**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 characterized 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 underwent isovolemic hemodilution, which is performed by removing 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. Participants’ blood gases, cardiac output, and total oxygen and consumption were measured from rest to peak exercise. 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, the latter being an antihypertrophic molecular switch in animal models (Maillet 2009) and associated with high-altitude polycythemia in humans (Jiang 2011). This suggests that CMS subjects may be more prone to potential cardiovascular complications via transcriptional changes. 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 conclusion, alterations in transcriptomic profiles in highlanders with CMS could be attributed to excessive immune responses and chronically damaged cardiovascular functions. Hemodilution helps alleviate CMS symptoms likely by decreasing blood-vessel viscous sheer and therefore decreasing activation of key inflammatory pathways.