**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 common among highlanders, is usually categorized by excessive production of red blood cells. In addition, patients generally suffer from sleep disorders, pulmonary hypertension, and exercise intolerance. Hemodilution, or “bloodletting” has been anecdotally reported to alleviate CMS symptoms. However, the transcriptomic 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 in PAXgene Blood RNA Tubes at rest, peak exercise, and fasting. Blood samples were then sent for library preparation and RNA sequencing. Raw gene expression was 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 was quantified by combining biological signals (log fold changes) and statistical significance (p values). The top 10% overexpressed and underexpressed genes were considered as significantly differentially expressed and were further analyzed via Ingenuity Pathway Analysis (IPA) to predict differentially regulated pathways.

Comparing pre- and post- exercise, 774 genes were significantly differentially expressed among CMS subjects, as opposed to 82 genes among hemodiluted CMS (CMSHem) subjects, and 227 genes among healthy Andeans (CON) subjects. For second-order comparisons, 1414, 291 and 493 genes were differentially expressed when comparing CMS to CON, CMS to CMSHem, and CMSHem to CON. This suggests that CMS subjects after hemodilution were transcriptomically more similar to healthy subjects (CON) than before hemodilution. Biological pathway analysis indicated upregulation of inflammatory pathways (Neuroinflammation signaling, IL-8 signaling and Natural Killer Cell signaling, CDC42) 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. In addition, since pathogenesis of pulmonary hypertension involves immune response and deregulated autonomic nervous system, the inflammatory pathways such as Neuroinflammation is found to induce the pathogenesis of neurological diseases (Hilzendeger, 2014), and the cytotoxicity of Natural Killer Cell in young male are promoted post-hypoxic exercise training (Wang, 2011). Following hypoxia, IL-8 expression is observed to increase three-fold, and IL-8 signaling significantly contributes to survival advantage of PC3 cancer cells (Maxwell, 2007). Furthermore, the pulmonary hypertension in control subjects exposed to acute hypoxia is found to be related to an increase in Endothelin-1 (Modesti, 2006). The CDC42 pathway was upregulated in CMS individuals prior to non-hemodilution, and CDC42 expression is significantly lower in CMS patients showing high altitude polycythemia, a common CMS characteristic. In turn, decreasing the expression of CDC42 will promote erythropoiesis in bone marrow of HAPC patients, and (Jiang, 2012). The pathway regulation pattern for fasting baseline comparisons were inconclusive due to large noise to signal ratio and limited sample size.

-cdc42: KO in mice results in cardiac hypertrophy; upregulated in HAPC Chinese highlanders compared to controls

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752064/>

<https://www.spandidos-publications.com/mmr/5/1/287>

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.

Chronic Mountain Sickness (CMS), or Monge’s disease, is characterized by polycythemia, severe hypoxemia, and pulmonary hypertension that affects many individuals living at high altitude (above 2500m). Secondary complications, such as cor pulmonale and congestive heart failure, may develop in individuals with CMS. Phlebotomy can be used to combat excessive erythropoiesis (EE) and in some cases blood is replaced with plasma expanders to maintain blood volume. This treatment has been anecdotally reported to alleviate symptoms associated with CMS, but the mechanism by which this occurs is unknown. EE is characterized by viscous blood, which may damage tissues and blood vessels, particularly during exercise, inducing an inflammatory response.