GAS PRESSURES ASSOCIATED WITH DIVING TRIGGER MICROPARTICLE PRODUCTION BY NEUTROPHILS

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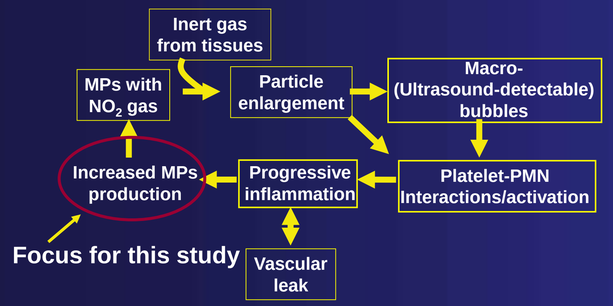
BACKGROUND:

Circulating microparticles (MPs) appear to cause some of the insults following decompression stress. Initially, we thought this was a bubble-mediated process. The mechanism for elevated MPs was questioned due to MPs elevations seemingly occurring during pressurization. This led to our hypothesis: MPs generation 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 115, 1481 (2013)

MOUSE MODEL

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

METHODS:

This study involves human & mouse PMN, isolated and exposed to gas ex vivo, looking at dynamics of MPs production.

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Figure 1. Human PMN make MPs during exposure

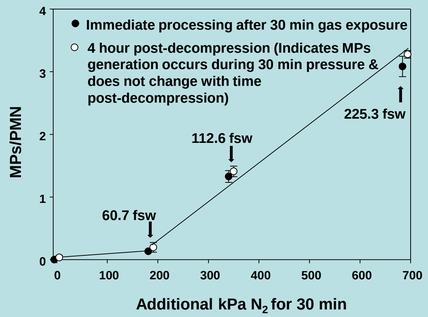
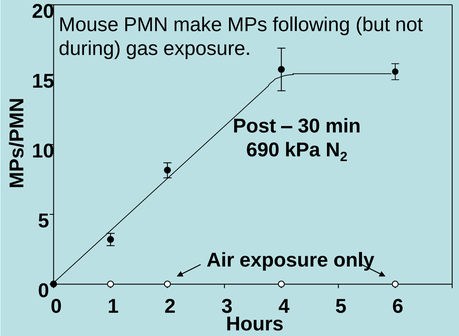


Figure 2. Murine PMN make MPs after exposure



MPs production requires NADPH oxidase (NOX) & nitric oxide synthase (iNOS)

PMN source	PBS	+ Nox Inhib	+ iNOS Inhib	+ Antioxidant
Human (MPs/PMN/30min)	3.34 ± 0.17 (7)	0.19 ± 0.19 (3)	0.37 ± 0.27 (5)	0.23 ± 0.13 (3)
Mouse (MPs/PMN/1hr)	3.84 ± 0.21 (8)	0.05 ± 0.03 (3)	0.13 ± 0.06 (5)	0.06 ± 0.04 (3)

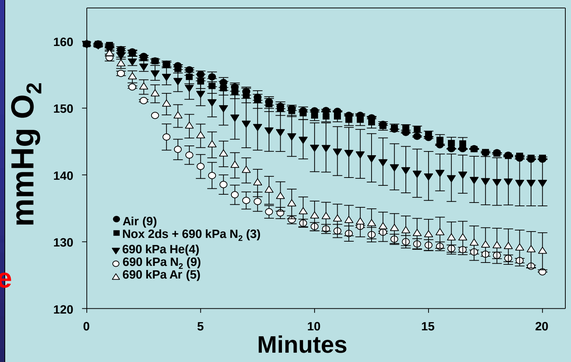
Table 1. Data show MPs production by PMN exposed to air + 690 kPa N_2. PBS, phosphate buffered saline; NOX = Incubation with 10μM Nox-2ds during the air/N_2 exposure; iNOS = Incubation with 1 mM 1400W during air/N_2 exposure; Antioxidant = Incubation with 1mM ebselen during the air/N_2 exposure.

Table 2. DOSE-RESPONSE
N_2 activates nitric oxide synthase & NADPH oxidase

Air + kPa N_2	MPs/PMN/hr	iNOS	NOX
0	0.0003±0.0002 (24)	0.19 ± 0.02 (21)	1.37 ± 0.14 (11)
186	0.08 ± 0.02 (4)*	0.47 ± 0.12 (3)*	1.95 ± 0.12 (4)*
228	0.72 ± 0.10 (4)*	0.73 ± 0.13 (7)*	2.42 ± 0.23 (4)*
345	1.14 ± 0.08 (8)*	0.83 ± 0.22 (6)*	3.57 ± 0.14 (4)*
455	2.08 ± 0.41 (4)*	1.45 ± 0.15 (3)*	4.09 ± 0.28 (4)*
690	3.84 ± 0.21 (8) *†	1.76 ± 0.08(24) *†	5.00 ± 0.36(10) *†

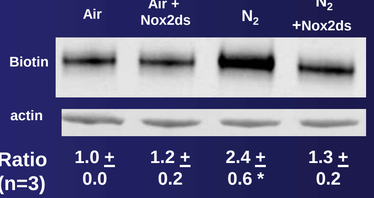
Near linear relation MPs, iNOS & NOX

Potency (Ar~ N_2>He) shown with NADPH oxidase



Potency same for MPs & iNOS (data not shown)

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



Air + 690 kPA N_2 x 30 min → SNO-actin formation

SNO-actin will increase actin turnover
Fold-increase With 690 kPa gas + air:

He	7.1 ± 1.2 (4)
N_2	18.6 ± 2.4 (12)
Ar	18.3 ± 2.7 (4)

CONCLUSION:

Inert gases (Ar~N_2>He) cause oxidative stress which alters actin turnover leading to MPs