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Microparticle production, neutrophil activation, and intravascular bubbles following open-water SCUBA diving

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Thom SR, Milovanova TN, Bogush M, Bhopale VM, Yang M, Bushmann K, Pollock NW, Ljubkovic M, Denoble P, Dujic Z. Microparticle production, neutrophil activation, and intravascular bubbles following open-water SCUBA diving. *J Appl Physiol* 112: 1268–1278, 2012. First published February 9, 2012; doi:10.1152/japplphysiol.01305.2011.—The goal of this study was to evaluate annexin V-positive microparticles (MPs) and neutrophil activation in humans following decompression from open-water SCUBA diving with the hypothesis that changes are related to intravascular bubble formation. Sixteen male volunteer divers followed a uniform profile of four daily SCUBA dives to 18 m of sea water for 47 min. Blood was obtained prior to and at 80 min following the first and fourth dives to evaluate the impact of repetitive diving, and intravascular bubbles were quantified by trans-thoracic echocardiography carried out at 20-min intervals for 2 h after each dive. MPs increased by 3.4-fold after each dive, neutrophil activation occurred as assessed by surface expression of myeloperoxidase and the CD18 component of β_2-integrins, and there was an increased presence of the platelet-derived CD41 protein on the neutrophil surface indicating interactions with platelet membranes. Intravascular bubbles were detected in all divers. Surprisingly, significant inverse correlations were found among postdiving bubble scores and MPs, most consistently at 80 min or more after the dive on the fourth day. There were significant positive correlations between MPs and platelet-neutrophil interactions after the first dive and between platelet-neutrophil interactions and neutrophil activation documented as an elevation in β_2-integrin expression after the fourth dive. We conclude that MPs- and neutrophil-related events in humans are consistent with findings in an animal decompression model. Whether there are causal relationships among bubbles, MPs, platelet-neutrophil interactions, and neutrophil activation remains obscure and requires additional study.

decompression sickness; leukocytes; platelets; antigen sharing; CD41; integrins; ultrasound

DECOMPRESSION SICKNESS (DCS) is a systemic pathophysiological process that occurs after tissues become super-saturated with gas. Inert gases inhaled while breathing are taken up by tissues in proportion to the ambient pressure, and when pressure is reduced, some of the gas released from tissues forms bubbles due to the presence of gas cavitation nuclei. The central place of bubbles as an inciting factor for DCS is widely accepted, yet most decompression procedures generate asymptomatic blood-borne bubbles (9).

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Elevations in pro-inflammatory circulating microparticles (MPs) have been associated with decompression, and MPs were shown to be responsible for vascular injuries in a murine model (37). MPs are 0.1 to 1 μm encapsulated membrane fragments that are characterized by expression of antigenic markers from parent cells. As MPs bud from cells, negatively charged phosphatidylserine residues are exposed, which often leads to secondary binding of annexin V. We have reported that over 95% of MPs generated after decompression stress in the animal model are annexin V positive (39). Well known processes that generate MPs include oxidative stress, cell activation/calcium influx, and apoptosis (10, 14, 33). We hypothesized that bubble-mediated shear stress and stimulation of calcium-activated big conductance potassium channels, as well as oxidative stress from activated neutrophils may be mechanisms for MPs generation postdecompression (37, 39).

The pathophysiological events responsible for DCS are unclear. Platelets and leukocytes have been found to aggregate and adhere to intravascular bubbles, and the notion that this may induce pathological changes was discussed early in the 1970s (31). In clinical cases, DCS has been associated with endothelial dysfunction, platelet activation, occasional alterations in coagulation pathways, and rare reduction of circulating platelet counts (2, 29, 30, 32). Platelet and neutrophil activation followed by perivascular leukocyte adherence are associated with tissue injuries and functional deficits postdecompression in animal models (24, 28, 34, 37). Recent studies have shown that some annexin V-positive particles enlarge beyond 1 μm in diameter during decompression because they contain a gas phase (39). These large particles, in particular, pose a risk of causing inflammatory vascular injuries. Injecting these particles into naive mice will recapitulate the pathophysiological vascular changes observed with provocative decompression (39).

Endothelium-derived MPs have been reported to increase in humans subjected to air decompression from a simulated pressure of 18 m of sea water (msw) in a hyperbaric chamber (23, 38). Elevations in circulating MPs have not been reported in humans subjected to bona fide open-water SCUBA activities. The goal of this pilot investigation was to evaluate MPs, neutrophil activation, and also intravascular bubbles in a group of healthy volunteers performing a uniform pattern of SCUBA diving. Our hypothesis was that MPs elevations would occur and are associated with neutrophil activation. Furthermore, we hypothesized that changes would be correlated with intravascular bubble presence. MPs sizes were monitored postdecompression because MPs enlargement is linked to pathological

changes in an animal model (39). Because we were unsure about magnitude of changes and because there is a general belief that DCS risk increases with repetitive diving over several days, a 4-day experimental diving series was chosen with phlebotomy performed on the first and last day (18).

METHODS

Subjects. Sixteen male divers were recruited for this preliminary investigation; women were excluded to avoid confounding by the menstrual cycle. All experimental procedures in the study were completed in accordance with the Declaration of Helsinki and approved by Ethical Committees of the University of Split School of Medicine, the Divers Alert Network, and the University of Pennsylvania. All subjects provided written informed consent. Baseline screening was conducted prior to diving with standard techniques because there is evidence to suggest that some anthropomorphic features and physical fitness may alter the risk of DCS (6, 25). Body mass index (BMI) was derived from mass and height measures. Waist-to-hip ratio was computed using circumference measures captured with a spring-tensioned soft measuring tape at the narrowest part of the waist and the widest part of the hips. Percent body fat was estimated by standardized seven-site skin fold techniques using a Harpenden skinfold caliper (Baty National, West Sussex, UK) with all measures taken by a single, experienced technician (16). Aerobic capacity ($\dot{V}O_{2\,max}$) was estimated with the Houston non-exercise test (15). Maximum grip strength was determined for both right and left hand with a hand dynamometer (model 78010; Lafayette Instrument). Health status and previous diving experience was self-reported. Anthropometric characteristics are shown in Table 1.

Study protocol. All subjects were certified divers with diving experience ranging from 4 to 25 yr. They provided their own diving equipment; all wore wetsuits between 5 and 8 mm in thickness. The dives were conducted in protected ocean water immediately adjacent to a shore laboratory. Water entry was through a rocky cobble beach or cement ramp. A 2- to 3-min surface swim through calm water was required for divers to reach the descent point and again to return to the exit point. The water temperature for all dives was ~16°C with minimal current. Divers had refrained from any diving and swimming activities for at least 3 days before the control dive and before the first dive in the experimental series. Diving profiles were monitored electronically (Sensus, Mississauga, Ontario, Canada).

The experimental dives were conducted once per day for 4 days following a dive leader. On each day the profile was a direct descent to 18 m for an actual bottom time of 47 min, then 2 min to ascend to the surface. Subjects swam continuously while at depth at a pace intended to represent a sustained moderate to moderately heavy work rate. A control dive was performed 7 mo after the experimental four-dive series by 10 of the initial 16 divers. The surface swim, submerged swimming intensity, and time at depth were the same as in the experimental series but divers were at a depth of only 5 msw.

Trans-thoracic echocardiography (TTE) imaging was performed with a phase array probe (1.5–3.3 MHz) using a Vivid q scanner (General Electric, Waukesha, WI) on each subject postdive every 20 min for 2 h in a beachside room ~50 steps from the dive site. They were asked to refrain from any strenuous exercise throughout the monitoring period. Subjects were positioned left lateral on a cot for all scanning. Each TTE scan included continuous measurement capturing a rest period followed by sequential movement/recovery periods of right arm and then right leg. The "movement" case consisted of effort to contract every muscle and move every joint of the target limb through the full flexion/extension range three times in series before returning to a neutral resting position. Each movement series was completed in 5–7 s. Cardiac scanning was maintained throughout and followed until a return to baseline conditions was confirmed, typically for a minimum of 10 cardiac cycles postmovement. Movement was employed to mobilize gas bubbles presumably lodged or generated in the venous pathway.

