

Bubble-induced platelet aggregation in a rat model of decompression sickness

Jean-Michel Pontier,^{1,2,3} Nicolas Vallée,^{2,3} and Lionel Bourdon^{2,3}

¹Medicine Department, French Navy Diving School, Toulon Army; ²Institut de Recherche Biomédicale des Armées—antenne Toulon and Institut de Médecine Navale du Service de Santé des Armées, and Underwater and Marine Research Department, Toulon Cedex; and ³Laboratoire de Physiologie et de Physiopathologie en Condition d'Oxygénation Extrême, Université de la Méditerranée, Marseille, France

Submitted 24 December 2008; accepted in final form 15 October 2009

Pontier JM, Vallée N, Bourdon L. Bubble-induced platelet aggregation in a rat model of decompression sickness. *J Appl Physiol* 107: 1825–1829, 2009. First published October 22, 2009; doi:10.1152/jappphysiol.91644.2008.—Previous studies have highlighted that bubble-induced platelet aggregation is a predictor index of decompression sickness (DCS) severity in animals and bubble formation after a single air dive in humans. The present study attempted to investigate plasmatic indexes of the coagulation system and platelet activation in our rat model of DCS. Male Sprague-Dawley rats were assigned to one experimental group with a hyperbaric exposure and one control group maintained at atmospheric pressure. Rats were compressed to 1,000 kPa (90 m saltwater) for 45 min while breathing air. The onset of death time and DCS symptoms were recorded during a 30-min observed period after rats had surfaced. Plasmatic indexes were platelet factor 4 (PF4) for platelet activation, soluble glycoprotein V (sGPV) for thrombin generation, and thrombin-antithrombin complexes for the coagulation system. Blood samples for a platelet count and markers were taken 3 wk before the experimental protocol and within the 30 min after rats had surfaced. We confirmed a correlation between the percent fall in platelet count and DCS severity. Plasmatic levels of sGPV and PF4 were significantly increased after the hyperbaric exposure, with no change in the control group. The present study confirms platelet consumption as a potential index for evaluating decompression stress and DCS severity. The results point to the participation of thrombin generation in the coagulation cascade and platelet activation in bubble-induced platelet aggregation. In our animal model of DCS, the results cannot prejudge the mechanisms of platelet activation between bubble-induced vessel wall injury and bubble-blood component interactions.

bubble formation

WHEN AMBIENT PRESSURE DECREASES, as at the end of a dive, the release of inert gas into the tissues produce bubbles. Generally, bubbles are drawn out from the tissues by the venous bloodstream and then carried to the pulmonary microvasculature, where they are eliminated from the body through the lungs. Depending on the number of bubbles and where they form, acute manifestations could occur, producing decompression sickness (DCS) (21). The occurrence of many bubbles is clearly linked to a high risk of DCS (24).

The presence of bubbles leads to the development of DCS through venous gas embolism and, sometimes, arterial embolism and ultimately vessel obstruction. When severe, one or more of the major symptoms are either respiratory or neurologic in origin (7). In animal models, the symptoms of neurological DCS may include weakness to permanent disability with paralysis, mainly through a spinal cord vascular stroke (6, 23).

As DCS is partly the consequence of the bubble-induced mechanical obstruction of vessels, blood platelets are likely to play a key role in the pathogenesis of the disease (22). Changes in the blood platelet count (PC) after hyperbaric exposure and decompression have been reported in an animal study (30) and in divers without clinical symptom (3, 9, 20, 26, 31, 37–39). In a previous study (33), we highlighted the relationship between the postdive decrease in PC and the magnitude of bubble formation in divers without DCS.

In DCS, experiments have strongly suggested a role for coagulation system activation as disseminated intravascular coagulation (15) and blood platelet aggregation in the pathogenesis of the disease. In a rat model of DCS, Philp et al. (28) demonstrated the central role of platelets in the formation of microthrombi in lung vessels after decompression. Moreover, adherence and aggregation of platelets to the bubble surface have been demonstrated in severe cases of DCS (29). In a previous study (32), we highlighted a relationship between the postdive decrease in PC and DCS severity in a rat model.

