

Expression of Endothelial Selectin Ligands on Human Leukocytes Following Dive

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The fact that impaired endothelial-dependent vasodilatation after scuba diving often occurs without visible changes in the endothelial layer implies its biochemical origin. Since Lewis x (CD15) and sialyl-Lewis x (CD15s) are granulocyte and monocyte carbohydrate antigens recognized as ligands by endothelial selectins, we assumed that they could be sensitive markers for impaired vasodilatation following diving. Using flow cytometry, we determined the CD15 and CD15s peripheral blood mononuclear cells of eight divers, 30 mins before and 50 mins after a single dive to 54 m for 20 mins bottom time. The number of gas bubbles in the right heart was monitored by ultrasound. Gas bubbles were seen in all eight divers, with the average number of bubbles/cm² 1.9 ± 1.9 . The proportion of CD15+ monocytes increased 2-fold after the dive as well as the subpopulation of monocytes highly expressing CD15s. The absolute number of monocytes was slightly, but not significantly, increased after the dive, whereas the absolute number of granulocytes was markedly elevated (up to 61%). There were no significant correlations between bubble formation and CD15+ monocyte expression ($r = -0.56$; $P = 0.17$), as well as with monocytes highly expressing CD15s ($r = 0.43$; $P = 0.29$). This

study suggests that biochemical changes induced by scuba diving primarily activate existing monocytes rather than increase the number of monocytes at a time of acute arterial endothelial dysfunction. *Exp Biol Med* 233:1181–1188, 2008

Key words: scuba diving, endothelial function, monocyte, sialyl-Lewis x, ultrasound

Introduction

While diving with compressed air, inert gas (nitrogen) is taken up by tissues during a dive proportionally to the depth and the uptake is exponentially related to the time spent under pressure. Upon the diver's return to the surface, this accumulated gas must be eliminated; this process follows an exponential curve, with time constants determined by blood flow to different tissues. If the ambient pressure is reduced faster than the gas can be eliminated, the partial pressure of gas in the tissue will be higher than the environmental pressure. This supersaturation can lead to gas coming out of solution, forming bubbles. Such bubbles grow from so-called bubble nuclei, which are attached to the vessel wall. These bubbles are considered to be the cause of the clinical symptoms associated with decompression sickness (DCS). Once detached from the endothelial cells, bubbles are carried by the bloodstream toward the heart and are finally entrapped in the pulmonary circulation, which serves as a filter to prevent spillover of the bubbles to the arterial side. We have shown previously that even a small bubble load is associated with delayed endothelial dysfunction 6 hours after exposure in the rabbit (1). Since the bubbles are finally trapped in the pulmonary circulation, it was assumed that they would have no further effects on the arterial circulation. However, we have recently shown that

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simulated diving can lead to acute arterial dysfunction in humans (2). This asymptomatic attenuation of the endothelial function of the brachial artery (flow-mediated dilatation) lasted for 3 days after a single air dive with compressed air and was partially prevented by acute- and long-term pre-dive supplementation of antioxidants (3, 4), implicating the negative effects of oxidative stress.

Brubakk *et al.* (5) proposed that vascular bubbles play an important role in the pathophysiology of DCS by altering endothelial cell phenotypes. Endothelial selectin (E-selectin), which mediates leukocyte adhesion and rolling along the vascular wall, is located in lipid microdomains, so-called lipid rafts (6). Endothelial membranes are always prepared for the formation of more stabilized domains and molecular clusters, with enhanced sizes and lifetimes, upon adequate triggering. This concept is a key toward understanding how the raft domains may be involved in the signaling and trafficking of raftophilic molecules; that is, upon an extracellular or intracellular stimulus, the formation of more stabilized rafts may be induced, which might function as a (temporary) platform or scaffold to gather the required molecules for signaling (7). Caveolae are a subset of lipid rafts that are particularly prevalent on the plasma membrane of endothelial cells (8). Caveola are invaginations in the vascular wall and could represent a stabilizing mechanism for bubbles (9). E-selectin is compartmentalized in cholesterol-enriched membrane microdomains; following ligation, it colocalizes with caveolin-1, a marker of caveolae (6).

