The Absence of Innate Inflammatory Gene Response to Acute Hyperbaric Stress in Non-Divers Following Heat Acclimation

B.A. Cameron*, T.M. McLellan, D.J. Eaton and S.G. Rhind

Defence R&D Canada – Toronto

*Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada

Background

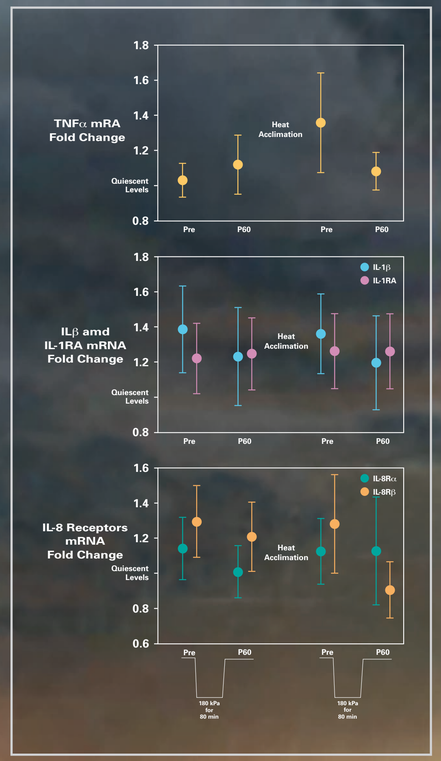
In a previous microarray study we measured significantly increased mRNA transcription of several inflammatory genes in the peripheral blood leukocytes (PBLs) of experienced divers over that in non-divers. These findings together with the growing body of evidence showing a common inflammatory response to various forms of stress and recent reports that heat and/or exercise acclimation reduces hyperbaric stress, prompted the examination of inflammatory gene response during decompression stress before and after heat acclimation after heat acclimation in non-divers.

Materials and Methods

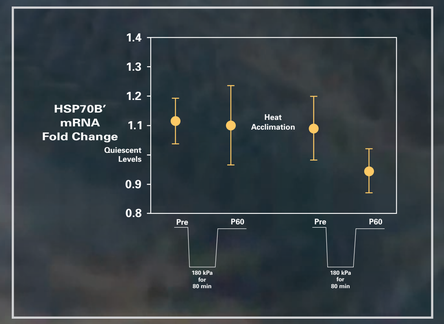
Peripheral blood samples were drawn from non-divers (n=9) pre-dive and 60 min after surfacing (P60) from hyperbaric exposure at 18 msw for 80 min before and after a 9-day heat acclimation protocol known to promote rheological and physiological adaptations in humans. Transcribed mRNA levels from the PBLs of non-divers were screened for a range of inflammatory markers using Agilent two-colour microarray analysis and normalized to mRNA levels from quiescent reference cells.

Results

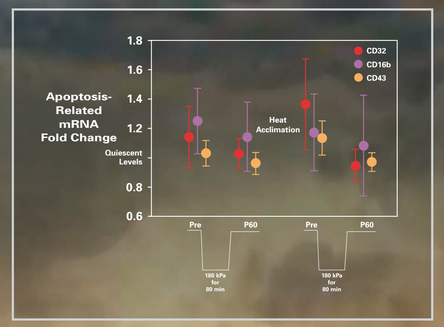
Doppler Ultrasound-detected bubble scores were low in all but one subject.



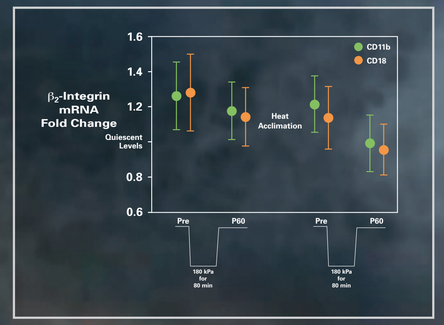
Tumour necrosis factor-α, IL-1 and IL-8 are thought to be the first three cyto-chemokines to serially appear during inflammatory response. Ligation of TNFα and IL-1 to their respective membrane receptors stimulate NF-kβ activation, and increased IL-8 receptor expression in polymorphonuclear neutrophils (PMN) membranes serve an important role in migration during extravasation. Decompression stress did not significantly alter mRNA transcription levels of TNFα, IL-1β and IL-1RA. IL-8Rα and IL-8Rβ in the PBLs of non-divers before or after heat acclimation.



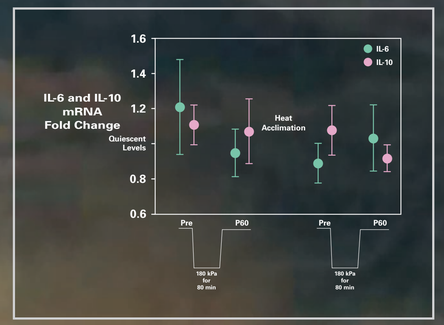
Rapid increase of the PMN heat shock protein (HSP70B') is a hallmark of inflammatory response. Decompression stress did not significantly alter mRNA transcription levels of HSP70B' in the PBLs of non-divers before or after heat acclimation.



