

The association of blood-borne microparticles and neutrophil activation with decompression sickness

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BACKGROUND:

Circulating microparticles (MPs) appear to cause some of the insults following decompression stress. MPs are elevated in animals and humans after simulated or *bona fide* underwater diving. However, data supporting a relationship between MPs, neutrophil activation and DCS in human divers are lacking

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)
Eur J Appl Physiol 114: 1955 (2014)
Med. Sci. Sports Exerc. 46: 1928 (2014)

MOUSE MODEL

J Appl Physiol 110, 340 (2011)
J Appl Physiol 112, 204 (2012)
J Appl Physiol 114, 550 (2013)
COMBINED HUMAN + MOUSE
J Biol Chem 280, 18831 (2014)

HYPOTHESIS- MPs and neutrophil activation will differ between asymptomatic divers and those with DCS.

METHODS & MATERIALS:

Blood was analyzed from 280 divers who had been exposed to maximum depths from 7 to 105 meters; 185 were asymptomatic and 95 presented to hospitals with signs/symptoms thought to be due to DCS. Divers who improved with recompression (n=90) were defined as suffering DCS. Blood was mailed and analyzed by published methods and results correlated with diving and DCS variables.



This work was supported by funds from ONR



Characteristics of study population

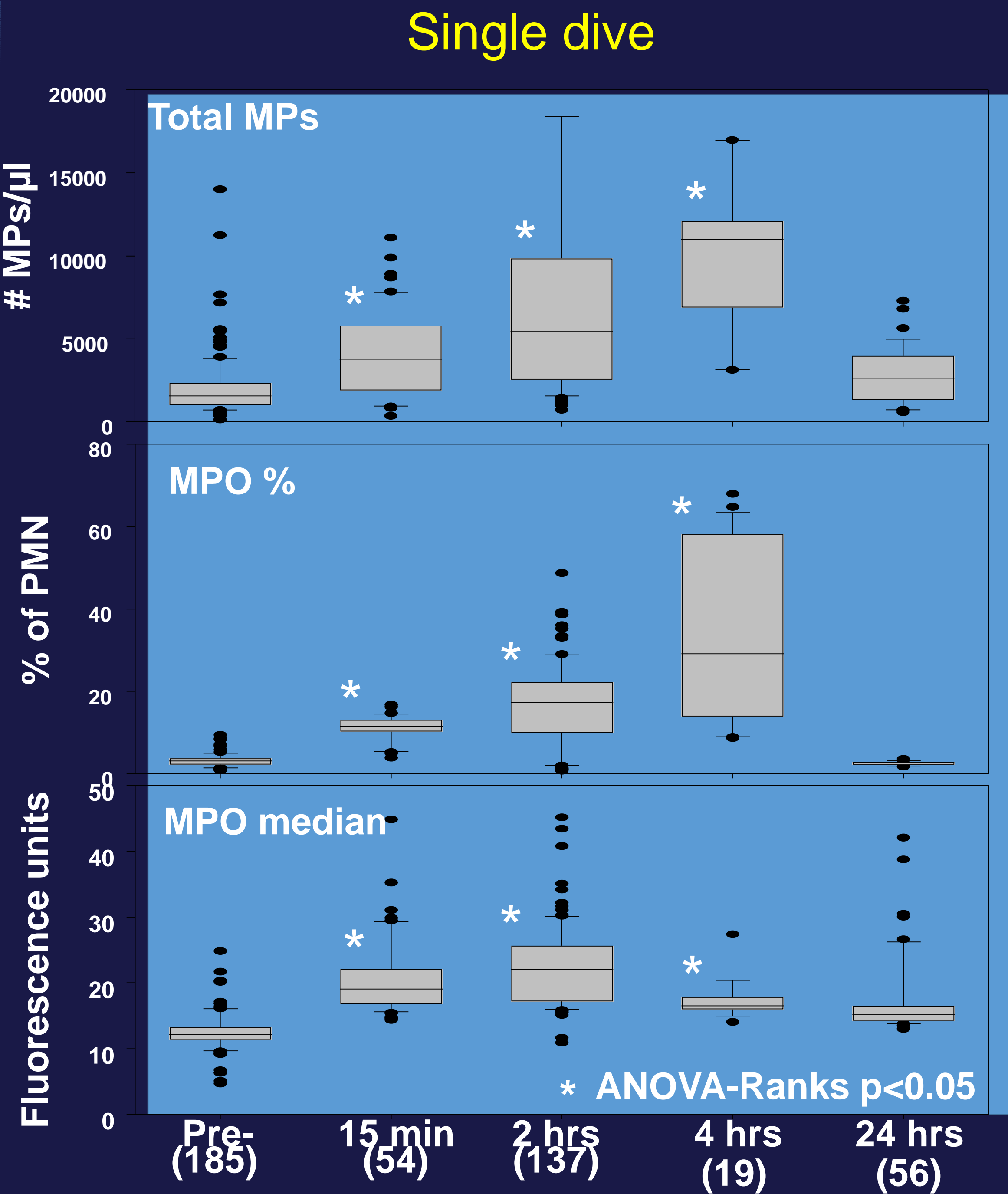
	DCS (90)	Research Subjects (185)
Age (years, median, 25 th & 75 th % ile)	34 (27, 42.3) Range: 16 - 73	40 (37, 44) Range: 21 - 72
Dive depth (meters)	22 (16, 34)	18 (18, 33)
# Female	23 (34%)	30 (20 %)
Compressed air use	69 (76.7%)	130 (70.3%)
EAN use	19 (21.1%)	28 (15.1%)
Tri-mix use	2 (2.2%)	27 (14.6%)*

