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

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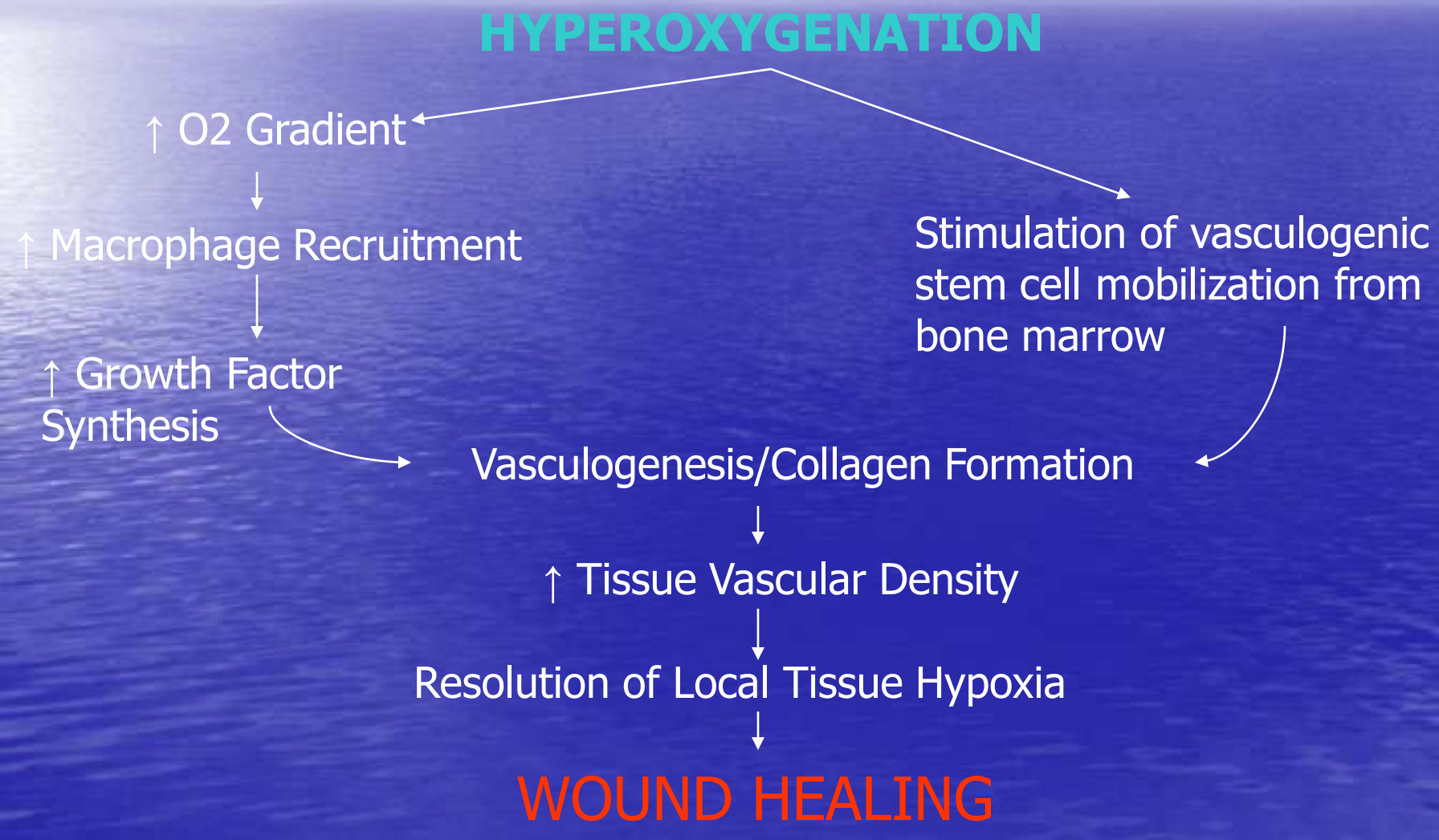
Background

