



DEPARTMENT OF
Pediatrics

UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE AND PUBLIC HEALTH

EFFECT OF INTERMITTENT HYPEROXIA ON STEM CELL MOBILIZATION AND CYTOKINE EXPRESSION

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Acronym “CD”

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

- ▶ CD34
- ▶ CD133
- ▶ CD45

- ▶ “pro-angiogenic stem cells”

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

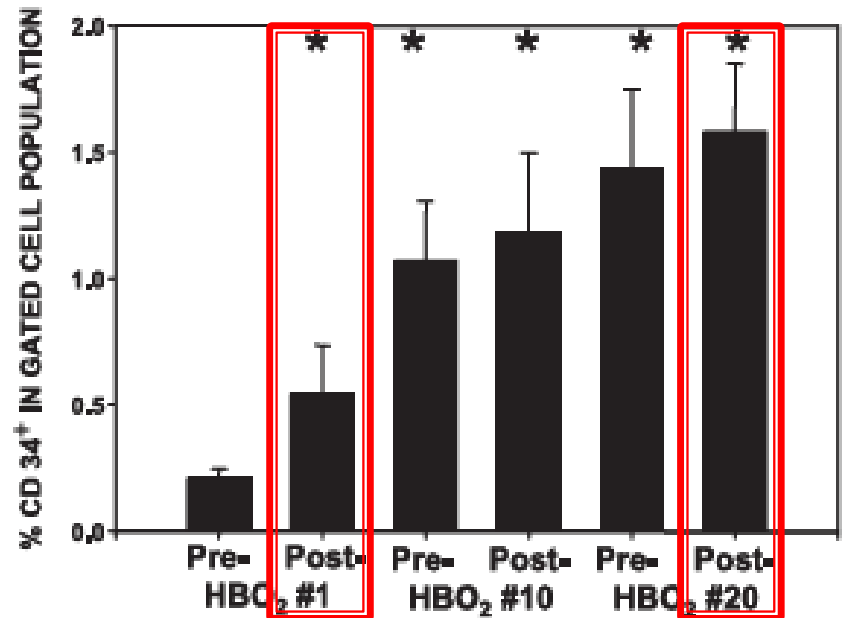


Fig. 4. Mean CD34⁺ population in blood of humans before and after HBO₂ treatments. Data are the fraction of CD34⁺ cells within the gated population using leukocytes obtained from 26 patients before and after their 1st, 10th, and 20th HBO₂ treatment. *Repeated-measures one-way ANOVA, $P < 0.05$ vs. the pre-HBO₂ first treatment value.

(Thom, Bhopale et al. 2006)



Question

- ▶ Will similar physiologic activity (pro-angiogenic stem cell mobilization) be seen at $PiO_2 \leq 760$ Torr (100% O_2 @ 1 ATA)
- ▶ 319 PiO_2 (42% O_2)

Specific Aims

Specific Aim 1:

Determine the extent of proangiogenic stem cell mobilization in venous plasma in response to intermittent hyperoxia ($\uparrow \text{PiO}_2$ 319 torr) compared to control (room air PiO_2 159.6 torr).

Hypothesis:

Proangiogenic stem cells will be significantly mobilized

Specific Aims

Specific Aim 2:

Determine the expression of VEGF and TNF α in venous plasma after exposure to intermittent \uparrow PiO $_2$ 319 torr.