TTE monitoring was conducted by two individuals trained in cardiac imaging. Individual subjects were scanned by the same technician throughout the study. Bubble signals were graded in real time through consensus of two evaluators, the person conducting the scanning and an observer, also extensively trained and experienced in evaluating TTE bubble studies. Grading employed a modified Brubakk scale that has been used in several studies (20). The grading system is as follows: 0, no bubbles; 1, occasional bubbles; 2, at least one bubble every four cardiac cycles; 3, at least one bubble every cardiac cycle; 4, continuous bubbling with modifiers [(a = at least one bubble per cm^2 in all frames), (b = at least three bubbles per cm^2 in all frames), or (c = almost complete whiteout but individual bubbles

Table 1. *Anthropometric data for each diver*

Subject	Age, yr	Body Mass, kg	Height, m	BMI, kg/m^{-2}	WHR	Body Fat, %	Est. $\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	Max Handgrip Force, R/l, kg
1	38	88.3	1.78	27.9	0.85	15.6	45.3	54.5/54.0
2	43	97.5	1.88	27.7	0.95	15.7	43.5	63.0/60.0
3	37	93.6	1.84	27.6	0.93	25.2	45.9	57.0/54.0
4	21	94.6	1.88	26.9	0.82	17.5	52.5	59.0/54.0
5	39	72.0	1.79	22.5	0.91	11.6	49.0	46.0/46.0
6	36	78.7	1.81	24.0	0.89	18.9	49.0	50.0/42.0
7	45	99.4	1.83	29.7	0.97	16.8	41.3	55.0/53.0
8	44	98.3	1.90	27.4	0.92	16.1	39.6	49.0/42.0
9	34	74.6	1.70	25.8	0.91	21.9	48.4	54.5/45.0
10	40	81.3	1.83	24.4	0.99	18.4	39.5	45.0/43.5
11	38	88.2	1.81	26.9	0.96	20.8	44.1	58.0/47.0
12	39	92.4	1.78	29.2	0.93	22.0	43.9	38.5/24.0
13	33	97.3	1.85	28.4	0.87	11.2	46.8	77.5/72.0
14	37	91.1	1.85	26.6	0.93	17.4	46.6	52.5/41.0
15	42	108.4	1.80	33.5	0.99	22.9	37.6	59.0/54.0
16	23	80.5	1.86	23.3	0.88	13.7	54.5	40.0/33.0
Mean ± SE	36.8 ± 1.7	89.8 ± 2.5	1.82 ± 0.01	27.0 ± 0.7	0.92 ± 0.01	17.9 ± 1.0	45.5 ± 1.2	53.7 ± 2.4/7.8 ± 2.8
Min	21	72.0	1.70	22.5	0.82	11.2	37.6	38.5/24.0
Max	45	108.4	1.90	33.5	0.99	25.2	54.5	77.5/72.0

Values are means ± SE. Body mass index (BMI) = weight in kg/(height in m)^2; waist-to-hip ratio (WHR) = narrowest waist circumference / widest hip circumference; maximum aerobic capacity estimated by predictive Eq. 9; body fat estimated by 7-site skinfold assessment (10).

can still be discerned)]; and 5, whiteout in which individual bubbles cannot be discerned. Resting scores were recorded as the lowest stable score maintained during the observation period. Movement scores reflected the highest score achieved following the movement series for each of the two limbs tested.

Venous blood was collected by a trained phlebotomist prior to the control dive and the experimental dives on *days 1* and *4* and at 80 min after the dives. Blood was drawn into Cyto-Chex BCT test tubes that contain a proprietary preservative (Streck, Medimark Europe, Grenoble, France). The volume drawn per sample (two tubes) was ~10 ml.

Materials. Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Annexin binding buffer and the following antibodies purchased from BD Pharmingen (San Jose, CA): fluorescein isothiocyanate (FITC) conjugated anti-annexin V, FITC-conjugated anti-human myeloperoxidase (MPO), R-phycoerythrin (PE) conjugated anti-human CD142, PerCP/Cy5.5 conjugated anti-human CD41, PerCP/Cy5.5 conjugated anti-human CD14, allophycocyanin (APC) conjugated anti-human glycophorin A, APC conjugated anti-human vWF. R-PE-conjugated anti-human CD41 was purchased from e-Biosciences (San Diego, CA), PerCP Cy5.5-conjugated anti-human CD66b from Biolegend (San Diego, CA), and Alexa 647-conjugated anti-human CD18 from Serotec (Raleigh, NC).

Standard procedures for MPs and neutrophil acquisition and processing. Blood samples in tubes containing preservative were sent by express mail to the University of Pennsylvania where all analyses were performed within 24 h after arrival, ~2–6 days from time of collection. Preliminary data have shown that MPs and neutrophil characteristics remain unchanged when samples stored at either 4°C or at room temperature are processed in a time span of 3 wk from time of collection (data not shown). All reagents and solutions used for MPs analysis were sterile and filtered (0.2 μm filter). Samples were processed following published procedures (37). In brief, a small sample of the whole, fixed blood was set aside for evaluation of neutrophils, to count leukocytes and platelets, and to calculate hematocrit by microhematocrit centrifuge. The remaining blood was centrifuged for 5 min at 1,500 *g*, the supernatant was made 0.2 M EDTA and then centrifuged at 15,000 *g* for 30 min. Aliquots of the 15,000 *g* supernatant were stained with antibodies for analysis by flow cytometry and confocal microscopy.

To provide a comparison for assessing the degree of neutrophil activation caused by diving, blood samples from three healthy nondiving volunteers, one woman and two men aged 50 ± 3.9 yr were obtained in heparinized tubes. Whole blood was incubated at room temperature with 4 mg/ml zymosan, 0.1 or 1.0 μM formyl-methionyl-leucine-phenylalanine (FMLP), 10 ng/ml phorbol 12-myristate 13-acetate (PMA), or with the same volume of PBS as used to add stimulating agents (control). Samples were taken at intervals up until 20 min; stained with antibodies to CD66b, CD18, MPO, and CD41 exactly as performed with samples from divers; and evaluated by flow cytometry.

Flow cytometry. Flow cytometry was performed with a 10-color FACSCanto (Becton Dickinson, San Jose, CA) using standard acquisition software. Gates were set to include 0.3- to 1.0-μm particles, with exclusion of background corresponding to debris usually present in buffers. MPs were stained with annexin V antibody and analyzed as previously described, including microbeads with diameters of 0.3 (Sigma), 1.0, and 3.0 μm (Spherotech, Lake Forest, IL) to carefully assess the size of particles. Analysis of neutrophils was performed on fixed blood samples as previously described (37).

Confocal microscopy. The sizes of annexin V-positive particles were measured following published procedures (39). In brief, images were acquired using a Zeiss Meta510 confocal microscope equipped with a Plan-Apochromat 63×/1.4NA oil objective. Particle suspensions stained with RPE-conjugated anti-annexin V antibody were combined with a small number of FITC-containing 0.86 μm beads (Sigma) to provide comparison and visually inspected to be sure no particle aggregates were present. Mean number of annexin V-positive particles evaluated among the 64 measurements (16 divers × 4 samples) was 741 ± 28 (SE, range 502 - 1429). Digital images were obtained and analyzed using Image J software.

