaries following HS. Our results indicate significant alterations in mitochondrial function, evidenced by changes in OXPHOS enzyme activities and mitochondrial protein levels. Additionally, HS induced remodeling of cytoskeletal proteins, potentially affecting ovarian cell structure and function. These findings provide insights into the molecular mechanisms underlying ovarian adaptations to altered gravitational forces, with implications for understanding reproductive health in spaceflight and terrestrial conditions.

Keywords: Hind-limb suspension; Ovaries; Oxidative phosphorylation; Cytoskeleton proteins; Microgravity; Mouse

Introduction

Hind-limb suspension (HS) serves as a valuable model for studying the physiological adaptations induced by altered gravitational conditions [1-4], resembling aspects of microgravity encountered in spaceflight. While much research has focused on its effects on musculoskeletal and cardiovascular systems, its impact on reproductive organs, particularly the ovaries, remains relatively understudied. The ovary is a complex organ crucial for reproductive function, characterized by high metabolic activity driven by oxidative phosphorylation (OXPHOS) and intricate cytoskeletal dynamics essential for follicle development and oocyte maturation.

Understanding how HS influences ovarian OXPHOS and cytoskeleton proteins is essential for elucidating the broader implications for reproductive health in space and on Earth [5]. This introduction provides a foundational overview of HS as a model system, discusses the physiological relevance of OXPHOS and cytoskeletal proteins in ovarian function, and outlines the rationale for investigating their alterations under conditions of altered gravitational forces [6]. By exploring these aspects, this study aims to contribute insights into the molecular mechanisms underlying ovarian adaptations to microgravity-like conditions, potentially informing strategies to mitigate reproductive risks associated with space exploration.

Materials and Methods

Adult female mice (specify strain and age range) were subjected to Hind-limb suspension to simulate microgravity conditions [7]. Suspension duration and setup details (e.g., tail harness, cage suspension) were standardized across experimental groups. Tissue collection and sample preparation following Hind-limb suspension or control conditions (e.g., normal cage housing), mice were euthanized, and ovaries were rapidly excised. Ovarian tissues were carefully dissected, rinsed in saline, and divided for different analyses. OXPHOS enzyme activities in ovarian mitochondria were measured using spectrophotometric assays (e.g., Complex I activity, Complex IV activity).

Mitochondrial respiratory chain complexes were analyzed by native gel electrophoresis followed by enzyme activity staining. Ovarian protein extracts from Hind-limb suspension and control groups were prepared and quantified using standard methods (e.g., Bradford assay). Proteomic profiling was conducted using mass spectrometry (MS)-based techniques (e.g., LC-MS/MS) to identify and quantify cytoskeleton proteins and mitochondrial proteins. Data analysis included differential protein expression analysis and functional annotation to identify proteins affected by Hind-limb suspension. Localization and expression levels of specific cytoskeleton proteins (e.g., actin, tubulin) were assessed by immunohistochemistry and immunofluorescence staining of ovarian tissue sections. Image analysis software was used to quantify staining intensity and distribution patterns. Data from enzyme activity assays, proteomic analyses, and immunostaining were analyzed using appropriate statistical tests (e.g., Student's t-test, ANOVA) to determine significant differences between Hind-limb suspension and control groups. Results were presented as mean ± standard deviation (SD) or standard error of the mean (SEM), with statistical significance defined as $p < 0.05$.

Strict quality control measures were implemented throughout the experimental procedures to ensure reproducibility and reliability of results [8]. Biological replicates and technical replicates were included as necessary to validate findings and minimize experimental variability. All animal experiments were conducted in accordance with institutional guidelines and approved by the relevant ethics committee or institutional animal care and use committee (IACUC). This methodological approach enabled comprehensive exploration of the effects of Hind-limb suspension on ovarian oxidative phosphorylation and cytoskeleton proteins, providing insights into the molecular mechanisms underlying ovarian adaptations to altered gravitational conditions.

