

GAS PRESSURES ASSOCIATED WITH DIVING TRIGGER MICROPARTICLE PRODUCTION BY NEUTROPHILS

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Microparticles (MPs) = blood-borne 0.1 – 1.0 µm vesicles

HYPOTHESIS:
MPs production occurs DURING
high pressure gas exposure.

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

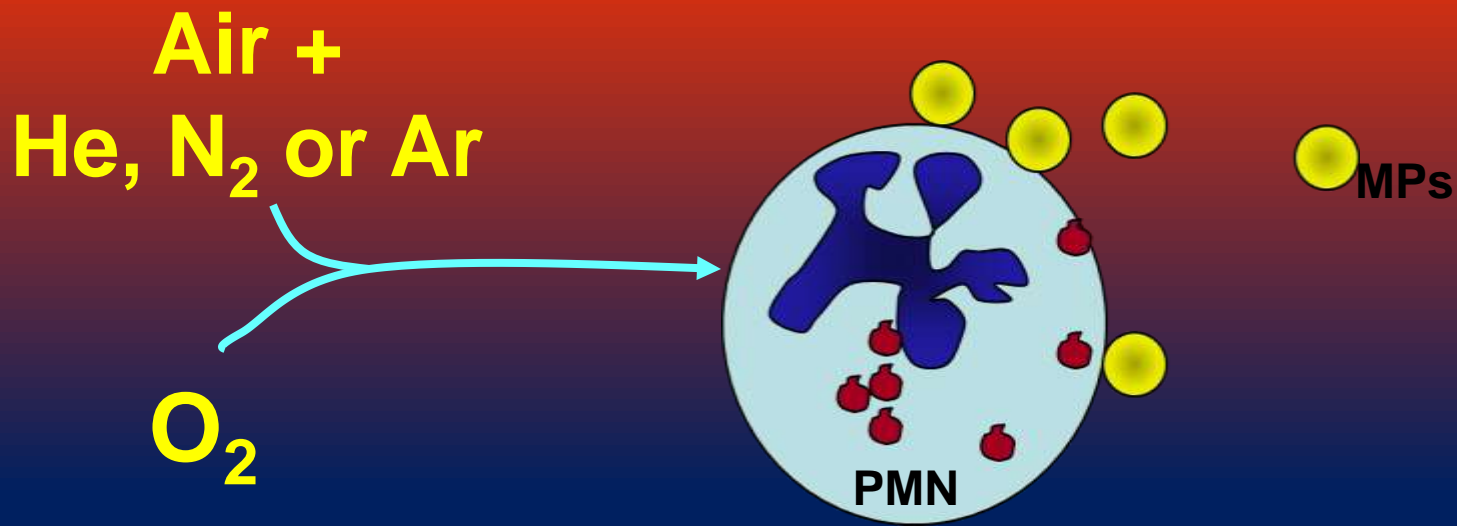
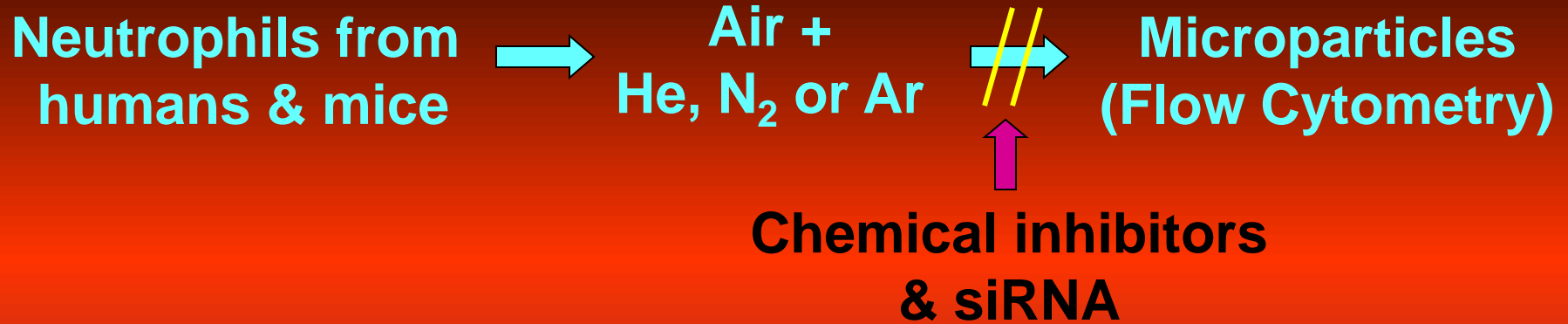
J Appl Physiol 110, 340 (2011)
J Appl Physiol 112, 204 (2012)
J Appl Physiol 114, 550 (2013)

MPs elevated with pressure/decompression.