Serum surface tension has an important role in bubble formation. The results of Hjelde *et al.* (10) indicate that small surface tension differences among individuals may influence vascular bubble formation and that formation of venous gas emboli may itself lower surface tension. Lewis x (CD15) and sialyl-Lewis x (CD15s) are expressed on circulating granulocytes and monocytes and, upon recognition by endothelial selectins, mediate initial leukocyte-endothelium interactions. These two molecules differ significantly in their hydrophilic properties. Under physiologic pH, terminal sialic (neuraminic) acid on the carbohydrate residue of CD15s is dissociated and carries a negative charge of extremely hydrophilic terminal neuraminat (11). The change of CD15s expression must be accompanied by water redistribution and surface tension changes within the blood.

We propose that membrane CD15s and CD15 expression varies during diving and thereby influences surface tension and bubble formation. Since we are aware that the majority of granulocytes express high levels of CD15 and CD15s, and it is already known that blood granulocytes increase significantly after a scuba dive (12), we propose that CD15s and CD15 could influence bubble production. Furthermore, we propose that a postdive increase in granulocytes expressing CD15 and CD15s will occur at a time of endothelial dysfunction. Levin *et al.* (13) have suggested that endothelial-monocyte interaction may be involved in the pathogenesis of DCS. To test our

hypotheses, we performed an analysis of peripheral blood samples to estimate the increase in the granulocyte population that normally carries CD15 and CD15s. Furthermore, we performed flow cytometry analysis of CD15s and CD15 on peripheral blood mononuclear cells (PBMCs) to estimate whether there is any alteration in the membrane expression of those markers. We collected blood samples 30 mins before and 50 mins after a dive to 54 m for 20 mins bottom time.

Materials and Methods

Study Population. All experimental procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Split School of Medicine. Each method and its potential risks were explained to the participants in detail and they gave written informed consent before the experiments, with the opportunity to withdraw at any point with no consequences.

The study was carried out by eight experienced divers (hours of diving ranging from 2000 to 5000) aged 32.4 ± 6.1 years (mean $\pm$ SD). Their height and weight were 1.82 ± 0.06 m and 86.2 ± 6.5 kg, respectively, body mass index was 26.1 ± 2.4 , and body fat index was 19.8 ± 6.8 (% body fat/kg). Mean forced vital capacity (FVC) was $122.3 \pm 14.6\%$ of the predicted values, forced expiratory volume in 1 sec (FEV₁) was $114.0 \pm 17.4\%$, and FEV₁/FVC ratio was $93.2 \pm 9.4\%$. All lung function values were within normal ranges.

At the time of the study, all subjects had a valid medical certificate for diving and had no symptoms of acute illness.

The dives were performed over one day. Figure 1 panel A shows the decompression procedure for this diving protocol. This dive was set to 54 m of sea water, with 20 mins bottom time and a total decompression time of 74 mins.

Field Diving Protocol. All dives were performed in accordance with Croatian Navy and U.S. Navy diving manuals and the divers were equipped with either wet or dry suits ($n = 5$ and $n = 3$, respectively). Six divers were supplied with a dive computer (Galileo, Uwatec, Thalwil, Switzerland) interfaced with a personal computer for later verification of the dive profile (Fig. 1A), sea temperature, scuba tank pressure, and heart rate (Polar Belt, Polar, Oulu, Finland) (Fig. 1B). Exercising during a decompression stop can reduce bubble formation (14); therefore, to avoid this effect, no exercise was performed during the bottom phase and decompression phase of the dive. Sea temperature at the bottom and at the decompression stop was 14° – 15° C for all dives, while outside temperature varied between 13° C and 14° C.

Postdive Monitoring and Bubble Analysis. After completion of the dives, the divers were transported to the facility for observation and monitoring. Subjects were placed in the supine position, and a phase array ultrasonic probe (1.5–3.3 MHz) was placed in a position to obtain a

