Very Few Exercise-Induced Arterialized Gas Bubbles Reach the Cerebral Vasculature

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ABSTRACT

BARAK, O. F., D. MADDEN, A. T. LOVERING, K. LAMBRECHTS, M. LJUBKOVIC, and Z. DUJIC. Very Few Exercise-Induced Arterialized Gas Bubbles Reach the Cerebral Vasculature. *Med. Sci. Sports Exerc.*, Vol. 47, No. 9, pp. 1798–1805, 2015. **Introduction**: Arterialization of venous gas emboli (VGE) formed after surfacing from SCUBA diving can become arterial gas emboli (AGE) through intrapulmonary arterial-venous anastomoses that open with exercise. **Methods**: We recruited twenty patent foramen ovale–negative SCUBA divers and conducted a field and a laboratory study with the aim of investigating the appearance of AGE in intracranial vessels. At the field, they performed a single dive to a depth of 18-m sea water with a 47-min bottom time and a direct ascent to the surface. Transthoracic echocardiography was used to score VGE and AGE, and transcranial Doppler was used to visualize middle and posterior cerebral arteries with automated objective bubble detection. Observations were conducted for 45-min after dive at rest and at the laboratory after agitated saline injection at rest and throughout an incremental cycle supine exercise test until exhaustion and for 10 min of recovery. **Results**: After resurfacing, all divers presented endogenous VGE and arterialization was present in three divers. Saline contrast injection led to AGE in nine of 19 subjects at rest. AGE that reached the cerebral arteries after dive were recorded in two divers at 60 W, three at 90 W, five at 120 W, six at 150 W, and four at 180 W and in three, four, five, nine, and nine, respectively, after saline contrast injection in the laboratory. All divers had AGE grades of 1 or 2, and only single AGE reached the cerebral vasculature. **Conclusions**: These data suggest that few emboli of venous origin reach the brain through exercise-induced intrapulmonary arterial–venous anastomoses but cerebral embolization is not high risk in the studied population. **Key Words:** SCUBA DIVING, GAS EMBOLI GRADING, ARTERIAL GAS EMBOLI, INTRAPULMONARY ATERIAL-VENOUS ANASTOMOSES, TRANSCRANIAL DOPPLER

During a SCUBA dive, breathing at increased ambient pressure results in augmented tissue gas uptake. Because of decrease in ambient pressure during ascent, gas supersaturation commonly occurs, allowing dissolved inert gases to be eliminated from the tissues, resulting in the formation of venous gas emboli (VGE). Asymptomatic VGE are common after resurfacing from SCUBA diving and are normally trapped and eliminated in the pulmonary microcirculation during gas exchange and ventilation (19). Arterialization, i.e., the crossover of these emboli from the right to the left side of the circulation (arterial gas emboli (AGE)) can appear because of intracardiac septal defects (like patent foramen ovale (PFO)), passage through the pulmonary microcirculation or large intrapulmonary arterial–venous anastomoses (IPAVA) (6,9). To have a crossover in PFO-negative divers, one has to produce sufficient endogenous bubble load and have open shunts (21). IPAVA are closed at rest but open during submaximal and maximal exercise in 90% of humans without intracardiac shunts (22). A recent study on SCUBA divers concluded that exercise directly increases vulnerability to arterialization of VGE after diving (26). However, this study did not address the question of whether this arterialization is right heart bubble load dependent.

AGE can reach the cerebral circulation and be trapped in the brain tissue, causing neurological injuries, which is one of the most serious decompression-related problems in sport divers (38). Contrast transcranial Doppler (TCD) has been

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extensively used to detect right-to-left shunts by visualizing AGE as microembolic signals (MES) in cerebral arteries, although TCD by itself does not reveal the anatomic origin of the shunt (5,15,37). In the diving community, attempts have been made to connect right-to-left shunts to neurological forms of decompression sickness (DCS) with inconclusive outcomes (3,10,14). One of these studies was designed to follow postdive arterializations but did not succeed in visualizing AGE in the cerebral vasculature (11). In this study, shunting was defined as the appearance of bubbles in the left heart within five cardiac cycles of the right heart opacifying, thus suggesting intracardiac passage of contrast. Four of their divers had evidence of these shunts on the TCD after saline injection (three of them only after provocative Valsalva maneuvers), and no bubbles were detected in the cerebral circulation after surfacing.

