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

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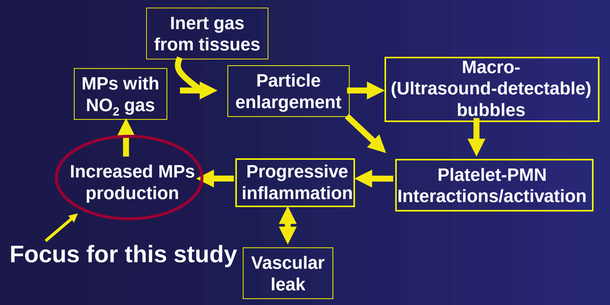
BACKGROUND:

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 mouse studies showing nitric oxide is required. This led to our hypothesis: MPs generation with high pressure gas exposure 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)

METHODS:

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

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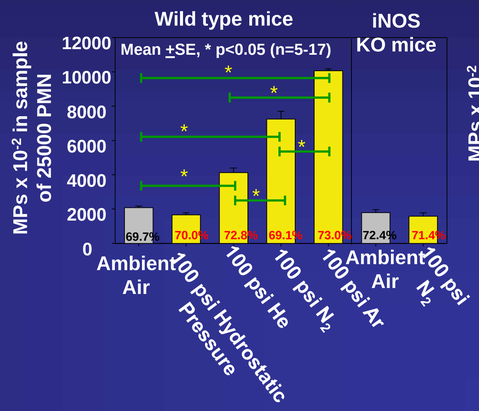
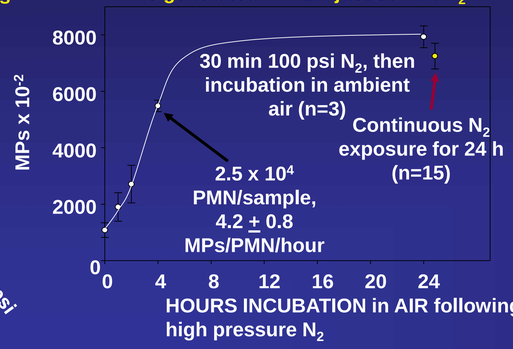
PMN incubated with gas for 24 h generate MPs

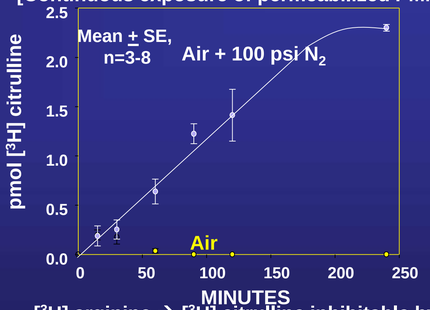


MPs generated AFTER just 30 min N_2



Nitric oxide synthase-2 activation by N_2

[Continuous exposure of permeabilized PMN]

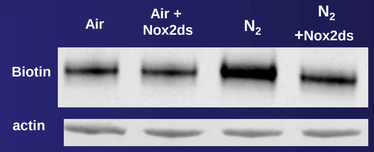


[^{3}H] arginine → [^{3}H] citrulline inhibitable by 1400 W (equal to that seen in iNOS KO mice)

Rows related to these issues

	MPs Production (#/µl)	iNOS activity pmol citrulline /hr	F-actin turnover Fluor/minx10^{-3}
100 psi N_2 only	5482 ± 205* (n=3)	0.61 ± 0.13* (n=5)	2.61 ± 0.11* (n=5)
iNOS	1400 W	-107 ± 60	0.00 ± 0.00	0.43 ± 0.03
F-actin	Cyto D	-93.8 ± 24	0.08 ± 0.05	0.21 ± 0.09
NADPH oxidase	Nox2ds	8 ± 4	0.01 ± 0.01	0.31 ± 0.05
SNO-protein	UV	4 ± 3	0.05 ± 0.04	0.61 ± 0.08
RNS	Ebselen	44 ± 12	0.09 ± 0.08	0.50 ± 0.11
Air only	-104 ± 55	0.03 ± 0.02	0.41 ± 0.03

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



Ratio (n=3)	1.0 ± 0.0	1.2 ± 0.2	2.4 ± 0.6 *	1.3 ± 0.2

100 psi N_2 x 30 min → SNO-actin formation

CONCLUSIONS:

1. MPs production by PMN is oxidative stress response.
2. Potency: He<N_2<Ar;
3. 100 psi (224 fsw) N_2 > 32 psi (72 fsw), none @ 15 psi for 1 hr
4. Partial effect, 15 min exposure, 30 = 60 min = 24 h exposure

POSSIBLE MECHANISM:

Inert gases enhance free radical production in isolated enzyme systems & unicellular organisms. Potency series is He< N_2< Ar< Kr<Xe; thought via 3-body chemical interaction: e^- + gas + O_2

Appl Environ Microbiol 47, 780, 1984
Undersea Biomed Res 14, 485, 1987
Arch Biochem Biophys 295, 391, 1992