- HBO known to promote wound healing
  - Advanced diabetic foot ulcers
  - Late effect radiation injury
- Mechanism of action includes stimulation of progenitor stem cells

HBO Mechanism – Vasculogenesis/Wound Healing



Secondary Effect – Vasculogenesis/Wound Healing -Stem Cell Mobilization-

- Normal Stem Cell Mobilization
  - Cytokine + receptor -> activates NOS
  - NOS → increased NO production
  - NO causes MMP-9 to cleave membrane bound cKit (stem cell factor) → soluble cKit
  - Soluble cKit causes stem cell mobilization

Secondary Effect – Vasculogenesis/Wound Healing -Stem Cell Mobilization-

- HBOT causes increased production of NO
  - Increases NOS III (EC cell NOS) production of NO
  - Increased NO signals skipping need for cytokine + receptor

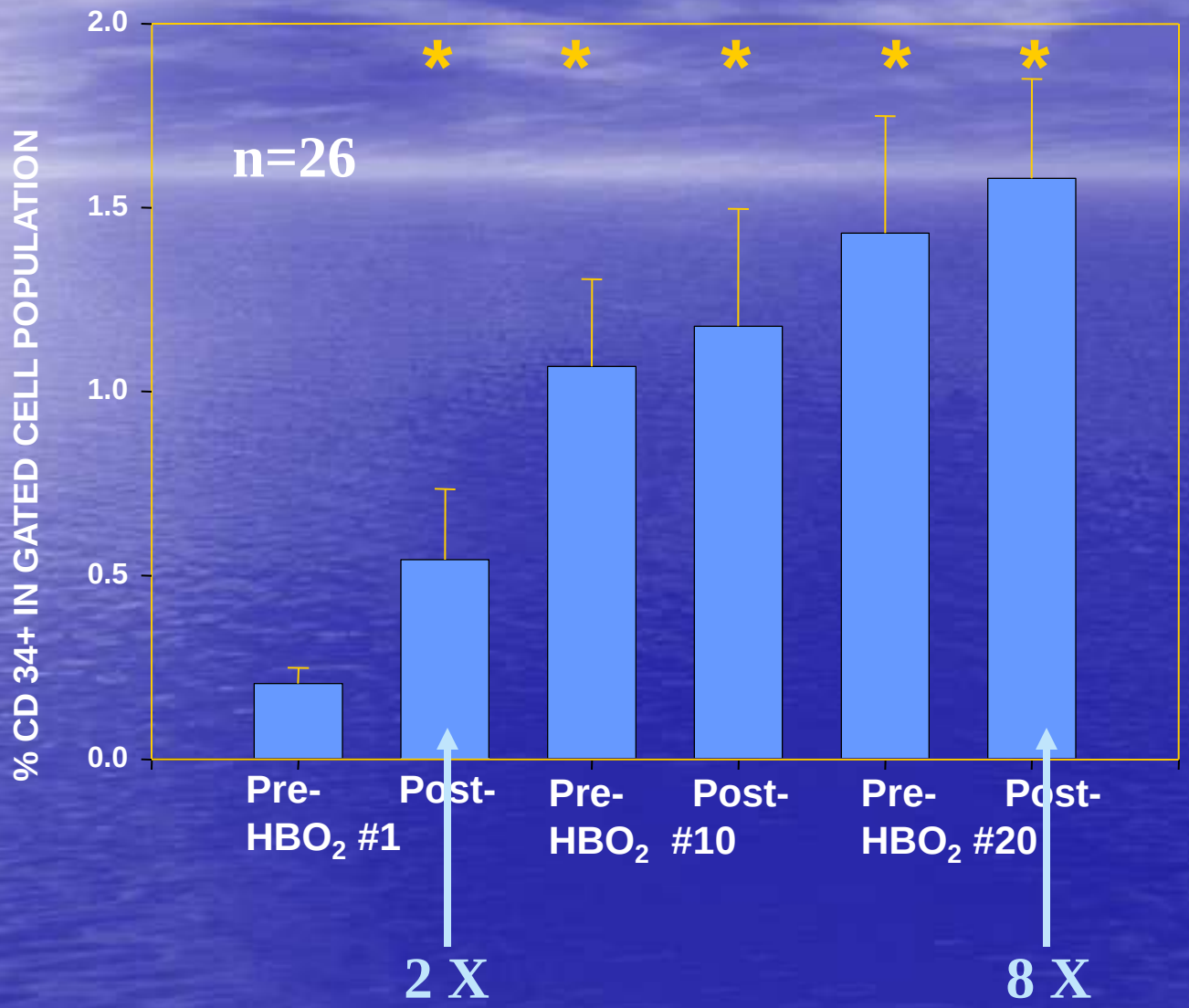
Thom et al, 2006; Goldstein et al, 2006)

