Eur J Appl Physiol (2017) 117:335–344
DOI 10.1007/s00421-017-3537-9

ORIGINAL ARTICLE

Venous gas emboli are involved in post-dive macro, but not microvascular dysfunction

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Received: 3 September 2016 / Accepted: 3 January 2017 / Published online: 21 January 2017

Abstract
Purpose Previous studies have shown vascular dysfunction of main conductance arteries and microvessels after diving. We aim to evaluate the impact of bubble formation on vascular function and haemostasis. To achieve this, we used a vibration preconditioning to influence bubble levels without changing any other parameters linked to the dive.
Methods Twentty-six divers were randomly assigned to one of three groups: (1) the "vibrations–dive" group (VD; $n=9$) was exposed to a whole-body vibration session 30 min prior the dive; (2) the "diving" group (D; $n=9$) served as a control for the effect of the diving protocol; (3) The "vibration" protocol (V; $n=8$) allowed us to assess the effect of vibrations without diving. Macro- and microvascular function was assessed for each subject before and after the dive, subsequently. Bubble grades were monitored with Doppler according to the Spencer grading system. Blood was taken before and after the protocol to assess any change of platelets or endothelial function.
Results Bubble formation was lower in the VD than the diving group. The other measured parameters remained unchanged after the "vibration" protocol alone. Diving alone induced macrovascular dysfunction, and increased PMP and thrombin generation. Those parameters were no longer changed in the VD group. Conversely, a microvascular dysfunction persists despite a significant decrease of circulating bubbles.
Conclusions Finally, the results of this study suggest that macro- but not microvascular impairment results at least partly from bubbles, possibly related to platelet activation and generation of pro-coagulant microparticles.

Keywords SCUBA · Decompression · Brachial artery · Cutaneous microcirculation · Vibration preconditionning · Platelets

Communicated by Guido Ferretti.

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Introduction

When SCUBA (self-contained underwater breathing apparatus) divers stay at depth, tissues uptake additional dissolved breathing gases which are then released into the blood as vascular gas emboli (VGE) during and following ascent, when the ambient pressure drops (Papadopoulou et al. 2014). Although decompression-induced bubble formation is a pivotal event in decompression sickness (DCS), the exact pathophysiological mechanisms linking VGE to DCS are still unclear. Impaired flow-mediated dilation (FMD) after diving has been repeatedly reported in humans, suggesting endothelial dysfunction of large conductance arteries (Brubakk et al.

2005; Marinovic et al. 2012; Lambrechts et al. 2013a). Later on, the impact of diving has been extended to the microvascular endothelium (Lambrechts et al. 2013a) as well as the macro- and microvascular smooth muscle (VSM) (Lambrechts et al. 2013a, c). Administration of antioxidant before the dive partially prevents the post-dive alteration of FMD (Obad et al. 2007a, b), indicating that oxidative stress is involved but that other factors also contribute to the post-dive alteration of blood vessels functions.

On the one hand, it has been shown that circulating bubbles can lead to endothelial dysfunction (Nossum et al. 2002) through either direct contact between microbubbles and endothelial cells (Klinger et al. 2011; Sobolewski et al. 2011) or activation of the coagulation cascade (Pontier et al. 2008a, 2009; Lambrechts et al. 2015). Activation of haemostatic pathways participates in the post-dive increase of circulating microparticles which in turn leads to leukocytes activation (Ersson et al. 2002; Yang et al. 2015a) and adhesion to the endothelium (Thom et al. 2015). On the other hand, impaired FMD (Brubakk et al. 2005; Theunissen et al. 2013a) and increased oxidative stress (Obad et al. 2007a; Theunissen et al. 2013b) were also reported post-dive even in the absence of VGE. It has been reported that an increased production of reactive oxygen species (ROS) by cultured endothelial cells during exposure to hyperbaric air elicits cell death, independently of the presence of bubbles (Wang et al. 2013, 2015).

Therefore, in this study we aimed to investigate in SCUBA divers the role of decompression-induced bubbles on vascular function by manipulating the amount of circulating VGE formed during decompression, independently of other diving parameters. Endothelium-dependent and -independent vasomotion was explored both at the macro- and microvascular levels. Because haemostasis and microparticles are possible links between VGE and vascular dysfunction, we also measured fibrin monomer, thrombin generation and procoagulant micoparticles (PMP). Von Willebrand factor (VWF) was also measured as a marker for platelet–endothelium interaction.

Among various strategies known to reduce VGE (Gempp and Blatteau 2010), we used whole-body vibrations because it was also shown not to change predive FMD (Germonpré et al. 2009; Balestra et al. 2016). This preconditioning protocol allowed us to compare changes induced by the same dive profile, but different amounts of VGE.