Apoptosis occurs in conjunction with decreased expression of CD-32, CD-16b and CD43 on the plasma membranes of PBLs. Decompression stress did not significantly alter mRNA transcription levels of CD-32, CD-16b and CD-43 in the PBLs of non-divers before or after heat acclimation.

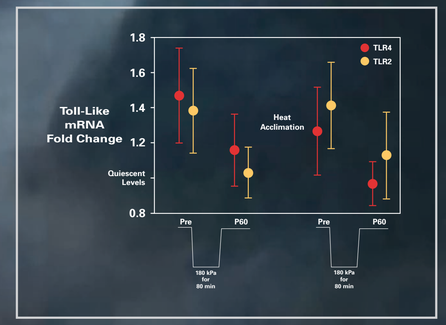


Increased copies of β_2-Integrin proteins on the PMN membranes provide additional sites for PMN-EC adhesion during inflammatory response. Decompression stress did not significantly alter mRNA transcription levels of β_2-Integrin component proteins, CD11b and CD18, in the PBLs of non-divers before or after heat acclimation.

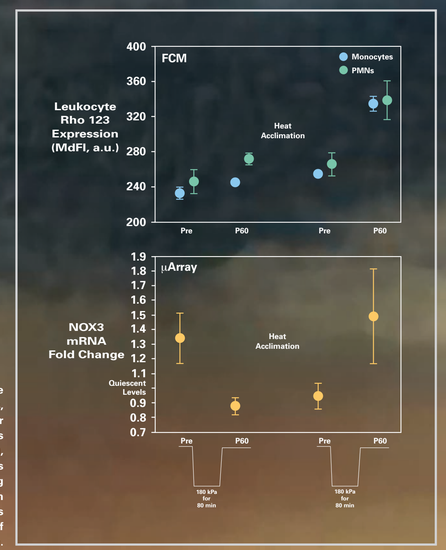


NF-κB activation and translocation leads to up-regulation of the inflammatory cytokine IL-6 and the anti-inflammatory cytokine, IL-10. IL-6 is essential for mediating systemic acute-phase response, antibody production, Tcell function and differentiation of cytokine producing lymphocytes. IL-10 is thought to protect the host by controlling the pathology associated with an over-exuberant inflammatory response. Decompression stress did not significantly alter mRNA transcription levels of IL-6 or IL-10 in the PBLs of non-divers before or after heat acclimation

The production of reactive oxygen species (ROS) by ECs and PBLs, reported to occur shortly after vascular disturbance, is thought to be the primary stimulus of inflammatory response. NADPH oxidase 3 (NOX3), a well-known integral membrane protein in ECs and PBLs has been reported to be responsible for ROS production following vascular disturbances leading to inflammatory response. Decompression stress significantly increased flow cytometry-detected ROS production in PMNs and monocytes and mRNA transcription levels of NOX3 in the PBLs of non-divers following heat acclimation.



Recent studies reported that significantly up-regulated expression of Toll-Like Receptor proteins TLR2 and TLR4 in the monocytes and endothelial cells of septic individuals may play a role in PMN activation. Moreover, TLR4 expression is probably closely associated with the magnitude of physiological response to recurrent stimulation by both LPS and non-pathogenic agents such as heparin sulphate and hyaluronate, products of EC damage. Decompression stress did not significantly alter mRNA transcription levels of TLR2 and TLR4 in the PBLs of non-divers before or after heat acclimation.



Summary

Decompression stress following a dive to 18 msw for 80 minutes did not elicit mRNA expression in a broad range of immuno-inflammatory markers in the PBLs of non-divers before or after heat acclimation. The absence of mRNA transcription of immuno-inflammatory markers in non-divers may be due to insufficient decompression stress following the dives. On the other hand, whereas a previous study showed that the innate immune system of experienced divers reacted to the presence of VGE, the results of this study suggests that the vasculature of non-divers, completely naïve to VGE, may not be prepared for the presence of inert gas bubbles. Finally, ROS production (FCM) in the monocytes and PMNs (FCM) and elevated mRNA transcription of NOX3 in PBLs (μarray) in non-divers, indicate some form of response to decompression stress after the second dive following heat acclimation. We are presently investigating the mRNA transcription of immuno-inflammatory markers in experienced divers following Trimix dives as a means to clarify the mechanisms involved in inflammatory response following decompression stress.

Acknowledgements

The authors thank the US Navy Office of Naval Research, Defence Research and Development Canada and The Natural Sciences and Engineering Research Council of Canada.

Defence Research and Development Canada | Recherche et développement pour la défense Canada

Canada