To our knowledge, no article that quantified AGE count in the cerebral arteries after exercise-induced IPAVA recruitment has yet been published. Having in mind that divers do exercise after resurfacing (e.g., carrying heavy equipment), AGE might pose higher risk by reaching the cerebral vasculature. In this study, we wanted to revisit the question of to what extent divers shunt after resurfacing and do they create a sufficient number of AGE that might reach the cerebral vasculature. We also wanted to see how workload-dependent IPAVA recruitment changes AGE formation and how these bubbles reach the CNS. As some dives might result in minimal VGE load, it would make potential open shunts impossible to detect through the observation of arterializing gas emboli. Using saline contrast injection as a "control," the large bolus of bubbles provides better means to detect open shunts through arterialization and sorts out divers who shunt, regardless of the amount of bubbles they produce after dive. As endogenous bubble production and shunting after saline injection might not go hand in hand, comparison of the two conditions will allow us to assess individual scenarios and see how they affect the appearance of bubbles in the cerebral circulation.

METHODS

Subjects

Twenty-five healthy male divers volunteered to participate in the study. After screening for PFO, five of them were excluded as positive and 20 PFO negative were recruited for the study. PFO testing was conducted by a certified anesthesiologist and a trained ultrasonographer using previously described procedures (21). All subjects were nonsmokers, were not receiving any medication, and were apparently healthy with no record of respiratory or circulatory disease. A written informed consent was acquired on the first visit when anthropometric measures were taken and pulmonary function was measured (Table 1). This study was approved by the University of Split School of Medicine ethics committee, and all procedures were conducted in accordance with the Declaration of Helsinki.

TABLE 1. Anthropometric and pulmonary function data.

Parameter	Mean ± SD
Age (yr)	35.2 ± 8.5
Height (cm)	185.8 ± 5.3
Weight (kg)	91.1 ± 11.4
FVC (L)	6.50 ± 0.86 (120.7 ± 15.3)
FEV_1 (L)	5.10 ± 0.67 (115.5 ± 13.5)
FEV_1/FVC (%)	79.63 ± 6.22 (97.9 ± 7.1)

Values in parentheses are percentage predicted.
FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

All preliminary measurements were taken 1 wk before the experiment. Before testing, anthropometric measurements of height and weight were determined. Pulmonary function assessment included forced vital capacity test carried out on Quark b^2 (COSMED, Rome, Italy).

Field Study

Diving protocol. The study was performed at a military base of the Croatian Navy Force. The dive site was about 30 m from the location where the experiments took place. All divers performed a single dive at a depth of 18 m sea water (msw) with a 47-min bottom time. Decompression was performed at a rate of 9 msw·min^{-1}, with direct ascent to the surface. Sea temperature at the bottom was 16°C. Throughout the dives, divers performed swimming of moderate intensity. Each diver was accompanied with a safety diver, and both of them were equipped with a diving computer (Uwatec Galileo sol; Johnson Outdoors, Inc., Racine, WI) interfaced with a laboratory computer for later verification of the diving profile.

Transthoracic echocardiography. Within 10 min of surfacing, the divers were placed in the supine position, and an apical four-chamber echocardiography with harmonic imaging and ECG guiding was performed (Vivid Q; GE, Milwaukee, WI) by the same ultrasonographer who performed the PFO tests. Initial bubble images were recorded 15, 30, and 45 min after surfacing, followed by recordings at the end of each exercise stage and at the end of a 10-min recovery after exercise cessation.

TCD. A 2-MHz pulsed Doppler ultrasound system (Spencer Technologies ST3, Seattle, WA) was used for objective automated bubble counting throughout the experiment (45-min postdive rest, incremental exercise until exhaustion, and 10-min postexercise recovery). TCD probes were mounted over the temporal window of the middle cerebral artery (MCA) on one side and the posterior cerebral artery (PCA) on the other side. In half of the subjects, MCA was registered on the right size and PCA on the left and *vice versa*. MCA was insonated at about 1 cm distal to the MCA and anterior cerebral artery bifurcation, and PCA at the P1 segment, corresponding to the segment between the tip of the basilar artery and the posterior cerebral collateral artery (40). Standardized search techniques were used to optimize signal quality, as recently described (40). The probes were fixed and held in place using a specialized headband fixation device (Marc 600; Spencer Technologies).

Supine exercise protocol. After 45 min of postdive rest, subjects remained in the supine position, and their feet were placed on the pedals of an electronically braked cycle ergometer (900 ERG; Marquette Hellige Medical Systems, Milwaukee, WI). When a clear image of the heart for transthoracic echocardiography (TTE) was obtained, participants began the exercise. They completed a single incremental exercise test with a starting workload of 60 W and a 30-W increase every 2 min until exhaustion, followed by 2 min of cooldown and 10-min supine recovery.