Secondary Effect – Vasculogenesis/Wound Healing -Stem Cell Mobilization-

- HBOT Stem Cell Mobilization
  - Activates NOS and increases bone marrow NO without the need for cytokine + receptor complex
  - Increased NO → MMP-9 → soluble cKit
  - Stem cell mobilization

Thom et al, 2006; Goldstein et al, 2006)

Secondary Effect – Vasculogenesis/Angiogenesis
Human Stem Cell Mobilization by HBO_2



- PATIENTS PREVIOUSLY EXPOSED TO RADIATION -

Am J. Physiology 2006. 290:H1378

Background

- Cells with surface markers consistent with progenitor stem cells:
  - CD34+
  - CD45-dim
- HBO mobilized stem cells contain higher content of:
  - HIF (hypoxia inducible factors)
  - Trx (Thioredoxin-1)

Background

- HBO protocols vary by treatment depth of 2 ATA to 2.5 ATA at different institutions
- We hypothesized that there may be differences in mobilization effect based on different treatment pressures

Materials and Methods

- Blood from 20 consecutive patients from each of 2 different clinical sites being treated for late effect radiation injury:
  - Before and after 1st, 10th, and 20th treatments
  - 2 clinical sites with different treatment pressures:
    - 2 ATA x 120 minutes
    - 2.5 ATA x 90 minutes

Materials and Methods

- Progenitor stem cells evaluated by:
  - CD34+
  - CD45-dim
  - Intracellular proteins
- Flow cytometry
  - Following previously published techniques (Thom et al, 2011; Milovanova et al, 2008, 2009)

Materials and Methods

- Statistical analysis:
  - Stem cell numbers and quantitative changes in wound protein markers
  - Repeated measures analysis of variance followed by the Tukey test for multiple comparisons
  - $P<0.05$ considered significant
  - Results displayed as +/-SE
  - Pre- and post-treatment comparisons made within each type (2 and 2.5 ATA) and between 2 and 2.5 ATA treatments of each number (1^{st},10^{th},20^{th}) by two-tailed t-test

Results

- No significant differences in age, gender, or radiation dose between the 2 groups
- Statistically significant increase in circulating progenitor stem cells at 2.5 vs 2 ATA:
  - Post-10, Pre-20, Post-20
- Intracellular regulatory proteins without difference:
  - Tested difference between pre- and post-HBO only and not across treatment course due to testing limitations

Figure 1. Leukocyte mobilization by HBO_2. 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-HBO_2 values are significantly different from pre-HBO_2 values at each treatment time in both groups (t-test).