Statistical analysis. Parametric data are expressed as means ± SE, nonparametric data are expressed as median, 25th, and 75th percentile values. We used Sigmastat software (Systat, Point Richmond, CA) for the statistical analysis. Microparticle numbers, neutrophil activation, and size of annexin V-positive particle populations, all parametric data, were analyzed by repeated-measures ANOVA followed by the Holm-Sidak test. Single comparisons were performed using Student's *t*-tests, and correlations among the data sets were analyzed by Pearson product moment calculations. Bubble scores (nonparametric data) were analyzed using Friedman repeated-measures analysis of variance, and correlations involving the bubble data sets were evaluated by the Spearman rank order test. Significance was accepted at $P < 0.05$.

RESULTS

Diving and intravascular bubbles. Divers reported no adverse effects from any of the SCUBA activities. Hematocrit, leukocyte, and platelet counts were not significantly different pre-/postdive (Table 2). Circulating bubbles quantified by trans-thoracic echocardiography were documented in all subjects after the 18 msw experimental dives. Bubble scores for experimental *dive 1* are shown in Fig. 1. As patterns were similar after *dives 2* through *4* the data are not shown. Bubble scores at rest or after arm or leg exercise that were taken at early times (20, 40, 60, and 80 min) were significantly different from scores at 100 and 120 min. As shown in the Fig. 1, there were also significant differences in the scores recorded at rest vs. exercise at each time point. Data sets for each diver followed consistent patterns; most resting and postexercise scores obtained early postdiving were highly correlated, whereas they were not well correlated with later scores (Table 3).

Scant intravascular bubbles were documented in the 10 subjects who performed the control dive to 5 msw, and the median scores were all 0.0. These values are shown on Fig. 1. Bubbles were detected in only three divers. One diver had

Table 2. *Hematocrit, leukocyte, and platelet counts*

	CONTROL		*Dive 1*		*Dive 4*	
	Pre	Post	Pre	Post	Pre	Post
WBC, x10^3	7.6 ± 0.9	7.9 ± 0.7	7.0 ± 0.3	7.5 ± 0.3	7.3 ± 0.5	8.2 ± 0.5
Platelets, x10^5	2.9 ± 0.2	3.1 ± 0.2	2.9 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	3.3 ± 0.2
Hct, %	48.6 ± 0.7	49.6 ± 0.7	47.0 ± 0.5	48.2 ± 0.6	48.1 ± 0.7	48.6 ± 0.4

Values are mean ± SE. Leukocyte count (WBC) expressed as cells/μl x 10^3, platelets/μl x 10^5, and hematocrit (Hct) as %. There are no significant differences across rows.

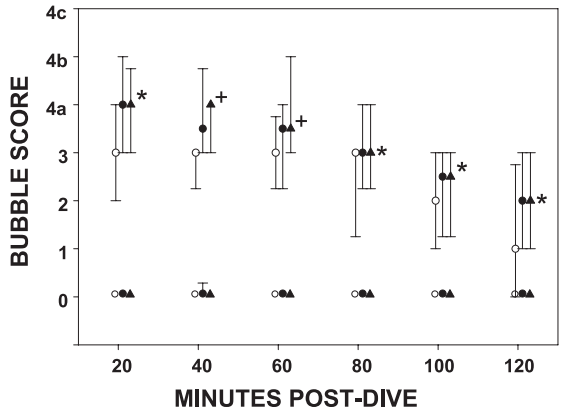

Fig. 1. Bubble scores following the control and *day 1* dives. Data show median, 25th, and 75th percentile values for these nonparametric data. Measurements were taken at 20-min intervals for 2 h while divers were at rest (○), after arm exercise (●), and leg exercise (▲). Symbols in the figure at the 0 median bubble score are data from the control 5 msw dive. Symbols reflecting higher bubble scores are from the experimental 18 msw dive. Within the 18 msw dive data sets the scores taken at early time points (20 through 80 min) were significantly different from the 100 and 120 min values. Other symbols in the figure demonstrate additional significant differences. *Postdiving arm and leg exercise values differ from the resting value; +postdiving leg exercise values, but not arm exercise values, differ from the resting value.

grade 1 bubbles with exercise at 20 min. One had *grade 1* at rest and *grade 2* bubbles with exercise at 40 min, and one had *grade 1* bubbles with exercise at 40 and 60 min. No bubbles were detected in subjects at later times.

MPs elevations and bubble scores. Circulating MPs counts are shown in Fig. 2. No significant changes occurred with the control dive. Increases occurred after each experimental dive, and the differences (post vs. pre) for each day were virtually identical. The mean elevation in MPs per microliter after the experimental dive on *day 1* was 19,164 ± 5,164 and on *day 4* it was 18,848 ± 3,971.

Interestingly, there were significant negative correlations between divers' bubble scores and MPs numbers after the 18 msw experimental dives. The relations after diving on the first day were somewhat erratic; the resting scores vs. total MPs numbers at 20 min exhibited a correlation coefficient of −0.630 ($P = 0.008$), and at 60 min the correlation coefficient was −0.606 ($P = 0.013$). At 80 min, the time when the blood sample was obtained, the correlation coefficient was −0.426 ($P = 0.0005$). Similar associations and *P* values were noted at 60 and 80 min postdive for the post-arm exercise bubble scores. The only post-leg exercise bubble score to have a significant correlation with MPs numbers was at 20 min ($r = -0.505$, $P = 0.044$). Values after the dive on *day 4* exhibited a consistent pattern and are shown in Table 4. There were no significant correlations for early time points postdiving, but significant negative correlations were observed between MPs number and bubble scores at 80, 100, and 120 min postdiving.

MPs surface protein expression pattern postdecompression. MPs subtypes were characterized by surface markers for vascular cell proteins. Figure 3 shows the percent of annexin V-positive MPs coexpressing each surface protein marker. The fraction of MPs expressing platelet-derived CD41 increased following the 5 msw control dive. This elevation was significantly different from the fraction of CD41-positive MPs seen before and after the 18 msw experimental dives. There were no other significant changes in MPs subtypes following the control dive, but elevations across all subtypes occurred after experimental dives. The elevations postdiving on *day 1* vs. *day 4* were not significantly different. Similarly, the predive values before the control, *day 1*, and *day 4* profiles were not significantly different.

The populations of annexin V-positive MPs exhibiting surface markers for two vascular cell types were evaluated because interactions and thus sharing of markers was described in the animal model (37). Serial measurements of this type have not been reported in the literature. Values obtained in the control series were generally one order of magnitude lower than the pre-and post-experimental dive values obtained 7 mo earlier (Fig. 4). All control values were significantly different from those obtained during the experimental 18 msw dive series but there were no significant differences pre- vs. post-control dive. Following the experimental 18 msw dives, significant differences were present across all subtypes (pre vs. post). There were no significant differences in MPs populations prior to *day 1* and *day 4* 18 msw dives.

MPs enlarge after decompression. Recent work in a murine model has shown that annexin V-positive particles enlarge with decompression, and this enlargement is closely associated with neutrophil activation and vascular injury (39). Therefore, MPs were examined using a confocal microscope. Once assured there were no particle aggregates, particle diameters were evaluated using computer imaging software. The distribution of annexin V-positive particles is shown in Fig. 5. No significant differences in MPs sizes were noted among the samples taken before and after the control dive and the sample obtained prior to the first 18 msw dive. The fraction of particles ≤1.0 μm decreased significantly after the *day 1* 18 msw dive. The fraction ≤1.0 μm pre- and post-*day 4* dive was significantly different from the pre-*day 1* dive, but the *day 4* values were not significantly different from each other.

A reciprocal pattern was observed after the experimental dives with particles having diameters >1.0 μm. The fraction of particles over 1.0 μm increased significantly after the *day 1* dive. The fractions >1.0 μm prior to and post-*day 4* dive were significantly different from the pre-*day 1* dive, but the values were not significantly different from each other. The mean diameter of these larger particles was 1.40 ± 0.28 μm prior to the *day 1* dive. The mean diameter of the larger annexin V-positive particles after this dive was 1.67 ± 0.43 μm ($P <$ 0.05 vs. predive value). The mean diameter of these particles prior to *day 4* dive was 1.62 ± 0.47 (NS vs. prior to *day 1*) but after *day 4* diving the mean diameter of the larger particles was 1.91 ± 0.59 μm ($P < 0.05$ vs. predive on *day 1* and *day 4*).