Methods

Study population

Twenty-six experienced male divers (mean age 38 ± 8 years; body mass index 25 ± 3 kg m^{-2}) were recruited for this study. None had experienced DCS in the past, and all had a valid medical clearance for diving at the time of the study. All participants were non-smokers and none were taking medication that could interfere with our measurements. They were asked to abstain from any physical activity and diving for 72 h (Obad et al. 2007a) prior to the experimental protocol; tea, coffee and alcohol were prohibited for 6 h prior to the test. All participants provided fully informed, written consent before the experiment. The protocol was approved by the Ethical Committee of the University Hospital of Brest and performed in accordance with the guidelines of the Helsinki Declaration for human research.

Study design

Divers were randomly assigned to one of the three following groups. Subjects in the "diving" group (D; $n = 9$) performed an open-sea air dive. They were transported to the dive site by a power boat during a maximum of 10 min ride. The dive lasted 30 min (bottom time), mostly at 30 m of seawater (msw) depth, with moderate standardized fining exercise. Then, the ascent was completed at a rate of 15 m min^{-1} with a 9 min stop at 3 msw according to the French Navy procedure (MN90). Each diver used his own equipment, including his personal dive computer to monitor depth and dive time. Divers in the "vibrations-dive" group (VD; $n = 9$) performed the same dive after they had been exposed to a whole-body vibrations session ending 30 min prior the dive. Vibrations were applied during 30 min using a commercially available vibration mattress (VM 9100RMw, HHP Products, Karlsruhe, Germany), and their frequencies ranged from 35 to 40 Hz along the whole body. Subjects were lying motionless on the mattress during the entire vibration session. A third "vibrations" protocol (V; $n = 8$) was used to assess the effect of vibrations on the studied parameters not related to diving. Subjects in this group were exposed to the same vibrations session than the VD group but did not dive. All pre-tests were done in a quiet controlled temperature room (22 ± 1 °C) before the dive ("diving" group) or vibrations ("vibrations–dive" and "vibrations" groups). Then, post-tests were performed in the same conditions at the end of each protocol so that each subject could be his own control. To account for any possible differences due to diving conditions, which could influence VGE production, or circadian variations in cardiovascular and haemostatic functions (Mheid et al. 2014), each diving team was composed of one subject from the "Diving" group and one from the "Vibrations–Dive" group.

Bubble analysis

VGE were monitored on the precordial area by an experienced operator using a pulsed Doppler equipped with a 2 MHz probe (Pioneer®). Measurements were obtained at 30, 60 and 90 min after surfacing, at rest and after three knee flexions. The production of VGE was quantified as described previously (Lambrechts et al. 2013b), according to the Kisman Integrated Severity Score (KISS) calculated according to the following formula:

$$\mathrm{KISS} = \left[100/4^{\alpha}\left(t_4 - t_1\right)\right] \cdot \left[\left(t_2 - t_1\right)\left({d_2}^{\alpha} + {d_1}^{\alpha}\right) + \left(t_3 - t_2\right)\left({d_3}^{\alpha} + {d_2}^{\alpha}\right) + \left(t_4 - t_3\right)\left({d_4}^{\alpha} + {d_3}^{\alpha}\right)\right]/2,$$

where t_i is the time of observation in minutes after reaching the surface, d_i is the Spencer scale bubble grades (grades 0–4) observed at time $t_{i,}$ and $\alpha = 3$ (the parameter α takes into account the fact that the bubble grade is not a linear measure of bubble quantity). The KISS was assumed to be a meaningful linearized measure of post-decompression intravascular bubble activity status that may be treated statistically.

Brachial artery ultrasound

FMD, an established measure of the endothelium-dependent vasodilation mediated by nitric oxide (NO) (Pyke and Tschakovsky 2005), was used to assess the effect of diving on the main conduit arteries. Subjects were at rest for 15 min in a supine position before the measurements were taken. Brachial artery diameter was measured by means of a 5.0–10.0 MHz linear transducer using a Mindray DP-2200 digital diagnostic ultrasound system immediately before and 1 min after a 5 min ischemia induced by inflating a cuff placed on the arm to 180 mmHg, as described previously (Corretti et al. 2002). When the images were chosen for analysis, the boundaries for diameter measurement were identified manually with an electronic caliper in a threefold repetition pattern to calculate the mean value. In our laboratory, the mean intra-observer variability for FMD measurement recorded on the same day, same site and same subject was $1.7 \pm 0.2\%$.

