

Preconditioning to Reduce Decompression Stress in Scuba Divers

Peter Germonpré; Costantino Balestra

- BACKGROUND:** Using ultrasound imaging, vascular gas emboli (VGE) are observed after asymptomatic scuba dives and are considered a key element in the potential development of decompression sickness (DCS). Diving is also accompanied with vascular dysfunction, as measured by flow-mediated dilation (FMD). Previous studies showed significant intersubject variability to VGE for the same diving exposure and demonstrated that VGE can be reduced with even a single pre-dive intervention. Several preconditioning methods have been reported recently, seemingly acting either on VGE quantity or on endothelial inflammatory markers.
- METHODS:** Nine male divers who consistently showed VGE postdive performed a standardized deep pool dive (33 m/108 ft, 20 min in 33°C water temperature) to investigate the effect of three different preconditioning interventions: heat exposure (a 30-min session of dry infrared sauna), whole-body vibration (a 30-min session on a vibration mattress), and dark chocolate ingestion (30 g of chocolate containing 86% cocoa). Dives were made one day per week and interventions were administered in a randomized order.
- RESULTS:** These interventions were shown to selectively reduce VGE, FMD, or both compared to control dives. Vibration had an effect on VGE (39.54%, SEM 16.3%) but not on FMD postdive. Sauna had effects on both parameters (VGE: 26.64%, SEM 10.4%; FMD: 102.7%, SEM 2.1%), whereas chocolate only improved FMD (102.5%, SEM 1.7%).
- DISCUSSION:** This experiment, which had the same subjects perform all control and preconditioning dives in wet but completely standardized diving conditions, demonstrates that endothelial dysfunction appears to not be solely related to VGE.
- KEYWORDS:** decompression illness, diving, preconditioning, prevention, protective interventions, vibration, sauna, chocolate.

Q1

Germonpré P, Balestra C. *Preconditioning to reduce decompression stress in scuba divers*. *Aerosp Med Hum Perform*. 2017; 88(1):1–7.

Vascular gas emboli (VGE) are routinely observed after asymptomatic scuba dives using ultrasound imaging or precordial Doppler. Although the presence of VGE is by itself not predictive for decompression sickness (DCS),¹⁰ a statistical link has been observed between the amount of observed VGE and the risk for development of DCS.²⁶ Therefore, and in the absence of better biomarkers, VGE are still considered a key element in the potential development of decompression sickness, which is potentially life threatening.^{25,38} In addition, the presence of VGE and/or microparticles and/or other circulating factors seems to be at least partially responsible for functional changes in the vascular wall postdive. Although completely asymptomatic, this vascular dysfunction can be clearly objectivized by measurement of brachial artery flow-mediated dilation (FMD).²¹ Although the magnitude of FMD changes postdive cannot be placed in correlation with the risk of developing DCS, it is clearly an indicator of so-called “decompression stress” and has been linked to the presence of

VGE, of endothelial microparticles,¹ and oxidative stress induced by high partial pressures of oxygen during the dive.³⁵ Administration of antioxidants before the dive only partially prevents postdive alteration of FMD,^{27,28} indicating that oxidative stress is not the only factor contributing to the postdive vascular dysfunction.

The exact mechanisms of VGE formation are not known. Observable VGE form during decompression after a more or less saturating dive, and are observed more frequently and abundantly after dives with higher “decompression stress.”

From Haute Ecole Bruxelles-Brabant (HE2B), ISEK, Brussels, Belgium.

This manuscript was received for review in April 2016. It was accepted for publication in October 2016.

Address correspondence to: Prof. Costantino Balestra, Ph.D., Environmental, Occupational & Integrative Physiology Laboratory, Haute Ecole Bruxelles-Brabant (HE2B), ISEK, 1160 Brussels, Belgium; daneuben@skynet.be.

Reprint & Copyright © by the Aerospace Medical Association, Alexandria, VA.

DOI: <https://doi.org/10.3357/AMHP:4642.2017>

However, for a given dive and decompression profile (depth, breathing gas, ascent rate, decompression stops), there is considerable variability in the number of VGE observed, both between subjects and also intra-individually.²⁴ Personal factors have been postulated such as age, sex, and body fat mass, but the relationship is insufficiently clear to be used as a prediction tool even if some data are available in rodents.⁵ Subjects of younger age and in good physical condition have been shown to be at lesser risk of postdive VGE than older and less fit divers.⁶

This uncertainty limits the practical interpretation of post-dive VGE-based research. While the bulk of past research was directed at reducing DCS risk (as evaluated by VGE detection) by modifying the decompression procedures, it has become clear in the last decade that, perhaps surprisingly, pre-dive interventions (“preconditioning”) seem to be able to influence the level of postdive VGE.

