

# Enhanced aggregability of human red blood cells by diving

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Taylor WF, Chen S, Barshtein G, Hyde DE, Yedgar S. Enhanced aggregability of human red blood cells by diving. *Undersea Hyper Med* 1998, 25(3):167-170.—In vitro studies have shown that mild pressure increases red blood cell (RBC) aggregation. Enhanced RBC aggregation in pathologic states can drive the circulation into stasis. This investigation examined the effects of pressure on RBC aggregation in human volunteers. The hypothesis tested is that RBC aggregation is increased during hyperbaric exposure. Eleven subjects participated in dives to 300 feet of seawater (fsw) in a man-rated chamber complex. Blood samples were taken at the surface, at 66 fsw, and at 300 fsw. Data were analyzed with a repeated measures one-way analysis of variance for a complete randomized design. Statistical significance was achieved at  $P < 0.05$ . Data are expressed as mean  $\pm$  SEM. The median aggregate size (number of RBC/aggregate) of RBCs was significantly increased at depth. At a shear stress of 0.1 dyne/cm<sup>2</sup>, median aggregate size was 12.0  $\pm$  2.1, 33.0  $\pm$  7.3, and 48.8  $\pm$  10.8 at the surface, at 66 fsw, and at 300 fsw, respectively. These results show that mild pressure increases RBC aggregation in the human circulation.

*hyperbaria, erythrocyte, peripheral circulation*

Red blood cell (RBC) aggregation plays a major role in blood flow, particularly in the microcirculation (1). Increased aggregation markedly enhances blood viscosity, which slows blood flow. This in turn further increases RBC aggregation, initiating a self-accelerating cycle leading to "sludge blood", stasis, and ischemia (2,3). Accordingly, impaired RBC aggregation has been implicated in various diseases associated with microcirculatory disorders such as cardiovascular diseases, diabetes, hemorrhagic shock, sickle cell disease, hyperlipidemia, retinal vein occlusion, and thalassemia (1,2,4-7).

High pressure, in the range of tens of atmospheres [1 atm abs = 33.9 feet of seawater (fsw) or 14.7 psi], has been shown to affect various functions, such as ion transport and ATP metabolism (8,9). In a previous study it was shown that in vitro application of hydrostatic pressure of several atmospheres enhances RBC aggregability (10). A pressure of several atm abs is applied to divers undersea or in hyperbaric pressure chambers, as well as to blood cells during routine centrifugation procedures (10,11). The pressure-induced enhancement of RBC aggregability may thus be pertinent to microcirculatory function in operational diving scenarios.

Professional divers have been reported to suffer from impaired microcirculation, specifically retinal vein occlusion and aneurysm (12). Red blood cell aggregation, which is a major determinant in microcirculation and considered

a predisposing factor to circulatory stasis (13), has been directly linked to retinal vein occlusion (12). The elevated RBC aggregability induced by hyperbaric exposure may then be implicated in the microcirculatory disorders observed in divers.

The present study was undertaken to test the hypothesis that mild hyperbaric pressure may increase RBC aggregation in divers. Investigation of this phenomenon will increase our understanding of the role of hyperbaric pressure in erythrocyte function both undersea and during hyperbaric treatment.

## METHODS AND PROCEDURES

Eleven volunteer U.S. Navy certified divers were subjected to simulated hyperbaric pressure in the man-rated chamber complex (MRCC) of the Naval Medical Research Institute. The subjects' physical characteristics were as follows (mean  $\pm$  SEM): height = 178.8  $\pm$  1.6 cm, weight = 90.2  $\pm$  3.1 kg, and age = 31  $\pm$  1 yr. All subjects provided informed consent to participate after being thoroughly acquainted with all procedures and risks. This protocol was approved by the Committee for the Protection of Human Subjects at the Naval Medical Research Institute and at the Naval Medical Research and Development Command. All subjects refrained from caffeine, nicotine, alcohol, and strenuous exercise 24 h before and during these procedures. Each diver received a postdive physical exam and was

found to be in excellent physical health. There were no untoward incidents and no cases of decompression illness.

