

INERT GASES STIMULATE MICROPARTICLE GENERATION BY NEUTROPHILS: A NITRIC OXIDE-MEDIATED OXIDATIVE STRESS RESPONSE

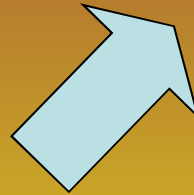
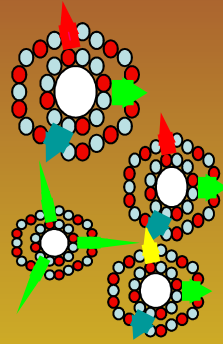
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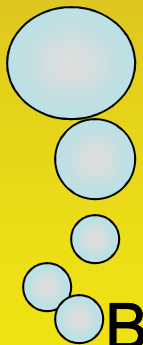


HYPOTHESIS

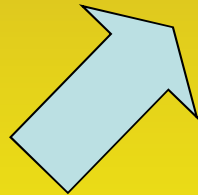
INFLAMMATORY
RESPONSES



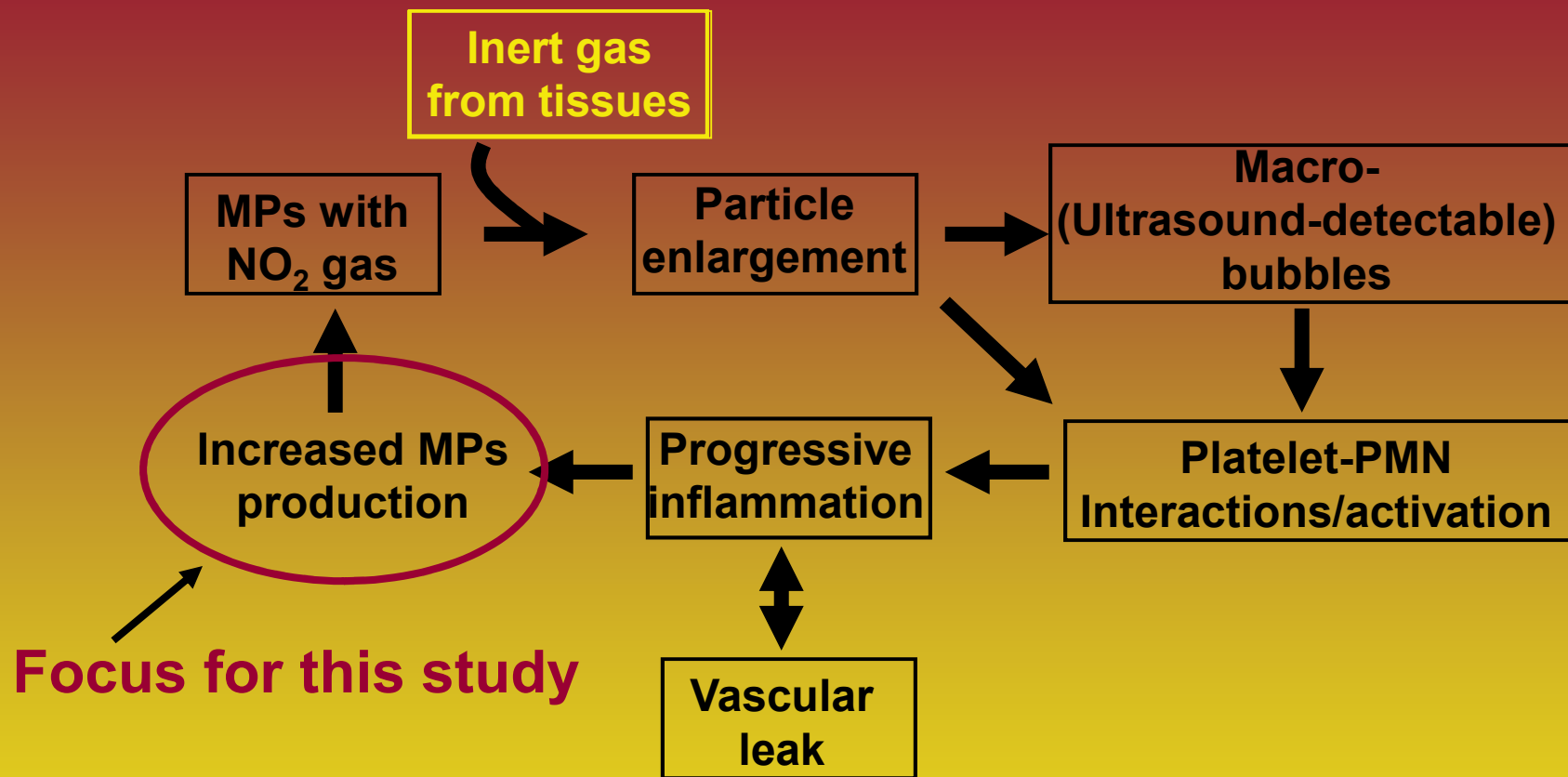
MICROPARTICLES
(0.1 – 1 μm spheres)