Endothelium-independent dilation was obtained by measuring brachial artery diameter immediately before and 2 min after administration of 0.6 mg nitroglycerin by oral spray (Natispray, trinitrine, Teofarma, Italy).

For FMD and NMD (nitroglycerin-mediated dilation), post-dive values were obtained 90 min after surfacing, i.e. after the last VGE measurement. FMD and NMD were calculated as the percent increase in arterial diameter from the resting state to maximal dilation. For FMD, control values were obtained 1 h before diving. Because nitroglycerin reduces bubble formation in divers (Dujic et al. 2006), the control values for NMD were obtained at least 72 h after the dive.

Laser Doppler flowmetry (LDF)

Microvascular function was assessed at the cutaneous level by LDF coupled with iontophoresis as previously described (Lambrechts et al. 2013a, c). Cutaneous blood flow (CBF) was recorded before the protocol and 30 min after surfacing. Subjects remained in the supine position. A multifiber laser probe (780 nm) (PF 450-PI; Perimed) specially designed to allow for current application and simultaneous CBF recording was placed at the ventral side of the non-dominant forearm, 5 cm below the elbow bend to avoid site to site variation. The probe was connected to an LD Flowmeter (Periflux PF 5001, Perimed). A battery-powered current supplier (Perilont PF 382; Perimed) was used to provide direct current (0.10 mA for 35 s) for iontophoresis.

Endothelium-dependent vasodilation was first induced with a 1% acetylcholine (ACh) chloride solution administered with an anodal current. After CBF had returned to baseline values, a cathodal current was used to deliver a 1% sodium nitroprussiate (SNP) solution to assess endothelium-independent vasoreactivity. The baseline CBF was the mean value of all readings over a stable 2 min period. The amplitude of the response to each substance was measured at the peak blood flow after the stimulation. CBF was indexed as cutaneous vascular conductance (CVC), calculated as CBF/mean arterial pressure. Blood pressure was the mean value obtained from two measurements recorded at rest just before each LDF assessment. Responses to ACh and SNP were presented as percentage of CVC variation from baseline after iontophoretic stimulation.

Blood sampling technique and measurements

Blood samples were drawn 60 min before and 90 min after surfacing (i.e. after all other measurements) from a clean median antecubital venipuncture using 4.5 ml Vacutainer® tubes (Becton Dickinson) with CTAD or EDTA. Samples were centrifuged at 3000*g* and 4 °C for 10 min. Then, plasma was aliquoted and stored at −80 °C until the assay.

Plasmatic concentrations of NO were assessed by measuring nitrite concentrations (µg/ml) with a colorimetric method and the Griess reagent. ELISA kits were used according to provider instructions for determination of plasmatic levels of 3-nitrotyrosine (OxiSelect Nitrotyrosine ELISA kit; Cell Biolabs Inc, San Diego, California), VWF (Von Willebrand Factor Human ELISA kit; Abcam, Cambridge, UK), and PMP (ZYMUPHEN MP-Activity kit; West Chester, USA).

Plasmatic fibrin monomer was determined by immunoturbidimetric method with STA Liatest kit and STA-R

Evolution analyser (Stago, France). Thrombin generation was quantified by fluorometric method using a fluorometer/thrombinoscope software (Fluoroskan, Thermo Fisher Scientific, USA) with reagent (Stago PPP Reagent Low), internal standard (Stago Pool Norm), calibrator (Stago Thrombin Calibrator) and thrombin substrate (Stago Flucakit) (Stago France).

Statistical analysis

Statistical analysis were performed with the Statistica 10 software program (Tulsa, Oklahoma, USA). For all data, Kolmogorov–Smirnov test was first used to test normality. When normality was confirmed, data were presented as mean ± SEM. In this case, a paired *t* test was used to compare values obtained for each diver before and after the protocol. Otherwise, median (1st and 3rd quartile) are presented and non-parametric tests were performed. This was the case for KISS scores only. Statistical significance was set a priori at $p < 0.05$.

Results

Circulating bubbles detection (KISS)

After the control dives, the median KISS scores ranged from 27.3 (9.75–34.71) at rest to 42.12 (27.3–87.41) after knee flexion. In the VD group, the median KISS scores were 0 (0–3.9) and 0 (0–9.36), respectively, without and with knee flexions. Mann–Whitney test for independent samples showed that KISS values were significantly lower after the "vibrations–dive" than after the control dive, both without and with knee flexions ($p = 0.010$ and $p = 0.005$ respectively) (Fig. 1).

