

Inflammatory Gene Expression in the Leukocytes of Naive Subjects and Experienced Divers Following Acute Hyperbaric Stress

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Introduction

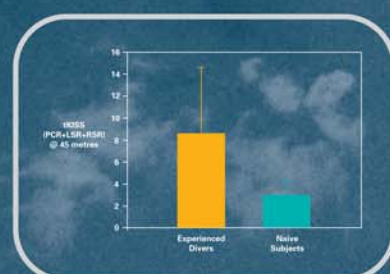
Peripheral blood leukocytes can be exploited as windows of the inflammatory response as they use cellular multi-pathway mechanisms to scrutinize, respond and orchestrate adaptive tolerance during physiological stress such as decompression sickness (DCS). mRNA microarray (μ -array) and flow cytometry (FC) analyses were used to monitor genomic transcription and protein expression in leukocytes of experienced divers and naive subjects following decompression from a 45 MSW dive for 30 minutes.

Methods

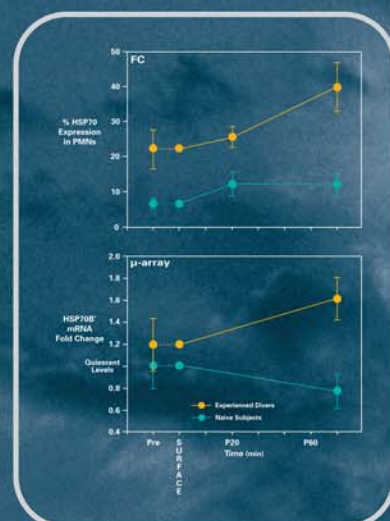
Peripheral blood samples were drawn from experienced divers (n=6) and naive subjects (n=6) prior to (Pre) and 20 and 60 minutes after surfacing (P20; P60) from a 45 MSW dive for 30 minutes according to DCIEM dive tables. Doppler ultrasound was used to measure venous gas emboli (VGE) at rest 20, 40, 80 and 120 minutes after the dive. VGE ratings were integrated for the four sampling times at the precordium and subclavian veins to produce Kisman Integrated Severity Scores (KISS) and summed together (tKISS). Fold changes in expression levels of a range of inflammatory markers were screened from purified leukocyte mRNA using Agilent two-colour μ -array analysis and normalized to mean mRNA expression levels from ten quiescent representative human cells. Monoclonal antibodies against several inflammatory markers used with whole blood FC analysis measured mean % positive and median fluorescence intensity (MFI) in peripheral blood neutrophils and monocytes.

Results

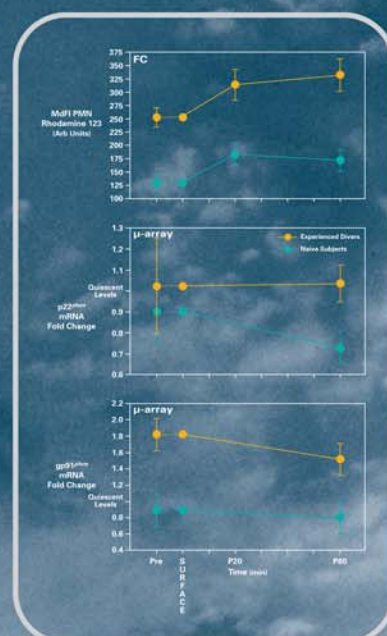
Results (mean values \pm SEM).



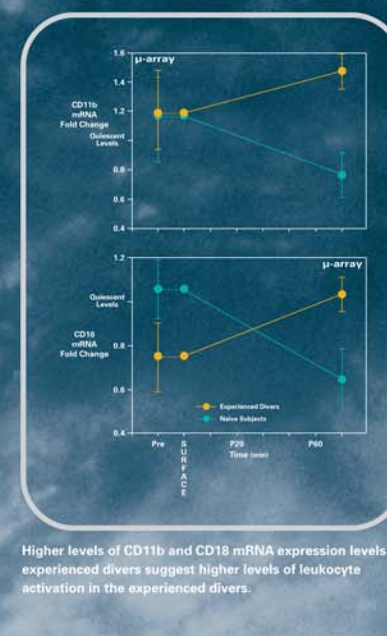
Although the experienced divers and naive subjects were not significantly different in age and BMI, total Kisman Integrated Severity Scores (tKISS) were moderately higher in the experienced divers following the 45 metre dive.