Laboratory Study

Contrast TTE (cTTE). Nineteen participants underwent a contrast echosonography test under laboratory conditions. A 20-gauge catheter was placed in the left cubital vein, and a three-way stopcock was attached with two 10-mL syringes connected to its ports. One syringe contained 1-mL air, and the other contained 5-mL sterile saline mixed with 1 mL of the participants' blood. Flushing the saline solution from one syringe to the other created the contrast bubbles, and they were forcefully injected as a bolus while images were taken simultaneously in the apical four-chamber view (8,23). The agitated saline was injected at rest and at the end of each exercise stage, and 20 ECG-guided cardiac cycles were recorded for further analysis (Vivid E9; GE, Milwaukee, WI). The delayed appearance of bubbles in the left heart (after >3 cardiac cycles) was indicative of their transpulmonary passage (8,22). Alongside contrast TTE, TCD bubble count was recorded and the exercise protocol from the field study was replicated under laboratory conditions.

Analyses

Bubble grading (BG). Cine-loops were retrospectively analyzed in the laboratory by two independent and previously trained observers. The grading system used the following definition according to the modified scale by Eftedal and Brubakk (7): 0, no bubbles; 1, occasional bubbles; 2, at least one bubble/fourth heart cycle; 3, at least one bubble/heart cycle; 4, continuous bubbling, with at least one bubble per square centimeter in all frames; and 5, "white out," where individual bubbles cannot be seen (7,30). Recently, a subdivision of grade 4 into the following grades was proposed (19): 4A, continuous bubbling, with one to two bubbles per square centimeter in all frames (same as the current grade 4); 4B, continuous bubbling, with at least three bubbles per square centimeter in all frames; 4C, almost complete white out in the right heart, but individual bubbles can still be discerned; and 5, complete white out.

MES. The absolute number of MES in the MCA and PCA was noted for the postdive resting period, for each stage of the exercise protocol and the postexercise recovery, and for each 2-min period, agitated saline was injected during the laboratory study.

Statistics. Descriptive and physiological data are presented as mean ± SD. Analysis of bubble scores was made using the Friedman test with Dunn multiple comparison posttest. Significance was set to $P < 0.05$.

RESULTS

Field study. All twenty divers successfully completed the diving protocol, and no signs of DCS were observed or reported. Figure 1 shows the bubble grade values of the field study assessed in the right (VGE) and left heart (AGE) at rest 30 min after surfacing, at different levels of cycling exercise, and after recovery. No statistical difference in VGE grades was found at different exercise levels, but there was a significant decrease in the recovery phase ($P = 0.012$). Higher AGE grades were present only at the 150-W workload compared with resting values ($P = 0.028$), and there were no significant differences among other workloads. Arterialization was not present after recovery. Table 2 shows individual data of VGE, AGE, and MES obtained at the field study and during cTTE. Thirty minutes after surfacing at resting conditions, all divers presented VGE with a range of 1–4B and arterialization was present in three divers who had bubble grades over 4A. At rest, MES was recorded in the MCA only in one diver with VGE grade of 4B and AGE grade of 2, and he did not continue with the exercise protocol. The incidence of arterialization increased with increasing workloads (four divers at 60 and 90 W, six at 120 W, eight at 150 W, and six at 180 W, with the notion that two of them could not complete the whole exercise protocol because of volitional exhaustion). Arterialization during exercise appeared mainly in divers with 4A VGE grades, but in three cases, it was present in divers with VGE grade 3 as well. MES was recorded in two divers at 60 W, three at 90 W, five at 120 W, six at 150 W, and four at 180 W. All these divers had AGE of grades 1 or 2. The total amounts of MES in the MCA and PCA were two, three, five, seven, and five at the different workloads, respectively. Separate MES in MCA and PCA are presented in Table 2.

Laboratory study. Some of the divers did not present high VGE load, which would make potential open shunts

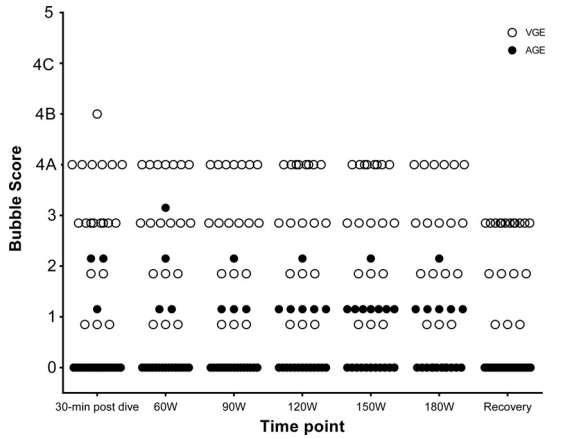

FIGURE 1—Bubble grades of the field study at rest 30 min after surfacing, at different levels of cycling exercise, and after recovery.

TABLE 2. Individual data of bubble grades and MES in the cerebral arteries obtained at the field study and during laboratory cTTE.