Age, diving depth and the gender distribution between divers with DCS and Research Subjects were not statistically significantly different. The last three rows indicate the breathing gas used by the divers. *p<0.001

With DCS, changes persist beyond 24 hours

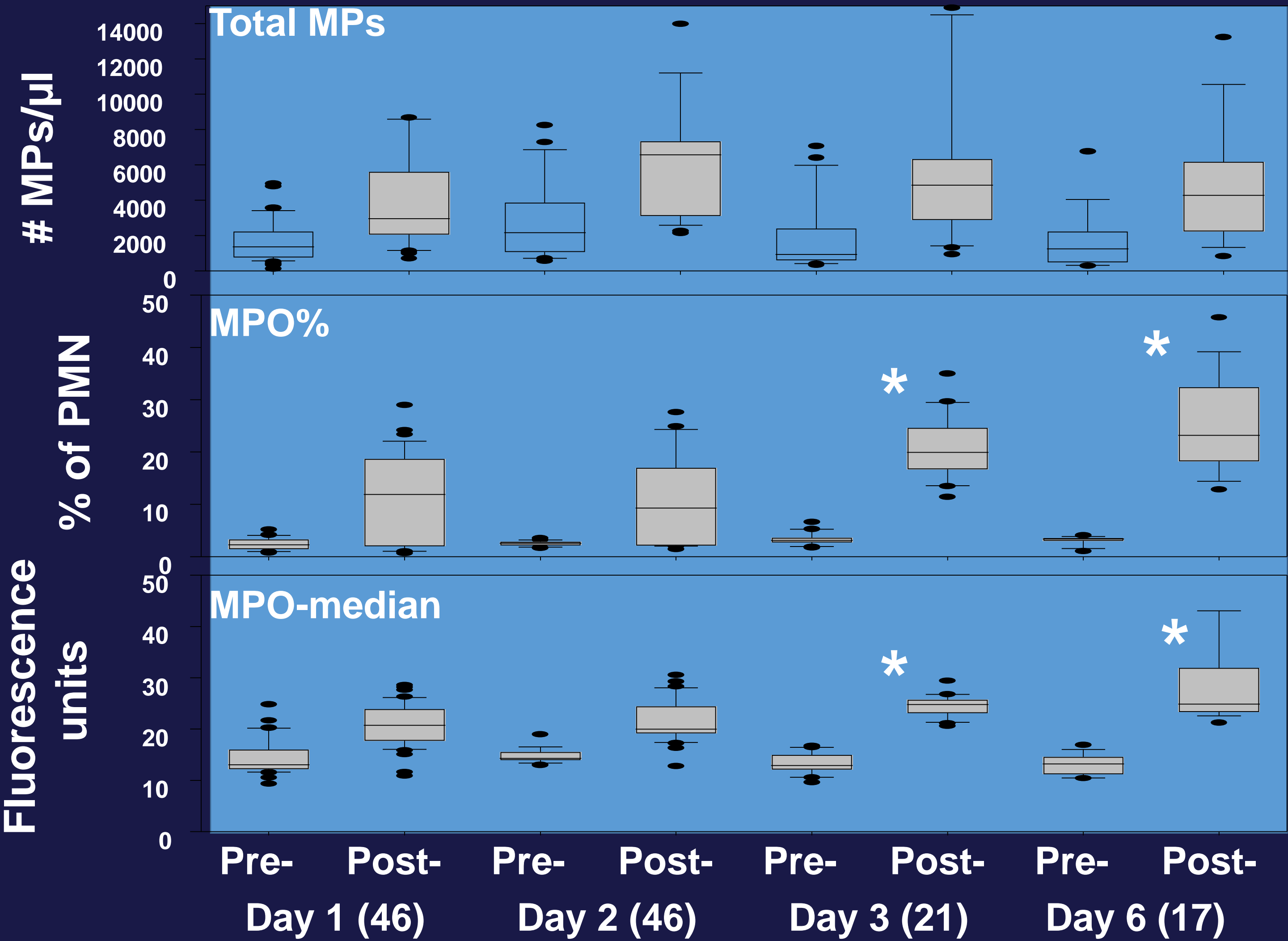
	Pre-dive Research subjects (n=185)	Post-dive Research Subjects (n=162)	DCS Divers- Acute (n=90)	DCS Divers- f/u (n=35)
Total MPs/μl	1448 (946, 2165)	2391 (1258, 5123)*	2716 (945, 6920)*	2047 (779, 3682)
MPO %	2.6 (1.9, 3.5)	5.8 (2.7, 12.2)	11.5 (4.3, 24.4)*	10.3 (4.6, 18.3)*
MPO-median	12.2 (11.4, 14.0)	15.3 (13.5, 21.6)*	16.4 (13.8, 19.9)*	14.9 (12.8, 22.3)
