CROSS-TOLERANCE: HYPOXIA STRESSES PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) MORE THAN HYPERTHERMIA

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ABSTRACT

Recent work has substituted hyperthermic exercise for hypoxic preconditioning prior to ascent to altitude. **PURPOSE**: This work investigated whether hyperthermic and hypoxic exercise elicit an equivalent stress response in PBMC. **METHODS:** Subjects (N = 4) ran (65% VO_{2max}) for 60min under three environmental conditions: Control (20°C / F_IO_2 = 20.9%), Hypoxia (20°C / F_IO_2 = 13.5%), and Hyperthermia (38°C / F_IO_2 = 20.9%). Core temperature (Tc) and peripheral oxygen saturation (SpO_2) were measured during exercise. PBMC were isolated from blood samples taken at Pre, Post, 1-Post, and 4-Post exercise. Protein content of markers along the TLR4 signaling pathway (TLR4, *p*-NFκB, NFκB) and indicators of cellular energy status (SIRT1 & *p*-AMPK) were determined via western blot. Group differences were determined with 2-Way (Condition x Time) RM ANOVAs with statistical significance set at $p \leq 0.05$. *Post hocs* were run where appropriate. **RESULTS:** SpO_2 averaged 79 ± 1% in Hypoxia. Maximal Tc in Hyperthermia was 39.2 ± 0.2ºC. *p*-NFκB was elevated ($p < 0.01$) at 1-Post in Hypoxia (+94%) but not in Hyperthermia. *p*-AMPK was reduced ($p < 0.01$) at 1-Post in Hypoxia (-66%) and Hyperthermia (-50%). SIRT1 was reduced ($p < 0.01$) at 1-Post in Hypoxia (-68%) and Hyperthermia (-47%). **CONCLUSION:** Hypoxic exercise increased inflammatory signaling in PBMC. AMPK & SIRT1 may have been downregulated to help PBMC maintain inflammatory signaling in response to increased circulating concentrations of LPS. Collectively, these data suggest that exercise at altitude stresses PBMC more than exercise under hyperthermic conditions. Further research on the mechanisms behind hypoxia/hyperthermia cross-tolerance in human PBMCs is warranted.

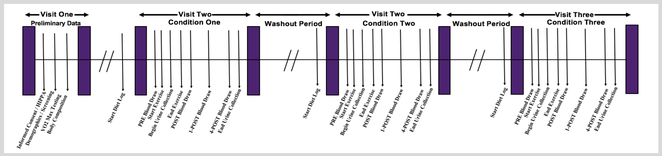
INTRODUCTION

- Blood flow is distributed away from the intestinal cavity during exercise stress. This causes ATP depletion in enterocytes and destabilizes the epithelial tight junction complex between enterocytes.[1,2] Gastrointestinal (GI) barrier damage allows endotoxin translocation into circulation, which activates inflammatory cascades by way of a TLR4-mediated signaling pathway.[1,3]
- When exercise is performed under severe environmental conditions GI barrier damage is increased. Hypoxia, similar to that which occurs during acute altitude exposure, has been shown to increase inflammatory signaling in immune and epithelial cells.[4,5] Severe hyperthermia increases the ratio of pro- to anti-inflammatory cytokines in circulation, which can lead to disseminated intravascular coagulation and multiple organ failure in the pathophysiology of exertional heatstroke.[2,6]
- Heat acclimation has been shown to afford similar improvements in systems-level physiologic variables as compared to altitude acclimatization. Whether or not this "cross-tolerance" extends to changes in protein expression within PBMC is unknown.

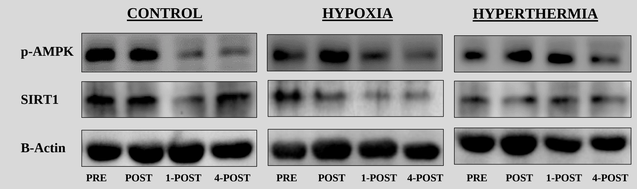
METHODS

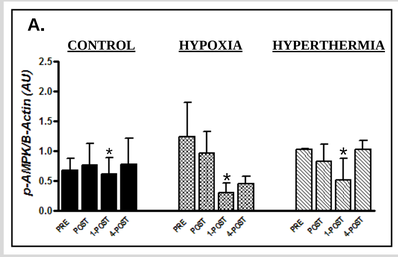
This study investigated the effect of exercise under control, hypoxic, and hyperthermic conditions on changes in protein expression within peripheral blood mononuclear cells (PBMC). Metabolic signaling proteins, heat shock proteins, and markers along the TLR-4 signaling pathway were assessed. PBMC were isolated from whole blood samples collected before (PRE), after (POST), 1 hour a (1-POST) and 4 hours after (4-POST) exercise. The study schematic (below) provides an overview of the study protocol.