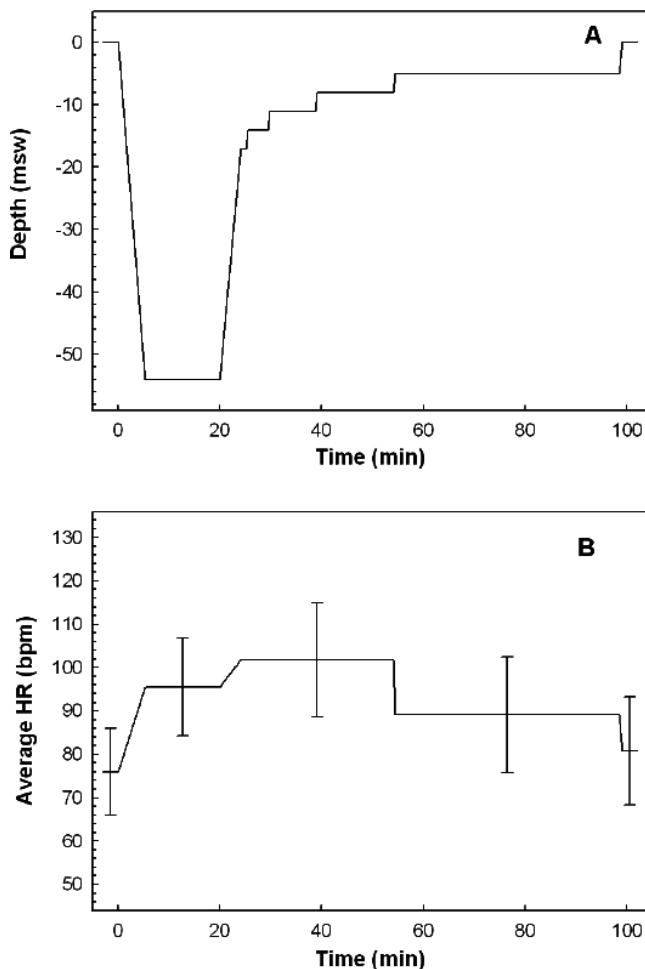


Figure 1. Diving profile (panel A) and average heart rate (HR) profile (panel B) of all divers.

clear view of the right and left ventricles and atria. The transducer was connected to a Vivid 3 Expert ultrasonic scanner (GE, Milwaukee, WI). The same experienced cardiologists performed all echocardiographic investigations. Monitoring was performed 40 mins after surfacing and repeated every 20 mins for a total of 2 hrs. The presence of a patent foramen ovale in our subjects was excluded by ultrasound.

Gas bubbles were observed in the right ventricle as high-intensity echoes. The cardiac images were recorded on S-VHS videotape for 60 secs at rest and after two coughs. The bubbles were graded using the method described by Eftedal and Brubakk (15). This grading system has been used extensively in several animal species as well as in humans. The grading system uses the following definition: 0 = no bubbles, 1 = occasional bubbles, 2 = at least one bubble/4th heart cycle, 3 = at least one bubble/cycle, 4 = continuous bubbling, at least one bubble/cm² in all frames, and 5 = "white-out" (individual bubbles cannot be seen). High-quality images were obtained in all subjects. After grading, the values were transferred to a linear scale (bubbles/cm²) as previously described (16).

Hematology Measurements. Blood samples were collected from the antecubital vein with BD Vacutainer Systems (Becton Dickinson, UK Ltd, Cowley, Oxford, England) 10 mins before and 20 mins after the dive, with subjects in a relaxed supine position.

Blood chemistry (sodium, potassium, chloride, magnesium, lactate dehydrogenase, creatine kinase, creatine kinase MB fraction, troponin I, glucose, lactate, C-reactive protein), and hematology, including white blood cell (WBC) count and differential (neutrophils, lymphocytes, monocytes), red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), platelets, and mean platelet volume, were analyzed several hours after sampling at the University Hospital Split Central Laboratory Department.

The spectrophotometric method was used to measure Hb concentrations (Hb cyanide method, Cell-Dyn 3700 System, Abbot Diagnostics Division, Abbot Park, IL). Hct was calculated from the RBC count and mean corpuscular volume (MCV) with following formula: $(RBC \times MCV) / 10$, and was expressed as a percentage of the whole blood volume. Total blood cell and differential counts were measured using two methods: the impedance count and the optical count by Cell-Dyn 3700 system. Glucose, lactate, sodium, potassium, chloride, magnesium, C-reactive protein, lactate dehydrogenase, creatine kinase, and creatine kinase MB fraction were measured with Olympus tests (Olympus Diagnostics, Cork, Ireland) on an analytical device Olympus AU 2700 (Olympus Mishima Co., Ltd., Shizuoka, Japan). Troponin I was measured on the analytical device Dimension Xpand (Dade Behring, Newark, DE) with an immunochemical method.

