Environmental stress on diving-induced platelet activation

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Bosco G, Yang Zj, Savini F, Nubile G, Data PG, Wang Jp, Camproesi EM. Emvironmental stress on diving-induced platelet activation. Undersea Hyper Med 2001; 28(4):207–211.—Platelet activation has been suggested to play an important role in the pathogenesis of prethrombotic states and thus may be responsible for decompression illness during compressed air (scuba) diving. To investigate the effect of physical, mental, and environmental stress on platelet activation during immersion in ice-cold water, we examined 10 male breath-hold divers (BHD), 10 elite BHD (eBHD), and 10 scuba divers during immersion in an ice-covered lake at moderate altitude. Platelet activation was examined by surface expression of activation-dependent glycoproteins CD62p, CD63, and CD42a with flow cytometry 10 min before and 1 min and again 24 h after diving. Plasma epinephrine level was also measured. In addition, the relationship between the activated platelets and the epinephrine level was evaluated. The percentage of platelet activation increased from 2.1 ± 0.4 to 5.7 ± 0.3, 1.8 ± 0.3 to 12.9 ± 0.8, and 3.7 ± 0.9 to 31.2 ± 0.8 in BHD, eBHD, and scuba divers, respectively. The percentage of platelet activation returned to pre-immersion levels in BHD and eBHD divers 24 h after diving, but was still higher in scuba divers. A positive relationship exists between the plasma epinephrine level and the percentage of the platelet activation. This study suggests that physical and mental stress enhance platelet activation during diving in ice-cold water.

ice-cold water diving, flow cytometry, epinephrine, platelet activation

Decompression illness (DCI) generally occurs in occupational groups such as compressed air workers, divers, aviators, and astronauts. The patient may manifest a spectrum of symptoms ranging from minimal to neurologic consequences, including cognitive impairment, motor-sensory dysfunction, or death. Acute changes of hydrostatic pressure may induce the production of circulating microbubbles (1,2). In general, these microbubbles do not produce noticeable illness. Conversely, sickness may occur even after a satisfactory decompression procedure. These facts suggest that not only microbubbles, but other factors may contribute to DCI (3).

Activated platelet may be involved in prothrombotic and pro-inflammatory processes (4) and may be respon-sible for the development of DCI. Activation of the plate-lets may be induced by microbubbles and environmental stress (5,6). Flow cytometry has been widely used as an assay of platelet function, e.g., activation (7). CD62P (also known as P-selectin) is a granule membrane pro-tein. CD62P mediates adhesion of activated platelets to neutrophils and monocytes and has been widely used for detecting circulating activated platelets by flow cytom-etry. Using this method, several studies have found platelet activation in divers subjected to air or nitrox compression (8), in patients with coronary artery disease (9).

Stress always exists during diving. To our knowledge, platelet activation during diving has not been reported. We examined the effect of diving in an iced lake at moderate altitude on platelet activation and bubble production. The changes of blood epinephrine level were also examined, and the relationship between epinephrine level and platelet activation was evaluated.

MATERIALS AND METHODS

Subjects: The study was approved by Chieti University Ethical Committee. Thirty healthy, non-smoking male divers consented to participate in the study: 10 breath-hold divers (BHD), 10 elite breath-hold divers (eBHD), and 10 scuba divers. There were no differences in age, weight, and height among the three groups. The mean age (range), weight, and height for all divers were 35.5 (30–42) yr, 78 (53–98) kg, and 177 (161–193) cm, respectively. All divers were allowed to acclimatize to an altitude of 3,000 m for 15 days.

The study was performed in Verney Lake (2,030 m at Monte Bianco, Italy) with the temperature of water of 0.6–1.2°C. The lake was covered by ice 1.5-m thick. All divers used masks and fins and were dressed in a 5-mm neoprene wet suit with hood, boots, gloves, and a thin neoprene top. The under-ice dives were performed one at a time with assistance of an experienced diver team (six

scuba divers and physicians) for safety purposes. On average, pre-immersion (with head out) time was 15 min for all divers. For the BHD and eBHD groups, three deep breaths were taken with head out and wearing a wet suit without mask before diving. The activity of BHD divers was limited in an open area. The eBHD divers had to dive under the ice between two holes 60 m apart. On average, diving time was 1 min with 60-m diving distance in the eBHD group. The scuba divers dived the same distance and stayed in 15–20 m depth of water for the entire study.

Bubble detection: Bubble detection was performed with Doppler device in all subjects by one of the investigators immediately after they left the water.

Laboratory analysis

Blood sampling and platelet preparation: Blood samples were carefully drawn from an antecubital vein through a 20-gauge needle by a specially trained staff to minimize artificial platelet activation. The samples were collected from all divers 10 min before and 1 min and 24 h after the dive and injected into Vacutainer tubes (Becton Dickinson) containing 3.2% trisodium citrate. The samples were centrifuged at room temperature for 10 min at 200 × *g*. Platelet-rich plasma was obtained with a Pasteur pipette and then fixed by adding an equal volume of 2% paraformaldehyde (vol/vol) in phosphate buffered saline (PBS). The samples were washed twice with 1 ml of PBS.