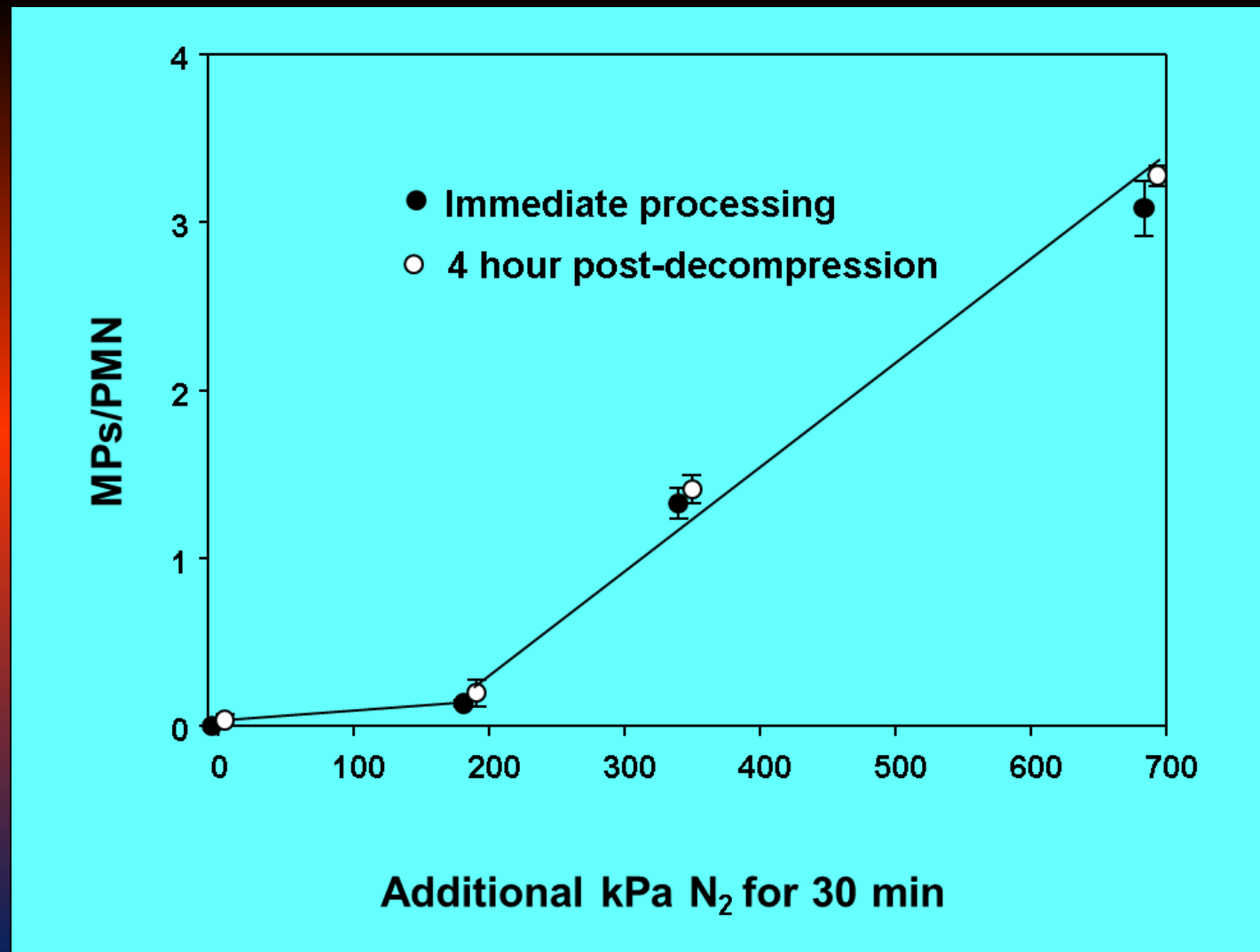
Murine model – MPs association with DCS-like injuries.