Antibodies. CD15s was detected with mouse anti-human CD15s antibody of the IgM isotype (Pharmingen, San Diego, CA) and visualized using secondary fluorescein-isothiocyanate (FITC)-conjugated rat anti-mouse IgM antibody (Pharmingen). Unlabelled mouse IgM (Caltag, Burlingame, CA) was used as an isotype control. Monoclonal mouse anti-human CD15, conjugated with FITC (IQ, Groningen, Netherlands), was used to detect CD15. Monoclonal anti-human CD19 antibody and monoclonal anti-human CD3 antibody, both conjugated with phycoerythrin, were used for the lymphocyte labeling (Pharmingen).

Flow Cytometry. PBMCs were isolated by density gradient centrifugation (Histopaque 1,077, Sigma-Aldrich, St. Louis, MO). Cells (1×10^6) were suspended in 100 μ l of phosphate-buffered saline (PBS) with 0.1% NaN₃ and incubated in the dark for 30 mins on ice with 0.5 μ g of primary anti-CD15s antibodies and/or FITC-conjugated antibody reactive to human CD15. After two washes in 0.1 M PBS with 0.1% sodium azide, 0.5 μ g of secondary FITC-conjugated, affinity chromatography-purified rabbit anti-mouse was added to cells previously incubated with anti-CD15s and incubated on ice for 30 mins. For double lymphocyte labeling, cells were incubated with 1 μ g of phycoerythrin-conjugated antibodies reactive to human

Table 1. Changes in Blood Sample Parameters Before and After the Dive^{a,b}

Parameter	Before dive	After dive
General chemistry		
Glucose (μM)	4.6 $\pm$ 0.6	5.2 $\pm$ 0.8
Lactate (μM)	1.3 $\pm$ 0.3	1.4 $\pm$ 0.4
CRP (mg/l)	1.9 $\pm$ 3.8	1.9 $\pm$ 3.9
Troponin (ng/ml)	0.02 $\pm$ 0.04	0.02 $\pm$ 0.04
Enzymes		
LDH (IU/l)	175.3 $\pm$ 39.5	206.1 $\pm$ 44.8*
CK (IU/l)	158.0 $\pm$ 55.1	242.3 $\pm$ 75.4*
CK MB (IU/l)	4.0 $\pm$ 2.1	11.3 $\pm$ 2.1*
Electrolytes		
Sodium (μM)	137.8 $\pm$ 1.0	139.5 $\pm$ 1.1*
Potassium (μM)	4.8 $\pm$ 0.5	4.3 $\pm$ 0.3*
Chloride (μM)	101.4 $\pm$ 1.7	102.8 $\pm$ 2.8
Magnesium (μM)	0.82 $\pm$ 0.06	0.82 $\pm$ 0.08
Blood count		
WBCs ($\times 10^9/\text{l}$)	6.4 $\pm$ 1.6	8.0 $\pm$ 1.9*
Neutrophils ($\times 10^9/\text{l}$)	3.8 $\pm$ 1.4	5.7 $\pm$ 1.9*
Lymphocytes ($\times 10^9/\text{l}$)	2.3 $\pm$ 0.5	1.8 $\pm$ 0.6*
Monocytes ($\times 10^9/\text{l}$)	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2
RBCs ($\times 10^{12}/\text{l}$)	5.0 $\pm$ 0.4	5.1 $\pm$ 0.3
Hb (g/l)	152.0 $\pm$ 9.9	155.0 $\pm$ 9.2
Hct (%)	0.44 $\pm$ 0.03	0.44 $\pm$ 0.03
PLTs ($\times 10^9/\text{l}$)	257.6 $\pm$ 38.3	271.6 $\pm$ 54.5
MPV (fl)	9.3 $\pm$ 0.8	9.0 $\pm$ 0.9*

^a Data are reported as mean $\pm$ SD for samples from eight divers.

^b CRP, C-reactive protein; LDH, lactate dehydrogenase; CK, creatine kinase; CK MB, creatine kinase MB fraction; WBCs, white blood cells; RBCs, red blood cells; Hb, hemoglobin; Hct, hematocrit; PLTs, platelets; MPV, mean platelet volume.

* Differences between samples; $P < 0.05$.

CD19 and CD3. Finally, cells were resuspended in 0.3 ml of 0.1M PBS with 0.1% sodium azide.

Two-color fluorescence was measured at the excitation wavelength of 496 nm using a FACSCalibur (Becton-Dickinson, San Jose, CA). Fluorescence was further quantified on the forward scatter/side scatter dot plots. A total of 5×10^5 cells were acquired. Nonspecific binding of secondary antibodies was excluded by incubating the cells only with the FITC-labeled secondary antibody.

