Extended Oxygen Decompression Increases Transcription Acitvity In Peripheral Blood Leukocytes In Navy Clearance Divers During TRIMIX Diving

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Background

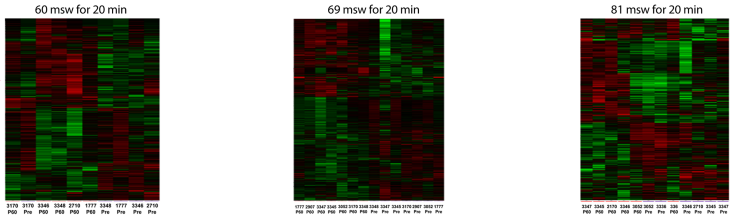
Decompression stress is thought to significantly alter vascular homeostasis. Peripheral blood leukocytes (PBLs) are important members of the innate immune system that influence and respond to vascular homeostasis. Agilent two-colour microarray analysis of PBLs in divers during earlier experimental Trimix dives revealed higher numbers of significantly expressed genes (transcription activity) in a more ordered genomic expression pattern after a 20 minute dive to 69 msw than that after dives to 60 or 81 msw for 20 minutes. We also noted that the 69 msw dives generated the highest levels of Doppler-detected VGE in the divers. Other studies have reported increased surface density of the protein components of the β2-Integrin molecule to indicate leukocyte activation status during decompression stress. VGE represent a significant component of decompression stress and oxygen decompression at 9 msw (PO_2 = 1.9 ATA) for 70 minutes without air breaks could conceivably be considered similar to a normal HBOT session. Since "oxidative stress is fundamental to hyperbaric oxygen therapy" (Thom, 2009), we suspected that transcription activity, Doppler-detected VGE and surface density of CD11b during the Trimix dives were indications of the combined effects of decompression stress and oxidative stress during decompression. We hypothesized that in addition to resulting in higher transcription activity, high levels of decompression stress due to VGE may promote more ordered patterns of genomic expression because it may overwhelm inter-individual variations in genomic response. In contrast, lower levels of decompression stress may result in lower transcription activity and less ordered patterns of genomic expression.

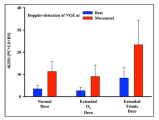
Methods

To examine this hypothesis, we attempted to reduce decompression stress after the 20 minute dive to 69 msw by altering its decompression schedule. The normal decompression schedule for a 69 msw dive for 20 minutes is composed of two components breathing Trimix for 22 minutes followed by 100% oxygen for 54 minutes at 9 msw. Using the normal 69 msw dive as a control we substituted extended Trimix or oxygen decompression periods from the 81 msw dive into the decompression schedules of two experimental dives for 20 minutes to 69 msw. As a result, one experimental dive's Trimix decompression period after the 69 msw dive was 30 minutes and the other dive's oxygen decompression period at 9 msw was 70 minutes. All dives were carried out with CUMA using a 50/50 mix of helium/nitrogen and 100% oxygen producing PO_2 average = 1.5 ATA at all depths. Pre- and post-dive whole blood samples were withdrawn into PaxGene (PreAnalytiX) and heparin vacutainer tubes from seven navy clearance divers. mRNA from PBLs was purified using PAXgene blood RNA kits (Qiagen). We used flow cytometry to assess membrane surface density of CD11b in the neutrophils of the divers. Doppler ultrasound was used to characterize the levels of venous gas emboli at rest and movement at post 20, 60, 100 and 140 minutes following the dives. Finally, two-colour Agilent microarray analysis probed with a human genome chip was used to characterize transcription activities in the PBLs in the clearance divers before (Pre) and 75 minutes after the dive (P75).

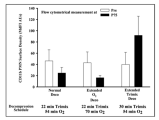
Analysis

The surface densities of neutrophil CD11b were analyzed using CellQuest® (Becton Dickenson). Resting and movement Doppler-detected VGE were analyzed according to Kisman using calculation of Z scores followed by summation of Kisman Integrated Severity Scores in the precordium and left and right subclavian veins (tKISS). Two-colour Agilent microarray analysis was performed in a parallel process by comparing a gene's raw experimental transcription activity to that of its quiescent activity in a universal PBL control. Then, GeneSpring® was used to normalize each gene's experimental transcription activity to its quiescent value after dives in each decompression schedule. Finally, GeneSpring® compared each gene's normalized transcription activity at P75 with that at Pre (Post – Pre) filtering out the lower 20th percentile in each decompression schedule. Each gene in the filtered upper 80th percentile was examined for significance by an uncorrected t-test ($p \leq 0.05$), and Venn analysis was used to determine common genes between each decompression schedule. Most microarray studies find that the resultant numbers of genes with significant transcription activities between two comparative groups are unmanageably high and uninformative. For example, approximately 40,000 probes used in the present study revealed 5275 significantly transcribed genes after the extended oxygen dives, an order of magnitude reduction. In terms of numbers of genes to investigate, microarray studies find it helpful to impose an initial step in analysis to identify genes that may realistically indicate the stress that caused their transcription. As a result, additional stringency is enforced by an arbitrary level of transcription activity between two groups of at least 2 fold higher or 2 fold lower, termed Biological Significance. Some microarray studies have used 3 fold or higher levels of stringency. The biologically significant genes in each of the three decompression schedules were examined for enrichment of Gene Ontology (GO) categories (Benjamini et al., 2001). Genomic expression heat maps were produced using GeneSpring®.