Leukocyte activation in divers. Figure 6 shows a series of measurements of neutrophils obtained pre- and postdiving. The neutrophil population was identified by surface expression of CD66b, and activation was assessed in two ways. One method was by measuring geometric mean fluorescence intensity of CD18 (a component of β_2-integrins) and myeloperoxidase (MPO) on the cell surface. The proportion of circulating neutrophils that became activated was also evaluated by measuring the percent of the total CD66b-positive cells with mean fluorescence for MPO or CD18 above 10 arbitrary fluorescence units. Following the control 5 msw dive the mean values for surface fluorescence of CD18 and MPO were not significantly different from predive values (Fig. 6). There was, however, a

Table 3. *Correlations among bubble scores*

	R-40	R-60	R-80	R-100	R-120	A-20	A-40	A-60	A-80	A-100	A-120	L-20	L-40	L-60	L-80	L-100	L-120
R-20	**0.620**	**0.588**	**0.586**	**0.670**	**0.583**	**0.806**	0.311	0.328	0.306	0.100	0.346	**0.804**	**0.504**	0.404	0.434	0.404	0.432
	0.010	**0.016**	**0.017**	**0.004**	**0.018**	**0.001**	0.233	0.207	0.242	0.705	0.182	**0.001**	**0.046**	0.118	0.089	0.118	0.091
R-40		**0.709**	**0.735**	**0.711**	**0.673**	**0.711**	**0.773**	**0.615**	**0.675**	0.388	0.434	**0.640**	**0.637**	**0.623**	**0.720**	**0.525**	**0.547**
		0.002	**0.001**	**0.002**	**0.004**	**0.002**	**0.001**	**0.011**	**0.004**	0.133	0.089	**0.007**	**0.008**	**0.010**	**0.001**	**0.035**	**0.028**
R-60			**0.867**	**0.763**	**0.729**	**0.733**	**0.681**	**0.769**	**0.728**	0.490	0.261	0.479	**0.649**	**0.750**	**0.668**	**0.685**	0.477
			0.001	**0.001**	**0.001**	**0.001**	**0.003**	**0.001**	**0.001**	0.052	0.319	0.058	**0.006**	**0.001**	**0.005**	**0.003**	0.060
R-80				**0.878**	**0.829**	**0.624**	**0.663**	**0.646**	**0.819**	**0.720**	**0.551**	0.425	**0.685**	**0.692**	**0.782**	**0.907**	**0.747**
				0.001	**0.001**	**0.005**	**0.009**	**0.007**	**0.001**	**0.001**	**0.027**	0.096	**0.003**	**0.003**	**0.001**	**0.001**	**0.001**
R-100					**0.931**	**0.611**	0.459	0.416	**0.670**	**0.640**	**0.615**	0.359	**0.553**	0.438	**0.592**	**0.697**	**0.717**
					0.001	**0.012**	0.072	0.107	**0.004**	**0.007**	**0.011**	0.167	**0.026**	0.087	**0.016**	**0.002**	**0.001**
R-120						**0.626**	0.437	0.460	**0.700**	**0.735**	**0.693**	0.284	**0.649**	**0.535**	**0.613**	**0.652**	**0.770**
						0.009	0.087	0.072	**0.002**	**0.001**	**0.003**	0.277	**0.006**	**0.032**	**0.012**	**0.006**	**0.001**
A-20							0.431	**0.551**	**0.515**	0.208	0.356	**0.708**	**0.539**	**0.538**	**0.553**	0.369	0.402
							0.091	**0.027**	**0.040**	0.429	0.171	**0.002**	**0.031**	**0.031**	**0.026**	0.153	0.118
A-40								**0.800**	**0.687**	0.407	0.202	**0.562**	**0.515**	**0.737**	**0.649**	**0.557**	**0.553**
								0.001	**0.003**	0.115	0.442	**0.023**	**0.040**	**0.001**	**0.006**	**0.025**	**0.026**
A-60									**0.688**	**0.480**	0.147	0.403	**0.555**	**0.747**	**0.668**	**0.573**	**0.509**
									0.003	**0.058**	0.578	0.118	**0.025**	**0.001**	**0.004**	**0.020**	**0.043**
A-80										**0.739**	**0.602**	0.284	**0.629**	**0.739**	**0.900**	**0.720**	**0.691**
										0.001	**0.013**	0.278	**0.009**	**0.001**	**0.001**	**0.002**	**0.002**
A-100											**0.757**	−0.10	**0.502**	0.457	**0.590**	**0.740**	**0.799**
											0.001	0.705	**0.046**	0.074	**0.016**	**0.001**	**0.001**
A-120												0.0920	0.321	0.284	**0.502**	0.470	**0.744**
												0.730	0.220	0.277	**0.046**	0.064	**0.001**
L-20													0.488	**0.506**	0.441	0.291	0.331
													0.053	**0.044**	0.084	0.267	0.202
L-40														**0.801**	**0.798**	**0.645**	0.481
														0.001	**0.001**	**0.007**	0.057
L-60															**0.825**	**0.658**	0.486
															0.001	**0.005**	0.055
L-80																**0.748**	**0.617**
																0.001	**0.011**
L-100																	**0.751**
																	0.001

Values show Spearman rank order correlation coefficient (top number in each row) and *P* value (lower number). Values in bold are statistically significant, *P* values <0.001 are listed as 0.001 (bottom number in each row). R-# indicates rest bubble score and time of measurement (e.g., 20, 40, 60, 80, 100, or 120 min postdive); A-# indicates bubble scores after arm exercise; L-# indicates bubble scores after leg exercise.

significantly increased fraction of the neutrophil population having MPO surface staining; but the fraction was less than occurred following the experimental dives. Neutrophils exhibited evidence of activation after each experimental dive to 18 msw based on both CD18 and MPO fluorescence intensity and percentage of cells with values above 10 arbitrary units.

The animal model suggests one mechanism that causes neutrophil activation is interactions with platelets or platelet-derived particles (37). This was evaluated in divers by measuring surface expression of CD41, the platelet-specific integrin α_{2b}-protein, on the CD66b cell population. As shown in Fig. 7, there was evidence of increased interactions postdiving. The fraction of neutrophils exhibiting CD41 above 10 arbitrary units was significantly elevated after the control and experimental dives. Mean CD41 surface fluorescence was significantly elevated after the experimental dives to 18 msw but not following the control dive (Fig. 7).

To provide a comparison for evaluating the postdive elevation in neutrophil activation and neutrophil interactions with platelet membranes, blood from healthy nondiving adults was incubated with various agents as described in METHODS. Substantially higher mean fluorescence values for CD18 and MPO occurred with all stimuli than occurred postdiving (Table 5). The value for CD41 mean fluorescence postdiving was similar to that seen with 0.1 μM FMLP and greater than the expression caused by zymosan and PMA. The fraction of the neutrophil population expressing elevated CD18 and MPO on the cell surface postdiving was similar to that seen with 0.1 μM FMLP

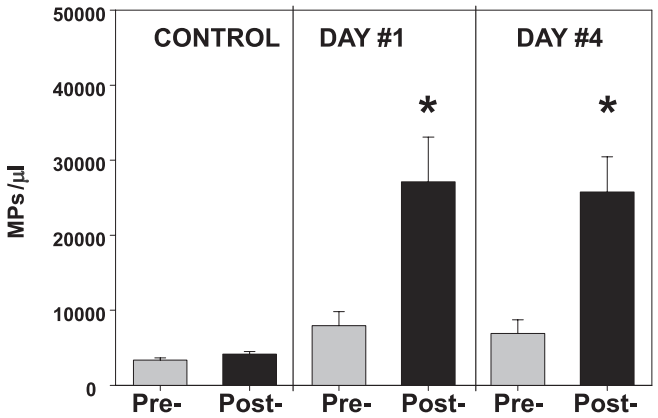

Fig. 2. Microparticles (MPs) number before and after each dive. Data show MPs numbers before and after the control 5 msw dive and 18 msw dives on *days 1* and *4* (means ± SE). *$P < 0.05$ vs. pre- and postcontrol dive and predive sample on *day 1* and *day 4*.

but less than that caused by the other agonists. The fractions of neutrophils showing elevations of CD41 on the cell surface were generally greater due to chemical agonists than what occurred postdiving.