Statistical Analysis. Data in the text and tables are presented as mean $\pm$ SD. Differences in pre- and postdive values were determined using the Student's t test for paired

samples. Associations between CD15+ monocyte expression and bubble formation and monocytes highly expressing CD15s and bubble formation were evaluated by Pearson's coefficient of correlation. Statistical significance was set at $P < 0.05$. All analyses were done using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK).

Results

All subjects completed the protocol without reporting any DCS signs/symptoms. During the bottom phase of the in-water dive, the divers did not exercise, as is supported by HR data (Fig. 1, panel B). The mean HR during the bottom phase was 95.6 ± 11.2 bpm. Bubbles were observed in the right side of the heart, with none apparent in the left side of the heart. The maximal mean bubble grade over the observation period was 1.9 ± 1.9 bubbles/cm² (without cough) and 2.4 ± 2.2 bubbles/cm² (with cough).

There was a significant increase in total WBCs and neutrophils after the dive, while lymphocytes as well as MPV significantly decreased. There were no significant changes in the RBC and platelet counts (Table 1). Regarding enzymes, there was a significant increase in lactate dehydrogenase, creatine kinase, and creatine kinase MB fraction after the dive.

The proportion of CD15+ monocytes differed significantly before and after the dive. CD15+ monocyte proportion was 38.4 ± 19.3 (mean $\pm$ SD) before the dive, whereas after the dive it increased to 67.3 ± 34.2 ($P < 0.01$; t test) (Table 2). Representative histograms of monocyte labeling with anti-CD15 antibody in representative samples from 2 divers are shown in Figure 2B. Marker M1 delineates the CD15+ population, according to the signal of isotype control (overlay). Furthermore, we observed a significant increase in the small monocyte subpopulation intensively labeled with CD15s after the dive (CD15s^{high}). The proportion of this small fraction was 6.7 ± 4.0 after the dive compared with 3.2 ± 1.4 before the dive ($P < 0.05$, t test, Table 2, Fig. 2A). Monocyte labeling with anti-CD15s antibody in representative samples from two divers is shown in Figure 2A. The CD15s^{high} population is delineated with marker M1, and all positively stained cells, according to the signal of isotype control (overlay), are delineated with marker M2. The proportions of CD15 and CD15s on monocytes and granulocytes of a single diver are shown in Table 3. There were also alterations in scatter properties,

Table 2. Expression of CD15 and CD15s on Different Leukocyte Subpopulations^a

Leukocyte subpopulation CD	Monocytes CD15+	Monocytes CD15s ^{high}	Granulocytes CD15+	Granulocytes CD15s+	Lymphocytes CD3+CD19+CD15s+
Before dive	38.4 $\pm$ 19.3	3.2 $\pm$ 1.4	91.8 $\pm$ 6.0	91.3 $\pm$ 7.6	4.1 $\pm$ 1.2
After dive	67.3 $\pm$ 34.2	6.7 $\pm$ 4.0	90.6 $\pm$ 8.0	87.1 $\pm$ 6.5	5.1 $\pm$ 1.7
P^b	0.0044	0.0195	0.3345	0.1407	0.1152

^a Data are reported as mean $\pm$ SD for samples from eight divers.

^b Bold-faced values are significant.