Several plasma markers can be used to characterize thrombosis occurring in the arterial network (coronary syndrome, ischemic stroke, etc.) or in veins (deep vein thrombosis, etc.) (2, 4, 5). Three markers have been studied particularly closely in the literature on this subject (1, 18, 34). Platelet factor 4 (PF4) is secreted by platelets, and its presence in the bloodstream can be correlated to the degree of platelet activation (34). The thrombin-antithrombin complex (TAT) and soluble glycoprotein V (sGPV) are both markers that reflect thrombin production (1). sGPV reflects the binding of platelets with the von Willebrand factor receptor when they adhere to the sub-endothelium during normal hemostasis. This marker has been studied in experimental models of acute thrombosis in rats and humans. Its detection is a recognized marker of thrombosis in atherosclerosis, atrial fibrillation, stroke, and myocardial infarction (34).

Few studies so far have examined the relationship between the occurrence of DCS and a thrombotic event resulting from the effects of bubbles on figured elements of the blood and vascular walls. Therefore, the objective of this study was to investigate platelet activation by measuring the PC and PF4, TAT, and sGPV levels in our experimental rat model of DCS (32).

MATERIALS AND METHODS

Study population. Fifty-seven male Sprague-Dawley rats (Charles River Laboratories) weighing 373 ± 18 g (mean \pm SD) were used. Rats were kept at $22 \pm 1^\circ\text{C}$ under a 12:12-h light-dark cycle (lights on at 7:00 AM) with standard laboratory food and water available ad libitum. Before the experiments, rats were housed in an accredited animal care facility. Procedures were in accordance with the European Communities Council rules (Brussels, Belgium) directive of Novem-

Address for reprint requests and other correspondence: J.-M. Pontier, French Navy Diving School, BP 311, Toulon Army 83800, France (e-mail: jm.pontier@free.fr).

ber 24, 1986 (86/609/EEC), as incorporated in French law (Decree 87/848).

Experimental protocol. Rats were randomly divided into two groups: one experimental group with a hyperbaric exposure and decompression procedure protocol ($n = 49$) and one control group without hyperbaric exposure ($n = 8$).

In the experimental group, rats were numbered for identification and individually weighed 30 min before and immediately after diving. They were placed in a cage containing five or six animals, and the whole group was submitted to high pressure in a 200-liter hyperbaric chamber (DCN, Toulon, France). Animals were unrestrained and could move freely inside the cage. They were observed for the duration of the dive via portholes on the chamber for any signs of distress during compression and decompression. Rats underwent the compression procedure at a rate of 100 kPa/min to a pressure of 1,000 kPa (90 m saltwater) maintained for 45 min while breathing air. At the end of the exposure period, rats were decompressed down to 200 kPa at a rate of 100 kPa/min with a 5-min stop at 200 kPa, a 5-min stop at 160 kPa, and a 10-min stop at 130 kPa. Decompression between 200 kPa and the surface was performed at a rate of 10 kPa/min. In a previous study (32) using this hyperbaric exposure and decompression profile, we found that 50% rats died within 5 min after they surfaced with pulmonary DCS symptoms, 32% rats presented neurological DCS symptoms before dying within 24 min, and 18% rats survived with no DCS symptoms (32). CO₂ levels were kept below 300 ppm by the continuous circulation of gases in the chamber through a soda-lime canister. A powerful fan ensured that oxygen was well mixed with the gases. Humidity (40–60%) was controlled with silica gel, and the ambient temperature was adjusted to 27°C to prevent hypothermia and to ensure that the rodents remained comfortable. Light was on during the whole procedure. After decompression, rats were separated by transferring them into individual cages and then weighed. Animals were observed for the time of onset of DCS and pulmonary/neurological symptoms during a 30-min period by a dedicated observer. Symptoms of pulmonary DCS can range from respiratory manifestations such as tachypnea to cardiorespiratory arrest and death, when all movements ceased. Symptoms of neurological DCS included walking difficulties and forelimb and/or hind-limb paralysis.

In the control group, rats experienced exactly the same procedure except that the hyperbaric chamber was maintained at atmospheric pressure for the same duration as the hyperbaric episode.

