



Title: CD34+/CD45-dim stem cell mobilization by hyperbaric oxygen – changes with oxygen dosage.



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Introduction: Hyperbaric oxygen treatment mobilizes bone marrow derived-stem/progenitor cells by a free radical mediated mechanism. We hypothesized that there may be differences in mobilization efficiency based on exposure to different oxygen partial pressures.

Materials and Methods: Blood from twenty consecutive patients was obtained before and after the 1st, 10th and 20th treatment at two clinical centers using protocols involving exposures to oxygen at either 2.0 or 2.5 atmospheres absolute (ATA).

Results: Post-treatment values of CD34+ and CD45-dim leukocytes were always 2-fold greater than the pre-treatment values for both protocols. Values for those treated at 2.5 ATA were significantly greater than the 2.0 ATA treatment group by factors of 1.9 to 3-fold after the 10th and before and after the 20th treatments. Intracellular content of hypoxia inducible factors -1,-2, and -3, thioredoxin and poly-ADP-ribose polymerase assessed in permeabilized CD34+ cells with fluorophore-conjugated antibodies were twice as high in all post- versus pre-treatment samples with no significant differences between 2.0 and 2.5 ATA protocols.

Summary: We conclude that putative progenitor cell mobilization is higher with 2.5 versus 2.0 ATA treatments, and all newly mobilized cells exhibit higher concentrations of an array of regulatory proteins.

Table 1. Intracellular protein content (fold-elevation post- versus prior to HBO₂).

Protein	Treatment #	2.0 ATA Protocol	2.5 ATA Protocol
HIF-1	1	2.35 ± 0.24	3.29 ± 0.55
	10	2.65 ± 0.21	2.67 ± 0.22
	20	2.54 ± 0.38	2.77 ± 0.26
HIF-2	1	2.33 ± 0.24	2.68 ± 0.30
	10	2.48 ± 0.15	2.54 ± 0.20
	20	2.54 ± 0.23	2.60 ± 0.21
HIF-3	1	2.27 ± 0.22	2.67 ± 0.31
	10	2.38 ± 0.24	2.29 ± 0.15
	20	2.43 ± 0.26	2.27 ± 0.15
Trx	1	2.34 ± 0.24	2.51 ± 0.26
	10	2.36 ± 0.22	2.28 ± 0.13
	20	2.44 ± 0.24	2.50 ± 0.29
PARP	1	2.36 ± 0.22	2.64 ± 0.26
	10	2.39 ± 0.22	2.42 ± 0.19
	20	2.57 ± 0.27	2.47 ± 0.22

Data show mean ± SE fold-differences in fluorescence of post-versus pre-HBO₂ permeabilized CD34+ cells using fluorophore-conjugated antibodies to proteins shown in column 1. All post-HBO₂ values are significantly different from pre-HBO₂ and there are no significant differences between the 2.0 and 2.5 ATA protocols.

Figure 1. Leukocyte mobilization by HBO₂. The number of circulating CD34+,CD45-dim cells in blood before and after the 1st, 10th and 20th treatment of 20 patients exposed to either 2.0 or 2.5 ATA. Data were normalized to blood volume and are mean ± SE, * indicates significant difference between 2.0 and 2.5 ATA groups (ANOVA). All post-HBO₂ values are significantly different from pre-HBO₂ values at each treatment time in both groups (t-test).