Experimental studies in rats have shown that a single bout of exercise 20 h pre-dive reduces VGE postdive and also DCS occurrence and DCS mortality.⁴⁰ In humans, the role of exercise has been debated¹² and depending on its timing and intensity seems to be able to increase or decrease bubbles.^{3,7,9} A nitric oxide (NO) mediated change in the surface properties of the vascular endothelium, favoring the elimination of gas micronuclei, has been suggested to explain this protection from bubble formation.¹¹ It was demonstrated that NO synthase activity increases following 45 min of exercise and that NO administration immediately before a dive reduces VGE.⁴¹ Nevertheless, bubble production is increased by NO blockade in sedentary but not in exercised rats,⁴⁰ suggesting other biochemical pathways such as heat shock proteins, antioxidant defenses, or blood rheology.

A protective effect of pre-dive heat exposure was observed in rats and could be related to biochemical processes involving heat shock proteins (HSP). HSP are proteins contributing to increased cell survival after thermal or other stresses.²² Several types of HSP have been described; the ones of importance here seem to be of the 70-kDa range (HSP70). In humans a previous study with a “dry” hyperbaric chamber dive and a single exposure to infrared dry sauna for 30 min, ending 1 h before the dive, showed a significant decrease in VGE, a significant increase in plasma HSP70 2 h following the sauna treatment, an extracellular dehydration resulting in slight hypovolemia, and a significant rise in FMD. It was speculated that sweat dehydration, HSP, and the NO pathway are all involved in the protective effect of the dry sauna exposure. It has been demonstrated that HSPs are able to interact with the endothelial NO pathway.⁴ On the other hand, high environmental temperatures lead to a sweat response, resulting in dehydration and some authors¹⁵ have shown that a moderate pre-dive dehydration, as induced by exercise, could influence vascular bubble formation. Conversely, preventive hydration before the dive has also been shown to enhance the efficacy of decompression. Pre-dive fluid ingestion may prevent the lowering of cardiac preload at the end of a dive, resulting in an increased elimination of excess inert gas during the decompression phase of a dive.¹⁵

It was demonstrated that 30 min of whole-body vibration before a wet dive had preventive effects on postdive VGE.¹⁸ As there was no observed change in FMD after vibration, the authors did not believe a NO mediated mechanism was involved; rather, a mechanical dislodgement of VGE precursors (located in microcrevices between endothelial cells) or enhanced lymphatic elimination of gas nuclei (in the extracellular interstitial space) was hypothesized. Vibrations could induce, by force transmission, a modification of endothelial spatial conformation. Secondly, the increase in lymphatic circulation induced by vibration^{2,20} would allow the elimination of intercellular tissue-located micronuclei. The effectiveness of pre-dive vibration on VGE elimination might thus be explained by the mechanical action of vibration on endovascular and tissue localization of the micronuclei.

Biochemical interventions have been studied. The effect of chocolate on cardiovascular health has been extensively studied outside of the diving context; for instance, endothelial function is improved with high cocoa content chocolate supplementation.¹⁴ Recently, the effect of ingestion of 30 g of dark chocolate, 2 h pre-dive, on the reduction of endothelial function and VGE after diving was studied. It was shown that chocolate had a similar effect on FMD as pre-dive heat exposure, but its effect on VGE was less pronounced and not significant.³³ The action of dark chocolate seems to be mediated via flavonoids. Flavonoids contained in chocolate were shown to decrease platelet adhesion 2 h after their ingestion¹⁴ and increase NO secretion by activation of endothelial nitric oxide synthetase.³⁴ NO has an inhibiting action on platelet adhesion and aggregation.³⁹ All of these properties would prevent the stability of VGE. In addition, NO secretion causes arterial vasodilatation and, thus, could reduce the risk of injury from any arterialized VGE emboli.

From the brief overview above, it is clear that several different preconditioning interventions may have a favorable influence on VGE and/or endothelial dysfunction postdive. These interventions, however, have not been consistently compared in real diving conditions and some of these previous studies have found conflicting results.