**Dive profile:** This dive profile consisted of 9 days at depth in the MRCC. Data reported in this manuscript were collected immediately before the dive and on the 1st and 2nd days of the dive (Days 1 and 2 of the dive). The dive began at 0700 h on Day 1 and the MRCC was pressurized to 36 fsw on air then compressed to 66 fsw on helium at 30 fsw/min at 0800 h. At 1600 h on that same day (Day 1) the chamber was compressed to 250 fsw at 10 fsw/min and then 300 fsw at 3 fsw/min with He. The chamber remained at 300 fsw until Day 9 (the final day of the dive) when decompression proceeded according to established Navy tables.

**Red blood cells:** The MRCC was pressurized to 66 fsw (2.95 atm abs) on the morning of Day 1 and then increased to 300 fsw (9.85 atm abs) at 1600 h that afternoon. Blood samples (4 ml) were taken at the surface (1 atm abs) immediately before the dive and at each storage depth, specifically 2 h after arriving at 66 fsw on the Day 1 at 1000 h) and then at 300 fsw at 1000 h of Day 2. Red blood cells were isolated at depth by mild centrifugation, which was not sufficient to induce a significant pressure effect (10), and resuspended in Tris-HCl buffer (pH = 7.4) while still at depth. Red blood cells were suspended at 10% hematocrit in a Tris-HCl buffer (pH = 7.4) supplemented with 1% albumin. Hematocrit was established with precise volumetric dilution of the RBC pellet. Aggregation was induced by the addition of dextran-500 (molecular weight 500,000) to a final concentration of 0.5%, according to a procedure established in previous studies (5,14). Each fraction was then brought to the surface via a small pressure locker at a rate of 1 fsw/min. The rate of decompression of the samples was such that there was no cell lysis in the samples and the samples contained no plasma proteins. Hematocrit was verified with a tabletop centrifuge and RBC aggregation was immediately determined in the laboratory.

Red blood cell aggregation (specifically aggregate size distribution and the shear stress required to disperse the aggregates) was determined in a narrow-gap (30  $\mu$ m) flow chamber fitted with a thermostat to control temperature (37°C) by using customized software and the computerized Cell Flow-properties Analyzer (CFA) as previously described (14,15). Briefly, a CCD camera was mounted on an inverted microscope to record the single layer flow of RBCs through the flow chamber. The shear stress was controlled with a peristaltic pump attached to the flow chamber.

**Data analysis:** Data were analyzed with a repeated measures analysis of variance (ANOVA) for a complete randomized block design. When the results of the ANOVA were significant, differences among data were detected with Fisher's Least Significant Difference Multiple Comparison

Test (16). Values are expressed as means  $\pm$  SEM. Statistical significance was achieved at  $P < 0.05$ .

## RESULTS

Hyperbaric exposure increased RBC aggregation in most divers and markedly augmented it in some of them. A demonstration of this phenomenon is depicted in Fig. 1, which is a micrograph of RBC aggregates as visualized in the CFA, at the surface, and following exposure to 66 fsw. The corresponding aggregate size distribution diagram is also shown in this figure. It reveals that cells are in very large aggregates and aggregate networks after the pressure treatment as compared with the control RBC.

Table shows the median aggregate size for all the divers. As noted above, the aggregation was increased at depth in most subjects and markedly enhanced in some of them. Median aggregate size was significantly increased at 66 fsw and there was no significant difference in the median aggregate size at 66 and 300 fsw. Examination of the data from each subject shows that exposure to hyperbaric pressure produced only a slight effect in those who had high aggregation at the surface (subjects 10 and 11). These data indicate that in most cases further exposure to 300 fsw produced only a small additional increase in the aggregation, suggesting that most of the effect is expressed at