DISCUSSION

General observations. Results from this study offer a number of insights regarding human responses to swimming and SCUBA diving. The control dive data indicate that stresses due to diving at 5 msw while breathing from a SCUBA apparatus in 16°C sea water when there are scant intravascular bubbles will cause: *1*) a shift in the population of circulating MPs such that more express platelet-derived CD41 without a significant elevation in total number of MPs; *2*) increased interactions between neutrophils and platelets or platelet-derived MPs to cause an elevation in fraction of CD66b-positive cells expressing CD41; and *3*) subtle evidence of neutrophil activation in that a higher fraction exhibits elevated surface expression of MPO. Others have reported that moderate or strenuous physical exercise will increase annexin V-positive MPs from leukocytes and platelets but not from endothelium or erythrocytes, and in some studies changes were concurrent with an elevation in leukocyte and platelet counts (3, 27, 35, 36). In these reports, MPs elevations were found immediately after exercise and at 2 h postexercise; one group has shown different rates of elevation up to 2 h between trained and untrained subjects (35). We obtained blood at 80 min postdive only, so we cannot say whether changes would have been greater with later samplings. We interpret the elevation observed in CD41-expressing MPs without a significant rise in total MPs number (see Figs. 2 and 3) as evidence of rapid MPs turnover. Surface phosphatidylserine, as is present on most MPs, constitutes a recognition signal that enables clearance/phagocytosis of particles (1). In the mouse model we found marked differences in clearance rates among MPs, but data in humans are lacking (39).

Decompression stress from 18 msw will cause a number of more overt changes than occur following 5 msw diving: *1*) increase the number of intravascular bubbles (Fig. 1); *2*) increase the number of annexin V-positive MPs by ~3.4-fold that are derived from leukocytes, erythrocytes, platelets, and endothelial cells (Figs. 2 and 3); *2*) enhance sharing of multiple surface proteins among MPs reflected by the populations of dual-positive particles (Fig. 4); *3*) increase the fraction of annexin V-particles larger than 1 μm (Fig. 5); *4*) increase neutrophil activation based on both the mean fluorescence for CD18 and MPO and the fraction of the population expressing over 10 arbitrary units (Fig. 6); *5*) increase neutrophil interactions with platelets or platelet-derived membranes based on both mean fluorescence for CD41 and the fraction of the population expressing over 10 arbitrary units (Fig. 7). The results demonstrate that MPs and neutrophil-related events are similar between humans and the mouse decompression model (37, 39).

To our knowledge, this is the first report demonstrating elevations in circulating MPs following open-water SCUBA diving. Endothelial cell-derived MPs have been reported to increase threefold following pressurization of human volunteers to 18 msw in a hyperbaric chamber (23, 38). Some of the quantitative differences among studies of MPs are likely to be related to sample preparation techniques and flow cytometry gating procedures. With regard to MPs derived from endothelium in our study, most CD31+ MPs are likely of endothelial origin, as this protein makes up a large portion of vascular intercellular junctions. We found that CD31+ MPs increase by as much as 12-fold (mean of post- vs. predive total MPs multiplied by fraction CD31+); however, some CD31+ MPs could also be derived from platelets, monocytes, and neutrophils.

MPs changes with diving. The results demonstrate that changes in MPs number and surface marker expression patterns with the 18 msw dives are transient and reproducible. That is, values prior to diving on *day 4* were not statistically different from *day 1*, and the patterns seen after dives on *days 1* and *4* were similar. Intravascular phenomena associated with MPs are complex. The mouse model has demonstrated that vascular injuries linked to their presence involve more than merely an increase in number. Following provocative decompression, pro-inflammatory events and vascular damage occur due to enlargement of annexin V-coated particles and/or changes in their surface marker protein pattern (39). Changes in MPs size were not detected following the control 5 msw dive, which is consistent with the nominal increase in hydrostatic pressure and therefore an absence of change in inert

Table 4. *Correlations among bubble scores and MPs number after day 4 diving*

	Resting		Arm Exercise		Leg Exercise	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
80 min	−0.576	0.019	−0.444	0.022	−0.597	0.014
100 min	−0.595	0.015	−0.557	0.025	−0.743	0.0006
120 min	−0.658	0.005	−0.761	0.0002	−0.665	0.005

Spearman rank order correlation coefficients and *P* values were calculated for bubble scores obtained at 80, 100, and 120 min postdive and divers' MPs counts after diving on *day 4*.

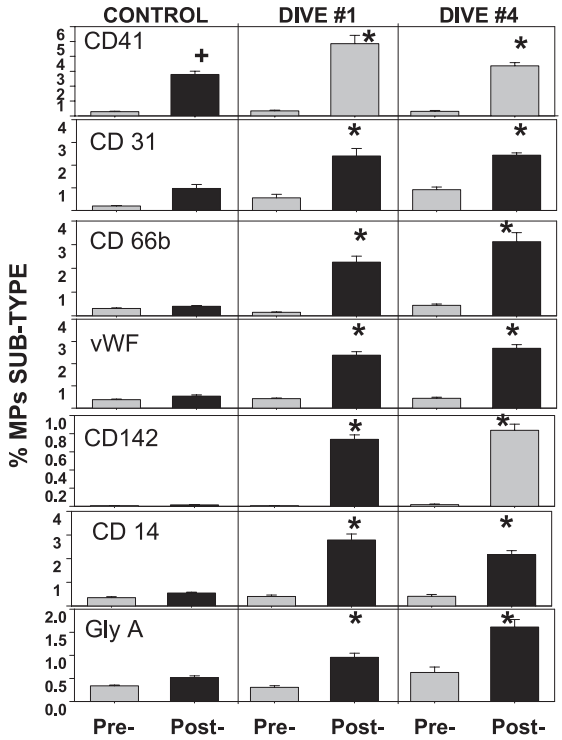

Fig. 3. MPs expressing surface markers for various antigens. MPs expressing the platelet-specific CD41 are shown in the first panel, those expressing CD31 (PECAM) in the second, the neutrophil protein CD66b in the third, von Willebrand factor (vWF) in the fourth, CD142 or tissue factor in the fifth, the common leukocyte antigen CD14 in the sixth, the erythrocyte-specific protein glycophorin A (Gly A) in the seventh. $+P < 0.05$ vs. the pre-5 msw control dive; $*P < 0.05$ vs. pre- and postcontrol dive and predive sample on *day 1* and *day 4*.

gas load. However, after the *day 1* 18 msw dive there was a significant increase in annexin V-positive particles greater than 1 μm diameter, and this enlargement persisted through the pre- and post-*day 4* dive. After diving, the fraction of annexin V particles larger than 1 μm diameter exceeded 20%, about the same proportion as in the mouse model (39). Further work will be needed to assess whether more provocative diving causes greater changes in MPs sizes or surface protein expression pattern in humans.