The aim of the present study was to investigate the effect of three different preconditioning interventions (chosen because of their easy practical application) on the same group of divers performing the exact same “wet” dive. By selecting divers who consistently show VGE after a selected dive profile, we sought to eliminate the influence of intra-individual variability. By comparing each diver to his own “control dive” VGE and FMD results, we sought to eliminate the influence of interindividual variability.

METHODS

Subjects

Volunteer divers were selected according to the following criteria: male, ages 25–45 yr, body mass index 20–25, good general health (at least three times per week some physical exercise),

nonsmoking, and certified as “advanced divers” with at least 50 logged dives. The study protocol was approved by the Local Ethical Committee Brussels (Academic Bioethical Committee, Brussels, Belgium), and each subject gave written informed consent before participation. All studies were performed in accordance with the Declaration of Helsinki. The study is part of a series of ongoing field studies on VGE.

Procedures

Dives were performed one day per week, with a minimum of three previous nondiving days. All dive profiles were identical: square profiles to 33 m (108 ft) of fresh water in a swimming pool (Nemo33, Brussels, Belgium) at a constant water temperature (33°C) for 20 min bottom time, without decompression stops. No physical exercise was performed during the dive: divers moved around freely in the restricted area (30 m²) on the bottom of the pool or sat on their knees to perform a variety of psychometric tests. Dives were performed in a randomized order to be “control” dives (no preconditioning), “vibration” dives, “sauna” dives, or “chocolate” dives. The randomization was done according to a randomized number generator (Microsoft Excel) to comprise at least three control dives and at least one of each preconditioning dive. All divers were scheduled to perform a minimum of six dives (three control dives and three intervention dives) over a period of 9 wk; those divers that, for personal reasons, could not perform all dives were excluded in the final analysis; also, those divers that did not, in the course of the three control dives, present any VGE were excluded from the analysis.

Vibration dives were preceded by 30 min of whole-body vibration, on a commercially available vibrating mattress (VM9100RM, HHP Products, Karlsruhe, Germany). Vibration frequencies ranged from 35 to 40 Hz (rms 2.0–4.0 m · s⁻¹) along the whole body thanks to 11 motors embedded in the mattress. Subjects lay motionless on the mattress during the whole vibration session, which ended 1 h before the start of the dive.

Sauna dives were preceded by a 30-min resting exposure in an infrared dry sauna (Model Ecozen Duo, Biozendo, Belgium) at 60°C ending 1 h before the dive, with mandatory ingestion of 500 ml of still water immediately after the sauna session.

Chocolate ingestion, taken orally 2 h before the dive, consisted of 30 grams (3 squares) of a commercially available Belgian dark chocolate (Cote d’Or Fin 86% Noir Brut; Cote d’Or, Belgium), containing 86% of cocoa; the amount of polyphenols was 135.8 ± 2.9 μmol of catechin equivalents per gram.

Postdive VGE were observed using transthoracic echocardiography and a “frame-based” counting method for VGE recently described, allowing continuous values and parametric statistical approaches.¹⁷ Echocardiography was done with a Vivid-i portable echocardiograph (GE Healthcare, UK) used poolside; echocardiography loops were recorded on hard disk for offline analysis by three evaluators blinded to the nature of the preconditioning. VGE numbers were counted at 30 min and 90 min postdive. NO mediated endothelial function

was measured by means of FMD of the brachial artery with a 5–10 MHz transducer (Mindray M9, Mindray, China), as previously described,³⁰ in parallel with VGE detection 30 min post dive.

Any diver not consistently showing VGE after the three control dives was excluded for the purpose of this study. In our dive series, 11 out of 24 divers were thus excluded. Of the 13 “bubblers” (54%), 9 divers (age: 33.8 ± 0.66 yr, BMI = 23.7 ± 0.19 kg · m⁻²) took part in all preconditioning dives and were selected for this analysis.

Statistical Analysis

Every preconditioning procedure was assessed according to relative changes of VGE counts as well as FMD variation, with each diver serving as his own control. For each diver, mean values of VGE and FMD after the control dives were referred to as “100%” so a percentage of variation could be used for comparative analysis of each preconditioning method. Data are thus given as a percentage of pre-dive values and quoted as means and 95% confidence interval (CI).

Statistical analyses were conducted using the software GraphPad Prism 6 (La Jolla, CA). The differences between the percentage of postdive and pre-dive FMD values (taken as 100%) and the difference in VGE after each preconditioning procedure compared to the control dives (taken as 100%) were compared by a two-tailed one-sample *t*-test after normality of the sample was assessed by the Kolmogorov-Smirnov test. The differences between postdive FMD variation with each preconditioning intervention compared to the control dive were tested with ANOVA for repeated measures with Dunnett post-test. Statistical significance level was set a priori at *P* < 0.05.