Blood sampling techniques. To obtain the basal values of plasmatic indexes, samples were collected in all rats (experimental and control groups) 3 wk before the experimental day. This sample was performed by a jugular vein exposure. During anesthesia with an intraperitoneal injection of a mixture of 16 mg/kg xylazine (2% Rompum, Bayer Pharma) and 100 mg/kg ketamine (Imalgène 1000, Laboratoire Rhône-Merieux), animals were kept in a padded box to avoid a fall in body temperature. Their necks were shaved, and they were fixed on the operating board in a supine position. The second sample was collected 30 min after rats had surfaced by an abdominal aortic puncture during anesthesia with an intraperitoneal injection of the same mixture. For measurements of sGPV, PF4, and TAT, a blood sample (900 μ l) was taken carefully into a disposable syringe with CTAD (citrate, theophylline, adenosine, and dipyridamole) and immediately kept on ice to avoid platelet activation. Plasma was obtained within 1 h by an initial centrifugation at 3,500 g and 4°C for 5 min in an Eppendorf tube. The supernatant was centrifuged at 10,000 g and 4°C for 5 min to prepare the platelet-poor plasma, which was stored at minus 80°C until the assay. This procedure has been previously found to minimize platelet activation and PF4 secretion. PF4, sGPV, and TAT were assayed using previously described techniques (26) [Asserachrom sGPV (Stago, Asnières, France) for PF4 and sGPV; and Enzygnost (Dade Behring, Glasgow, DE) for TAT].

For PC analysis, blood samples were drawn sequentially through small incisions at the tip of the rat's tail. To avoid thrombin generation

and PF4 secretion and to minimize platelet activation, samples were not drawn from an arterial puncture. Stroking the rat's tail gently resulted in blood droplets forming at the incision. Up to 20 μ l of blood were collected within 90 s by specially trained staff. Samples were collected into an Eppendorf tube 3 wk before the experimental protocol and within 30 min after the exposure in both groups. Samples were anticoagulated by EDTA (12 mM) to avoid coagulation and assayed for the PC with an Animal Blood Counter (Scil vet ABC, Scil, France).

At the end of the protocol, animals were anesthetized with halothane (5% with O₂, Halothane Belamont) and killed by a lethal injection of pentobarbital (200 mg/kg ip, Sanofi Santé Animal).

Statistical analysis. Data are presented as means \pm SD throughout. For statistic processing, we used the Sigmastat 3.0 software program (SPSS, Chicago, IL). Data were normally distributed. We used non-parametric statistics because of the small sample size. A Wilcoxon signed-rank test was used for paired data, whereas a Mann-Whitney test was used for comparisons between the different groups. Spearman's test was performed for the regression analysis of the percent fall in platelets between decompression and death time. The level of significance was set at $P < 0.05$.

RESULTS

In the experimental group ($n = 49$), death occurred inside the hyperbaric chamber during the decompression phase or immediately after the rats had surfaced in 29 rats. They were excluded from the experimental protocol because of a too short latency time to death for the blood sampling technique. Blood samples for the PC and platelet activation markers were therefore performed in 20 rats (assayed rats).

In this group, blood PC values after hyperbaric exposure significantly decreased compared with the preexposure values ($506 \times 10^3 \text{ mm}^{-3} \pm 145$ vs. $771 \times 10^3 \text{ mm}^{-3} \pm 98$, mean \pm SD, $P < 0.001$) with no significant change in the control group ($682 \times 10^3 \text{ mm}^{-3} \pm 73$ vs. $658 \times 10^3 \text{ mm}^{-3} \pm 130$, mean \pm SD, $P = 0.74$).

Pulmonary DCS symptoms with abnormal breathing, respiratory arrest, and, ultimately, death occurred in 7 of the 20 assayed rats [deceased DCS (D-DCS) group] within the 30-min observation period. Neurological DCS symptoms, including limb paralysis and walking difficulties, occurred in 11 of the 20 assayed rats [neurological DCS (N-DCS) group]. Finally, two rats survived during the 30-min observation period after surfacing with no apparent DCS symptoms.

The percent fall in the PC was different in the D-DCS and N-DCS groups ($49.2 \pm 16.0\%$ vs. $28.6 \pm 10.9\%$, respectively, $P < 0.01$); it was only $20.4 \pm 6.1\%$ in the no DCS symptom group, but the sample size was too small to consider this value significant (Fig. 1).