Recent human & animal studies suggest
MPs production may occur during 'dive'.

METHODS



Human PMN response to 30 minutes of N₂

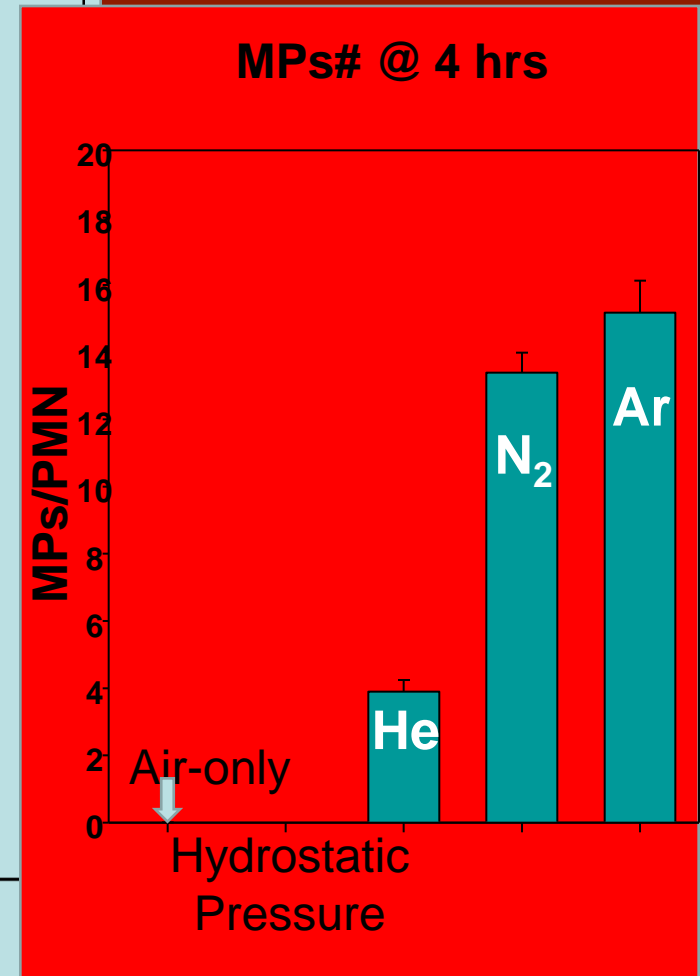
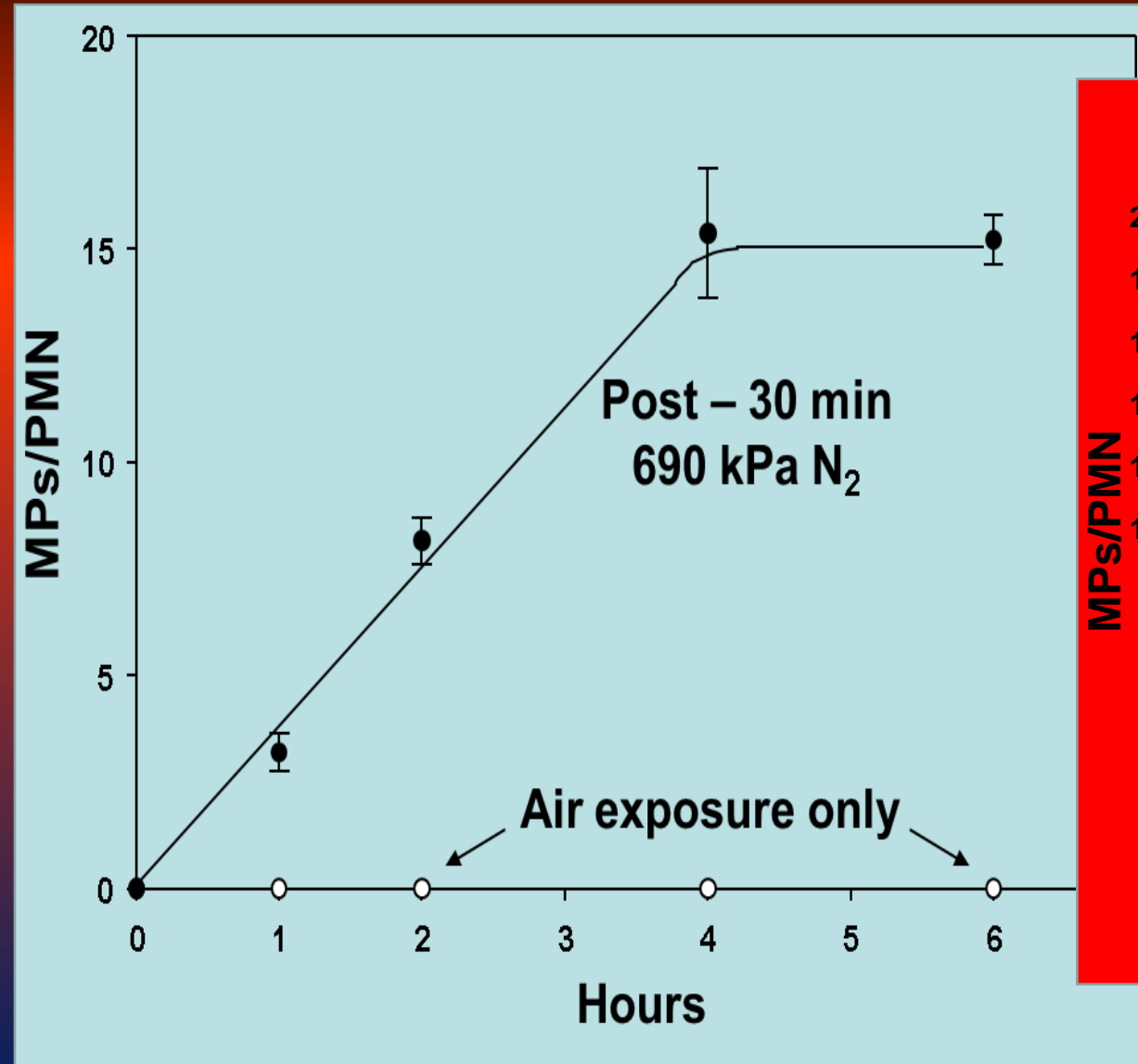