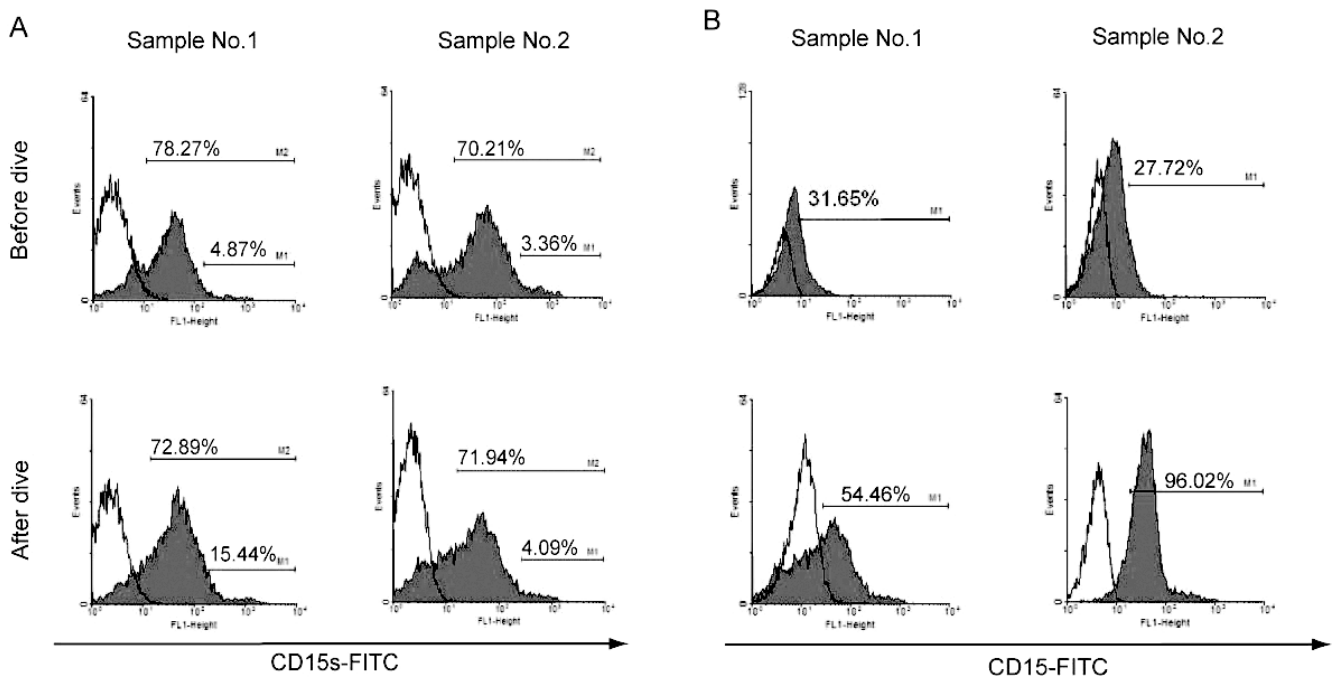


Figure 2. Representative histograms (black) showing monocyte labeling with anti-CD15s antibody in representative samples from two divers. Marker M1 delineates CD15s^{high} population and marker M2 delineates all positively stained cells, according to the signal of isotype control (overlay) (panel A). Representative histograms (black) showing monocyte labeling with anti-CD15 antibody in representative samples from two divers. Marker M1 delineates CD15⁺ population, according to the signal of isotype control (overlay) (panel B).

presented as an increase in forward scatter values of the monocyte fraction, which we observed in samples from 4 divers, who also exhibited the highest increase in the CD15s^{high} monocyte proportion (Fig. 3).

There were no correlations between CD15⁺ monocyte expression and average bubble formation ($r = -0.56$; $P = 0.17$) as well as with monocytes highly expressing CD15s ($r = 0.43$; $P = 0.29$).

The expression of CD15 and CD15s was continuously low on lymphocytes (CD3⁺CD19⁺) and was unaltered on

the residual granulocyte fraction remaining in the PBMC sample (Table 2).

Discussion

In our study, a dive for 20 mins at a depth of 54 m induced a significant increase in creatine kinase and lactate dehydrogenase, indicating possible muscle injury. This occurred even without significant physical activity during immersion and the following decompression, although the divers did perform slight swimming movements. Moreover,

Table 3. Expression of CD15 and CD15s on Monocytes and Granulocytes of Single Diver

Leukocyte subpopulation CD	Monocytes CD15 ⁺	Monocytes CD15s ^{high}	Granulocytes CD15 ⁺	Granulocytes CD15s ⁺
Before dive	12.2	3.6	94.0	93.0
	16.3	4.9	83.6	90.0
	31.7	4.4	85.0	84.5
	46.1	3.4	96.8	94.8
	53.5	3.0	98.3	98.1
	65.4	3.4	97.9	98.0
	27.7	2.7	86.3	95.8
	54.3	0.2	92.2	76.0
After dive	24.4	4.0	78.4	81.1
	20.3	15.4	79.2	94.1
	54.5	7.8	89.4	83.7
	49.5	4.1	90.5	89.4
	99.9	8.2	97.3	88.3
	98.4	7.1	98.3	95.3
	96.0	3.5	98.6	76.1
	95.8	3.8	92.9	88.9