RESULTS

As they had been selected according to strict biometric criteria to be part of a homogenous group in the first place (BMI not exceeding 26), biometric data of the nine divers were not significantly different (Table I). Control dives were characterized by a very significant decrease of FMD postdive (mean 93.45% of pre-dive values; SEM 1.8), which is in accordance with previously reported data in the literature.¹⁸ Vibration preconditioning also showed a significant FMD impairment postdive, although reduced compared to the control dive values (94.56% of pre-dive values; SEM 1.6). However, both sauna and chocolate prevented the FMD decrease postdive (Fig. 1) (sauna: 102.7% of pre-dive values, SEM 2.1; chocolate: 102.5% of

Table I. Biometric Data (N = 9).

	AGE (yr)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg/m ²)
Mean	35	76	178	24
Standard deviation	5.6	4.7	5.1	1.3
Lower 95% CI of mean	34	75	177	24
Upper 95% CI of mean	37	78	179	24

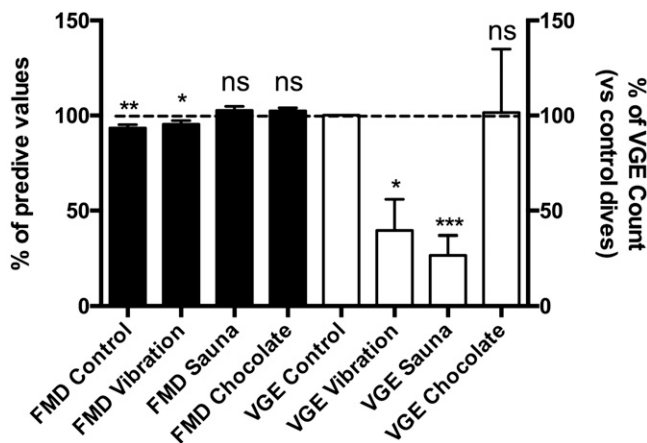


Fig. 1. Effects of different preconditioning methods on endothelial function preservation as measured by FMD (dark bars and left Y axis) and on postdive VGE counts (white bars and right Y axis). FMD = flow mediated dilation, VGE = vascular gas emboli. Data are expressed as means ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. The differences between postdive FMD values and pre-dive FMD (taken as 100%) were compared by a two-tailed one-sample *t*-test after normality of the sample was assessed by the Kolmogorov-Smirnov test. The difference in VGE counts compared to the individual mean VGE count after control dives (taken as 100%) was compared by a two-tailed one-sample *t*-test after normality of the sample was assessed by the Kolmogorov-Smirnov test.

pre-dive values, SEM 1.7). The differences between parameters measured with each preconditioning intervention compared to each diver’s own control postdive FMD variation (taken as 100%) showed that the vibration dive did not significantly alter FMD decrease (94.97%, *P* > 0.05); however, both sauna (86.85%, *P* < 0.01) and chocolate (86.83%, *P* < 0.01) prevented the postdive FMD decrease in a very significant way.

Data are summarized in Table II. mean VGE counted on the three control dives was taken as 100% for each diver separately. Chocolate preconditioning did not show a significant difference in number of postdive VGE (101.5%, SEM 33.4%). However, VGE counts were significantly reduced after the vibration dive (39.54%, SEM 16.3%) (*P* < 0.05) as well as following the sauna preconditioning (26.64%, SEM 10.4%) (*P* < 0.001). Fig. 1 shows the comparative effects of all three preconditioning procedures on FMD and VGE.

Table II. FMD Variation After Preconditioning (Pre-Dive FMD = 100%).

	VALUE (%)	95% CI	P-VALUE
Pre-dive FMD	100%		
Postdive FMD			
Control dives	93.45%	89.7–97.2%	< 0.01
Vibration	94.56%	90.7–98.4%	< 0.05
Sauna	102.7%	97.7–107.7%	N.S.
Chocolate	102.5%	98.7–106.3%	N.S.
Change in postdive FMD variation after preconditioning (individual postcontrol dive FMD decrease = 100%)			
Control dive	100%		
Vibration	94.97%	85.5–104.43%	N.S.
Sauna	86.85%	77.69–96.00%	< 0.01
Chocolate	86.83%	77.68–95.99%	< 0.01

