Prevention of decompression sickness (DCS) with graded pre-oxygenation

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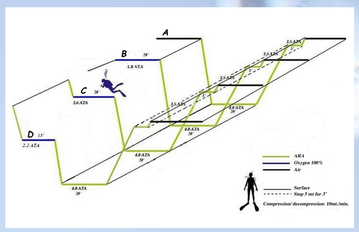
Background:

Micronuclei reduction may be beneficial in gas bubbles formation during decompression. Platelets and lymphocytes reciprocally regulate mutual functions and participate in both patho-physiological processes, as thrombosis or inflammation (Nailin Li. J Leukoc Biol. 2008) so both could be involved in bubbles formation and thus also in DCS manifestations. Aim of the study is to test the hypothesis that normo (NBO) and hyperbaric (HBO) oxygen pre-treatments immediately before an open water scuba diving might reduce bubble formation, platelets activation, affect antioxidant systems and intracellular Ca^{2+} homeostasis in peripheral blood lymphocytes in order to decrease the risk of DCS.



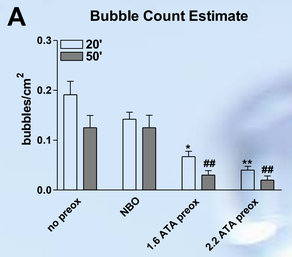
Materials and Methods:

Six healthy volunteers (age: 38.2±11.0 yr, BMI: 27,1±5,4, body fat 21,6±7,5) participated in this study provided by a valid fit-to-dive medical certificate and according the declaration of Helsinky and the local ethical committee.
The study is based on four day protocol:
A. Compression at 4 ATA for 20';
B. Normobaric preoxygenation (NBO) for 20' and compression at 4 ATA for 20';
C. Preoxygenation at 1.6 ATA for 20' with 100% oxygen (HBO), immediately compression at 4 ATA for 20';
D. Preoxygenation at 2.2 ATA for 15' with 100% oxygen (HBO), immediately compression at 4 ATA for 20';

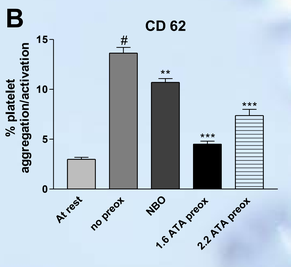
The diving procedure at 4 ATA was performed in open water (Tremiti islands, FG-Italy, may-september 2008) with the same water temperature (20±2°C) and work load of exercise (underwater bicycle by OKEO, GE-Italy).Once the surface was reached, they were monitored with precordial ultrasonic doppler (Hadeco SonoMate 300G-2Mhz probe) at 20 min, 50 min. Digitazion of the Doppler signal by sonogram is used to assist in the identification and scoring of *bubble* signals from the cardiac background. *Venous blood samples* were obtained immediately before and after pressure exposure to study: *platelets* by flow cytometric analysis (Coulter Eletronics, Miami, FL) -surface expression of activation-dependent glycoprotein, CD62; *Lymphocytes* isolated on a Ficoll- Paque PLUS (Amersham, Piscataway NY) gradient using the Boyum method (Belia et al , Free Radic Res. 2009)-*H_2O_2 levels and catalase activity* following standardized protocols from M'Bemba-Meka et al. (Chemico-Biological Interactions 156:69-80, 2005) and Belia et al. (Free Radic Res. 43(2):138-148,2009) respectively; and intracellular calcium on single cells using Fura2/AM as calcium fluorescent probe and a videomicroscopy system applied to an inverted Olympus X50 microscope (Beccafico S et al. Ann. N.Y. Acad. Sci. 2007). Data were expressed as mean±s.e.m. and analyzed with ANOVA.

Results:

Bubble Count Estimate and platelets

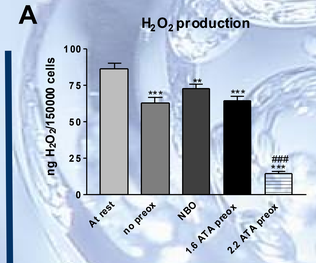


Panel (A) HBO significantly reduced deco induced air bubbles at 20' and 50'
* p<0.01 vs 20'-no-preox.
** p<0.001 vs 20'- no-preox
p<0.001 vs 50'-no-preox

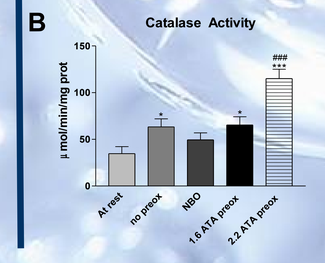


Panel (B) Percentage platelet activation compared to at rest condition was increased after the diving section (no preox), in contrast it was reduced in NBO AND HBO conditions
# p<0.001 vs at rest
** p<0.05 and *** p<0.001 vs no preox.

Oxidative stress in lymphocytes

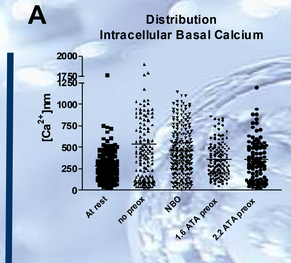


Panel (A) represents H_2O_2 production in lymphocytes at rest, before and after HBO session. In the different scuba diving sessions, H_2O_2 production was reduced compared to control (at rest).
** p< 0.01 and ***p< 0.001 vs at rest
p< 0.001 vs no-preox

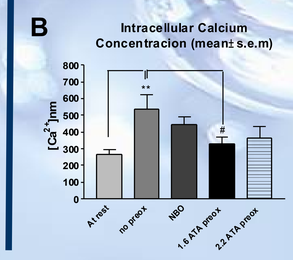


Panel (B) shows the acitivity of antioxidant enzyme catalase which significantly increased in almost all scuba diving sessions with the exception of NBO section.
*p< 0.05 and ***p<0.001 vs at rest
p< 0.001 vs no preox

Intracellular Calcium Homeostasis in lymphocytes



Panel (A) shows the basal $[Ca^{2+}]i$ values in analyzed cells under different conditions. Cell distributions revealed an heterogeneous population after diving and NBO sections.



Panel (B) represents values of basal $[Ca^{2+}]i$ in lymphocytes derived from cell population distributions. Compared to the rest condition, basal $[Ca^{2+}]i$ was increased after the diving session, in contrast to the 1.6 ATA preox, condition to which basal $[Ca^{2+}]i$ was reduced with respect to the no-preox session. **p<0.01 vs at rest, #p<0.05 vs no preox.

Conclusions:

This study indicates that both NBO or HBO pre-treatment might be beneficial in reducing DCS. Our data support that HBO might be more effective than NBO in reducing bubble numbers, reducing platelet activationas well in more pronounced activation of antioxidant enzymes in lymphocytes, and in maintaining intracellular Ca^{2+} closer to the normal basal levels.

Poster # F 1

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