Effect of Intermittent Hyperoxia on Stem Cell Mobilization and Cytokine Expression
Persistent post concussion research

| Table 2. Changes From Baseline in Postconcussion Symptom Scores Using the Rivermead Post-Concussion Symptoms Questionnaire and Subscales Among the Intent-to-Treat and Per-Protocol Populations |
|--------------------------------------------------|---------------------------------|-----------------|---------------------------------|-----------------|
| Intervention                                     | Intent-to-Treat Population      | Per-Protocol Population |
|                                                 | Baseline, Mean (SD) | After Intervention, Mean (SD) | Change Score (95% CI) | P Value | Baseline, Mean (SD) | After Intervention, Mean (SD) | Change Score (95% CI) |
| Rivermead Post-Concussion Symptoms Questionnaire 3 Subscale |
| Standard care                                    | 5.4 (2.7) | 5.1 (2.8) | 0.0 (-1.0 to 1.0) (n = 20) | .97 | 5.4 (2.7) | 5.1 (2.8) | 0.0 (-1.0 to 1.0) (n = 20) |
| HBO                                              | 5.5 (3.3) | 4.2 (3.0) | 1.2 (0.0-2.4) (n = 23) | .04 | 4.7 (3.2) | 3.1 (2.2) | 1.6 (-0.1 to 3.3) (n = 11) |
| Sham                                             | 4.7 (3.1) | 3.5 (3.3) | 1.5 (0.1 to 2.9) (n = 21) | .03 | 4.8 (3.7) | 2.7 (2.8) | 2.2 (0.7 to 3.6) (n = 13) |
| Rivermead Post-Concussion Symptoms Questionnaire 13 Subscale |
| Standard care                                    | 27.1 (12.2) | 25.5 (13.9) | 0.5 (-4.0 to 5.0) | .87 | 27.1 (12.2) | 25.5 (13.9) | 0.5 (-4.0 to 5.0) |
| HBO                                              | 27.5 (13.1) | 22.5 (12.4) | 4.2 (-0.8 to 9.1) | .02 | 25.0 (13.4) | 15.6 (10.9) | 9.4 (2.9 to 15.9) |
| Sham                                             | 25.5 (11.6) | 20.7 (12.8) | 5.5 (0.7 to 10.3) | .04 | 25.9 (14.0) | 17.4 (13.3) | 8.5 (2.8 to 14.2) |
| Total Rivermead Post-Concussion Symptoms Questionnaire |
| Standard care                                    | 32.5 (14.4) | 30.6 (16.1) | 0.5 (-4.8 to 5.8) | .91 | 32.5 (14.4) | 30.6 (16.1) | 0.5 (-4.8 to 5.8) |
| HBO                                              | 33.0 (15.8) | 26.7 (14.8) | 5.4 (-0.5 to 11.3) | .008 | 29.7 (16.3) | 18.7 (13.0) | 11.0 (3.2 to 18.8) |
| Sham                                             | 30.2 (14.2) | 24.2 (15.4) | 7.0 (1.0 to 12.9) | .02 | 30.8 (17.6) | 20.1 (15.7) | 10.7 (3.9 to 17.5) |

Abbreviation: HBO, hyperbaric oxygen.

* The 95% CIs were calculated using 95% binomial exact CIs.

*b Wilcoxon signed rank test.

1.5, 1.2 x40 n=71
Conclusions
Among service members with PCS, HBO showed no benefits over an air sham compression procedure, but symptoms in both groups improved compared with mTBI care without supplemental chamber interventions.

This outcome suggests that the observed improvements were not oxygen mediated but may reflect nonspecific improvements related to placebo effects. Taken with results from other concurrent investigations, our study does not support phase 3 trials of HBO for the treatment of PCS at this time.

Miller et al 2015
Experts would never consider room air (21% oxygen), delivered at 1.2 ATA, to be an acceptable therapeutic dose for any approved clinical indication.

It is illogical to think that this dose will have a biologically beneficial effect for any approved indication.

