EXERCISE AT SIMULATED ALTITUDE ALTERS PROTEIN EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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ABSTRACT

PURPOSE: This work investigated whether hepatopulmonary shunting during exercise at altitude influences protein expression in peripheral blood mononuclear cells (PBMC). METHODS: Subjects (N = 5) ran (60% VO2max) for 60 min under Normoxia (F02 = 20.93%) and Hypoxia (F02 = 13.5%). Tissue oxygen saturation levels were monitored via peripheral oxygen saturation (SpO2) and near-infrared spectroscopy (NIRS). Peripheral blood mononuclear cells (PBMC) were isolated from blood samples that were taken before (PRE), after (POST), 1 hr (1-POST), and 4 hrs after (4-POST) exercise. From these samples, western blot was used to analyze markers along the TLR4 signaling pathway (TLR4, p-NFκB, NFκB), indicators of cellular energy status (SIRT1 & p-AMPK), and mediators of the heat shock response (HSP32, HSP60, HSP70). Data were analyzed with Two-Way (Condition x Time) RM-ANOVA’s with significance set at p<0.05. Post hoc tests were run where appropriate.

RESULTS: SpO2 averaged 79 ± 1% and SpO2 averaged 61 ± 2% in Hypoxia. PBMC data indicated that HSP60, SIRT1-1 and p-AMPK were reduced at 1-Post (by 31%, 68%, and 66% respectively, all p<0.05), whereas the ratio of p-NFκB to NFκB at 1-Post was increased by 139% (p<0.05). CONCLUSION: Activation of AMPK has been shown to reduce inflammatory signaling via SIRT1 mediated deacetylation of p-NFκB. Downregulation of p-AMPK and SIRT1 following simulated altitude exposure may help PBMC maintain mitochondrial capacity. Export of HSP60 to circulation following hypoxic exercise could facilitate mitochondrial biogenesis in skeletal muscle via activation of PGC-1α.

INTRODUCTION

- Reduced gastrointestinal (GI) perfusion during exercise stress can cause ATP depletion in enterocytes. The tight junctions between enterocytes are also damaged, resulting in lipopolysaccharide (LPS) translocation into circulation1.
- LPS is immunogenic. Excessive LPS in circulation can lead to leukocytic sepsis, which manifests as systemic inflammation due to activation of a TLR4-mediated pathway. However, the onlyoccurs in extreme cases because LPS is normally cleared by hepatocytes and Kupffer cells in the liver2.
- Peripheral blood mononuclear cells (PBMC) are activated by circulating LPS and respond by secreting pro-inflammatory cytokines into circulation1. We have previously shown that exertional heat stress increases LPS in circulation and activates PBMC3.
- We suspect that exercise under simulated altitude (hypoxia) may confer a similar response in PBMC. For that reason we have exposed human subjects to exercise under hypoxic conditions. After this, we have analyzed the expression of proteins that regulate metabolism, the heat shock response, and inflammation in PBMC.

METHODS

This single blind, normoxia control protocol investigated the effect of exercise under Normoxic and Hypoxic conditions. Systems-level physiological responses were assessed throughout exercise. Alterations in the protein content of circulating PBMC were assessed from whole blood samples collected before (PRE), after (POST), hour after (1-POST) and 4 hours after (4-POST) exercise.

RESULTS

- Decreased nutrient availability during intense exercise can cause an increase in AMPK and SIRT1. The reduction in AMPK and SIRT1 at 1-Post would help PBMC maintain their pro-inflammatory status, which could assist in the clearance of elevated LPS in circulation (Figure 1).
- HSP60 and HSP32 were both shown to be decreased at 1-Post in Hypoxia. These proteins may be exported into plasma, where they could assist in the activation of mitochondrial biogenesis in skeletal muscle via the activation of PGC-1α (Figure 2).
- Reduced AMPK and SIRT1 signaling in conjunction with the loss of HSP60 and HSP32 would serve to increase the pro-inflammatory status of PBMC. In turn, this could explain the increase in the p-NFκB: NFκB ratio at 1-Post in Hypoxia, which is paradoxical considering that TLR4 was also reduced at this timepoint (Figure 3).

CONCLUSIONS

REFERENCES