Whereas the number of MPs sharing surface markers increased after each experimental dive (Fig. 4), the pattern of surface markers expression was roughly the same. That is, the patterns before dives on *days 1* and *4* were not significantly different. Similarly, the patterns after *dives 1* and *4* were quite similar. MPs interactions leading to sharing or blending of surface markers as depicted by data in Fig. 4 have been described ex vivo and we reported their in vivo occurrences in mice (22, 37). Results from this study provide the first evidence supporting an exchange of surface antigens among MPs in vivo in humans. Antigen sharing may have important clinical implications if a general phenomenon. For example, if targeted immunotherapy were considered in situations where MPs elevations occur, such as with inflammatory disorders and neoplasia, antigen sharing may obscure the targeting strategy (14). In this regard, it is notable that the dual-positive MPs populations identified before and after the control dive were one order of magnitude lower than those seen with the 18 msw experimental dives. It is important to stress that serial measurements of this type have not been reported previously. Whereas some variations in flow cytometry measurements can be ascribed to subtle alterations in laser intensity and gating, we expressed our findings as a percent of total, leading us to believe the differences are not due to technical details and that the variability we observed may be normal. The subject divers did not have discernible differences in their exertion, diving, or health status before blood was obtained for the control dive and before initiating the experimental dive series. Perhaps of greater importance with regard to the stress of diving, the magnitude of surface marker sharing postdive was markedly greater with the experimental vs. control dive.

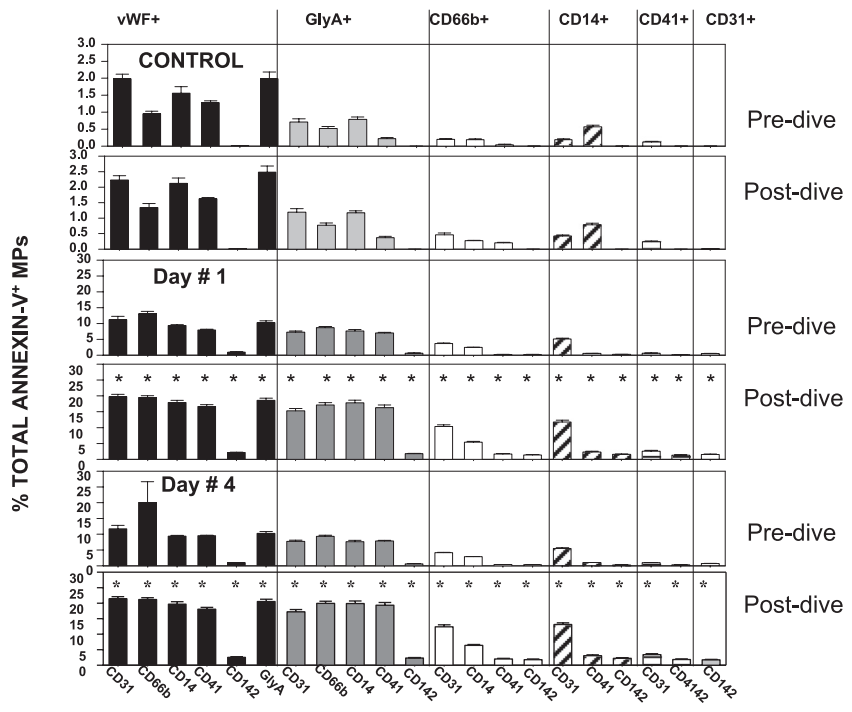

Fig. 4. MPs display multiple cell markers. MPs were stained to detect proteins from vascular cell sources. Data are the percentage of MPs expressing three markers: annexin V, the cell surface antigen shown at the *top* and the antigen shown at the *bottom*. Values are means ± SE. Values pre- and postcontrol 5 msw dive are not significantly different; control values are significantly different from all pre- and postdive values on *days 1* and *4*, $*P < 0.05$ vs. predive value on *days 1* and *4*; the predive values on *days 1* and *4* are not significantly different.

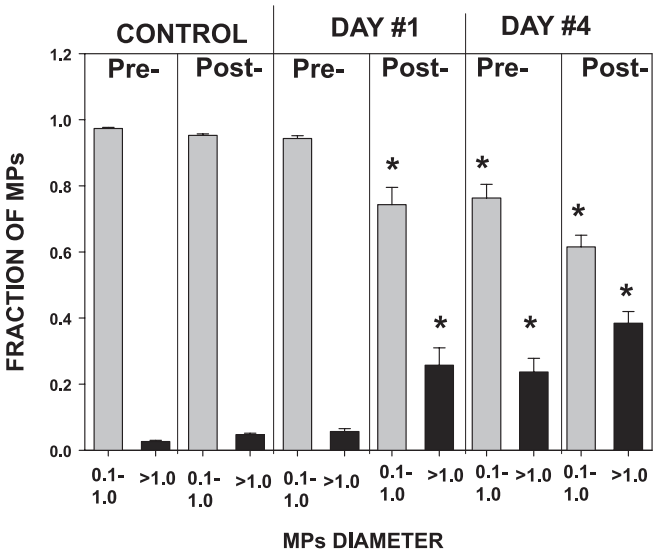

Fig. 5. Fraction of circulating annexin V-positive particles with ≤ or >1 μm diameter. Data show the fraction of particles that are, by definition, MPs (diameters ≤1 μm) or with diameters greater than 1 μm before and after each dive. Data are means ± SE, *$P < 0.05$ vs. the respective particle size for control and pre-*dive 1* values.

Platelet-neutrophil interactions and neutrophil activation. Intravascular neutrophil activation/degranulation occurs when cells interact with platelets. Platelet-derived MPs have been shown to stimulate leukocyte activation and aggregation and MPs can directly stimulate release of pro-inflammatory cytokines (17, 21, 26). Annexin V-positive platelet MPs also exhibit pro-coagulant activity (4). Mice decompressed from ~18 msw as well as after more stressful decompressions exhibit enhanced interactions between neutrophils and platelets or platelet MPs, as assessed by an increase in CD41 on the surface of neutrophils (37). These interactions mediate neutrophil activation documented as elevations in surface expression of β_2-integrins, measured as CD18 staining, and also degranulation, documented by cell-surface MPO because changes are much less prevalent when thrombocytopenic animals are subjected to the same decompression stress (37).

Neutrophil activation following each experimental dive to 18 msw was documented by CD18 staining and cell-surface MPO. MPO on the neutrophil surface can cause auto-activation; results from the murine model have demonstrated that neutrophil activation/degranulation following decompression contributes to generation of MPs from other cells, such as erythrocytes and endothelial cells, and to development of vascular injuries (19, 37). We believe these changes are important elements of decompression stress because the same events occur when MPs from decompressed mice are injected into naive animals (39). Notably, after the control 5 msw dive there also was an elevated proportion of neutrophils that exhibited increased surface expression of MPO. The magnitude of the elevation was small compared with the decompression stress from 18 msw; however, the mean MPO fluorescence on neutrophils did not significantly change after the control dive (Fig. 7). This response could be a consequence of the elevation in MPs expressing platelet-derived CD41 (as shown in Fig. 3) although there was no elevation in total number of circulating MPs. More to the point, this indicates that stimuli other than intravascular bubbles can trigger subtle changes in MPs and neutrophils.