DISCUSSION

VGE start forming during the off-gassing of tissues in the decompression (ascent) phase of a dive, and are believed to result from the triggering of bubble precursors (nuclei) into growth. The precise mechanism of micronuclei formation is still debated, with possible sites in facilitating endothelial surface regions having surfactants, hydrophobic surfaces, or crevices.^{29–31} Previous studies have pointed out a significant intersubject variability to VGE for the same diving exposure. There is also a large intra-individual variation, indicating that diving time and nitrogen pressure are not the only determinants of VGE formation. It has been shown that VGE formation can be reduced with a single pre-dive intervention;^{4,18} however, the precise mechanisms by which such preconditioning “works” have not yet been determined.

This large inter- and intra-individual variability make research into pre-dive interventions difficult, as there are always possible confounding factors. Therefore, in order to determine the effect of a certain preconditioning procedure, it is of paramount importance that not only the dive profile, but also the diver biometrics be standardized as much as possible; moreover, the diving experience, exercise, smoking, and possibly even dietary habits should also be controlled as much as possible.³² There appears to be a potentially large difference in VGE postdive for a similar pressure-nitrogen exposure, depending on whether the dive was performed “wet” or “dry.”²⁴

The presumed micronuclei-originated VGE production is consistent with the fact that a certain form of “acclimatization” to decompression stress seems to exist, with a higher probability of VGE for the first dives after a period of nondiving.^{1,42} In order to avoid this possible confounder, no diving or strenuous physical exercise were allowed in the 3 d before each research dive day. All dives were performed in the early afternoon, with divers being instructed to take a standard breakfast and a light, low-fat lunch.

Even when, as in our experimental dives, most of these factors are accounted for, there seems to exist a population of “non-bubblers,” “inconsistent bubblers,” and “consistent bubblers” for reasons as yet unknown. Extensive biochemical testing of all our divers before each dive failed to reveal a clear pattern as to a biochemical origin of this variation (data not shown). This has been reported before, a number of years ago.¹⁹ For this reason, it is necessary to assess any effect that preconditioning might have on postdive parameters on the same subjects, each acting as their own control, once a consistency of VGE levels for these subjects during control dives has been demonstrated. For this study and because of these unknowns, “inconsistent bubblers” have been excluded from analysis.

Finally, it is important to be able to detect VGE in an objective reproducible manner and, if possible, allow a true quantitative (continuous) counting of VGE. This is not only necessary in order to determine whether a diver is a “consistent bubbler” or not, but also to be able to discern a true difference in VGE counts. These are, in the range of diving where human

experiments can ethically be performed, usually mild to moderate, and existing grading scales (Spencer, Kisman-Masurel, Eftedal-Brubakk) are nonparametric in nature and/or have too large a step in categories exactly in the range observed. The Eftedal-Brubakk scale,¹³ for instance, “jumps” from 1 bubble per cardiac cycle (grade 3) to 1 bubble per square centimeter (grade 4). This is exactly the range where recreational and sports diving are expected to yield bubble counts. The recently validated technique of frame-based bubble counting¹⁷ is better suited to this goal; however, it requires as yet specific hardware and some manual labor, pending automatic video ingestion and VGE counting software.

FMD is a noninvasive technique allowing a longitudinal evaluation of endothelium-dependent vascular reactivity. The technique has been well described and standardized.³³ However, variations in the technique (e.g., placement of the occluding cuff at the level of the humerus or the forearm; duration of occlusion and interval between release of the cuff and measurements) and individual characteristics may influence the magnitude of the FMD.⁸ In this study, all divers were compared to their own control values and the technique used was identical for all divers, both before and after the dive (5 min total occlusion at the level of the mid-humerus, as described previously³³). It is argued that the imposed shear stress by occlusion and release may be different in different persons (owing to anatomical and physiological differences) and that it is thus necessary to normalize the results obtained by the shear stress. However, in the strict timing of our experiment, a prolonged measurement of the shear stress was not possible as this would interfere with the other measurements. Furthermore, it was not possible to have the equipment needed for this normalizing evaluation available at the dive site. While this may be viewed as a limitation to our study, it is not likely that anatomical or physiological characteristics of our (young and healthy) divers would change over the course of 7–8 wk in such a way as to exert a major influence on the results.