	Rest				60 W				90 W			
Subject	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L
101	1	0/4A	0/0	0/0	1	0/4B	0/0	0/0	1	0/4C	0/0	0/0
102	2	0/0	0/0	0/0	2	0/3	0/0	0/0	3	0/3	0/0	0/0
103	4A	0/0	0/0	0/0	4A	0/0	0/0	0/0	4A	0/0	0/0	0/0
104	3	0/2	0/0	0/0	4A	0/3	0/0	0/0	4A	0/3	0/1	0/0
105	3	0/2	0/0	0/0	2	0/2	0/0	0/0	2	0/3	0/0	0/0
106	3	0/0	0/0	0/0	3	0/2	0/0	0/0	3	0/3	0/0	0/0
107	4A	0/0	0/0	0/0	4A	0/0	0/0	0/0	4A	1/0	0/0	1/0
108	4A	2/3	0/0	0/0	4A	3/4A	0/0	0/1	4A	2/4B	0/0	0/1
110	3	0/3	0/0	0/0	2	0/4B	0/0	0/0	2	0/4B	0/0	0/0
112	4A	1/—	0/—	0/—	4A	2/—	0/—	1/—	4A	1/—	0/—	1/—
113	1	0/0	0/0	0/0	1	0/0	0/0	0/0	1	0/0	0/0	0/0
114	3	0/2	0/0	0/0	3	0/3	0/0	0/0	3	0/4A	0/0	0/0
115	1	0/2	0/0	0/0	1	0/4A	0/0	0/1	1	0/4A	0/1	0/0
117	4A	0/2	0/0	0/0	4A	0/3	0/1	0/1	4A	0/—	0/—	0/—
118	4A	0/0	0/0	0/0	4A	1/3	0/0	1/0	4A	1/4A	1/0	0/0
120	4B	2/0	1/0	0/0		—	—	—		—	—	—
121	3	0/0	0/0	0/0	3	0/3	0/0	0/0	3	0/3	0/1	0/0
122	3	0/0	0/0	0/	3	0/0	0/0	0/0	3	0/3	0/0	0/0
123	3	0/0	0/0	00/	3	0/0	0/0	0/0	2	0/0	0/0	0/0
124	2	0/3	0/0	00	3	1/4A	0/0	0/0	3	0/4A	0/0	0/0

	120 W				150 W				180 W				Recovery			
Subject	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L	VGE	AGE/ AGE_L	MCA/ MCA_L	PCA/ PCA_L
101	1	0/4C	0/0	0/0	1	0/5	0/0	0/0	1	0/5	0/0	0/2	1/0	0/0	0/0	0/0
102	3	0/3	0/0	0/0	3	1/3	2/1	0/1	3	1/3	2/2	1/1	3/0	0/0	0/0	0/0
103	4A	0/1	0/0	0/0	4A	0/3	0/1	0/1	4A	0/3	0/0	0/1	3/0	0/0	0/0	0/0
104	4A	1/3	0/0	1/1	4A	1/3	0/2	1/1	—	—	—	—	3/2	0/0	0/0	0/0
105	2	0/4A	0/0	0/0	2	0/4B	0/0	0/1	2	0/4C	0/1	0/2	3/2	0/0	0/0	0/0
106	3	0/3	0/0	0/0	4A	1/3	0/0	1/0	4A	1/3	0/0	0/0	3/0	0/0	0/0	0/0
107	4A	1/0	0/0	1/0	4A	1/0	0/0	1/0	4A	1/1	0/1	1/0	3/0	0/0	0/0	0/0
108	4A	2/4B	0/0	1/1	4A	2/4B	1/1	0/2	4A	2/4B	0/1	0/2	3/0	0/0	0/0	0/0
110	2	0/4B	0/0	0/0	2	0/4B	0/—	0/—	2	0/—	0/—	0/—	2/0	0/0	0/0	0/0
112	4A	1/—	0/—	0/—	4A	1/—	0/—	1/—	4A	1/—	0/—	0/—	3/—	0/—	0/—	0/—
113	1	0/NA	0/0	0/1	1	0/NA	0/1	0/1	1	0/NA	0/1	0/1	1/0	0/0	0/0	0/0
114	3	1/4A	0/0	1/0	4A	1/4B	0/0	0/2	4A	1/4B	1/1	0/0	2/1	0/0	0/0	0/0
115	1	0/4B	0/0	0/1	1	0/4B	0/1	0/1	1	0/4B	0/1	0/1	1/0	0/0	0/0	0/0
117	4A	1/—	1/—	0/—	4A	1/—	0/—	0/—	3	0/—	0/—	0/—	3/—	0/—	0/—	0/—
118	3	0/4A	0/0	0/0	3	0/4A	0/0	0/0	3	0/4A	0/0	0/0	3/2	0/0	0/0	0/0
120		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
121	3	0/3	0/2	0/1	3	0/3	0/2	0/0	3	0/—	0/—	0/—	3/0	0/0	0/0	0/0
122	3	0/3	0/0	0/0	3	0/3	0/0	0/0	3	0/3	0/0	0/0	3/0	0/0	0/0	0/0
123	2	0/0	0/0	0/0	2	0/0	0/0	0/0	2	0/0	0/0	0/0	2/0	0/0	0/0	0/0
124	3	0/4A	0/0	0/0	3	0/4A	0/2	0/1	3	0/4A	0/1	0/1	2/0	0/0	0/0	0/0