Because there are no data in the literature that provide a comparison between inflammatory responses caused by diving and those triggered by more conventional neutrophil agonists, we carried out a number of additional studies with neutrophils from nondivers (Table 5). The magnitude of neutrophil activation seen among the divers (Figs. 6 and 7) was a fraction of that caused by agonists such as FMLP, PMA, and zymosan, and generally a much higher proportion of the neutrophil population became activated with the chemical agonists. Our use of these agents was not to suggest that mechanisms of activation postdiving are necessarily similar; their use was merely to provide an index for comparison. That various agents cause gradations in neutrophil activation is well established, but few studies have used techniques similar to ours and in whole blood (5). We conclude that the stress associated with

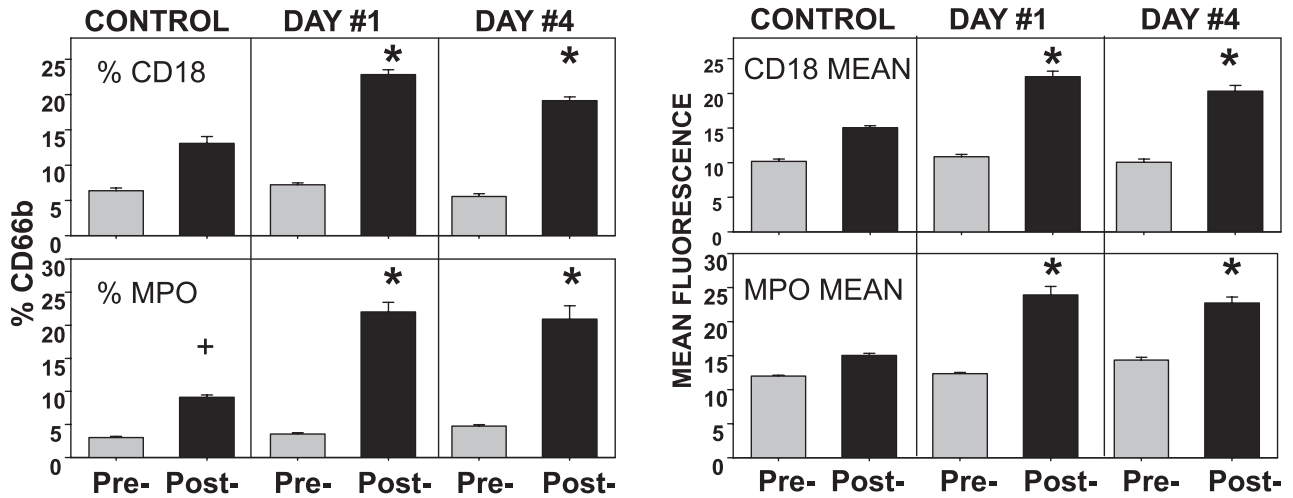

Fig. 6. Neutrophil activation. Neutrophils were identified by CD66b staining and coexpression of CD18 and MPO assessed by flow cytometry. *Left*, percent of CD66b-positive cells expressing a mean fluorescence value ≥ 10 arbitrary units for each surface marker; *right,* CD66b-positive cell geometric mean fluorescence for each marker. *$P < 0.05$ vs. pre- and postcontrol and the predive values on each day, +$P < 0.05$ for precontrol dive value.

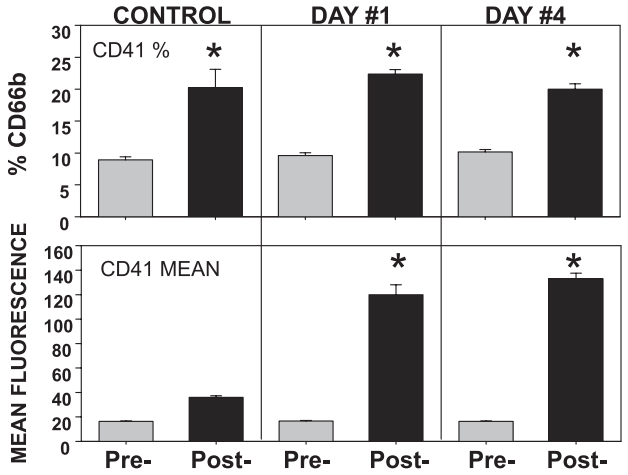

Fig. 7. Evidence of platelet-neutrophil interactions after diving. Neutrophils were identified by CD66b staining and coexpression of CD41, the platelet-specific integrin-α2b protein on the cell surface was assessed by flow cytometry. *Top,* percent of CD66b positive cells (neutrophils) expressing a mean CD41 fluorescence value ≥10 arbitrary units; *bottom*, CD41 geometric mean fluorescence before and after diving. *$P < 0.05$ vs. the predive value on each day.

the control dive causes subtle neutrophil activation characterized by an elevation of the fraction of cells with surface MPO and decompression stress from 18 msw causes a minority (~20%) of neutrophils to undergo mild/moderate activation. The mouse model demonstrates progressive elevations in neutrophil activation with more provocative decompression (37). It is conceivable that gradations occur in humans with decompression stresses and, in the extreme, contribute to the DCS syndrome.

Associations between bubbles, MPs, and neutrophil activation. The prime goal of this investigation was to assess MPs and neutrophil changes following open-water SCUBA diving. On the basis of published findings as discussed in the INTRODUCTION, we hypothesized there may be correlations among divers' bubble scores and blood-borne changes including MPs numbers, annexin V-positive particles larger than 1 μm, with interactions between neutrophils and platelet membranes and/or with neutrophil activation.

Bubble scores at early time points following the experimental dives were greater than those recorded at later time points. Correlations among individual divers' resting bubble scores and bubble scores after arm or leg exercise tended to be significant, especially when they were temporally close. Moreover, there were elevations in bubble scores after exercise at each time point (Fig. 1). The data sets (Fig. 1, Table 3) present a comprehensive analysis of echocardiographic events after open-water diving. The findings are consistent with previous reports following simulated decompression scenarios (7, 8).

We hypothesized that intravascular bubbles may be responsible for generating MPs postdecompression (37, 39). Interestingly, there were significant negative correlations between bubble scores and MPs after each dive (Table 3). Blood was obtained contemporaneous with the bubble score measurement at 80 min postdive. It was only at this time and later measurements following the fourth dive when consistent correlations were found between bubble scores and MPs number. Why there were only a few sporadic bubble scores that exhibited significant correlations with MPs number after the first dive is unknown.

It is clear that circulating MPs increase postdive and the data suggest that a lower MPs count (at least at 80 min postdive) portends higher bubble scores. Additional studies are planned to assess associations between MPs number and intravascular bubbles at times earlier than 80 min postdecompression and also to evaluate changes over time. Most studies of decompression-induced bubbles detected by ultrasound indicate they are in the range of 24 to 160 μm (11–13). In our previous paper, we discussed data supporting the presence of a gas phase in some MPs as the mechanism for MPs enlargement postdecompression (39). Two possible mechanisms were offered to explain this phenomenon: (*1*) a fraction of circulating MPs have a gas phase and thus serve as a nucleation site for bubble growth or (*2*) postdecompression bubbles cause lysis of MPs and some of the ruptured membranes re-anneal around micro-bubbles. Current findings could be interpreted as consistent with either mechanism. That is, MPs destruction may occur because intra-MPs gas expands postdive or if bubble-mediated shear stress causes MPs to rupture.