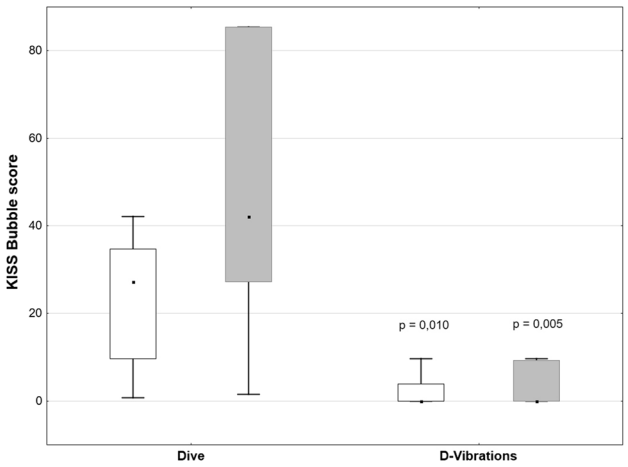

Fig. 1 Kissman Integrated Severity Score (KISS) after a dive without (*left panel*) and with (*right panel*) 30 min of whole-body vibration. Bubble scores are at rest (*white bars*) and after knee flexions (*black bars*). Data are presented as median (*black point*) and first and third quartile (*boxes*). $^{*}p < 0.05$

Brachial artery ultrasound (FMD and NMD)

Baseline predive brachial artery diameters were not different over these multiple protocols.

Diving alone significantly impaired FMD (10.9 ± 2.1 vs 3.8 ± 1%; $p = 0.004$) and NMD (14.2 ± 2.4 vs 3.6 ± 1%; $p = 0.000016$), and these changes were prevented by predive vibrations. Indeed, in the VD group, post-dive values were not significantly different than pre-dive ones for the FMD (7.08 ± 1.1 vs 6.11 ± 1.0%; $p = 0.68$) and the NMD (12.03 ± 0.7% vs 10.44 ± 0.9%; $p = 0.39$). The vibrations alone did not modify FMD (9.0 ± 2.9 vs 8.11 ± 2.2%; $p = 0.71$) or NDM (10.2 ± 2.0 vs 10.9 ± 2.1%; $p = 0.98$) (Fig. 2).

Laser Doppler flowmetry

Diving alone did not significantly alter the CVC response to ACh (9.3 ± 1.7 vs 7.6 ± 1.1%; $p = 0.452$), whereas it decreased significantly the CVC response to SNP (CVC = 7.1 ± 1.5 vs 2.9 ± 0.9%; $p = 0.034$). Vibration preconditioning did not modify the effect of diving on the response to ACh (10.6 ± 2.5 vs 6.0 ± 0.8%; $p = 0.102$) or to SNP (13.6 ± 2.5 vs 9.3 ± 1.3%; $p = 0.027$). The CVC responses to ACh (9.1 ± 1.2 vs 9.0 ± 1.6%; $p = 0.961$) and SNP (8.2 ± 1.1 vs 6.5 ± 1.3%, $p = 0.410$) were unchanged after vibrations alone (Fig. 3).

Plasmatic markers of haemostasis and endothelial activity

Concentrations of nitrites ($p = 0.64$) and VWF ($p = 0.18$), markers of endothelial activity, were not modified by diving, with or without vibrations.

Plasmatic markers of haemostasis were modified after the control dive only. We observed in this group significantly higher values of PMP (0.29 ± 0.1 vs 0.42 ± 0.08 nM; $p = 0.043$) and thrombin generation (0.34 ± 0.05 vs 0.55 ± 0.1 mM; $p = 0.004$) after than before the dive. Fibrin monomers remained unchanged ($p = 0.96$). All these dive-induced changes were prevented by preconditioning with vibrations (Fig. 4).

Finally, vibrations alone did not generate any significant variation in plasmatic markers.