The fixed platelets were placed in polypropylene tubes containing 10 µl of anti-human CD42a flourescein isothiocyanate (FITC), 10 µl of anti-human CD62p (PE), and 10 µl of anti-human CD63 PE. As a negative control, 10 µl mouse IgG1 PE was added in place of CD63 PE and anti-D62P PE, and 10 µl mouse IgG2a FITC was added in place of anti-CD42a FITC. All samples were immediately placed on ice for 30 min, in the dark. The stained cells were washed twice and the pellet finally resuspensed in 500 µl of PBS and analyzed by flow cytometry.

Flow cytometry: Flow cytometric analysis was performed with a Coulter Elite flow cytometry (Coulter Eletronics, Miami, FL) equipped with a 488-nm helium–neon laser. The flow cytometer was calibrated with microbeads (Coulter) to verify the light scatter and the fluorescence signal reproducibility. Analysis was performed by evaluation of at least 10,000 cells for each sample. Each region of interest (platelet population) was memorized by computer to analyze each platelet sample every time with the same parameters. The platelet population was identified by gating the CD42a-positive cells. The negative and positive delineator was determined by gating 2% background staining on the isotype control fluorescence. The percentage of PE (CD62p and CD63) positive events in this population was then determined. A separate linear gate was used to determine mean PE fluorescence in arbitrary units on a log scale.

Epinephrine: The Amicyl-Testa Epinephrine Radioimmunoassay Kit (MBH Flughafenstrade, Hamburg, German) provides material for the quantitative measurement of enzymatically and chemically derivatized epinephrine in plasma. The sample preparation was performed in two steps. First, epinephrine was extracted from the biological sample by use of a cis-diole-specific affinity extraction. Second, the extracted epinephrine was converted to metanephrine by rat liver catechol-*O*-methyltransferase as enzyme and S-adenosylmethionine as coenzyme and simultaneously derivatized to *N*-acylmetanephrine. The assay procedure followed the basic principle of radioimmunoassays.

Statistical analysis

Data were presented as mean ± SD and analyzed by paired Student's *t* test between the groups, whereas analysis of variance was used to analyze the time-course data within the group. A $P < 0.05$ was considered a significant difference.

RESULTS

We found no significant change in the hematologic profile in all subjects except for a slight increase in red blood cell number, from 4.9 × 106 to 5.3 × 106 (P = not significant) after 15 days acclimation at 3,000-m altitude.

As shown in Fig. 1, there was no different in the percentage of the platelet activation between the groups. The immersion induced a significant increase in the percentage of the platelet activation, from 2.1 ± 0.4, 1.8 ± 0.3, and 3.7 ± 0.9 to 5.7 ± 0.3, 12.9 ± 0.8, and 31.2 ± 0.8 in BHD, scuba, and eBHD, respectively. The percentage of the platelet activation returned to pre-

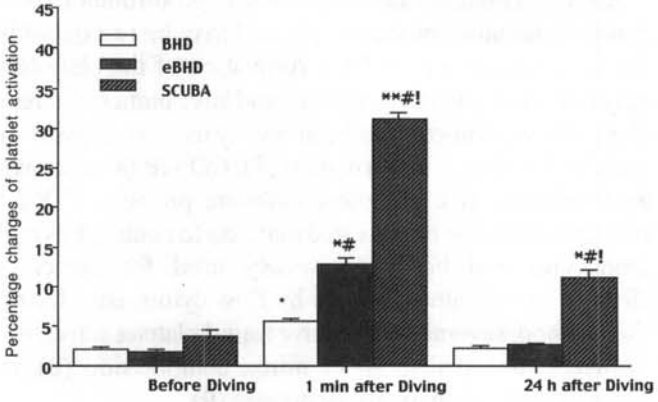

FIG. 1—Percentage of the platelet activation before, 1 min, and 24 h after diving.

immersion level in BHD and scuba 24 h after immersion, being 2.35 ± 0.3 and 2.83 ± 0.2, respectively. In eBHD that dived under the ice with times not less than 90 s and distances between 60 and 80 m, the percentage of the platelet activation was still significantly higher than pre-immersion level (11.3 ± 0.9) 24 h after immersion.

Figure 2 shows epinephrine levels, as stress index, before and after diving. The levels of epinephrine significantly increased from 10 ± 0.6 to 27.1 ± 0.7 pg · ml^{-1} ($P < 0.01$) in BHD and from 9.8 ± 0.8 to 28 ± 0.9 pg · ml^{-1} ($P < 0.01$) in scuba. In eBHD, the epinephrine level increased significantly from 10.1 ± 0.5 to 45.8 ± 0.6 pg · ml^{-1} ($P < 0.001$), the increase was greater than in BHD and scuba divers.

No bubbles were detected in any subjects in all three groups.

DISCUSSION

In our study, the effect of diving in ice-cold water on platelet activation was evaluated in BHD, eBHD, and scuba divers. Our main findings are that 1) the percentage of the platelet activation increased when diving in ice-cold water in all divers; 2) the increase in the percentage of the platelet activation was greater in eBHD divers than scuba and BHD divers; 3) diving in ice-cold water induced a significant increase in blood epinephrine level in all divers; 4) eBHD divers had the highest blood epinephrine level; and 5) no detectable air bubbles were observed in any divers.