AGE, arterial gas emboli; AGE_L, AGE after cTTE; MCA, bubble count in the MCA; MCA_L, bubble count in the MCA after cTTE; PCA, bubble count in the PCA; PCA_L, bubble count in the PCA after cTTE; "—", confer that data were not collected; VGE, venous gas emboli.

impossible to detect through the observation of arterializing gas emboli. To account for this, we repeated the same protocol in the laboratory with cTTE. The injection of a large bolus of bubbles provides better means to detect open shunts through arterialization. Figure 2 shows the bubble grade values of the cTTE test assessed at the left heart (AGE) at rest, at different levels of cycle exercise, and after recovery (with VGE grades). Nine of 19 subjects arterialized at rest. A statistically significant difference in AGE grades was present at every exercise stage ($P < 0.05$). No arterialization was found after recovery, but VGE were still present in four divers. In Table 2, next to the results of the field study, VGE, AGE, and MES obtained during cTTE are presented. No MES were recorded at resting conditions in any of the divers. All but one diver arterialized by the completion of the exercise protocol. MES was recorded in three divers at 60 W, four at 90 W, five at 120 W, nine at 150 W, and nine at 180 W. AGE grades varied between 3 and 5. The total amounts of MES in the MCA and PCA were four, four, seven, 22, and 20 at the different workloads, respectively. Separate MES in MCA and PCA are presented in Table 2.

DISCUSSION

In this study, we wanted to revisit the algorithms that connect VGE grades with arterialization after exercise-induced IPAVA recruitment and the appearance of arterialized gas bubbles in the cerebral vasculature. The main findings of this study were as follows: 1) incremental exercise did not increase postdive VGE production and subsequent AGE loads, but cTTE showed that exercise opens dynamic IPAVA in a load-dependent manner; 2) even lower VGE loads lead to arterialization at higher exercise levels; 3) emboli of venous origin can reach the brain through exercise-induced IPAVA; 4) postdive VGE loads were not large enough to lead to sufficient arterialization and a high number of gas emboli in the cerebral vasculature; 5) even high VGE loads with contrast bubble injection and increased arterialization at different workloads did not lead to large MES count in the brain.

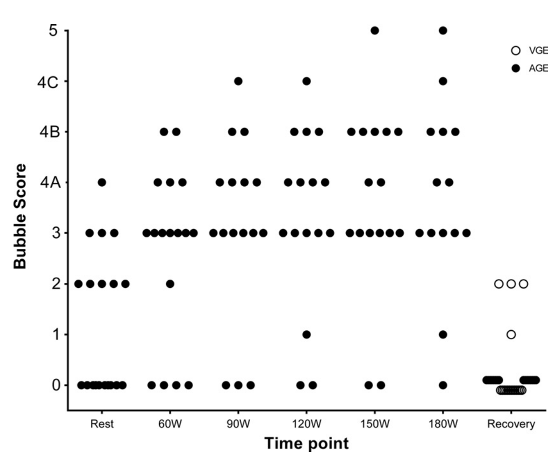

FIGURE 2—Bubble grades of the laboratory study with cTTE at rest, at different levels of cycling exercise, and after recovery.

BASIC SCIENCES

Intensity of exercise and AGE appearance. The traditional view that put VGE in the spotlight for being responsible for causing decompression injuries has already been disputed. Indeed, only weak positive correlation between VGE count and DCS exists. As our group indicated earlier, significant bubble loads are necessary for VGE to cross over to the arterial side of the circulation (20,21). Apart from presenting high bubble scores after dive, for the VGE to arterialize, one has to have open communications between venous and arterial circulation either at the level of the heart or in the lungs. Intracardiac shunts like PFO have usually been linked to increased VGE arterialization, and they are still considered an important risk factor for the development of DCS (4). The investigation of extracardiac shunts in the diving community has only lately received more focus after extensive research on the opening of IPAVA (8,22,24). According to these studies, large-diameter vessels in the lungs are closed at rest in most individuals but increasingly open as exercise intensity increases. A study conducted at our laboratory revealed that postdive exercise increases VGE arterialization and AGE were observed in the left heart after exercise intensities varying between 23% and 92% of $\dot{V}O_{2max}$ and cleared after 1 or 2 min after exercise cessation (26). In this study, exercise was suspended at workloads where pulmonary shunting was displayed in this study. We tried to extend the question to see whether IPAVA recruitment is workload dependent; that is, the AGE grade will increase with further exercise intensity increments after the point of initially observed arterialization. Although incremental exercise did not increase postdive VGE production, it increased the incidence of arterialization with higher workloads but not the AGE loads. The absence of arterialization or low AGE grades in earlier studies from this laboratory were explained with substantially low right heart bubble loads, suggesting that arterialization is load dependent (6,19). Without changes in the VGE grade throughout postdive exercise, no changes in arterialization could be expected in this study.