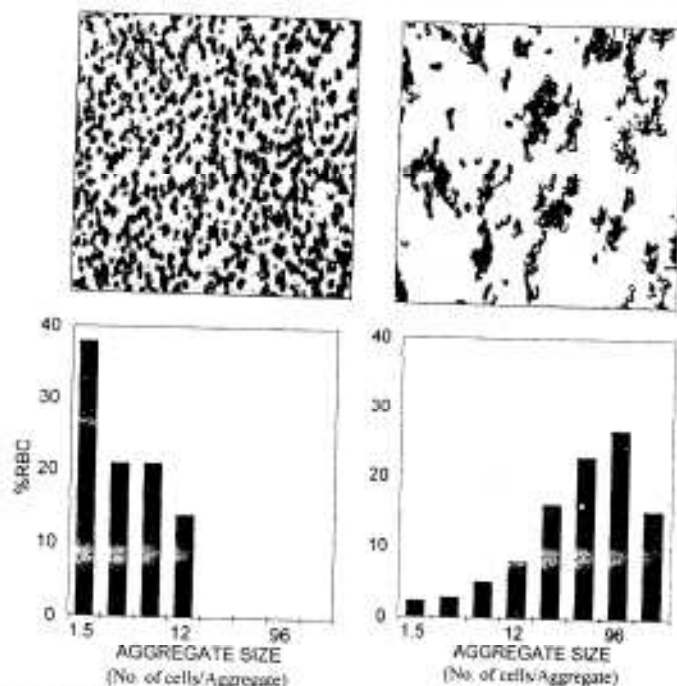


FIG. 1—Micrographs and corresponding size distribution diagrams of RBC aggregates before and after hyperbaric exposure. RBCs were isolated from blood samples drawn from a subject at the surface (left) and at 66 fsw (right), and aggregation was examined at low shear stress of 125 dyn/cm<sup>2</sup> in the CFA. Size distribution diagrams (bottom) depict the percentage of RBC population in an aggregate of the size range indicated in the abscissa (on a logarithmic scale).

**Table 1: RBC Aggregation for All Subjects Before and After Hyperbaric Exposure\***

Subject	Median Aggregate Size		
	Surface	66 fsw	300 fsw
1	2.3	20.2	25.7
2	7.1	50.7	37.3
3	9.4	19.4	26.4
4	8.4	19.4	25.6
5	16.9	40.4	62.2
6	6.2	95.2	97.0
7	11.0	40.0	39.5
8	8.0	15.7	23.4
9	16.8	16.8	135.0
10	23.1	12.0	29.5
11	23.4	33.0	34.6
Mean $\pm$ SEM	12.0 $\pm$ 2.1	33.0 $\pm$ 7.3 <sup>b</sup>	48.7 $\pm$ 10.8 <sup>b</sup>

\*Median aggregate size (the aggregate size above which 50% of RBC population exists) for each subject. Blood samples were taken at the surface, at 66 fsw, and at 300 fsw. RBCs were isolated at depth and subjected to aggregation analysis in the CFA. Median aggregate sizes at 66 and 300 fsw were no different from each other but both were significantly increased compared to the surface measurement.

<sup>b</sup>Significant at  $P < 0.05$ .

shallow depths. As noted in the introduction, enhanced RBC aggregation may facilitate microcirculatory obstruction. In this regard, the number of large aggregates in the RBC population, rather than the median aggregate size, may be more important. Accordingly, the distribution of the RBC aggregates into small (<8 cells/aggregate), medium (9-64 cells/aggregate), and large (>64 cells/aggregate) ranges was analyzed. Figure 2 shows this size

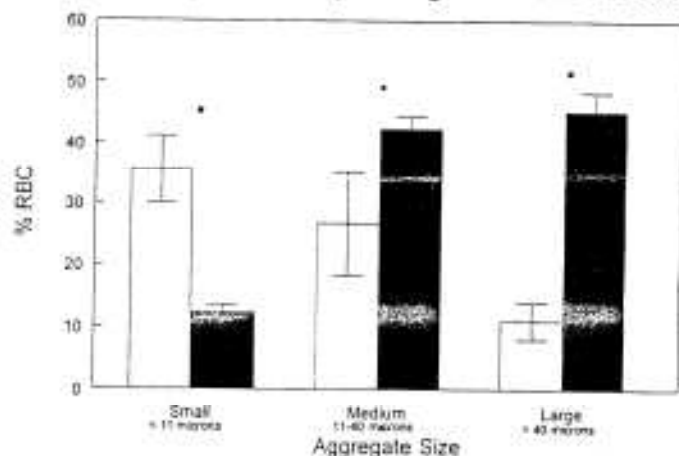


Fig. 2—Distribution of RBC aggregates into size range before and after hyperbaric exposure. Percentage of RBC population in small, medium, and large size ranges, corresponding to <8 cells/aggregate, 8-64 cells/aggregate, and >64 cells/aggregate, respectively. Data represented are mean  $\pm$  SEM for RBCs taken at surface (open blocks) and at 66 fsw (solid blocks). \*Values at depth significantly different from surface,  $n = 11$  ( $P < 0.05$ ).