MPs- CD66b/μl	4.8 (1.8, 38.2)	31.1 (15.9, 44.3)*	84.0 (22.8, 148.9)*†	55.7 (18.8, 128.9)*
MPs- CD41/μl	9.6 (4.5, 33.7)	45.0 (18.4, 87.5)*	110.4 (52.2, 400.9)*†	88.2 (19.5, 337.4)*
MPs- CD31/μl	13.1 (5.3, 64.2)	37.9 (21.0, 242.8)*	186.9 (70.3, 606.3)*†	137.6 (53.5, 298.3)* †
MPs- CD142/μl	1.4 (0.3, 16.9)	16.4 (2.1, 132.3)*	66.7 (24.5, 194.6)*†Δ	19.4 (8.1, 46.9)*
MPs- CD235/μl	6.4 (3.9, 15.8)	32.5 (9.0, 126.7)*	385.9 (56.6, 692.6)*†Δ	73.4 (34.1, 248.6)* †
MPs-vWF/μl	6.5 (4.0, 18.0)	38.2 (6.6, 148.3)*	248.9 (36.0, 558.0)*†Δ	58.1 (4.1, 272.0)*
MPs- CD14/μl	7.7 (4.1, 18.0)	25.5 (6.2, 81.8)*	271.2 (101.4, 765.0)*†	206.6 (26.5, 309.1)* †