From previous literature, the relative influence of NO mediated changes (FMD) and VGE have not been satisfactorily clarified:

- Changes in FMD (decreased reduction of FMD postdive) could modify VGE production (with increased NO levels acting on micronuclei sites, possibly in crevices or hydrophobic endothelial surface sites).^{11,37}
- Decreased VGE production might change FMD by provoking less mechanical or biochemical endothelial damage. Even though VGE are primarily detected in the venous vasculature, whereas FMD is a measure of arterial “stiffness,” the sensibility of the VGE detection methods is thus that small, undetected VGE (less than 22 μm ¹⁹) might pass the pulmonary vasculature (which acts as a bubble “filter”⁶) and exert influence in the arterial vascular bed. Even as VGE detection techniques improve (from acoustic Doppler to visual 2D echocardiography to second harmonics echocardiography), there obviously is a size limit below which no VGE will be detected.^{29,31}

- Finally, it is entirely possible that there is no direct relationship between VGE and FMD, but that both are simply caused by the same mechanism. A relationship between VGE levels and the presence of endothelial microparticles (MP) has been demonstrated.^{35,36} Either by shear forces or by mechanical/biochemical damage to the endothelial cell wall, VGE might cause release of endothelial MP, which will, unlike VGE, readily pass the pulmonary capillary filter.

Our results indicate various effects for each preconditioning method. Nitric oxide mediated changes do not necessarily reflect onto changes in VGE counts, indicating different mechanisms involved.

From our results, it is already possible to formulate clear hypotheses:

- Vibration and sauna might act by mechanical dislodging of VGE nuclei (either by transmission of energy shocks or by increased shear forces with increased circulation). Both factors might also be involved in the effects seen in pre-dive exercise preconditioning, as this provokes an increased cardiac output and mechanical movement—in this regard it is interesting to note that treadmill running seems to have a greater effect than bicycle ergometry.¹⁶ Sauna (heat exposure) might improve FMD by an NO-mediated mechanism or by intermediate action through HSP, both of which are not likely with vibration (however, this remains to be confirmed). The lesser improvement of FMD by vibration might be due to either a smaller number or to a smaller size range of VGE (not detected by echocardiography) still “irritating” the endothelium.
- Chocolate acts by improving NO-mediated changes, but does not seem to be influencing VGE at all; this shows that NO preconditioning might not have the same effect on reduction of DCS incidence as would be expected. Our results are in contrast with some previous studies using pure NO donors.^{23,37} However, these other studies did not standardize the dives, divers, and possible confounding factors as ours did. Also, consistency of bubbling was not tested over a series of control dives. On the other hand, a NO-mediated decrease of diving induced endothelial changes might, irrespectively of VGE counts, reduce further inflammatory changes by incomplete decompression. As there is more and more evidence that DCS is only partially a “bubble vascular blockade” disease and that many, if not most, of persistent symptoms can be related to inflammatory reactions caused by these bubbles, preconditioning “along the NO pathway” would most likely be beneficial in any case.

Contrary to what was previously hypothesized, we show that VGE and endothelial dysfunction after diving are not always concurrent and can be decoupled. Indeed, on the same subjects acting as their own control and in real wet diving conditioning, different preconditioning can selectively reduce VGE, endothelial dysfunction, or both. This demonstrates the need for an integrative personalized decompression stress assessment, redefining decompression stress to include both VGE

and endothelial function. Whereas the above-described preconditioning techniques may not protect the diver from all instances of inadequate decompression, we have shown that having a relaxing whole-body vibration session, a nice sauna, and a good taste of dark chocolate might be a good start to a dive day.

ACKNOWLEDGMENTS

The authors thank Ing J. Beernaerts for accepting us in his diving pool, Nemo 33; also, G. E. Belgium for the generous loan and technical support for the Vivid-i portable echocardiograph and probes, and of course all volunteers (divers and supporting staff, cardiologists, and nurses/technicians) for participating in this study.

The authors declare no conflicts of interest, financial or otherwise.

Costantino Balestra and Peter Germonpré conceptualized and designed the research; analyzed and interpreted the data; edited and revised the manuscript; and approved the final version of manuscript. Both authors were equal contributors to this work and should be considered as co-first authors.

Authors and affiliations: Peter Germonpré, M.D., Medical Director, Centre for Hyperbaric Oxygen Therapy, Military Hospital Burssels, Brussels, Belgium, and Costantino Balestra, M.Sc., Ph.D., Environmental, Occupational, Integrative Physiology Laboratory, Haute Ecole Bruxelles-Brabant (HE2B), ISEK, Brussels, Belgium.