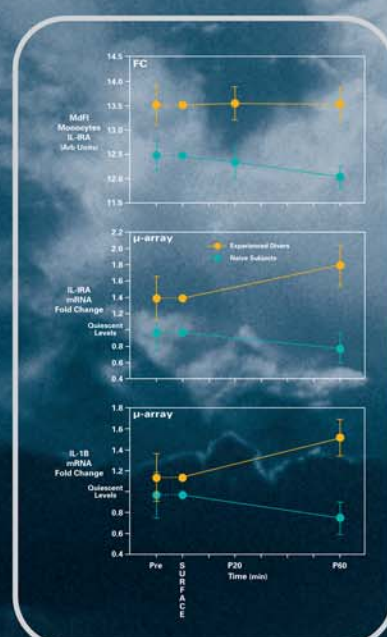
mRNA expression levels of HSP70B', the PMN HSP70, and HSP70 protein expression levels were higher in the PMNs of experienced divers.



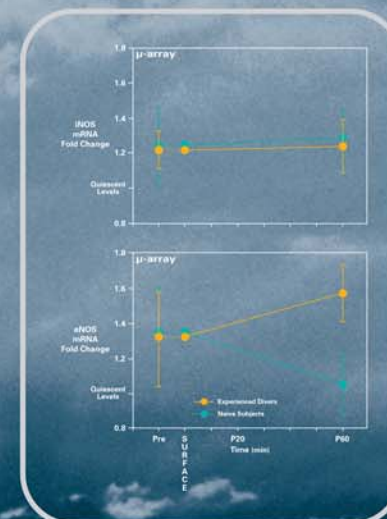
The higher mRNA expression levels of two critical subunits of PMN NADPH oxidase, gp91^{phox} and p22^{phox}, in experienced divers mirrored the higher levels of respiratory burst (rhodamine 123) measured by FC in the experienced divers.



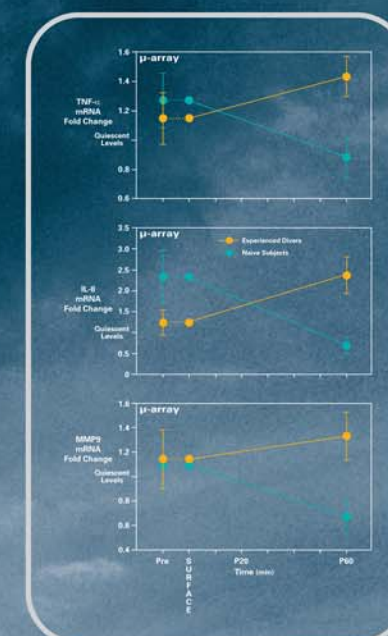
Higher levels of CD11b and CD18 mRNA expression levels in experienced divers suggest higher levels of leukocyte activation in the experienced divers.



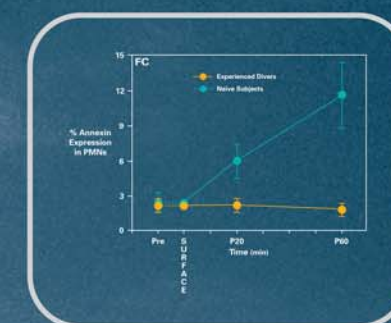
The higher mRNA expression levels of the anti-inflammatory cytokine IL-1RA in experienced divers closely reflected IL-1RA protein expression levels measured by FC in the monocytes of experienced divers. mRNA expression levels of the inflammatory cytokine IL-1 β were also higher in the experienced divers.



The higher levels of leukocyte activation in the experienced divers did not cause induction of iNOS but did result in higher levels of mRNA expression of eNOS in experienced divers.



IL-1 β , TNF α , and IL-8 have been reported to participate in the induction of the matrix metalloproteinase 9 (MMP9). mRNA expression levels of these three cytokines and MMP9, a proteolytic enzyme of collagen, were all higher in experienced divers.



Apoptosis was attenuated or delayed in the PMNs of the experienced divers after the dive.

Conclusions

This study showed several examples of a close relationship between protein expression and mRNA expression measured by flow cytometry and micro-array analysis, respectively. By revealing fold changes in thousands of mRNA expression levels, micro-array analysis acts as a molecular roadmap to biochemical pathways involved in the inflammatory response due to venous gas emboli following decompression. Several pro- and anti-inflammatory cytokines that were expressed and activated in the experienced divers remained inactive in the naive subjects following the dive. We also observed higher levels of antioxidants in the plasma of the experienced divers. The absence of iNOS transcription coupled with the higher levels of eNOS mRNA suggests a controlled inflammatory response to VGE in the experienced divers not observed in the naive divers. This controlled inflammatory response may have influenced the delay in apoptosis in the PMNs of the experienced divers.

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.