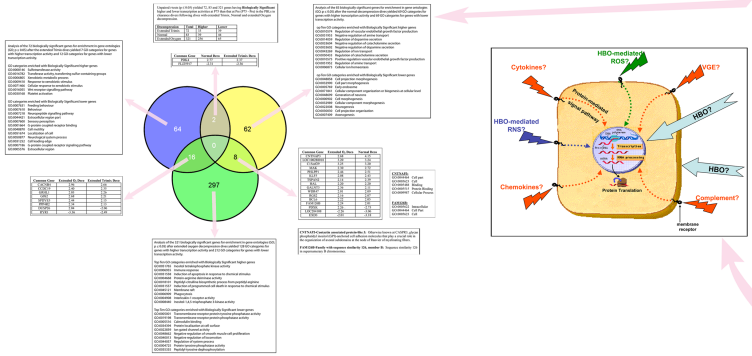


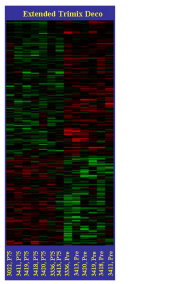


Doppler ultrasound indicated higher levels of VGE-related vascular decompression stress in 7 clearance divers at rest and movement following the extended Trimix dives over that following the normal and extended oxygen decompression dives.



Whereas decreased surface density of CD11b may indicate reduced neutrophil adhesion and/or suppression of immunoinflammatory response in 7 clearance divers at P75 following extended oxygen decompression, the increase of CD11b may indicate heightened neutrophil activation following extended Trimix decompression.





Representative heat maps of significant transcription activity show that the three decompression schedules after a 69 msw dive for 20 min produced ordered patterns of genomic expression in seven clearance divers.

Results

No divers were diagnosed with DCS during these experiments.

Unpaired t-tests ($p \leq 0.05$) yielded 618, 1733 and 5275 genes with transcription activities significantly higher and lower at P75 than that at Pre (P75 - Pre) in the PBLs in clearance divers following dives with extended Trimix, Normal and extended Oxygen decompression. Of the 618 significantly transcribed genes following the extended Trimix decompression dives, 72 genes were biologically significant at P75. Of these 72 genes, 33 had transcription activities 2 fold higher and 39 genes were 2 fold lower at P75. Of the 1733 significantly transcribed genes following the Normal decompression dives, 82 genes were biologically significant at P75. Of these 82 genes, 39 were 2 fold higher and 44 genes were 2 fold lower at P75. In contrast, of the 5275 significantly transcribed genes following the extended Oxygen decompression dives, 321 genes were biologically significant at P75. Of these 321 genes, 256 were 2 fold higher and 65 genes were 2 fold lower at P75.

Summary

The transcription activities following three different decompression schedules were not as straightforward as hypothesized. We predicted that lower transcription activities and less ordered patterns of genomic expression would occur following dives using extended Trimix and Oxygen decompression. Although lower transcription activity resulted from extended Trimix decompression, transcription activity following extended Oxygen decompression was significantly higher than the dives using the other decompression schedules. Furthermore, although they have not yet been completely analyzed, all the dives produced ordered genomic expression.

Doppler ultrasound and flow cytometry indicated that the highest level of decompression stress occurred following extended Trimix decompression, and the extended Oxygen decompression was the least stressful. In contrast, PBL transcription activity was relatively low following extended Trimix and significantly high following extended Oxygen decompression.

GeneSpring analysis showed that while transcription activities of biologically significant genes were relatively similar following the extended Trimix and Normal decompression dives, the GO categories enriched with biologically significant genes following those decompression schedules were entirely different. Moreover, transcription activities of biologically significant genes were approximately 4 times higher in the PBLs in clearance divers following extended Oxygen decompression than the extended Trimix or Normal decompression dives. Similar to HBOT, it is likely that oxidative stress causes significant alterations in genomic expression during oxygen decompression but little is known about the effects of its duration. In this respect, the effects of the additional 16 minutes of extended oxygen decompression at 9 msw over that following the normal decompression schedule suggest that duration of oxygen decompression profoundly affects genomic expression in PBLs. Interestingly, the numbers of common biologically significant genes were very low or zero when the decompression schedules were compared. Furthermore, the complete dissimilarity in GO categories enriched with biologically significant genes in each decompression schedule suggests substantial differences in genomic expression following each decompression schedule.

Previous studies have reported that HBO profoundly affected the performance of plasma membrane proteins including β2-Integrin. Others have shown that heightened immunoinflammatory response increased the surface density of β2-Integrin. The alterations in surface density of CD11b following the dives in the present study, appears to support those findings. But we examined the effects of decompression stress on transcription activity deep inside the nuclear envelope expecting that protein expression would somewhat reflect transcription activity. If genomic response to vascular disturbance due to VGE relies primarily on signal processing, it is possible that a significant expanse of time may be required for receptor interaction at the plasma membrane in the PBL through second messengers to the nucleus before transcription activity can be initiated in response to decompression stress. In contrast, genomic response to oxygen diffusion to the nucleus during oxygen decompression (or HBOT) is probably instantaneous. This in part may account for the profound transcription activity following extended oxygen decompression but presents the unforeseen problem of optimum sampling time of genomic response to decompression stress due to VGE. Moreover, we believe that the profile for genomic expression in PBLs during HBOT is also unknown. Nevertheless, P75 may not be the optimum time to sample genomic expression during decompression stress. As a result, a timed study of genomic expression over a 24 hour period to ascertain the dynamic profile of transcription activity due to decompression stress without oxygen decompression may be necessary. Furthermore, studies examining the effects of decompression stress due to VGE without the complicating factor of oxygen decompression will also be very important.

Finally, we wonder about the effects of decompression stress due to VGE that interact with the effects of oxidative stress on genomic expression during oxygen decompression. It is tempting to speculate that this interaction represents a continuum of physiological and biochemical mechanisms induced by increasing and decreasing levels of decompression and oxidative stress much in the same way as decompression tables are developed to minimize the risk of decompression sickness.

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