Hoge and Jonas, JAMA Internal Medicine 2015
Question

- Was this really a placebo effect or did both groups improve because of treatment with different doses of oxygen?
Acronym “CD”

- Cluster of differentiation
- Cell surface markers
- Identify stem cell sub-populations

- CD34
- CD133
- CD45

- “pro-angiogenic stem cells”
Adapted from Thom, S.R et al. 2011
Stem cell mobilization by oxygen

- CD34+ stem cell in humans
- 1520 torr PiO$_2$
  - (2.0ATA x 100%O$_2$ x 760 torr)
- Suggesting a dose response
- Modulating SC mobilization

(Thom, Bhopale et al. 2006)
Question

- Will similar physiologic activity (pro-angiogenic stem cell mobilization) be seen at $\text{PiO}_2 \leq 760$ Torr ($100\% \text{ O}_2 \text{ @ 1ATA}$)

- $319$ Torr $\text{PiO}_2$ ($42\% \text{ O}_2$)
Specific Aims

Specific Aim 1:
Determine the extent of proangiogenic stem cell mobilization in venous plasma in response to intermittent hyperoxia (↑PiO$_2$ 319 torr) compared to control (room air PiO$_2$ ~159 torr).

Hypothesis:
Proangiogenic stem cells will be significantly mobilized
Specific Aims

Specific Aim 2:
Determine the expression of VEGF and TNFα in venous plasma after exposure to intermittent ↑PiO₂ 319 torr.

Hypothesis
TNFα expression will significantly decrease and VEGF will increase
Treatment setup 319 torr \( \uparrow \text{PO}_2 \)
Treatment setup
12 Sprague Dawley Rats

**Control group**  
n=6 PiO\(_2\) 159 torr  
(21% oxygen)

- 2 hours daily for 10 days
- 24 hrs. delay
- Sacrifice and process samples
- Flow Cytometry and ELISA
- Data Analysis

**Treatment Group**  
n=6 \(\uparrow\)PiO\(_2\) 319 torr  
(42% oxygen)

- 2 hours daily for 10 days
- 24 hrs. delay
- Sacrifice and process samples
- Flow Cytometry and ELISA
- Data Analysis
Methodology – Sample preparation

- Draw >8 mL venous blood from IVC into heparin tubes
- Lyse red blood cells – ammonium chloride
- Centrifuge to separate cells from plasma
- Freeze plasma for cytokine ELISA
- Stain cells with antibodies
- Quantify stem cells with flow cytometry
- Quantify cytokine expression with ELISA
Data Analysis

- Non-parametric Mann Whitney test
- using a p of < .05 to indicate a difference between the groups
Results Pro-angiogenic stem cells

- Hematopoietic Pro-angiogenic
  - p = 0.0087
- Non-Hematopoietic Endothelial
  - p = 0.23
- Non-Hematopoietic Stem Cells
  - p = 0.16

CD45+/133+ Hyperoxia
CD45+/133+ Normoxia
CD45-/133+ Hyperoxia
CD45-/133+ Normoxia
CD45-34+ Hyperoxia
CD45-34+ Normoxia

Cytokines and growth factors
Summary

CD133+ neovascularization

immunohistochemical staining using antibodies specific EC marker isolectin B4

Suggesting CD133 modulates vascular healing

(Nakanishi, Ishikawa et al. 2009)
Results – Cytokine Expression
Tumor Necrosis Factor α (TNF α)

- signal protein
- macrophages
  - Induce fever / Systemic inflammation
  - Apoptosis
  - inhibit tumor growth
- Potent inducer of neo-vascularization
VEGF

- signal protein
- many cells
- stimulates the formation of blood vessels
Results – Cytokine Expression

TNFα expression

- p = 0.004

VEGF expression

- p = 0.17
Results – Cytokine Expression

More trends

- Leptin Expression
  - Absorbance (AU)
  - Hyperoxia: p = 0.09
  - Normoxia

- MIP-1α Expression
  - Absorbance (AU)
  - Hyperoxia: p = 0.07
  - Normoxia

Adipokine implicated in the pathogenesis of chronic inflammation
- Increased levels linked with risk of Type 2 diabetes
- Cardiovascular disease
- Cancer

Macrophage inflammatory protein
- Found to be elevated in patients with depression

Anti-inflammatory
Conclusions

- First study to demonstrate
  - at a $P_iO_2$ 320 Torr
  - pro-angiogenic stem cell mobilization
  - suppression of an inflammatory cytokine

- Suggests the possibility of proangiogenic stem cell mobilization in humans at much lower oxygen pressure than previously postulated.
Low intensity intermittent oxygen therapy may enhance re-vascularization

Increases questions regarding shams that intermittently increase PiO$_2$, even at relatively low levels
Future Directions

- Repeat this experiment in humans
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