BUBBLES



Initially thought a bubble-mediated process. Mechanism for elevated MPs questioned due to mouse studies showing $\cdot\text{NO}$ required.
Hypothesis: MPs generation during a dive is an oxidative stress response.



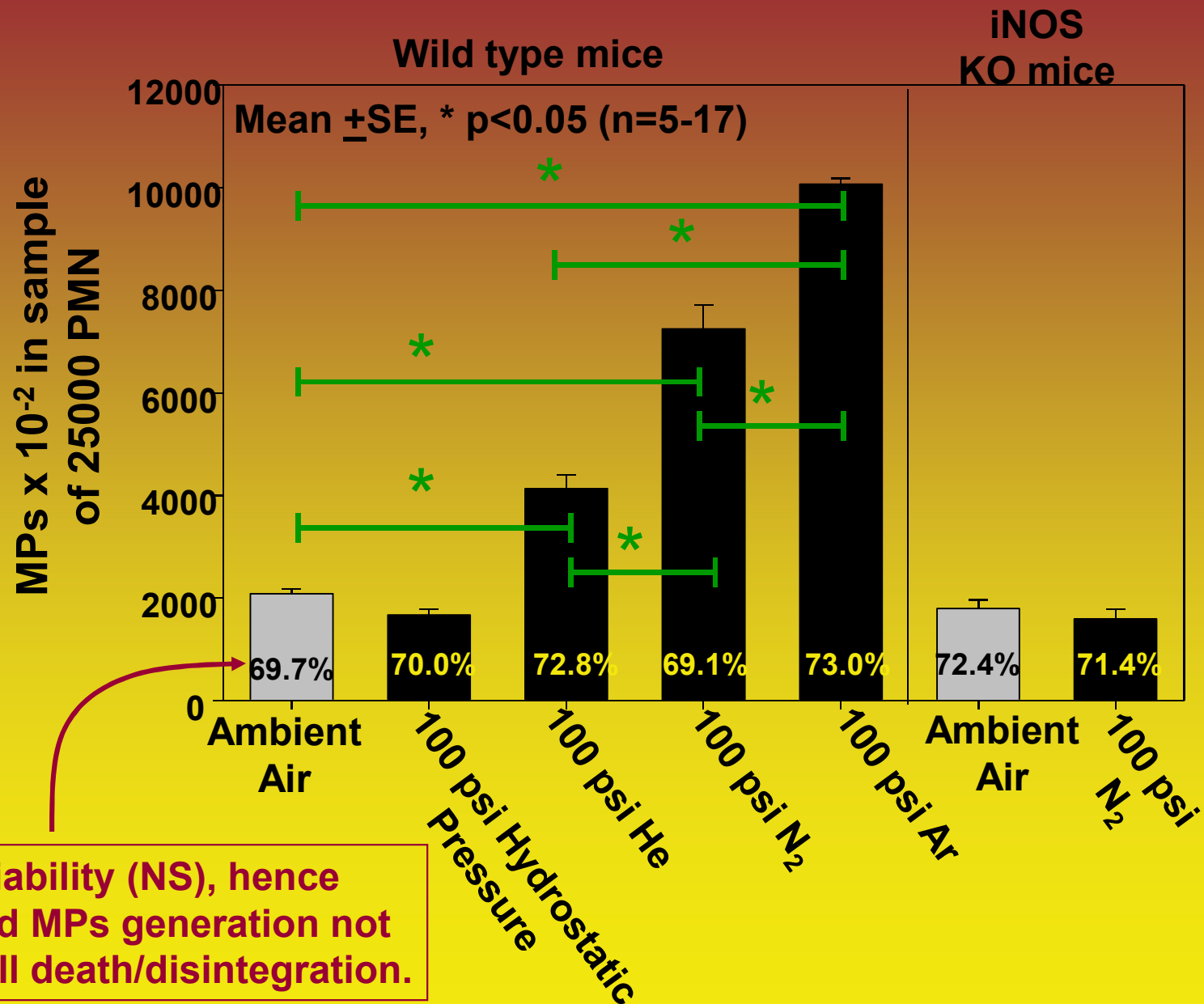
HUMAN STUDIES

Eur J Appl Physiol 105, 507 (2009)
