**Transcriptome response to high-altitude exercise in Andean Highlanders with Chronic Mountain Sickness before and after hemodilution**

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Chronic Mountain Sickness (CMS), a disease typically develops among highlanders, is usually categorized by excessive production of red blood cells. Patients generally suffer from sleep disorders, pulmonary hypertension, and exercise intolerance. As an empirical treatment, hemodilution has been reported to alleviate CMS symptoms. However, the transcriptomics differences between healthy and CMS individuals and the underlying biological mechanism of hemodilution are yet to be elucidated.

Healthy and CMS Andean males (, ) resident at Cerro de Pasco, Peru (~4300 m) were asked to peddle on a cycle ergometer until reaching peak exercise. Participants with CMS were then treated by isovolemic hemodilution, which is performed by draining a portion of the participants’ blood and replacing it with artificial plasma that contains no red blood cells. Participants were then asked to repeat the previous exercise protocol. During exercise, participants’ blood gas contents, cardiac functions, and total oxygen and consumption were measured in-situ. Blood samples were taken and contained in PAXgene Blood RNA Tubes at rest, at peak exercise, and at fasting state. Blood samples were then sent for RNA sequencing. Raw gene expressions were compared at fasting baseline among healthy, CMS, and CMS hemodiluted participants. Second-order comparisons were constructed by first profiling the transcriptomic changes during exercise and then comparing the difference-in-difference expression levels. Differential gene expression were quantified by combining biological signal (log fold changes) and statistical significance (p values). Top 10% overexpressed and underexpressed genes were considered as significantly differentially expressed and were further analyzed by Ingenuity Pathway Analysis to indicate regulations of biological pathways.

Comparing pre- and post- exercise, 774 genes were significantly differentially expressed among CMS subjects, as opposed to 82 genes among hemodiluted CMS subjects, and 227 genes among healthy subjects. For second-order comparisons, 1414, 291 and 493 genes were differentially expressed when comparing CMS to healthy, CMS to CMS hemodiluted, and CMS hemodiluted to healthy subjects, indicating that CMS subjects after hemodilution were transcriptomically similar to healthy subjects. Biological pathway analysis revealed upregulation of inflammation pathways including neuroinflammation signaling, IL-8 signaling and natural killer cell signaling pathways as a transcriptomic exercise response among CMS subjects. Cardiac hypertrophy signaling pathways were also upregulated in CMS subjects during exercise, suggesting potential cardiovascular complications due to CMS. After hemodilution, upregulation of inflammation pathways was less pronounced. The pathway regulation pattern of hemodiluted CMS subjects were similar to that of healthy subjects. The pathway regulation pattern for fasting baseline comparisons were inconclusive due to large noise to signal ratio and limited sample size.

In conclusion, the compromised exercise capacity of individuals with CMS can be potentially attributed to excessive immune response during exercise. Hemodilution helps alleviate CMS symptoms likely by decreasing blood-vessel viscous sheer and therefore decreasing inflammation.