Study Schematic



RESULTS





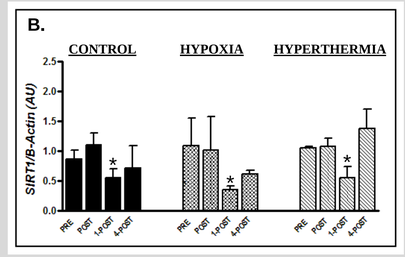
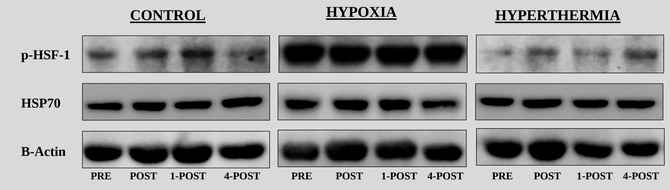
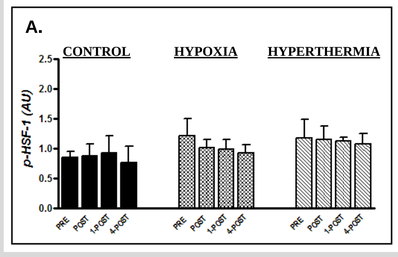


Figure 1. Alteration of metabolic status of PBMC's. [A] p-AMPK and [B] SIRT 1 responses to 60 min exercise (65% VO_{2max}) under various environmental conditions (as described in Methods). Expression of both proteins is reduced at 1-post in Hypoxia and Hyperthermia.





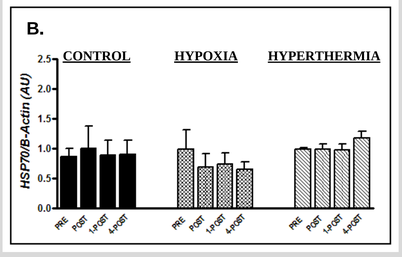
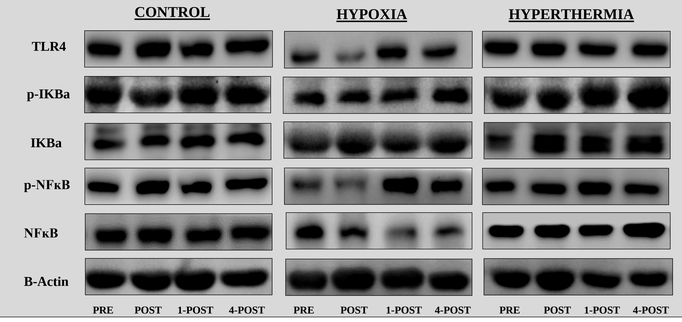
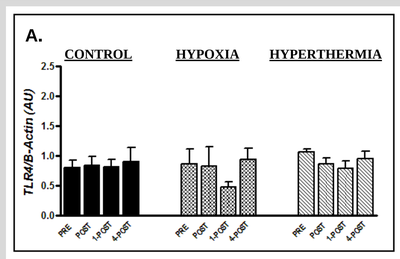
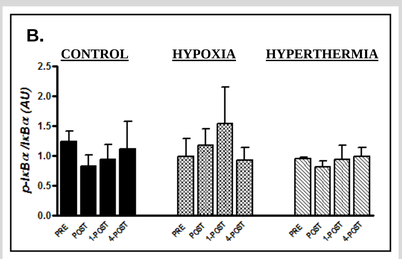


Figure 2. Protein markers of the heat shock response in PBMC. [A] p-HSF-1 and [B] HSP70 responses to 60 min exercise (65% VO_{2max}) performed under various environmental conditions (as described in Methods).







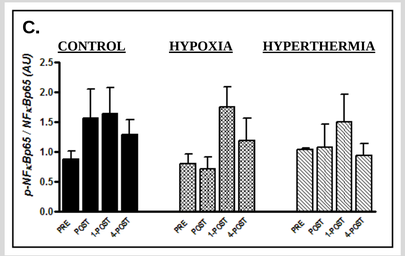


Figure 3. Exertional heat stress alters the TLR4 mediated signaling cascade in PBMC. [A] TLR4, [B] p-IKBa / IKBa, [C] pNFKB / NFKB responses to 60 min exercise (65% VO_{2max}) performed under various environmental conditions (as described in Methods). Elevations in *p*-IKB and *p*-NFKB were noted at 1-Post in Hypoxia (43% / 94%, respectively).

CONCLUSIONS

- Environmental stress increases signals for activation of PBMC. This causes greater energy demand and activation of stress responses in PBMC.
- Data suggest that improvements in cellular energy deficit may help PBMC maintain pro-inflammatory response during the post-exercise period.
- Past studies show that if there is limited access to altitude then hyperthermia can be an expectable alternative.[7] However, the present data suggest that hypoxia stresses PBMC more than hyperthermia, suggesting that further tests are warranted.[7]

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