No changes in the PF4, sGPV, and TAT plasma levels were observed in the control group after the simulated hyperbaric exposure (PF4: 1.3 ± 0.2 vs. 3.0 ± 0.8 ng/ml, $P = 0.289$; sGPV: 0.7 ± 0.4 vs. 5.6 ± 1.9 ng/ml, $P = 0.195$; and TAT: 4.4 ± 1.7 vs. 11.0 ± 2.1 ng/ml, $P = 0.23$). Plasma levels of platelet activation markers measured after the dive were significantly increased (Fig. 2) compared with the pre-dive values in assayed rats for PF4 and sGPV (3.7 ± 0.8 vs. 9.9 ± 2.1 ng/ml for pre-dive vs. post-dive values of PF4, $P = 0.014$; and 4.5 ± 2.0 vs. 17.3 ± 4.5 ng/ml for pre-dive vs. post-dive values of sGPV, $P = 0.011$). The increase observed for TAT was not significant (10.4 ± 2.9 vs. 27.0 ± 8.1 ng/ml for pre-dive vs. post-dive values, $P = 0.053$).

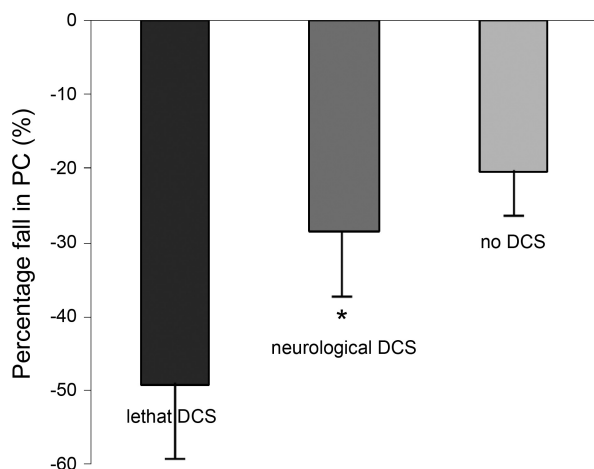


Fig. 1. Fall in blood platelet count (PC; in %) after decompression and decompression sickness (DCS) forms. In lethal DCS, death was preceded by pulmonary symptoms (abnormal breathing and respiratory arrest); in neurological DCS, the symptoms included limb paralysis and walking difficulties; and in no DCS, there were no symptoms during the 30-min observation period. *Significant difference ($P < 0.01$) between lethal and neurological DCS groups.

A negative correlation between PF4 activation marker values and the fall in the PC ($r^2 = 0.30$, $n = 19$, $P < 0.01$) was observed in the assayed rats; in this group, this relation was not observed between sGPV or TAT and the decreased PC.

DISCUSSION

We found that the blood PC measured immediately after the hyperbaric exposure was significantly decreased compared with the prediving values; no change was observed in the control group. This result is in accordance with previous studies (30, 32) in different DCS rat models. Rats suffering from severe DCS with a short latency to death presented a more pronounced decline in the PC than surviving rats. Our results suggest a possible dose-response relationship between DCS severity and the magnitude of the PC decrease. A previous study (19) has demonstrated a relationship between DCS severity and death latency. This result confirms the findings of our previous study (32) in which we suggested that the post-dive PC decrease could be a predictor of DCS severity after decompression in a rat model.

The results showed significantly increased plasma sGPV values in the experimental group after the hyperbaric exposure. This is an index of thrombin generation, which reflects the in vivo platelet activation and thrombotic status (34). In the bloodstream, circulating bubbles damage the vascular wall and activate endothelial cells. Mechanical damage to the vascular wall can go as far as a complete abrasion of the endothelium, revealing collagen and the subendothelial basal layer (25). Most tissues can be affected by these lesions. However, the pulmonary capillary network is the first to be locally affected by the formation of bubbles (7).