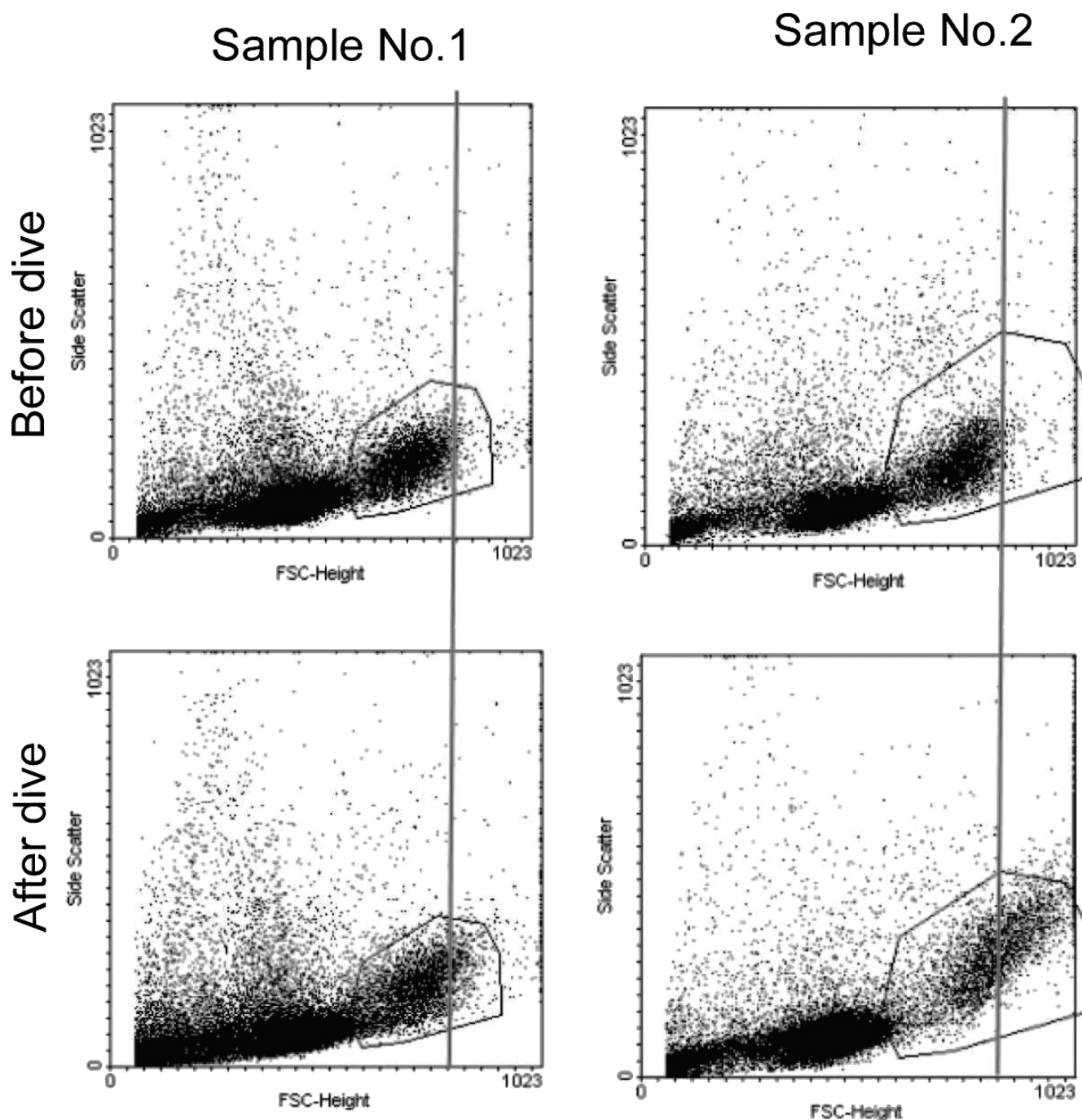


Figure 3. Representative dotplots showing alterations in monocyte scatter properties in representative samples from two divers (with and without change in the monocyte scatter properties). Gate delineates monocyte population. Black line delineates highest forward scatter values in the monocyte population before the dive.