Aviat Space Environ Med 81, 41 (2010)
Appl Physiol Nutr Metab 37, 1 (2012)
J Appl Physiol 112, 1268 (2012)
J Appl Physiol in press, (2013)

MOUSE MODEL

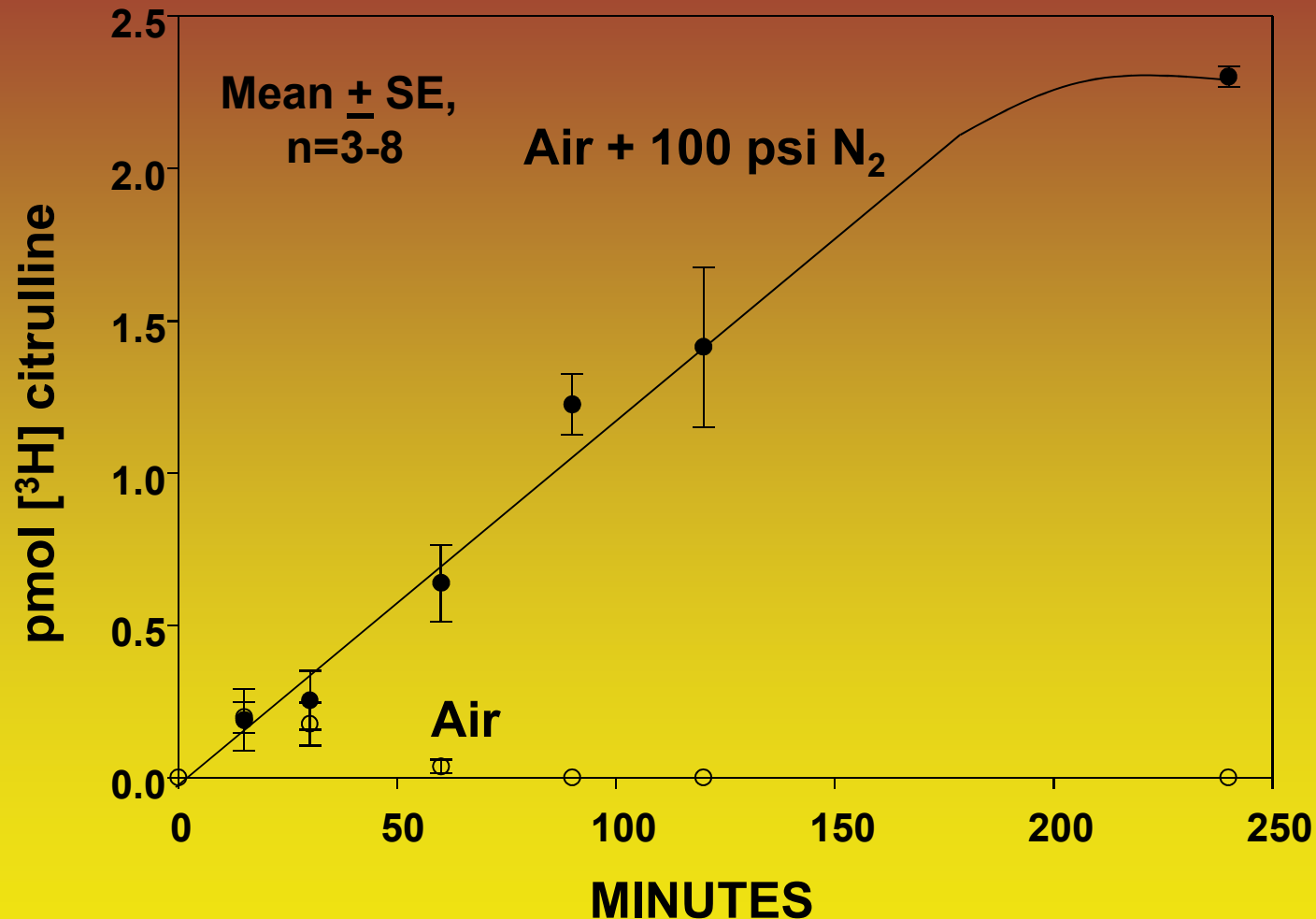
J Appl Physiol 110, 340 (2011)
J Appl Physiol 112, 204 (2012)
J Appl Physiol 114, 550 (2013)