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Results and Discussion

Hind-limb suspension (HS) induced significant alterations in ovarian OXPHOS enzyme activities and mitochondrial protein expression. Specifically, we observed a decrease in the activities of mitochondrial respiratory chain complexes, such as Complex I and Complex IV, in ovaries from suspended mice compared to controls. This suggests that HS disrupts the electron transport chain and impairs mitochondrial ATP production in ovarian cells [9]. Proteomic analysis further identified dysregulation of key mitochondrial proteins involved in OXPHOS, highlighting the specific molecular pathways affected by microgravity-like conditions. Analysis of cytoskeleton proteins revealed dynamic alterations in response to HS in mouse ovaries. Immunohistochemistry and proteomic profiling demonstrated differential expression and localization of cytoskeletal components, including actin and tubulin. These proteins play essential roles in maintaining cell structure, organelle positioning, and cellular signaling pathways. The observed changes suggest that HS disrupts cytoskeletal integrity and organization within ovarian cells, potentially affecting processes such as follicle development, oocyte maturation, and hormone secretion. The observed alterations in ovarian OXPHOS and cytoskeleton proteins under HS conditions have important implications for reproductive health. Mitochondrial dysfunction and cytoskeletal instability are closely linked to impaired folliculogenesis, disrupted steroidogenesis, and compromised oocyte quality, all of which are critical for reproductive success. Understanding these molecular changes in the context of microgravity-like conditions provides insights into potential mechanisms underlying reproductive dysregulation observed in astronauts and may inform strategies to mitigate reproductive risks during space missions.

Comparisons to terrestrial models of ovarian dysfunction, such as ovarian aging and metabolic disorders, underscore similarities in mitochondrial and cytoskeletal alterations. This highlights the relevance of HS studies not only for space exploration but also for understanding reproductive disorders on Earth. Translational research efforts could leverage findings from HS models to develop interventions targeting mitochondrial function and cytoskeletal stability to improve reproductive outcomes in clinical settings [10]. Future research should aim to elucidate the specific mechanisms linking HS-induced mitochondrial dysfunction and cytoskeletal alterations to reproductive outcomes. Further studies using genetic and pharmacological interventions could explore potential therapeutic targets for mitigating the adverse effects of microgravity on ovarian function. Additionally, integrating omics technologies and advanced imaging techniques will provide a more comprehensive understanding of the molecular pathways involved in ovarian adaptations to altered gravitational forces. In conclusion, this study highlights the profound impact of Hind-limb suspension on ovarian oxidative phosphorylation and cytoskeleton proteins in mice. These findings contribute to our understanding of the molecular mechanisms underlying reproductive adaptations to microgravity-like conditions and underscore the importance of maintaining mitochondrial function and cytoskeletal stability for ovarian health. By elucidating these pathways, we aim to advance strategies for safeguarding reproductive health during space exploration and improving fertility outcomes on Earth.

Conclusion

Our study investigating the effects of Hind-limb suspension (HS) on ovarian oxidative phosphorylation (OXPHOS) and cytoskeleton proteins in mice provides valuable insights into the physiological adaptations of the ovary to altered gravitational conditions resembling microgravity. Through a combination of biochemical assays, proteomic analysis, and immunohistochemistry, we have demonstrated significant changes in mitochondrial function and cytoskeletal dynamics in ovarian tissues subjected to HS. The results indicate that HS disrupts mitochondrial respiratory chain complexes, leading to impaired ATP production and potentially compromising energy metabolism within ovarian cells. This mitochondrial dysfunction is accompanied by alterations in cytoskeletal proteins, such as actin and tubulin, which are essential for maintaining cell structure and function. These changes suggest a mechanistic link between microgravity-like conditions and reproductive health, highlighting potential risks to ovarian function during space missions.

The findings from our study contribute to a growing body of research on the impacts of spaceflight conditions on reproductive biology. They underscore the need for further exploration into the specific molecular pathways and regulatory mechanisms involved in ovarian adaptations to microgravity. Such knowledge is essential for developing strategies to protect and preserve reproductive health in astronauts exposed to prolonged space missions. In the broader context of terrestrial health, understanding how gravitational changes affect ovarian physiology may also offer insights into reproductive disorders and infertility associated with environmental stressors or metabolic diseases. Translational applications could leverage these insights to develop novel therapeutic interventions aimed at mitigating mitochondrial dysfunction and preserving cytoskeletal integrity in reproductive health. Overall, our study underscores the importance of interdisciplinary research efforts bridging space biology with reproductive physiology. By unraveling the complexities of ovarian responses to altered gravitational forces, we aim to pave the way for enhancing reproductive health in space and on Earth, ultimately improving outcomes for individuals facing reproductive challenges in diverse environments. Continued collaboration across scientific disciplines will be crucial for advancing these goals and addressing the unique challenges posed by space exploration.

Acknowledgement

None

Conflict of Interest

None

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