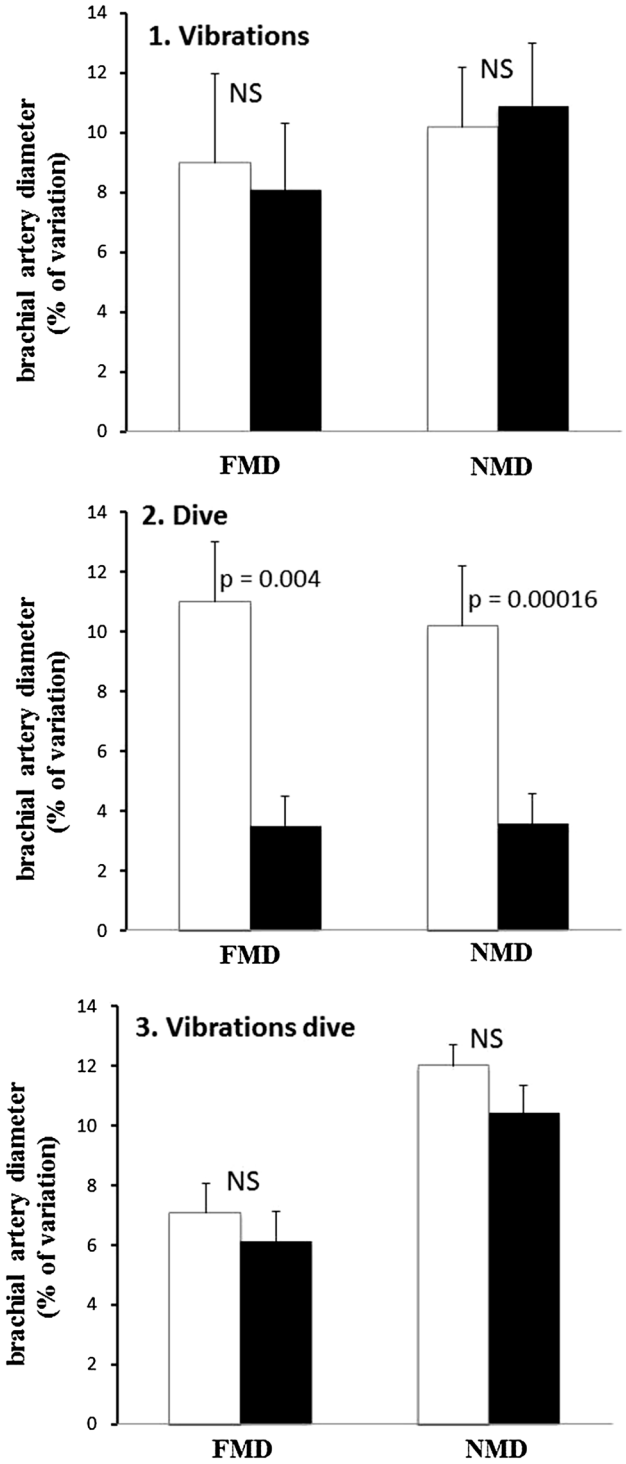

Fig. 2 Flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD) before (*white bars*) and after (*black bars*) each of the three protocols (vibrations, dive and vibrations–dive). Values are expressed as mean ± SEM of the percentage of variation form baseline in response to shear stress (FMD) or to nitroglycerin (NMD). *$p < 0.05$ between before and after the protocol