We also investigated correlations among the various MPs parameters and neutrophil measurements. These parametric data were analyzed with the Pearson product moment,

Table 5. *Neutrophil activation in blood after incubation with various agonists*

	Mean Fluorescence			% Population >10 Units		
	CD18	MPO	CD41	CD18	MPO	CD41
PBS	12.3 ± 1.1	13.9 ± 0.01	15.7 ± 0.3	5.7 ± 0.2	3.0 ± 0.1	7.8 ± 0.1
0.1 μM FMLP	102.8 ± 9.5*	87.4 ± 4.8*	143.9 ± 13.1*	15.3 ± 0.9*	7.5 ± 0.7*	9.7 ± 0.4
1.0 μM FMLP	410.7 ± 21.0*	245.7 ± 35.4*	451.4 ± 63.0*	93.8 ± 4.3*	74.8 ± 2.7*	21.7 ± 1.9*
Zymosan	148.8 ± 14.5*	113.5 ± 2.0*	69.8 ± 6.2*	70.1 ± 2.7*	45.4 ± 2.3*	13.8 ± 1.7*
PMA	433.7 ± 32.7*	302.9 ± 11.2*	52.1 ± 15.2*	53.3 ± 2.2*	44.3 ± 2.6*	18.5 ± 0.8*

Data are maximum values for blood incubated 20 min with either 4 mg/ml zymosan, 0.1 or 1.0 μM FMLP, or 10 ng/ml PMA as described in METHODS. Left shows neutrophils (CD66b positive cell) mean fluorescence for each marker, right shows the percent of CD66b positive cells expressing a mean fluorescence value > 10 arbitrary fluorescence units for each surface marker. *$P < 0.05$ vs. cells incubated with only PBS. Within each column multiple comparisons were performed and significant differences found. For columns showing mean fluorescence data, CD18 expression on neutrophils was significantly different in the following sequence: PMA >1.0 μM FMLP > zymosan =0.1 μM FMLP > PBS; for MPO on cells the sequence: PMA =1.0 μM FMLP > zymosan >0.1 μM FMLP > PBS; and for CD41 on cells the sequence: 1.0 μM FMLP >0.1 μM FMLP > zymosan = PMA > PBS. For columns showing % of neutrophil population with mean fluorescence > 10 units, CD18 expression on neutrophils was significantly different in the following sequence: 1.0 μM FMLP > zymosan > PMA >0.1 μM FMLP > PBS; for MPO on cells the sequence: 1.0 μM FMLP > zymosan = PMA >0.1 μM FMLP > PBS; and for CD41 on cells the sequence: 1.0 μM FMLP = PMA = zymosan >0.1 μM FMLP = PBS.

Table 6. *Correlations between MPs and neutrophils*

Dive 1	FXN MPs<1 μm	FXN MPs >1 μm	PMN-CD41	PMN-MPO	PMN-CD18
MPs#	0.348	−0.348	−0.013	−0.195	0.459
	$P = 0.186$	$P = 0.186$	$P = 0.963$	$P = 0.469$	$P = 0.074$
MPs<1 μm		**−1.000**	**0.553**	−0.145	0.397
		$P < 0.0001$	$P = 0.026$	$P = 0.591$	$P = 0.128$
MPs >1 μm			**−0.553**	0.145	−0.397
			$P = 0.026$	$P = 0.591$	$P = 0.128$
PMN-CD41				0.178	0.153
				$P = 0.510$	$P = 0.572$
PMN-MPO					−0.051
					$P = 0.851$
Dive 4	FXN MPs<1 μm	FXN MPs >1 μm	PMN-CD41	PMN-MPO	PMN-CD18
MPs#	0.226	−0.226	−0.056	**−0.718**	−0.111
	$P = 0.399$	$P = 0.399$	$P = 0.854$	$P = 0.002$	$P = 0.682$
MPs<1 μm		**−1.000**	−0.338	−0.372	−0.311
		$P < 0.0001$	$P = 0.200$	$P = 0.156$	$P = 0.241$
MPs >1 μm			0.338	0.372	0.311
			$P = 0.200$	$P = 0.156$	$P = 0.241$
PMN-CD41				0.180	**0.717**
				$P = 0.506$	$P = 0.002$
PMN-MPO					0.443
					$P = 0.086$

Correlation coefficients (top number) and *P* values (bottom number) for associations among post-*dives 1* and *4* MPs number, MPs fraction ≤ or >1 μm diameter, mean fluorescence value for the platelet CD41 protein on the neutrophil (PMN, CD66b+ cell) surface (PMN-CD41), mean fluorescence value for MPO on the PMN surface (PMN-MPO), and mean fluorescence value for CD18 on the PMN (PMN-CD18). Statistically significant coefficients are shown in bold.

although we recognize that this approach may fail to demonstrate a relationship if an interaction exists but is nonlinear. Given that oxidative stress, MPs size and surface protein patterns all appear to be important for neutrophil activation and postdecompression vascular injuries in the murine model (37, 39), calculations with our data sets must be interpreted with caution. With data from a relatively small sampling of divers, we also believe extensive discussion on analyses showing an absence of strong or statistically significant correlation coefficients has little merit. No significant correlations were identified among data sets from the control dive series.

Data displaying correlation coefficients and *P* values for a number of relationships associated with the 18 msw dives on *day 1* and *day 4* are shown in Table 6. Following the dive on *day 1* reciprocal positive and negative correlations ($\rho = 0.553$) were identified between annexin V-positive particles ≤1 μm, particles >1 μm, and neutrophil surface expression of CD41, a reflection of interactions with platelet membranes ($P = 0.026$). A positive correlation was identified for particles ≤1 μm diameter, suggesting that when this fraction is higher there are more platelet-neutrophil interactions. After the *day 4* dive there was a strong positive correlation ($\rho = 0.717$, $P = 0.002$) between neutrophils expressing platelet-derived CD41 and neutrophil activation assessed by surface expression of CD18. This is consistent with a view that platelet interactions may mediate neutrophil activation, as in the mouse decompression model (37). The relationship is shown in Fig. 8. The only significant correlation coefficient involving total number of MPs was related to MPO on the neutrophil surface after the *day 4* dive ($\rho = -0.718$, $P = 0.002$). The negative relationship–more surface MPO with lower MPs numbers—may suggest that MPs destruction/clearance is a concurrent event with neutrophil degranulation. Although these may be related mechanistically, it is also possible that particle sizes and surface protein expression pattern, as is indicated by the mouse model (39), or myriad other variables, are more important for neutrophil activation vs. merely absolute MPs number. Subtle alterations in MPs may occur with diving stressors other than decompression-induced bubble formation, as is suggested by findings from the control dive. Whether these changes contribute to postdecompression events will require further study.

In conclusion, our results provide additional insight into decompression pathophysiology and therefore they are relevant for SCUBA diving, high-altitude aviation, and space exploration. Questions persist regarding the mechanisms for MPs production and enlargement postdecompression, mechanisms for platelet-neutrophil interactions, and neutrophil activation. Further work will be needed to determine whether the responses identified in this study pose a health risk for divers and whether similar events arise in bona fide DCS.

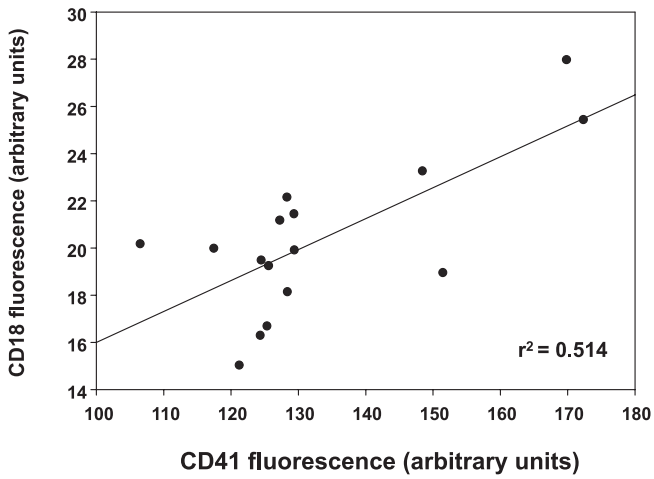

Fig. 8. CD41 mean fluorescence is plotted vs. the CD18 mean fluorescence on CD66b-positive neutrophils after the *day 4* dive. Data for each diver are plotted along with the linear regression line and its r^2 value.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.R.T. and Z.D. conception and design of research; S.R.T., T.N.M., M.B., V.M.B., M.Y., K.B., N.W.P., M.L., P.J.D., and Z.D. performed experiments; S.R.T. analyzed data; S.R.T. interpreted results of experiments; S.R.T. prepared figures; S.R.T. drafted manuscript; S.R.T., N.W.P., M.L., and Z.D. edited and revised manuscript; S.R.T., T.N.M., M.B., V.M.B., M.Y., K.B., N.W.P., M.L., P.J.D., and Z.D. approved final version of manuscript.

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