PMN-generated MPs – 24 hr incubation



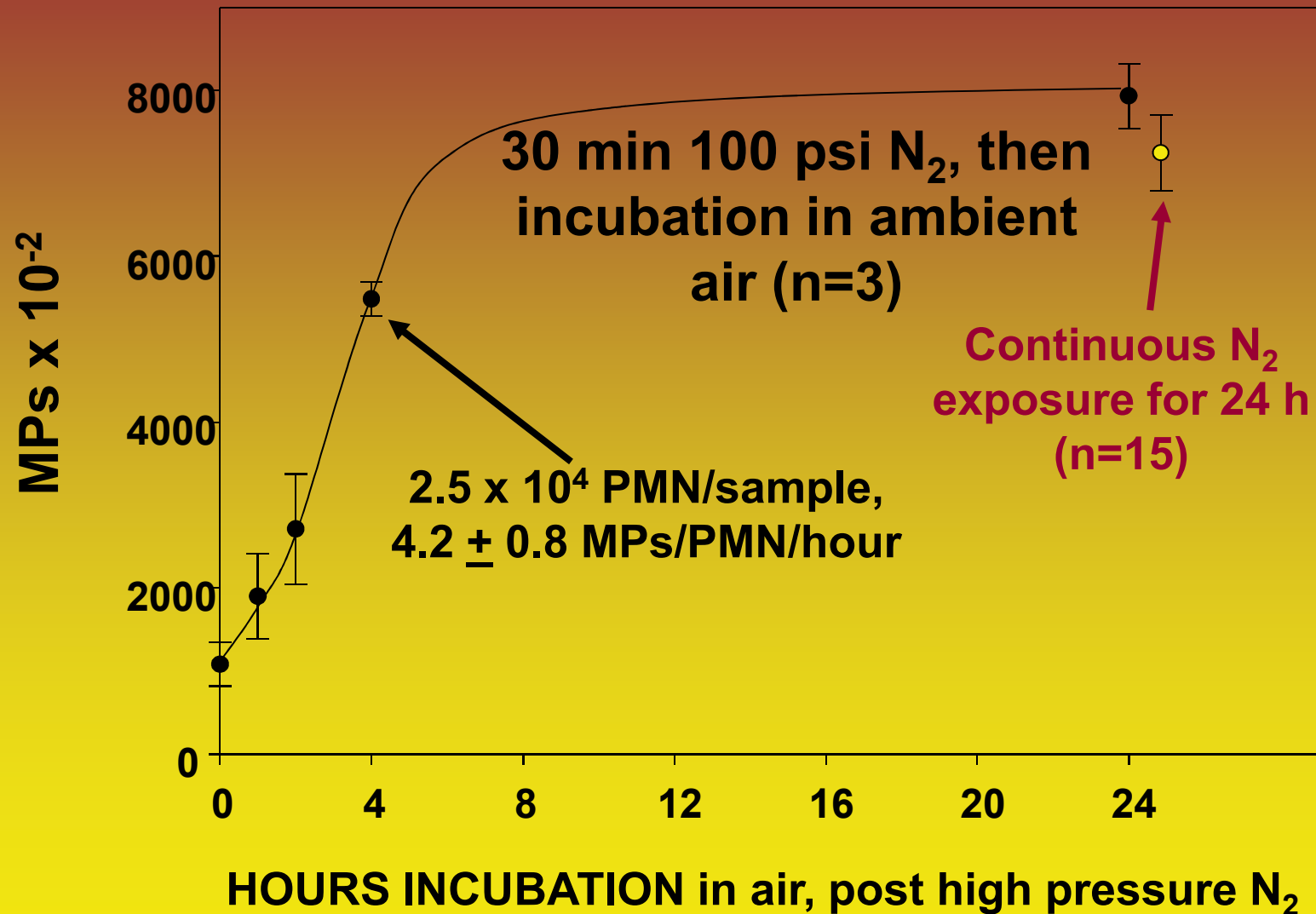
NOS-2 activation by N₂

Continuous exposures – OG permeabilized PMN



[³H] arginine → [³H] citrulline inhibitable by 1400 W
(equal to that seen in iNOS KO mice)

MPs generated AFTER just 30 min N₂



MPs production



iNOS activity



F-actin turnover



Δ **MPs/ μ l at 4 hr** **pmol citrulline /hr** **FBEs Fluor/minx10⁻³**

100 psi N₂ only

PBS

5482 \pm 205*
(n=3)

0.61 \pm 0.13*
(n=5)

2.61 \pm 0.11*
(n=5)