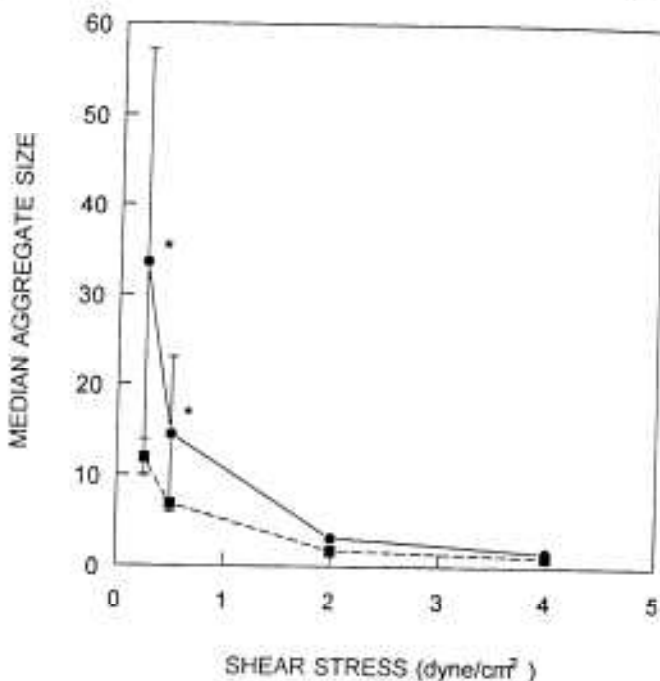


FIG. 3—Effect of shear stress on the aggregation of RBCs taken at the surface and at 66 fsw. Median aggregate size at varying shear stress was determined for RBCs taken at the surface (■) and at 66 fsw (●). \*Values at depth significantly different from surface,  $n = 11$  ( $P < 0.05$ ).

distribution at the surface and at 66 fsw. These data show that after exposure to pressure, over 40% of the cells (and very few of the untreated cells) are in large-sized aggregates, whereas a very small portion is in the low range.

A measure of the RBC aggregability is the shear stress required to disperse the aggregates (5,14). Figure 3 shows that higher than normal shear stress was required to disperse the pressure-treated RBC aggregates, indicating that exposure to pressure might induce the formation of aggregates that are more resistant to disaggregation by the blood flow.

## DISCUSSION

This study shows that exposure of humans to mild hyperbaric pressure at 66 fsw (2.95 atm abs for 2 h) induces a dramatic increase in the aggregability of their RBCs. This result is similar to the in vitro effect previously observed (10). The extent of the effect seems to depend on the basal status at the surface, as an increase in aggregability was minor in those who exhibited high RBC aggregation at surface. Although the subject pool consisted of a group of very specialized U.S. Navy saturation divers, the wide range of responses observed is an indication that no special adaptation has occurred with regard to the aggregability of their red blood cells at depth.

Of special interest is the finding that a dramatic increase in RBC aggregation was achieved at 66 fsw, whereas further exposure to 300 fsw produced a relatively small

additional effect. It seems that the effect is not proportional to the pressure but approaches a plateau at shallow depths. This supports the findings of the *in vitro* study where it was observed that the effect of pressure on RBC aggregability exhibits an asymptotic behavior as a multiplicative function of the level and duration of the pressure applied. In the *in vitro* study, we showed that the enhancement of RBC aggregation is due to hydrostatic pressure (10). In the present study, the hyperbaric pressure was induced in a chamber where inert gas is used to apply pressure. Thus, the distinction between pressure as a mechanical force and partial gas pressure should be considered. In the pressure chamber, fractions of gases are adjusted to maintain the same reactive partial pressure of oxygen and nitrogen at all depths beyond 66 fsw ( $O_2$  0.44–0.46 atm abs,  $N_2$  < 1.0 atm abs, and balance He). Therefore, as depth increases beyond 66 fsw, the He partial pressure increases. If the increase in RBC aggregation is in part caused by an increase in partial pressure of inert gas, one would expect a proportional increase of RBC aggregation at 300 fsw. That was not observed. Together with the *in vitro* experiment, these results suggest that the elevation of RBC aggregability is due to hydrostatic rather than to partial gas pressure, but this remains to be confirmed.

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