Hypothesis

TNF α expression will significantly decrease and VEGF will increase

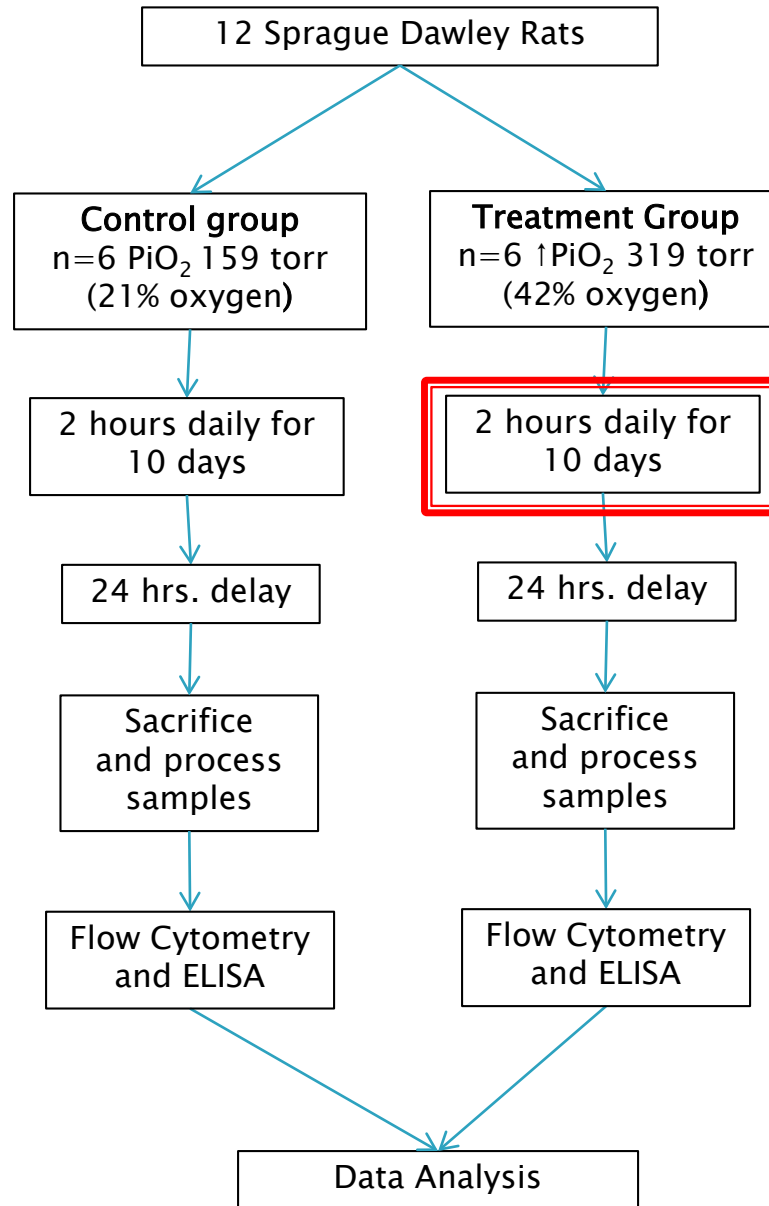
Treatment setup 319 torr \uparrow PO₂



Treatment setup



Approach



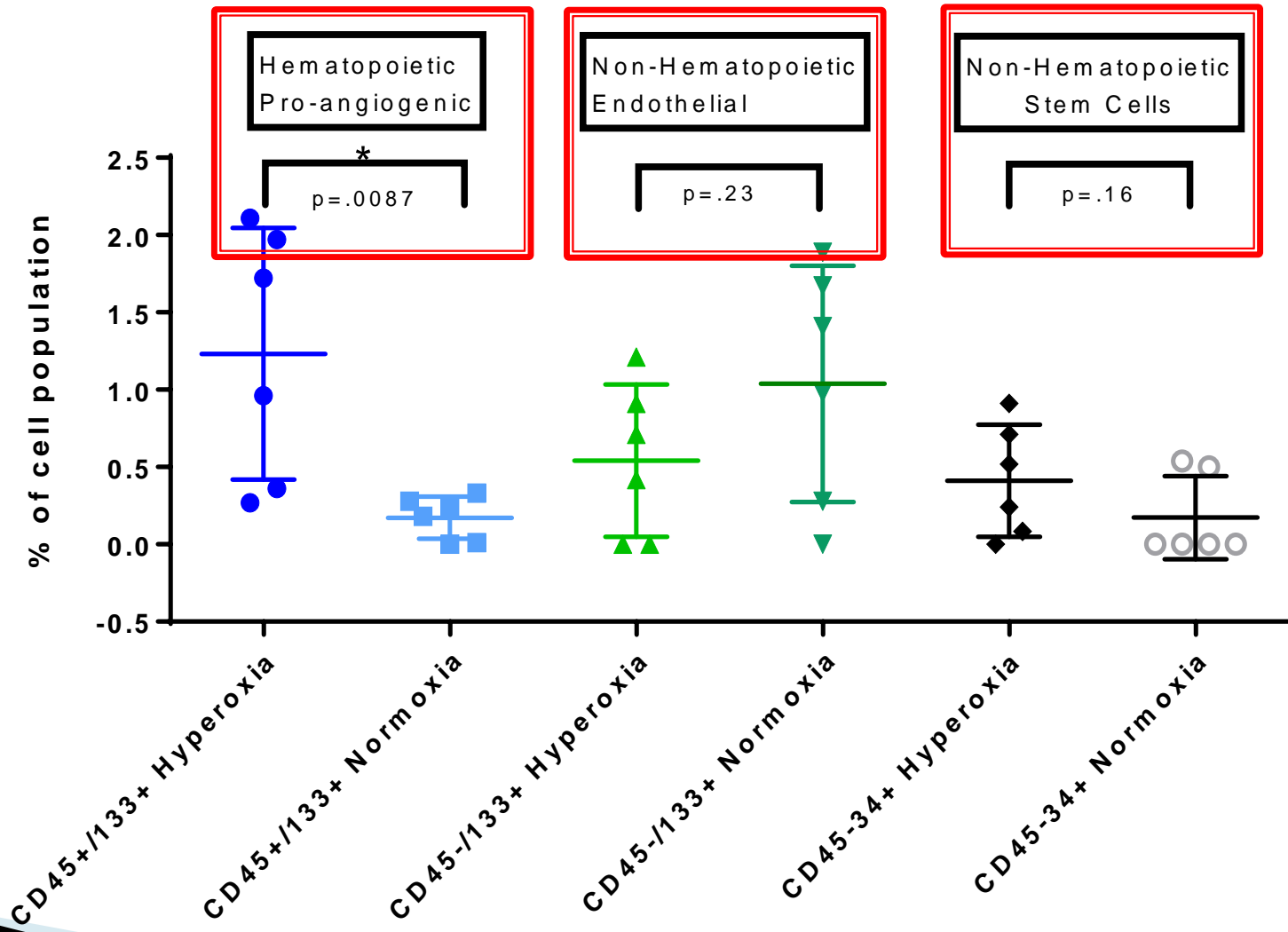
Methodology – Sample preparation

- ▶ 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

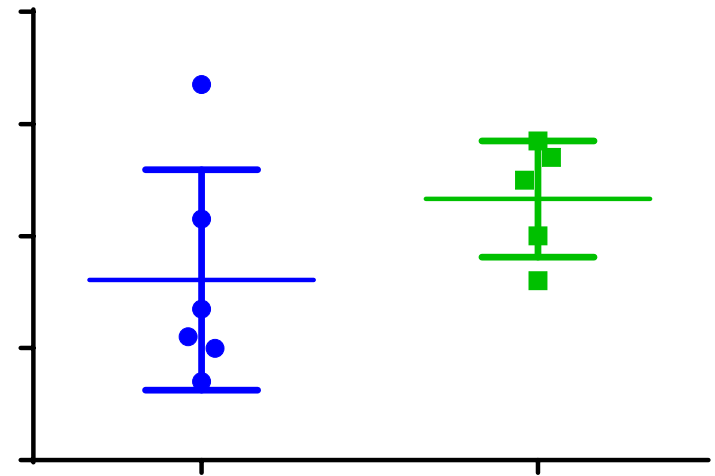
Data Analysis

- ▶ Non-parametric Mann Whitney test
- ▶ using a p of $< .05$ to indicate a difference between the groups

Results Pro-angiogenic stem cells



Results – Cytokine Expression



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.

Future Directions

- ▶ Compare the effect of pressure to concentration
 - 2 ATA of 21% air
- ▶ Repeat this experiment at P_iO_2
 - P_iO_2 191 Torr (25.2% O_2) (1.2 ATA)
 - P_iO_2 91 Torr (12% O_2) (.57 ATA)
- ▶ Include an injury cohort

Implications

- ▶ Intermittent hyperoxic therapy may enhance re-vascularization
- ▶ Increases questions regarding the use of shams that intermittently increase PiO_2 , even at relatively low levels

Thank you



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Questions?

