Transcriptomic response of *Drosophila melanogaster* pupae developed in hypergravity

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The metamorphosis of *Drosophila* is evolutionarily adapted to Earth's gravity, and is a tightly regulated process. Deviation from 1g to microgravity or hypergravity can influence metamorphosis, and alter associated gene expression. Understanding the relationship between an altered gravity environment and developmental processes is important for NASA's space travel goals. In the present study, 20 female and 20 male synchronized (Canton S, 2 to 3day old) flies were allowed to lay eggs while being maintained in a hypergravity environment (3g). Centrifugation was briefly stopped to discard the parent flies after 24hrs of egg laying, and then immediately continued until the eggs developed into P6-staged pupae (25 - 43 hours after pupation initiation). Post–hypergravity exposure, P6-staged pupae were collected, total RNA was extracted using Qiagen RNeasy mini kits. We used RNA-Seq and qRT-PCR techniques to profile global transcriptomic changes in early pupae exposed to chronic hypergravity. During the pupal stage, *Drosophila* relies upon gravitational cues for proper development. Assessing gene expression changes in the pupa under altered gravity conditions helps highlight gravity dependent genetic pathways. A robust transcriptional response was observed in hypergravity-exposed pupae compared to controls, with 1,513 genes showing a significant ($q < 0.05$) difference in gene expression. Five major biological processes were affected: ion transport, redox homeostasis, immune response, proteolysis, and cuticle development. This outlines the underlying molecular changes occurring in *Drosophila* pupae in response to hypergravity.

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