The aggregation of blood platelets is essential in maintenance of normal hemostasis. Circulating platelets are sensitive to a large variety of physiologic and non-physiologic stimulants. It has been observed that drastic pressure changes, such as deep diving, may activate platelets, mainly due to the formation of the bubbles. Circulating activated platelets has been reported to be associated with many common clinical disorders such as coronary artery disease (10) and during decompression (8). Our study demonstrates that diving in ice-cold water

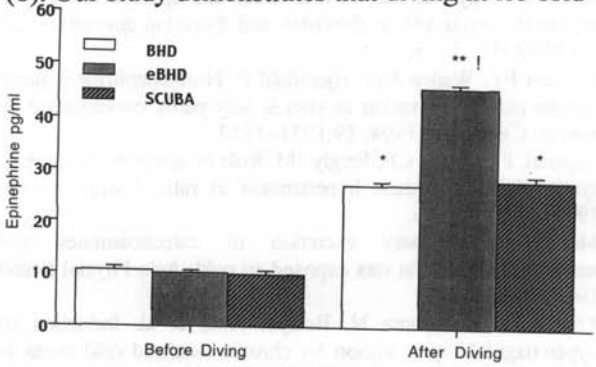

FIG. 2—Epinephrine levels, as stress index, before and 1 min after diving. *$P < 0.05$; **$P < 0.01$ compared to before diving; !$P < 0.05$ compared to BHD and scuba.

at moderate altitude was also associated with platelet activation, and suggests that the platelets may be activated during diving without existence of the bubbles.

Prolonged exposure to a high-altitude hypoxia environment may affect platelet function. Zaccaria and colleagues (11) reported that a 15-day stay at 4,400 m induced a down-regulation of platelet alpha-2 receptors in six men and two women. In our study, no apparent change in platelet function was noticed in any diver who stayed at 3,000 m for 15 days. The difference between the two studies may be due to the different altitude, thus the oxygen partial pressure. According to the Zaccaria study, chronic hypoxia exposure would have no apparent effect on basal catecholamine levels. Therefore, the levels of blood epinephrine before diving in the present study represented the true basal values.

Physical exercise and mental stress have been observed to activate platelet (12,13). The mechanisms underlying stress-induced platelet activation may be due to 1) catecholamine-induced sensitization of platelet (14,15); 2) exercise-induced hyperdynamic circulation, which led to shear-stress (16,17); release of ADP from erythrocytes and platelets during exercise (18), exercise-induced inflammatory reaction (19). The present study provides further evidence that diving in ice-cold water may induce platelet activation. The present study also suggests that the elevated blood epinephrine level may be a contributing factor. Although we did not measure the workload (due to technical difficulty) the divers performed, the intensity of activities was certainly different among three groups. BHD divers were allowed to stay in an open water area and exercised on the surface of water for most of the time. Scuba divers were immersed in the water for most of the time and assisted the eBHD divers. The eBHD divers were required to swim under the ice between two holes 60 m away from each other. Our data are in agreement with other studies that exercise-induced platelet activation may be intensity dependent (20,21).

It is of interest that the more experienced eBHD divers had the highest percentage of activated platelets. Even before diving, the percentage of activated platelets was markedly higher in eBHD divers than in other divers. This is probably caused by mental stress. Mental stress has been reported to induce platelet activation (21). The effect of mental stress is believed to be mainly caused by surges of plasma catecholamines. Endogenous and exogenous catecholamines have significant platelet activating effects (15,22). In the present study, the eBHD divers were instructed before diving that they had to swim under the 1.5-m-thick ice and exit from a hole 60 m away. Visibility under water was limited. The situation

was explained to them and their nervousness was obvious. This potentially dangerous situation induced mental stress and probably contributed to a higher percentage of platelet activation. Our study also suggests that mental stress-induced platelet activation may not be epinephrine dependent. This is in accordance with the notion that neither exercise nor mental stress elevates plasma epinephrine to levels high enough to have platelet-activating effects, and other catecholamines such as norepinephrine are also important for stress-induced platelet activation (21).

Exposure to cold environment (cold stress) stimulates the sympathetic nervous system as evidenced by increased level of blood (23) and urine catecholamines (24). Cold stress per se may activate platelet (25). The present study demonstrates that diving in ice-cold water significantly increased blood epinephrine level and is associated with increased platelet activation. Platelet activation was greater in scuba divers than in BHD divers; however, epinephrine level was no different. Clearly, other factors may contribute to platelet activation and need further investigations.

It is unclear whether cold water diving-induced platelet activation poses any danger to healthy divers. However, enhanced platelet activation has been suggested as one of the contributing factors in the development of most ischemic strokes (26). Platelet activation is also observed in patients with atherothrombosis lesions, arrhythmias (27), and renal insufficiency (28).

In summary, diving in ice-cold water induces activation of the platelet. Physical, mental, and environmental factors all contribute.

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