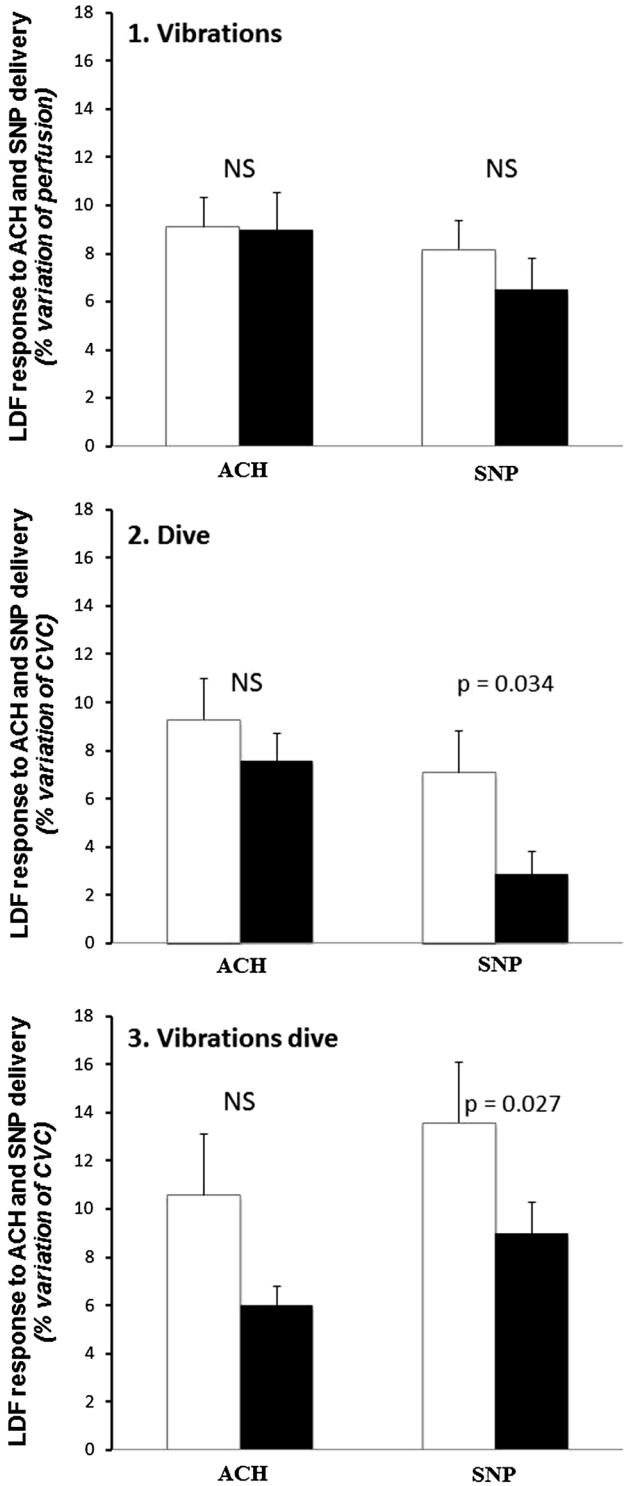

Fig. 3 Laser Doppler flowmetry expressed as a percentage (mean ± SEM) of cutaneous vascular conductance (CVC, see "Methods" section) variation from baseline to acetylcholine (ACH) or to sodium nitroprusside (SNP) iontophoresis stimulation, before (*white bars*) and after (*black bars*) each of the three protocols (vibrations, dive and vibrations–dive). *$p < 0.05$ between before and after the protocol