Same values if kept at pressure for 4 hours.
Viability always 85% or better, no loss of PMN #.

Inhibited by NOS-2 inhibitor (1400W), NOX inhibitor (Nox2ds) and ebselen

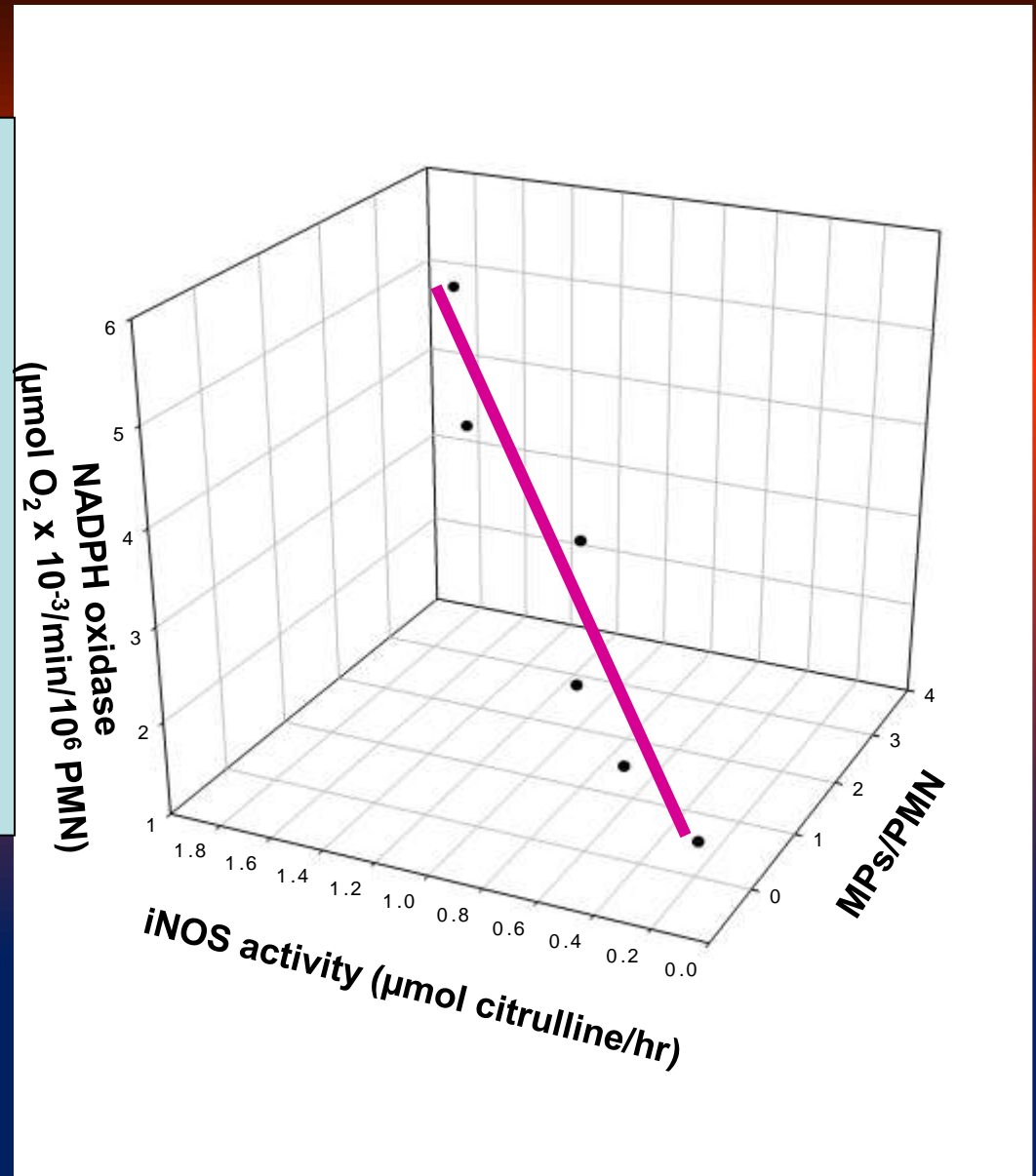
Mouse PMN responses