Asymptomatic divers exhibit MPs elevations and neutrophil activation assessed as surface myeloperoxidase (MPO) that resolves in 24 hours



Data are pre-dive values for research subjects (n=185), post-dive research subjects (n=162, matched to DCS divers for gas used) where blood was obtained at the longest time after diving to match the time when samples were obtained in the acute DCS group (column 3). Median time (25th and 75th %iles), post diving for research subjects: 24 (7.4, 96)hours; for DCS divers 24 (11.8, 55) hours. Column 4 displays data from divers with DCS who returned for follow-up evaluations at a median time of 28 (13.5, 35) days after treatment for DCS. (*) indicates p<0.001 versus pre-dive research subject values; †indicates p<0.05 vs post-dive research subject values, Δ=p<0.05 versus late follow-up DCS values. Rows are labeled as follows: MPO% indicates the fraction neutrophils exhibiting myeloperoxidase fluorescence above the FMO threshold, MPO-m indicates the geometric median fluorescence value for MPO. All other rows indicate the number/μl plasma for MPs manifesting the following surface markers: CD66b (neutrophil specific), CD41 (platelet specific), CD31 (platelet-endothelial cell adhesion molecule), CD142 (tissue factor), CD14 (leukocyte common antigen), CD235 (erythrocyte specific), vWF (von Willebrand factor).

Repetitive dives



All post-dive values are significantly different (p<0.001) from pre-dive values on the same day. The (*) indicates p<0.001 versus day 1 and day 2 post-dive values based on 2-way repeated measures ANOVA of log-transformed data.

Regression analysis: MPs & neutrophil activation association with DCS: Odds ratios & 95% confidence intervals

Adjustment	MPO%	MPOm	CD66	CD41	CD31	CD142	CD14	CD235	vWF
Unadjusted	1.6 (1.2, 2.0) p<0.00	6.9 (2.1, 23.1) p=0.00	1.4 (1.2, 1.8) p<0.00	1.5 (1.2, 1.8) p<0.00	1.7 (1.4, 2.2) p<0.00	1.4 (1.2, 1.7) p<0.00	2.0 (1.4, 2.7) p<0.00	1.6 (1.3, 2.2) p<0.00	1.7 (1.3, 2.3) p<0.00
Time blood sample obtained	1 (1.2, 2.1) p<0.00	1 (2.1, 24.8) p=0.00	1 (1.3, 2.1) p<0.00	1 (1.4, 2.2) p<0.00	1 (1.6, 2.8) p<0.00	1 (1.5, 2.4) p<0.00	1 (1.5, 3.1) p<0.00	1 (1.5, 3.2) p<0.00	1 (1.5, 3.0) p<0.00
Diver Age	1.6 (1.2, 2.1) p<0.00	8.6 (2.4, 31.5) p=0.00	1.4 (1.1, 1.7) p=0.00	1.4 (1.2, 1.6) p<0.00	1.6 (1.3, 2.1) p<0.00	1.4 (1.2, 1.7) p<0.00	2.0 (1.4, 2.9) p<0.00	1.6 (1.2, 2.2) p=0.00	1.7 (1.2, 2.4) p=0.00
Time, depth, gas, repeat dive, age	1.6 (1.2, 2.1) p=0.00	7.1 (1.8, 27.8) p=0.00	1.5 (1.2, 2.0) p=0.00	1.6 (1.2, 2.1) p<0.00	2.2 (1.6, 3.1) p<0.00	2.0 (1.5, 2.6) p<0.00	3.4 (1.8, 6.4) p<0.00	2.4 (1.4, 4.1) p=0.00	2.5 (1.5, 4.2) p<0.00

CONCLUSION:

We conclude that MPs production and neutrophil activation exhibit strong associations with DCS.