REFERENCES

- Arieli R, Arieli U, Marmur A. Bubble size on detachment from the luminal aspect of ovine large blood vessels after decompression: The effect of mechanical disturbance. *Respir Physiol Neurobiol.* 2015; 216:1–8.
- Balestra C. The lymphatic pathway for microbubbles. *Diving Hyperb Med.* 2014; 44(1):1.
- Blatteau JE, Boussuges A, Gempp E, Pontier JM, Castagna O, et al. Haemodynamic changes induced by submaximal exercise before a dive and its consequences on bubble formation. *Br J Sports Med.* 2007; 41(6):375–379.
- Blatteau JE, Gempp E, Balestra C, Mets T, Germonpre P. Pre-dive sauna and venous gas bubbles upon decompression from 400 kPa. *Aviat Space Environ Med.* 2008; 79(12):1100–1105.
- Buzzacott P, Theron M, Mazur A, Wang Q, Lambrechts K, et al. Age, weight and decompression sickness in rats. *Arch Physiol Biochem.* 2016; 122(2):67–69.
- Carturan D, Boussuges A, Burnet H, Fondarai J, Vanuxem P, Gardette B. Circulating venous bubbles in recreational diving: relationships with age, weight, maximal oxygen uptake and body fat percentage. *Int J Sports Med.* 1999; 20(6):410–414.
- Castagna O, Brisswalter J, Vallee N, Blatteau JE. Endurance exercise immediately before sea diving reduces bubble formation in scuba divers. *Eur J Appl Physiol.* 2011; 111(6):1047–1054.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002; 39(2):257–265.
- Dervay JP, Powell MR, Butler B, Fife CE. The effect of exercise and rest duration on the generation of venous gas bubbles at altitude. *Aviat Space Environ Med.* 2002; 73(1):22–27.
- Doolette DJ. Venous gas emboli detected by two-dimensional echocardiography are an imperfect surrogate endpoint for decompression sickness. *Diving Hyperb Med.* 2016; 46(1):4–10.
- Dujić Z, Palada I, Valic Z, Duplancić D, Obad A, et al. Exogenous nitric oxide and bubble formation in divers. *Med Sci Sports Exerc.* 2006; 38(8):1432–1435.
- Dujić Z, Valic Z, Brubakk AO. Beneficial role of exercise on scuba diving. *Exerc Sport Sci Rev.* 2008; 36(1):38–42.
- Eftedal O, Brubakk AO. Agreement between trained and untrained observers in grading intravascular bubble signals in ultrasonic images. *Undersea Hyperb Med.* 1997; 24(4):293–299.
- Flammer AJ, Hermann F, Sudano I, Spieker L, Hermann M, et al. Dark chocolate improves coronary vasomotion and reduces platelet reactivity. *Circulation.* 2007; 116(21):2376–2382.
- Gempp E, Blatteau JE, Pontier JM, Balestra C, Louge P. Preventive effect of pre-dive hydration on bubble formation in divers. *Br J Sports Med.* 2009; 43(3):224–228.
- Gennser M, Jurd KM, Blogg SL. Pre-dive exercise and post-dive evolution of venous gas emboli. *Aviat Space Environ Med.* 2012; 83(1):30–34.
- Germonpré P, Papadopoulou V, Hemelryck W, Obeid G, Lafere P, et al. The use of portable 2D echocardiography and ‘frame-based’ bubble counting as a tool to evaluate diving decompression stress. *Diving Hyperb Med.* 2014; 44(1):5–13.
- Germonpré P, Pontier JM, Gempp E, Blatteau JE, Deneweth S, et al. Pre-dive vibration effect on bubble formation after a 30-m dive requiring a decompression stop. *Aviat Space Environ Med.* 2009; 80(12):1044–1048.
- Hills BA, Butler BD. Size distribution of intravascular air emboli produced by decompression. *Undersea Biomed Res.* 1981; 8(3):163–170.
- Leduc A, Lievens P, Dewald J. The influence of multidirectional vibrations on wound healing and on regeneration of blood- and lymph vessels. *Lymphology.* 1981; 14(4):179–185.
- Mazur A, Lambrechts K, Wang Q, Belhomme M, Theron M, et al. Influence of decompression sickness on vasoconstriction of isolated rat vessels. *J Appl Physiol.* 2016; 120(7):784–791.
- Medby C, Bye A, Wisloff U, Brubakk AO. Heat shock increases survival in rats exposed to hyperbaric pressure. *Diving Hyperb Med.* 2008; 38(4):189–193.
- Møllerlökken A, Berge VJ, Jorgensen A, Wisloff U, Brubakk AO. Effect of a short-acting NO donor on bubble formation from a saturation dive in pigs. *J Appl Physiol.* 2006; 101(6):1541–1545.
- Møllerlökken A, Breskovic T, Palada I, Valic Z, Dujic Z, Brubakk AO. Observation of increased venous gas emboli after wet dives compared to dry dives. *Diving Hyperb Med.* 2011; 41(3):124–128.
- Møllerlökken A, Gaustad SE, Havnes MB, Gutvik CR, Hjelde A, et al. Venous gas embolism as a predictive tool for improving CNS decompression safety. *Eur J Appl Physiol.* 2012; 112(2):401–409.
- Nishi R, Brubakk AO. Bubble Detection. In: Brubakk AO, Neuman T, editors. *Bennett and Elliott’s physiology and medicine of diving*, 5th ed. Edinburgh: Saunders; 2003:501–529.
- Obad A, Marinovic J, Ljubkovic M, Breskovic T, Modun D, et al. Successive deep dives impair endothelial function and enhance oxidative stress in man. *Clin Physiol Funct Imaging.* 2010; 30(6):432–438.
- Obad A, Valic Z, Palada I, Brubakk AO, Modun D, Dujic Z. Antioxidant pretreatment and reduced arterial endothelial dysfunction after diving. *Aviat Space Environ Med.* 2007; 78(12):1114–1120.
- Papadopoulou V, Eckersley RJ, Balestra C, Karapantsios TD, Tang MX. A critical review of physiological bubble formation in hyperbaric decompression. *Adv Colloid Interface Sci.* 2013; 191–192:22–30.
- Papadopoulou V, Evgenidis S, Eckersley RJ, Mesimeris T, Balestra C, et al. Decompression induced bubble dynamics on ex vivo fat and muscle tissue surfaces with a new experimental set up. *Colloids Surf B Biointerfaces.* 2015; 129:121–129.
- Papadopoulou V, Tang MX, Balestra C, Eckersley RJ, Karapantsios TD. Circulatory bubble dynamics: from physical to biological aspects. *Adv Colloid Interface Sci.* 2014; 206:239–249.
- Schellart NA, Rozloznik M, Balestra C. Relationships between plasma lipids, proteins, surface tension and post-dive bubbles. *Undersea Hyperb Med.* 2015; 42(2):133–141.
- Theunissen S, Guerrero F, Sponsiello N, Cialoni D, Pieri M, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med.* 2013; 40(2):135–144.