...30 min exposure, monitor for 4 hours – MPs vary with gas...



Dose-response for MPs, NOS-2 and NOX

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) *†



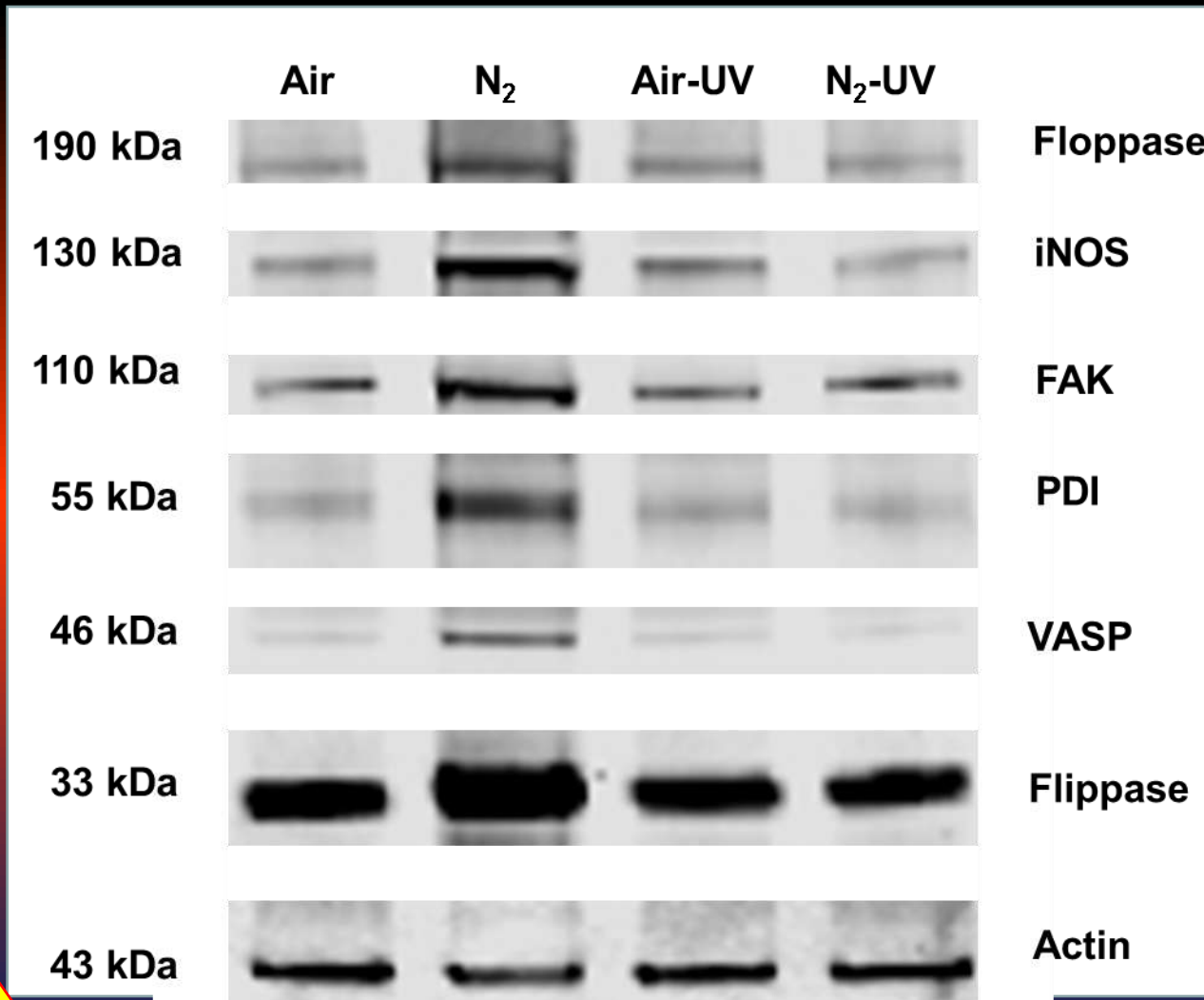
Gases cause actin turnover (FBEs) and same agents that inhibit FBEs inhibit MPs production

