**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 (CMSPre), as opposed to 82 genes among hemodiluted CMS subjects (CMSPost), and 227 genes among healthy Andeans subjects (CON). For second-order comparisons, 1414, 291 and 493 genes were differentially expressed when comparing CMS to CON, CMSPre to CMSPost, and CMSPost to CON. This suggests that CMS subjects after hemodilution were transcriptomically more similar to healthy subjects than before hemodilution. Biological pathway analysis indicated upregulation of inflammatory pathways (Neuroinflammation signaling, IL-8 signaling, and Natural Killer Cell signaling) as a transcriptomic exercise response among CMS subjects before hemodilution. The upregulation of Neuroinflammation Signaling pathway is driven by the differential expression of a series of immune response genes including human leukocyte antigen genes (HLA-B, HLA-C), Interleukin-1 receptor associated kinase genes (IRAK3, IRAK4), HMOX1, IFNGR2 and IL6R. Cardiac Hypertrophy Signaling and Cdc42 Signaling pathways were also upregulated in CMS subjects during exercise. Latter was shown in animal studies as the antihypertrophic molecular switch (Maillet 2009), and in humans to be associated with high-altitude polycythemia (Jiang 2011). This suggests that CMS subjects may be more prone to potential cardiovascular complications. 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 and chronically damaged cardiovascular functions. Hemodilution helps alleviate CMS symptoms likely by decreasing blood-vessel viscous sheer and therefore decreasing inflammation.