Physiologically, the purpose of platelet activation and aggregation is to immediately fill the vascular lesion by forming a clot of platelets. The interaction of platelets with the vascular wall plays a key role in normal hemostasis, ensuring vascular function and preventing hemorrhages in vessels in the micro-circulation. Damage to the vascular wall causes the vessel to

contract, triggers changes in local hemodynamic and rheological conditions, and, in particular, exposes the extracellular matrix of connective tissue. Although the resting endothelial cell presents a thromboresistant surface, the collagen-rich sub-endothelium can cause thrombosis. The effects of hemodynamic forces and von Willebrand factor cause the platelets to adhere to the subendothelium. This is the first stage of hemostasis. Alongside this platelet activation, the coagulation system is also activated. The damaged endothelial cells release tissue factor, which, in conjunction with factor VII, initiates the coagulation process and rapid thrombin formation. Activated platelets secrete the contents of their granules and initiate the arachidonic acid pathway. The ADP secreted and thromboxane A_2 formed induces platelet aggregation via their receptors, the GPIIb/IIIa complex, if fibrinogen is present. This creates a platelet plug, which fills the blood vessel lesion. In certain diseases, however, these mechanisms can be activated in an uncoordinated way and can lead to thrombosis. In DCS, the presence of thrombin is more likely linked to the adhesion of platelets to the vessel's damaged subendothelium. Thrombin, which is a powerful agonist, initiates platelet activation and thus the appearance of aggregates in the vascular system, which, in turn, leads to a thrombotic state, as is the case in disseminated intravascular coagulation (15, 28, 29).

In our experimental group, PF4 significantly increased after the hyperbaric exposure. PF4 is a specific platelet activation marker, and its presence in the plasma is a direct indicator of platelet activation. In vitro, platelet aggregation triggered by nitrogen bubbles causes blood platelets to decrease. The mechanisms of this aggregation are similar to those caused by platelet agonists such as ADP (37). Thorsen et al. (36) have suggested that bubbles are able to activate platelets in vitro by releasing ADP. Observations carried out in vitro using electron microscopes have shown that the induction of platelet aggregation by bubbles is linked to the contact and adhesion of plasma proteins and lipids to the interface between the liquid blood phase and the gaseous bubble phase (37). This process can be modified by pharmacological agents, in particular

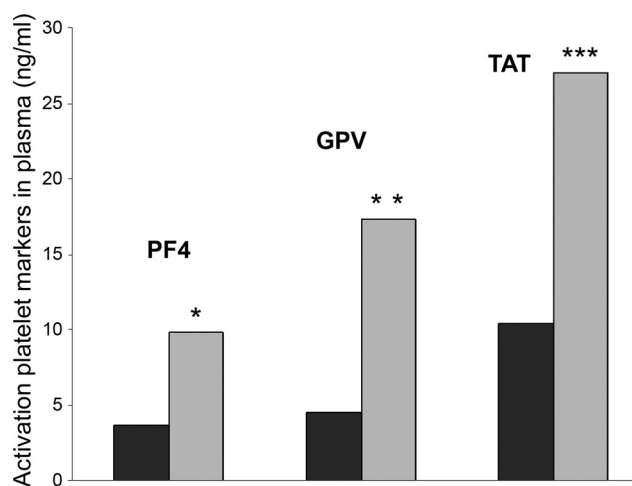


Fig. 2. Changes in the plasma levels of platelet activation markers [platelet factor 4 (PF4), soluble glycoprotein V (sGPV), and thrombin-antithrombin complexes (TAT)] in the experimental group before (solid bars) and after (shaded bars) the hyperbaric exposure at 90 m saltwater for 45 min. Significant differences are indicated as follows: * $P < 0.01$, ** $P < 0.01$, and *** $P < 0.01$.

those that increase the intracellular cAMP concentration in platelets (36).

In vivo, Philp et al. (29) have shown that bubbles in the bloodstream would have the same effects as foreign bodies that come into contact with the figured elements of the blood, leading to platelet adhesion and aggregation around the bubbles. Geller (11) was the first to suggest the possibility of close interactions between bubbles in the bloodstream and platelets, although formal experimental proof was not provided. Others have demonstrated the presence of platelet aggregation around bubbles in the bloodstream during explosive decompression in rats (16) and the formation of platelet thrombi on histological preparations of dog pulmonary tissue after pathogenic decompression (8). Studies have demonstrated that the initial DCS event is linked to agglutination of the formed elements of blood during decompression, and the researchers suggested that the aggregations of platelets then behaved like emboli (10, 14, 17, 30), causing platelet activation and aggregation around the bubbles (29). The interface between the gas and blood could bring about coagulation events, activating the complement system and fibrinolytic cascade (13, 27). At the blood bubble interface, platelets have been observed clustering or sticking to the bubble surface (12, 35).