there was a significant increase in total WBCs and neutrophils, which is in accordance with a previous report (17) that suggested a possible role of neutrophils in removing residual cell fragments and in reconstructing damaged muscle fiber. The absence of correlation between bubble formation and CD15s and CD15 expression could be explained by the fact that molecules that are responsible for specific differences between the divers, major histocompat-

ibility molecules, are located in lipid rafts (18) wherefrom they could influence lipid raft clustering. Therefore, the individual differences in lipid rafts clustering could influence bubble formation independently of the increase in the percentage of CD15s and CD15 monocytes following the dive. Still, it is possible that the reason there is no correlation could be caused by different phenomena. For the first time, we have shown the altered expression of

endothelial cell ligands CD15 and CD15s on peripheral blood monocytes, which may be involved in the pathogenesis of DCS. Still, bearing in mind the multifactorial nature of DCS, future studies should investigate this question in greater detail. A specific finding of our study is the 2-fold increase of CD15⁺ monocytes as well as the increase in the small CD15s⁺ monocyte subpopulation. This result corresponds to previous data obtained by Levin *et al.* (13) in dogs with DCS after exposure to a pressure equivalent to that of a 67-m dive. Scanning electron microscopy showed that the endothelium of the jugular veins and the carotid arteries was grossly intact, with no evidence of mechanical damage, but giant cells, derived from monocytes, adhered to the endothelium. Monocytes constitute 5%–10% of total human peripheral blood leukocytes and, after several days in circulation, they enter the tissues as macrophages. Monocytes display considerable heterogeneity in size, granularity, and nuclear morphology, as well as in the expression of cell membrane molecules. This has led to the suggestion that individual monocyte/macrophage populations have specialized functions within their microenvironments (19). Subpopulations of monocytes with higher scatter parameters, high capacity to produce reactive oxygen species, and stronger expression of CD15 and CD15s have been described (20). The size of this subpopulation increases after stimulation with lipopolysaccharide and it is composed of activated monocytes rather than representing a distinct subpopulation originating in the bone marrow. CD15 and CD15s may be present in the cytoplasm, and they could be expressed on the membranes of monocytes (20). These carbohydrate structures are involved in lipopolysaccharide priming of the monocyte oxidative burst. The hyperoxia associated with scuba diving leads to a condition of oxidative stress with increased lymphocyte H₂O₂ production, heme oxygenase-1 expression, nitric oxide synthesis by the inducible isoform of nitric oxide synthetase, and antioxidant enzyme adaptations in order to reduce cellular damage (17). The absolute number of monocytes identified in our study was not significantly changed, whereas a proportion of CD15⁺ and CD15s⁺ monocytes increased 2-fold after the dive. We may conclude that these are highly reactive and inducible subpopulations of monocytes in whole blood that share phenotypic (high CD15 and CD15s expression) and functional characteristics with neutrophils as described by Elbim *et al.* (20). These subpopulations of monocytes are proposed to play an important role in host inflammatory responses through strong production of reactive oxygen species (20).

Normally, about 90% of granulocytes express CD15⁺ and CD15s⁺. Based on the analysis of the residual fraction of granulocytes from the PMBC sample, these percentages were not significantly altered by the dive. However, due to the significant increase in the absolute number of granulocytes (average elevation up to 61%), the capacity for water uptake by hydration of their CD15s ligands is increased, causing spatial and temporal differences in the

surface tension of the vessel wall, which could contribute to bubble formation. Bubble nuclei and their adherence to the endothelium are critical for bubble formation in the blood (5). However, once bubbles become larger, a reduction in surface tension in the blood will reduce the bubbles' adhesiveness to the vessel wall, thus allowing more bubbles to enter the bloodstream (21). Their presence in free-flowing blood must indicate that the adhering forces have been reduced or that the bubbles have grown to a size that allows them to be washed away through the bloodstream. In addition to their effects on water redistribution and surface tension changes within the blood, granulocytes can influence bubble motion from the endothelium. Granulocyte CD15s and E-selectin interactions and bubble formation take place in the same hydrophobic lipid-rich endothelial domains, caveolae and lipid rafts.

In conclusion, the significant change of CD15⁺ monocytes and CD15s⁺ monocytes following a single field scuba dive is not critical for bubble formation, but may be involved in acute postdive-impaired vasodilatation of conduit arteries. The specific mechanisms involved in bubble formation await further examination, with particular attention to the molecules involved in lipid rafts, the site of a monocyte-endothelial selectin interaction. In addition, there were signs of muscle injury, even in this dive with mild exercise, a fact that supports the idea that inflammation may be involved in decompression injury.

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