iNOS

1400 W

-107 \pm 60

0.00 \pm 0.00

0.43 \pm 0.03

F-actin

Cyto D

-93.8 \pm 24

0.08 \pm 0.05

0.21 \pm 0.09

NADPH oxidase

Nox2ds

8 \pm 4

0.01 \pm 0.01

0.31 \pm 0.05

SNO-protein

UV

4 \pm 3

0.05 \pm 0.04

0.61 \pm 0.08

RNS

Ebselen

44 \pm 12

0.09 \pm 0.08

0.50 \pm 0.11

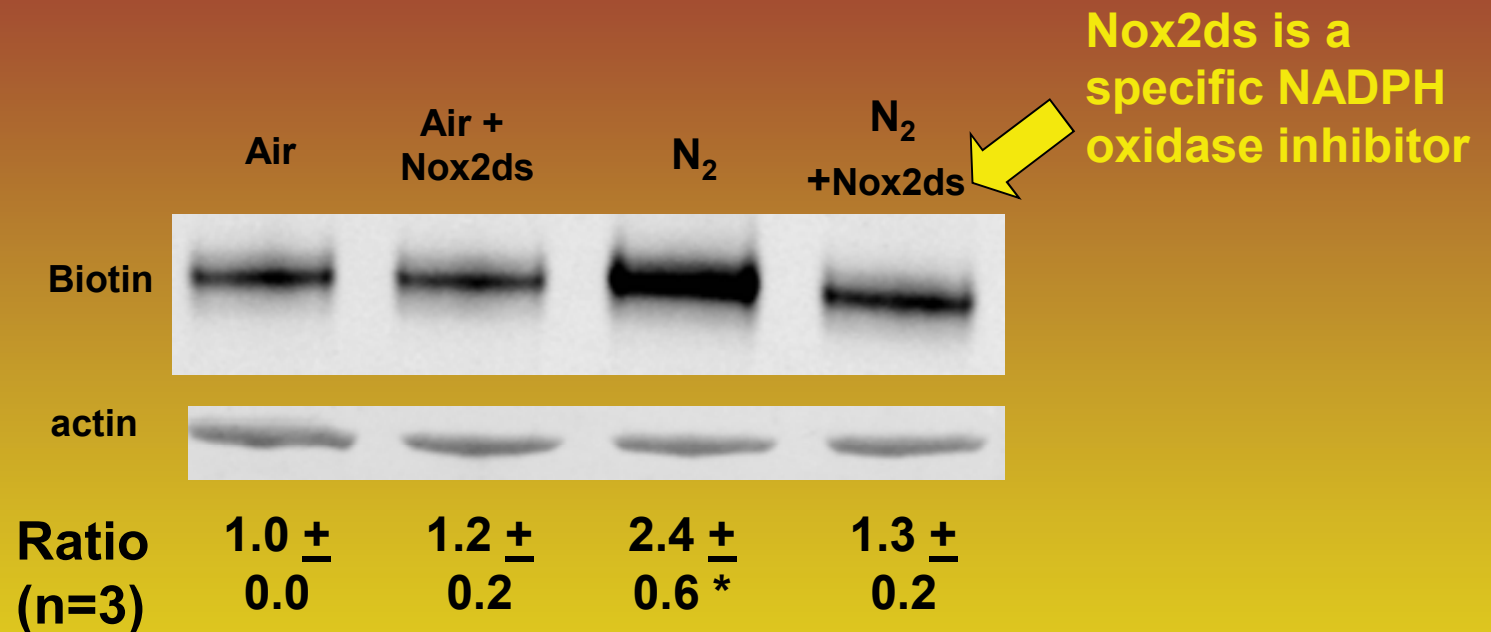
Air only

-104 \pm 55

0.03 \pm 0.02

0.41 \pm 0.03

Biotin-switch assay to detect S-nitrosylated proteins (there are several, but most prominent is actin)



100 psi N₂ x 30 min →
SNO-actin formation

MPs production by PMN is oxidative stress response.

Potency: $\text{He} < \text{N}_2 < \text{Ar}$;

100 psi (224 fsw) N_2 > 32 psi (72 fsw), none @ 15 psi for 1 hr

Partial effect, 15 min exposure, 30 = 60 min = 24 h exposure

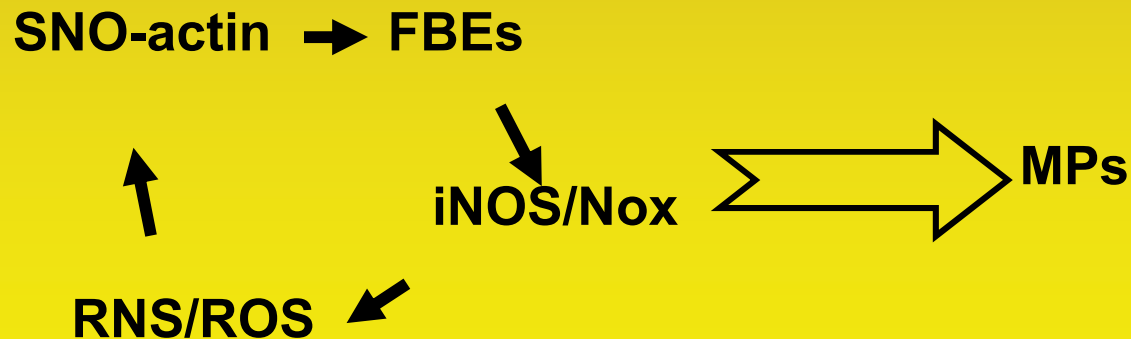
POSSIBLE MECHANISM:

**Inert gases enhance ROS in isolated enzyme systems,
unicellular organisms $\text{He} < \text{N}_2 < \text{Ar} < \text{Kr} < \text{Xe}$; 3-body
chemical interaction: $e^- + \text{gas} + \text{O}_2$**

Appl Environ Microbiol 47, 780, 1984

Undersea Biomed Res 14, 485, 1987

Arch Biochem Biophys 295, 391, 1992



For the future

- 1. If, indeed, MPs generate 'bubbles', DCS risk is under metabolic control?**
- 2. Do other cells/platelets behave like PMN?**
- 3. Why do MPs activate PMN?**
- 4. Do *ex vivo* generated MPs recapitulate 'DCS' if administered IV?**
- 5. What controls MPs production?**
- 6. Just how 'generalizable' are these findings?**
Looking for collaborators willing to send divers' blood, marine mammals, what about HBO₂ & inside tenders?