34. Theunissen S, Schumacker J, Guerrero F, Tillmans F, Boutros A, et al. Dark chocolate reduces endothelial dysfunction after successive breath-hold dives in cool water. *Eur J Appl Physiol.* 2013; 113(12):2967–2975.
35. Thom SR, Bennett M, Banham ND, Chin W, Blake DF, et al. Association of microparticles and neutrophil activation with decompression sickness. *J Appl Physiol.* 2015; 119(5):427–434.
36. Thom SR, Milovanova TN, Bogush M, Bhopale VM, Yang M, et al. Microparticle production, neutrophil activation, and intravascular bubbles following open-water scuba diving. *J Appl Physiol.* 2012; 112(8):1268–1278.
37. Valic Z, Palada I, Dujic Z. Short-acting NO donor and decompression sickness in humans. *J Appl Physiol.* 2007; 102(4):1725; author reply 1726.
38. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. *Lancet.* 2011; 377(9760):153–164.
39. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, et al. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. *J Cardiovasc Pharmacol.* 2006; 47(Suppl. 2):S177–S186; discussion S206–S209.
40. Wisløff U, Richardson RS, Brubakk AO. NOS inhibition increases bubble formation and reduces survival in sedentary but not exercised rats. *J Physiol.* 2003; 546(2):577–582.
41. Wisløff U, Richardson RS, Brubakk AO. Exercise and nitric oxide prevent bubble formation: a novel approach to the prevention of decompression sickness? *J Physiol.* 2004; 555(3):825–829.
42. Zanchi J, Ljubkovic M, Denoble PJ, Dujic Z, Ranapurwala S, Pollock NW. Influence of repeated daily diving on decompression stress. *Int J Sports Med.* 2014; 35(6):465–468.

Author Query sheet-AMHP4642

Q1 : Author: We normally provide both feet and meters for depth and altitude. Please check that conversions are acceptable.