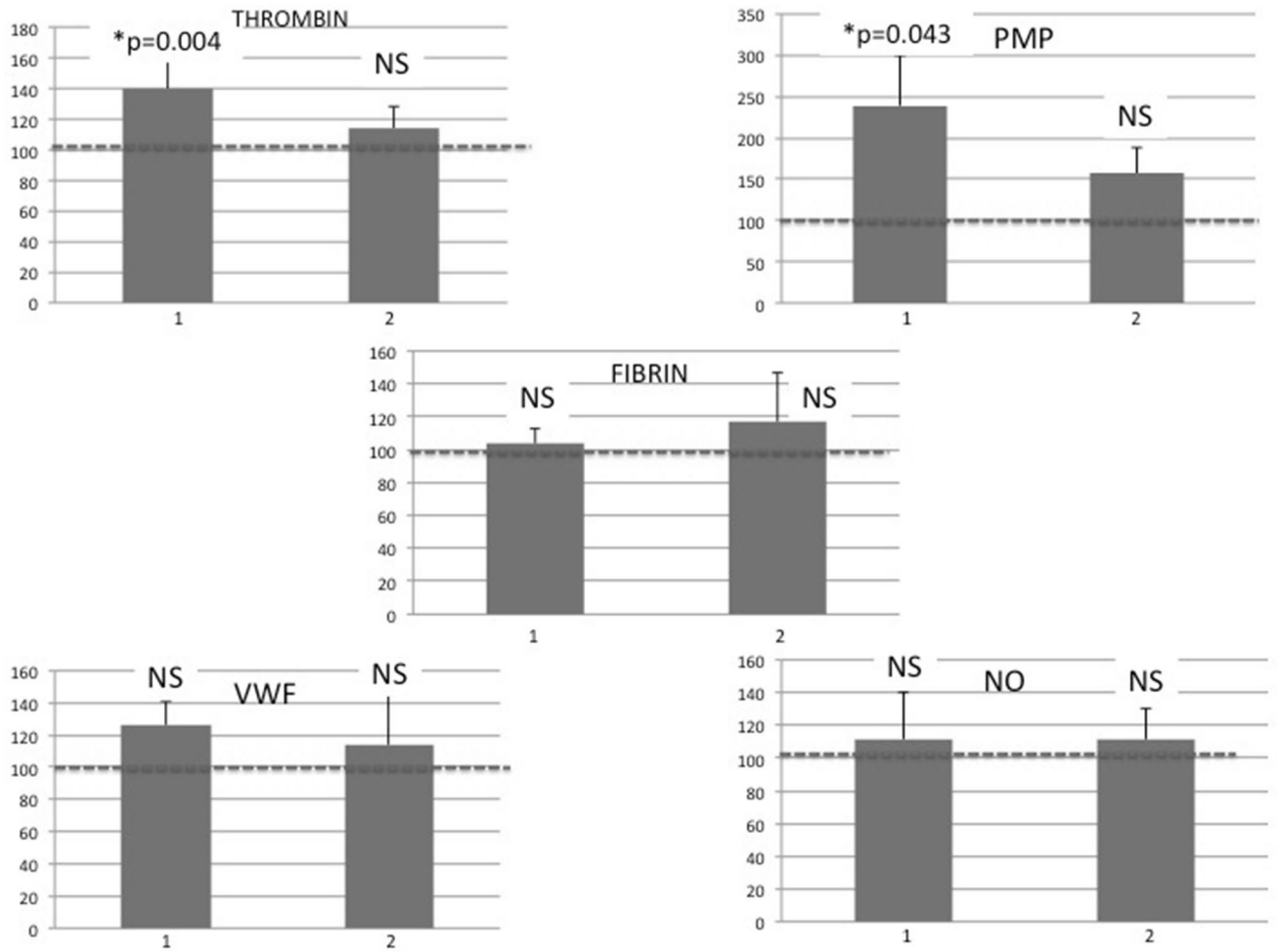

Fig. 4 Evolution (in % of baseline) for plasmatic markers (thrombin, PMP, fibrin, vWF and NO) after the dive protocol (*1*) or after the "vibrations + dive" protocol (2*)*. For each marker, the basal level represents the 100% value and is indicated by the *dashed line.* Data are presented as mean ± SEM. $*p < 0.05$

Discussion

In this paper, we took advantage of the protective effect of whole-body vibration preconditioning on post-dive bubble production to investigate the involvement of post-decompression intravascular circulating bubbles on the post-dive vascular dysfunction. Our data showed that conductance arteries were no longer impaired when the amount of VGE was lowered, whereas microcirculation alteration appears to be more likely independent.

The effect of vibration preconditioning on bubble formation

Our aim was to study which part of the impact of diving on the vascular system was specifically due to VGE to improve our understanding of the pathophysiology of decompression sickness. Thanks to vibration as preconditioning, we compared two groups of subjects who performed identical dives, which means that they were exposed to the same level and duration of hyperoxia, partial pressure of nitrogen and hydrostatic pressure. Matching these parameters between the groups was important, since we previously reported that hyperoxia exerted a greater impact on mitochondrial ROS generation in cultured endothelial cells but a lower impact on mitochondrial depolarization and cell death than hydrostatic pressure or nitrogen during diving (Wang et al. 2015). Similarly, Yang et al. (2015b) reported a more than threefold elevation in total circulating MPs in mice after exposure to 790 kPa on air or normoxic nitrogen, together with neutrophilic activation, platelet–neutrophil interactions and vascular injury. However, none of these changes occurred after exposures to the same partial pressure of oxygen that occurs during hyperbaric air exposures. Taken together, these data suggest that each environmental parameter related to diving acts on vessels through

different mechanisms. Thus, to use a single diving profile in the present study in association or not with preconditioning enabled us to focus specifically on the effect of VGE on the vascular function. In agreement with previous reports (Germonpré et al. 2009; Balestra et al. 2016), we found that the KISS was four times lower in subjects who received 30 min of whole-body vibrations prior to the same dive than in those who did not. Interestingly, we did not detect any difference for NO bioavailability after 30 min of vibrations alone which makes unlikely the possibility that lower amount of VGE results from increased endothelium-derived NO. On the other hand, Germonpré et al. (2009) hypothesized that whole-body vibrations might induce mechanical dislodgement or enhanced lymphatic elimination of gas nuclei which could prevent their transformation into circulating bubbles. This hypothesis was very recently reinforced by data showing that vibrations are more efficient to reduce VGE than pre-dive denitrogenation obtained by oxygen breathing (Balestra et al. 2016). Our present results also are more in favour of this hypothesis.