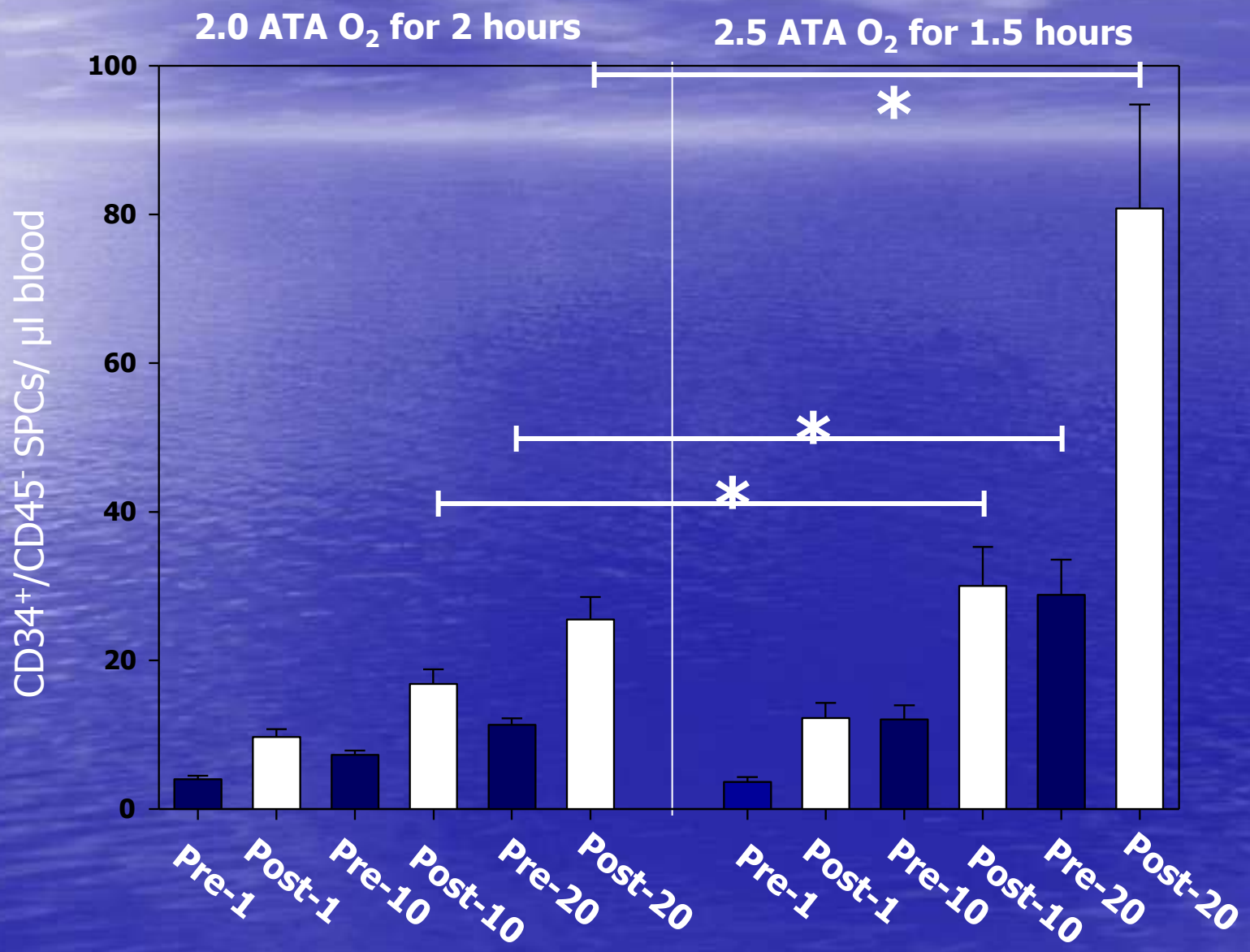


Table 1. Intracellular protein content (fold-elevation post- versus prior to HBO_2).

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-HBO_2 permeabilized CD34+ cells using fluorophore-conjugated antibodies to proteins shown in column 1. All post-HBO_2 values are significantly different from pre-HBO_2 and there are no significant differences between the 2.0 and 2.5 ATA protocols.

Summary

- Increased treatment pressure results in increased progenitor stem cell release
- Clinical efficacy implications of this finding require further study
- Further Details:
  - M Heyboer III, et al. Stem Cell Research. 2014, 12:638-645