Low VGE grades at the field study might have left us with false conclusions about low bubble producers that potentially open shunts that cannot be detected through the observation of arterializing gas emboli. To address this potential shortcoming, we repeated the same protocol in laboratory conditions using saline cTTE and demonstrated that exercise opens dynamic IPAVA in a load-dependent manner. A similar finding was acknowledged by Eldridge et al. (8) who demonstrated qualitatively greater density of contrast bubbles in the left ventricle at increasing workloads. Although cTTE is sensitive in detecting intrapulmonary shunts at rest (12), concerns are still present when this method is used during exercise; that is, contrast bubble size and pulmonary microcirculation morphology changes (8). The increasing bubble grades in the left heart with increasing workloads would support the hypothesis that workload determines the magnitude of IPAVA recruitment. Whether this magnitude involves increased number or size of the shunts is still not known. Earlier works had shown that pulmonary capillary distention is estimated to not exceed 20 μm at peak exercise intensities (39) and with the size distribution of injected contrast bubbles entering the pulmonary microcirculation of 60–90 μm does not offer an explanation of bubble passage to the arterial circulation. Whether similar changes in size happen to pulmonary AV shunts through exercise needs to be further explored. We failed to draw the same conclusion from the field study because of low and unchanged VGE loads throughout the incremental exercise. This might mean that in addition to workloads that determine IPAVA recruitment, VGE loads are also an important issue to consider for arterialization of gas bubbles.

As opposed to this notion, an interesting finding of this study was that even lower VGE loads could lead to arterialization at higher exercise levels. From previous experiments, we have observed that all cases of postdive arterialization were always associated with high VGE grades at rest (19,21). One of 20 divers in this experiment arterialized in postdive resting conditions, and increasing workloads increased the incidence of divers arterializing. Three of these divers displayed a crossover of VGE at bubble grades of 3 in the right heart. Madden et al. (26) have also shown that open IPAVA during postdive exercise may provide a potential path to arterialization at even lower VGE loads. The gradual increase of cardiac output (and pulmonary artery pressure to a lesser extent) during incremental exercise has been shown to correlate with the opening of IPAVA (16,36). Why do low VGE counts lead to arterialization? We hypothesize that to arterialize with a lower VGE grade, one would have to have a higher magnitude of IPAVA recruitment. Yet, with the current technical capabilities of TTE, lack of morphological visualization of these shunts prevents us from drawing solid conclusions on whether anatomical of physiological (cardiac output or pressure) changes allow bubble crossover with low VGE loads.

Cerebrovascular embolization with AGE. The main question of this experiment was to what extent the arterialized gas bubbles reach the cerebral vasculature. Although postdive arterialization was observed in three divers at rest and appeared in six more divers after exercise-induced IPAVA recruitment, the AGE load was not substantial enough to lead to a larger MES count in the cerebral arteries. Surprisingly, when the AGE load was much higher after agitated saline administration at the same exercise stages, MES count did not differ much from the postdive conditions; that is, even with large shunt openings at higher workloads, MES count did not correlate with the arterial bubble grades.

Efforts have been made to connect arterialization of VGE with DCS, which is the main decompression-related problem in SCUBA diving. Gerriets et al. (10) could not confirm an association between brain lesions and the presence of right-to-left shunts in sport divers. They screened 42 divers for shunts with echocontrast TCD, and of the 16 divers who were positive, only three of them arterialized after dive. More interestingly, they detected only one hyperintensive