	Agent	MPs/PMN/hr x 10 ²		FBEs (fluor/min) X 10 ³	
		Air	N ₂	Air	N ₂
	PBS	0.03±0.02 (24)	384 ± 21 (8) *	0.46±0.05 (12)	8.55±0.40 (12) *
	KO	0.00±0.00 (5)	0.00±0.00 (5)	0.39±0.06 (3)	0.59±0.16 (3)
Inhibit iNOS →	1400W	0.00±0.00 (4)	0.13±0.06 (5)	0.54±0.12 (5)	0.42±0.08 (3)
Inhibit NOX →	Nox2ds	0.04±0.02 (3)	0.05±0.03 (3)	0.40±0.10 (4)	0.53±0.16 (4)
Small Inhibitory RNAs	VASP-si	0.09±0.17 (4)	0.15±0.07 (4)	0.40±0.06 (3)	0.57±0.22 (3)
	FAK-si	0.05±0.02 (4)	0.08±0.04 (3)	0.40±0.17 (3)	0.67±0.09 (3)
	Flip-si	0.02±0.02 (4)	0.06±0.04 (4)	0.43±0.03 (3)	0.43±0.03 (3)
	Flop-si	0.04±0.04 (4)	0.05±0.04 (4)	0.47±0.03 (3)	0.53±0.09 (3)
	PDI-si	0.04±0.03 (4)	0.06±0.06 (4)	0.41±0.02 (3)	0.30±0.01 (3)