In conclusion, if DCS is partly the consequence of the bubble-induced mechanical obstruction of vessels, then a thrombotic event could play a key role in the pathogenesis of the disease. The results point to the participation of thrombin generation, a powerful platelet agonist, and platelet activation in bubble-induced platelet aggregation. In our animal model of DCS, the results cannot prejudge between bubble-induced vessel wall injury and bubble-blood component interactions in platelet activation.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Christian Gachet for the very helpful comments about the study and manuscript and to B. Zouani, S. Ciret, and M. Nicolas for the valuable technical help. The blood tests were carried out by Catherine Ravanat of the Institut National de la Santé et de la Recherche Médicale 311 unit at the Haemostasis-Thrombosis Biology and Pharmacology Laboratory (Etablissement Français du Sang, Strasbourg, France), whose director is Dr. Christian Gachet.

GRANTS

This work was supported by the Délégation Générale pour l'Armement (COSSA 2008).

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

1. Aleil B, Meyer N, Wolff V, Kienst D, Wiesel ML. Plasma glycoprotein V levels in the general population: normal distribution, associated parameters and implications for clinical studies. *Thromb Haemost* 96: 505–511, 2006.
2. Arzoa DO, Moog S, Ravanat C. Measurement of GPV released by activated platelets using a sensitive immunocapture ELISA: its use to follow platelet storage in transfusion. *Thromb Haemost* 81: 131–138, 1999.
3. Barnard EP, Weathersby PK. Blood cell changes in asymptomatic divers. *Undersea Biomed Res* 8: 187–198, 1981.
4. Bauer KA. Activation of markers of coagulation. *Baillieres Best Pract Res Clin Haematol* 12: 387–406, 1999.
5. Blann AD, Lanza F, Galajda P, Gurney D, Moog S. Increased platelet glycoprotein V levels in patients with coronary and peripheral atherosclerosis—the influence of aspirin and cigarette smoking. *Thromb Haemost* 86: 777–783, 2001.
6. Broome JR. Association of CNS hemorrhage with failure to respond to recompression treatment—implications for management of refractory cases of decompression illness. *Undersea Hyperb Med Soc* 12: 364–373, 1996.
7. Butler BD, Hills BA. Transpulmonary passage of venous air emboli. *J Appl Physiol* 59: 543–547, 1985.
8. Clay JR. Histopathology of experimental decompression sickness. *Aerospace Med* 34: 1107–1110, 1963.
9. Domoto H, Nakabayashi K, Hashimoto A. Decrease in platelet count during saturation diving. *Aviat Space Environ Med* 72: 380–384, 2001.
10. End E. Blood agglutination in decompression sickness. In: *Underwater Physiology IV. Proceedings of the Fourth Symposium on Underwater Physiology*, edited by Lambertsen CJ. New York: Academic, 1971, p. 235–238.
11. Geller F. Über die Blutgerinnung unter dem Einfluss von Gasen [Coagulation and circulating gas bubble]. *Arch Gesamte Physiol Menschen Tiere* 244: 687–695, 1941.
12. Giry P, Porlier G, Eastman D, Radomski MW. Dive-induced modifications in platelet kinetics in rats. *Undersea Biomed Res* 4: 147–157, 1977.
13. Hallenbeck JM, Bove AA, Moquin RB. Accelerated coagulation of whole blood and cell-free plasma by bubbling in vitro. *Aerospace Med* 44: 712–714, 1973.
14. Hjelde A, Bergh K, Brubakk AO. Complement activation in divers after repeated air/heliox dives and its possible relevance to DCS. *J Appl Physiol* 78: 1140–1144, 1995.
15. Holland HA. *Discussion of Disseminated Intravascular Coagulation in Decompression Sickness. Report 585*. Groton, CT: United State Naval Submarine Medical Center, 1969.
16. Jacobs MH, Stewart DR. Observations on the blood of albino rats following rapid decompression. *US Natl Res Council Comm Aviat Med*: 1–5, 1942.
17. Jacey MJ, Heyder E, Williamson RA. Biochemistry and haematology of decompression sickness: a case report. *Aviat Space Environ Med* 47: 657–661, 1976.
18. Kamath S, Blann D, Chin BSP, Lanza F, Aleil B. A study of platelet activation in atrial fibrillation and the effects of antithrombotic therapy. *Eur Heart J* 23: 1788–1795, 2002.
19. Lillo RS, Parker EC. Mixed-gas model for predicting decompression sickness in rats. *J Appl Physiol* 89: 2107–2116, 2000.
20. Martin KJ, Nichols G. Observations on platelet changes in man after simulated diving. *Aerospace Med* 43: 827–830, 1972.
21. Melamed Y, Shupak A, Bitterman H. Medical problems associated with underwater diving. *N Engl J Med* 326: 30–35, 1992.
22. Moon RE, Fawcett TA, Exposito AJ. Platelet count in deep saturation diving. *Undersea Biomed Res* 19: 279–286, 1992.
23. Newton HB. Neurologic complications of scuba diving. *Am Fam Physician* 63: 2211–2218, 2001.
24. Nishi RY. Doppler evaluation of decompression tables. In: *Man in the Sea*, edited by Lin YC, Shida KK. Honolulu, HI: Univ. of Hawaii Press, 1990, p. 297–316.
25. Nossou V, Koten S, Brubakk AO. Endothelial damage by bubbles in the pulmonary artery of the pig. *Undersea Hyperb Med* 26: 1–8, 1999.
26. Olszanski R, Radziwon P, Baj Z, Kaczmarek P, Giedrojc J. Changes in the extrinsic and intrinsic coagulation pathways in humans after decompression following saturation diving. *Blood Coagul Fibrinolysis* 12: 269–7420, 2001.
27. Pekna M, Ersson A. Complement response to decompression. *Undersea Hyperb Med* 23: 31–34, 1996.
28. Philp RB, Schacham P, Gowdey CW. Involvement of platelets and microthrombi in experimental decompression sickness: similarities with disseminated intravascular coagulation. *Aerospace Med* 41: 1358–1361, 1971.
29. Philp RB, Inwood MJ, Warren BA. Interactions between gas bubbles and components of the blood: implications in decompression sickness. *Aerospace Med* 43: 946–953, 1972.
30. Philp RB. A review of blood changes associated with compression-decompression: relationship to decompression sickness. *Undersea Biomed Res* 1: 117–150, 1974.
31. Philp RB, Benett PB, Andersen JC, Fields GN. Effects of aspirin and dipyridamole on platelet function, haematology, and blood chemistry of saturation divers. *Undersea Biomed Res* 6: 127–145, 1979.
32. Pontier JM, Blatteau JE, Vallée N. Blood platelet count and severity of decompression sickness in rats after a provocative dive. *Aviat Space Environ Med* 59: 761–764, 2008.