brain lesion with 1.5-T magnetic resonance imaging but in a diver with no evidence of right-to-left shunts. Knauth et al. (14) on the other hand found multiple brain lesions exclusively in divers with PFO of high hemodynamic relevance. Major right-to-left shunts were associated with increased incidence of cochleovestibular and cerebral DCS (3). They were using contrast TCD defining major right-to-left shunts as >20 hits and found that almost 60% of divers who had DCS were shunting. After analyzing subgroups of DCS, they showed an even higher incidence of shunters who had neurological forms of DCS. A similar screening method (cTCD) coupled with TTE was used to screen for PFO in divers, of whom 15 had neurological forms of DCS and eight of them were proven positive (13). Intracardiac shunts were diagnosed with contrast TCD and TTE in four of 17 divers, yet no bubbles were observed after dive (11). Most of the aforementioned studies defined the existence of right-to-left shunts after the appearance of bubbles in the left heart within five cardiac cycles of the right heart opacifying, indicating more toward intracardiac shunting and neglecting the possibility of late appearance of bubbles observed in intrapulmonary shunts. In our study, seven of 20 divers arterialized after dive and six of them had MES at some point of the experiment. After agitated saline injection, all but one subject arterialized and nine of them had MES in the cerebral arteries. In light of these results and the aforementioned studies, the issue of the sensitivity of TCD needs to be considered when looking for gas bubbles in the cerebral arteries. In clinical studies, TCD was shown to be superior to direct imaging of the heart for passage of bubbles (5,37) through intracardiac shunts. The Valsalva maneuver increased the number of MES even more (5) and made cTCD recommended as the first choice for PFO screening (15). When intracardiac shunts are excluded, the number of MES drops significantly. This could be explained with closed IPAVA at rest in most individuals, but even with exercise recruitment, the MES count does not reach those observed with intracardiac shunts. The use of TCD to account for arterialized gas bubbles after dive is even more tenuous, having inconsistent VGE loads to begin with. Low bubble producers have lower probability to arterialize, but given a high enough VGE load with cTCD may show as shunt openers, overriding the low probability for VGE crossover. In this study, two divers (number 101 and 115) seemed to belong to this group of divers and both of them had MES in the cerebral arteries only after saline injection. On the other hand, high bubble producers after dive might not be major shunt openers (subject number 103 and 107), yet at high exercise levels, they also showed MES.

We also addressed the potential regional differences where bubbles might end up after crossing over to the arterial side of the circulation by observing the anterior (through MCA) and posterior (through PCA) part of the cerebral vasculature. The rationale behind this idea was the data from clinical and epidemiological studies showing distinct regional distribution of ischemic lesions in different vascular regions. In most of the studies, the anterior circulation was the most common site of arterial occlusion (MCA occurring with 51%–64%) and only 7% had posterior embolization (PCA) (28,29). Having observed only one MES after dive and none after agitated saline injection at rest, we could not relate our results to the aforementioned studies. Although the incidence of DCS remains very low (0.01%–0.019% in recreational divers), gas emboli can end up in almost any part of the brain (38). A case control study on 101 consecutive dive accidents revealed that major right-to-left shunts were associated with increased incidence of cochleovestibular and cerebral DCS (3). Clinically manifested cerebral embolism is still just the tip of the iceberg. Regardless of the importance of different vital and less vital brain regions, subclinical cerebral gas embolism is an important issue in the diving community. In a cohort study of multiple brain lesions in sport divers, Knauth et al. (14) detected 41 brain lesions with magnetic resonance imaging in 11 of the 87 divers. In the absence of reported neurological types of DCS, that study supported the hypothesis that these lesions are the consequence of subclinical cerebral gas embolism. It is important to mention that most of the multiple lesions were observed in divers with verified PFO, but in seven of them without right-to-left shunts, single lesions were also present. Exact locations of these lesions were not presented in the article, but they were also explained as results of subclinical embolism. The authors acknowledge that although a large PFO seems to be a risk factor in developing multiple brain lesions, other cofactors are likely to play a role. In our study, divers were PFO negative and we were able to observe cerebral gas emboli, supporting the notion that emboli of venous origin can reach the brain through IPAVA.

With exercise-induced IPAVA recruitment and the increased incidence of arterialization, the number of MES also increased but did not follow the expected pattern of different vascular territories. Higher numbers of embolic hits were observed in the PCA at all stages of exercise. Nevertheless, this observation should be taken with caution because the number of MES was low and the differences were presented as absolute sums in all the divers. The differences would have been more representative had we found AGE hits in individuals presenting distinct territorial differences. Another important issue to consider is the change in cerebral blood flow during exercise. Although the ratio of anterior versus posterior cerebral circulation is approximately 73 to 27 (internal carotid artery (ICA) vs vertebral artery (VA)) at rest, a shift toward increased posterior circulation is observed at higher exercise intensities (33). Recent studies of cerebral blood flow distribution during exercise (27,35) observed linear increase in cerebral blood flow up to 60%–70% $\dot{V}O_{2max}$. Above these exercise levels, blood flow in the ICA and the MCA decreased but kept rising in the VA and the PCA. According to these data, we were expecting regional differences in MES appearance in MCA and PCA after exercise-induced IPAVA recruitment in SCUBA divers. Yet, the distribution of MES in the MCA

and PCA did not line up with the load-dependent changes in cerebral blood flow distribution.