Cell proteins can be linked to show associations with filamentous actin

DTSP link=
~12 Å°

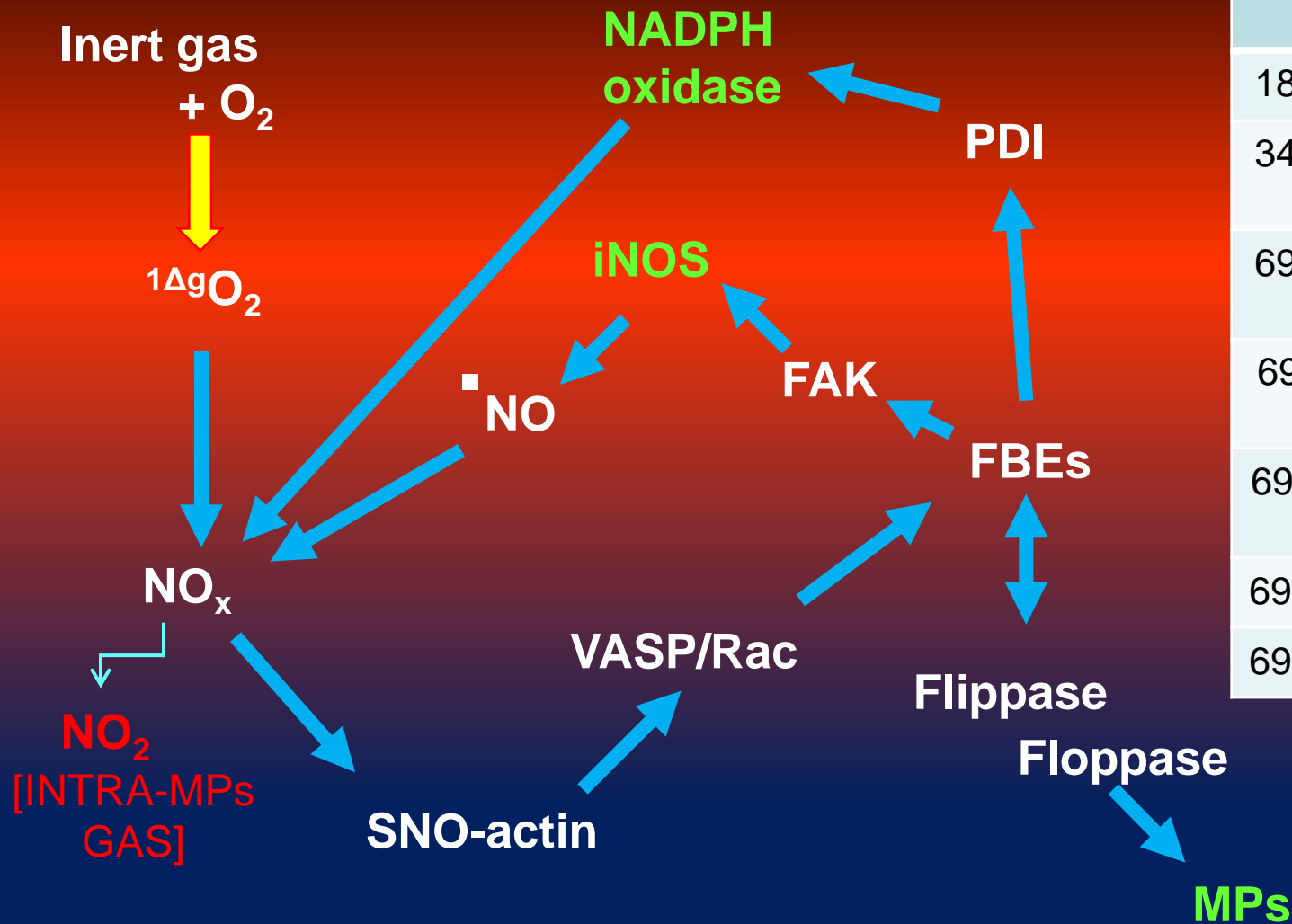
Fold increase
from just air
exposure



sF-actin	VASP/actn	FAK/actin	iNOS/actin	Flip/actin	Flop/actin	PDI/actin
690 kPa N ₂	4.68 ± 1.21*	2.91 ± 0.39*	2.79 ± 0.29*	2.48 ± 0.45*	3.28 ± 0.68*	4.03 ± 0.43*
Air + UV	1.10 ± 0.11	1.09 ± 0.08	1.22 ± 0.08	1.02 ± 0.07	1.19 ± 0.15	1.11 ± 0.04
N ₂ + UV	0.86 ± 0.11	0.96 ± 0.08	1.38 ± 0.37	1.01 ± 0.15	1.17 ± 0.26	0.88 ± 0.11

Microparticle Production Mechanism

Singlet O₂
sensor green



Air +	0 ± 0 (26)
186 kPa N ₂	441 ± 5 (3)
345 kPa N ₂	4101 ± 260 (3)
690 kPa N ₂	6237 ± 243 (11)
690 kPa Ar	5253 ± 259 (6)
690 kPa He	1309 ± 152 (6)
690 kPa SF ₆	27 ± 84 (6)
690 kPa N ₂ O	212 ± 97 (6)

**MPs are associated with high pressure/
decompression stress**

Some MPs act as bubble nucleation sites

**Inert gases increases MPs formation –
the higher the pressure, the more MPs.**

**Threshold pressure for human MPs
production is ~ 60 fsw.**

**Data suggest that via an oxidative stress response,
gas pressure increases
bubble nucleation sites which
precipitate decompression stress/ DCS(?)**