33. **Pontier JM, Jimenez C, Blatteau JE.** Blood platelet count and bubble formation after a dive to 30 msw for 30 min. *Aviat Space Environ Med* 79: 1–4, 2008.
34. **Ravanat C, Freund M, Mangin P.** GPV is a marker of in vivo platelet activation study in a rat thrombosis model. *Thromb Haemost* 83: 327–333, 2000.
35. **Tanoue K, Mano Y, Kuroiwa K.** Consumption of platelets in decompression sickness of rabbits. *J Appl Physiol* 62: 1772–1779, 1987.
36. **Thorsen T, Ovstedal T, Vereide A, Holmsen H.** Effects of platelet antagonist on the reduction in platelet density caused by microbubbles in vitro. *Undersea Biomed Res* 13: 289–303, 1986.
37. **Thorsen T, Dalen H, Bjerkvig R, Holmsen H.** Transmission and scanning electron microscopy of N₂ microbubble-activated human platelets in vitro. *Undersea Biomed Res* 14: 45–58, 1987.
38. **Valeri CR, Feingold H, Zaroulis CG, Sphar RL, Adams GM.** Effects of hyperbaric exposure on human platelets. *Aerospace Med* 45: 610–616, 1974.
39. **Ward CA, Mc Culloch D, Fraser WD.** Effect of mild pressure profiles on bubble induced platelet aggregation. *Undersea Biomed Res* 19: 51–53, 1992.