Because body posture strongly affects intracranial hydrodynamics and cerebral hemodynamics (1), we should not omit to point out that our measurements were carried out in the supine position and that might have also had some influence on the territorial distribution of MES. Alongside cardiac output (18), cerebral blood flow is greater when supine compared with that when seated or upright, with larger differences being reported between ICA and VA blood flow in the supine than those in the seated position (32,33). Neither the smaller diameter nor the minimal cerebral blood flow in the posterior segment of the cerebral circulation could explain the larger number of MES observed in the PCA.

With exercise, the intrapulmonary shunts open and one would expect an increase in the number of MES. In neither of the studies we conducted did we observe significant increase of bubbles in the cerebral arteries, even though there was evident crossover of VGE and increase of AGE grades with higher workloads. Even when a grade 5 AGE load was observed at high workloads (subject number 101), only two MES were recorded in the cerebral arteries. The potential explanation for this controversy is that the larger cardiac output with increased exercise loads will redistribute blood flow mainly to the skeletal muscles and reduce the chance of MES to appear in the cerebral vasculature. At rest, approximately 15% of cardiac output reaches the brain and just approximately 20% reaches the skeletal muscles. During exercise, the fraction of cardiac output distributed toward the brain drops to 3%–4% (even though absolute values increase) and increases up to 95% to skeletal muscles at maximal exercise (17,31). Evidently, future experiments are needed to visualize AGE distribution in other vascular compartments with exercise-increased cardiac output.

It is important to mention that besides arterialization of VGE, bubbles in the arterial circulation can arise from a tear of the pulmonary parenchyma with entry of gas into pulmonary venous outflow. Alveolar gas entering the left side of the heart appears as AGE that can reach different sites in the body.

Limitations. TTE bubble grading is a semiquantitative method and bubble grading retains a subjective component, which must be considered when drawing conclusions related to VGE loads in the heart. In addition, the position of the subjects during bubble grading is not optimal for visualization because of the exercise protocol (supine vs left side position). However, we obtained excellent images for review. Contrast TTE is limited to detect anatomic IPAVA in subjects who do not have a PFO, and it cannot be used to quantify the amount of blood flowing through IPAVA. There was low VGE load after dive, which may have prevented us from detecting open IPAVA; however, the work in the laboratory using the same subjects with saline contrast injection allowed us to determine the existence of and patency of IPAVA in these divers. Another limitation is the sensitivity of TCD. In our study, power M-mode Doppler image with concurrent single-gate spectrogram was used for calculations, where microemboli appear as high-power/high-intensity transient unidirectional signals. Bates et al. (2) measured the size of IPAVA in the rat and noted that 70-μm emboli could pass through the lung. In humans, these pathways were found to have a functional diameter of 25 and 50 μm (25). To our knowledge, no article has yet been published on the minimal size of a bubble required to have MES in the cerebral vasculature passing through IPAVA in healthy humans after dive. In a study by Schoenburg et al. (34), microbubbles over 15–25 μm effectively removed by a dynamic air bubble trap significantly reduced the number of MES in the MCA but smaller emboli were still detected with a 2-MHz pulse-wave TCD device. This suggests that Doppler detection is sensitive enough to detect MES that would originate from intrapulmonary anastomoses as the IPAVA measured in postmortem human lungs have been measured to be > 25 μm, although smaller ones may exist. Although automated bubble grading is more of a quantitative method, it registers MES only in the observed arteries. Gas emboli might find their ways into different vessels and fail to be registered, even though they reached the brain. Finally, whether a different diving protocol would result in altered results needs further investigations. In previous research, different no-decompression air dive profiles were tested with different depth and time of diving (19). All dives resulted in high incidence of grade 4 VGE. We have found this profile successful in producing visible VGE while still being a relatively mild profile even by recreational standards, and therefore, data collected are applicable to a wide range of divers and not just deep diving professionals.

In summary, we have observed that emboli of venous origin can reach the brain through exercise-induced IPAVA. Whether larger bubble stress with more severe dive protocols would lead to larger embolic load to the cerebral circulation still needs to be investigated. Clearly the increase in bubble load is different after dive and after contrast saline injection and demonstrates large individual variability. Divers who tend to have higher endogenous bubble grades might not shunt, and divers who show low postdive bubble grades might be "shunt openers." Regardless of dive protocols and bubble loads, even a single bubble of a sufficient size, reaching the right part of the brain could pose potential injury. With this in mind, we cannot state with certainty that low bubble counts that reach the cerebral vasculature might not pose a risk for the development of DCS or of asymptomatic silent embolization of the brain, but if appropriate decompression procedures are followed, DCS is uncommon.

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