Effect on conductance artery

Endothelial-dependent and -independent vasomotion of large conductance vessel was assessed non-invasively with high-resolution ultrasound imaging of the brachial artery during vasodilation induced by either an increased flow or the NO donor nitroglycerin, respectively, as first described by Celermajer et al. (1992). For occlusion shorter than 5 min, it has been shown that the post-occlusive increase in blood flow induces an endothelium-dependent vasodilation of the brachial artery which is NO dependent (Pyke and Tschakovsky 2005). Alternatively, under physiologic conditions, NO donors (such as nitroglycerin and SNP) produce NO that stimulates directly relaxation of vascular smooth muscle and can thus be used as an endothelium-independent control (Turner et al. 2008). In accordance with our previous results (Lambrechts et al. 2013a), we found that both FMD and NMD of the brachial artery are significantly reduced after the control dive, which suggests that the whole vascular wall is altered. On the contrary, decreasing VGE prevented this post-dive vessel dysfunction, since both the FMD and NMD tests did not reach statistical difference when vibrations were administered before the dive. Our data suggest that VGE are responsible for the post-dive alteration of vascular function. This is further supported by previous works which showed impaired vasoreactivity after administration of microbubbles at atmospheric pressure (Nossum et al. 2002; Fok et al. 2015) as well as a deleterious action of bubbles on cultured endothelial cells (Klinger et al. 2011; Sobolewski et al. 2011). Besides a direct mechanical action (Klinger et al. 2011; Sobolewski et al. 2011), putative mechanisms by which VGE could impair vascular function include ROS (Obad et al. 2007a, 2010; Theunissen et al. 2015). In this regard, previous studies showed that (1) both oxidative stress (Mazur et al. 2016) and platelet activation (Baj et al. 2000; Pontier et al. 2008b) correlate with decompression stress, (2) pretreatment with anticoagulant decreases oxidative stress after the dive, even in the absence of DCS (Lambrechts et al. 2015) and (3): MPs released during the dive predominantly originate from platelets (Pontier et al. 2012; Thom et al. 2015) and exert a deleterious effect on vessel walls (Thom et al. 2011). With these data in mind, we thus sought whether the preventive action of pre-dive vibrations on vascular dysfunction could be related to an action on haemostasis pathways. Indeed, the increase in the thrombin generation and procoagulant MPs we found after the control dive was no longer present when bubble formation was reduced by vibration preconditionning, as was the impairment of brachial artery relaxation. However, that the activation of the coagulation cascade coincides with the decrease in FMD and NMD observed in our diving group raises the question of the exact relationship between these two parameters. Previous research showed that VGE can form during decompression from circulating MPs (Thom et al. 2013). However, because vibrations alone did not modify the concentration of circulating PMP, it seems unlikely that the lower amount of VGE results from less circulating MPs. Our data rather support the idea that the increase of PMP could be induced by VGE during decompression. Furthermore, because it is well documented that alteration of the endothelium can promote a procoagulant state (van Hinsbergh 2012), we then assessed on which function, of the coagulation or endothelial one, predive vibrations could first act. The VWF is a glycoprotein that promotes primary platelet adhesion and aggregation following vessel injury and carries coagulation factor VIII (Bryckaert et al. 2015). Although VWF is produced by both megakaryocytes and endothelial cells, its plasma levels depend almost entirely on secretion by endothelial cells (Kanaji et al. 2012). It has been reported that the level of VWF in the blood plasma can increase as quickly as 1 h, for instance following acute myocardial infarction (Li et al. 2015). Based on these, we used VWF as a reliable marker of acute endothelial activation. The lack of changes in VWF even after the control dive suggests that activation of the coagulation cascade, as assessed by the increased thrombin generation after the dive, is not initiated by a bubble-induced activation of the endothelium, but rather that circulating bubbles activate platelets which in turn could lead to vascular dysfunction and thrombin generation through liberation of MPs. Our data thus suggest that VGE activate platelet function and the release of PMP, maybe through oxidative stress, which in turn results in impairment of large conductance arteries. However, decreased FMD mediated by oxidative

stress was previously reported after breath-hold diving also, while no bubbles were produced (Theunissen et al. 2013a, b, c). This suggests that post-dive vascular dysfunction results not only from bubble-related insult, but that other factor(s) are also involved. Such a conclusion would agree with data from another recent work which showed that pre-dive vibrations reduce VGE and partially prevents the dive-induced impairment of FMD (Germonpré and Balestra 2017). In this work, the same decrease of post-dive FMD (about 5.4%) as we found in our present study reached statistical significance in a homogenous group of divers selected according to very strict biometric criteria and after a dive in 33 °C water temperature. This deserves further investigations.

Effect on microcirculation

At the level of microcirculation, the response to ACh remained unchanged after the control dive despite a decrease of the response to SNP. These data are similar to our results previously obtained on trained divers (Lambrechts et al. 2013c). Although the mechanisms by which iontophoretic administration of ACh increases cutaneous blood flow are still under debate, both NO and COX-dependent pathways have been evidenced (Roustit and Cracowski 2012). Besides its action through endothelium, a C-fiber (axon reflex)-mediated mechanisms have also been reported during iontophoresis of ACh (Berghoff et al. 2002). However, this mechanism occurs only when the iontophoretic current is equal to or higher than 5.10^{-2} mC mm^{-2}. Given that the surface of the drug-delivery electrodes is 113 mm^{-2}, we delivered a charge density equal to 3.10^{-2} mC mm^{-2} only. Thus, it is unlikely that an axon reflex occurred in our study and the response to ACh is therefore predominantly (if not only) endothelium dependent. In these conditions, the lack of changes in the cutaneous response to ACh reflects the integrity of microvascular endothelium post-dive. This conclusion is further supported by the unchanged plasmatic concentrations of VWF and nitrites found in this study as well as in previous ones (Marinovic et al. 2012; Theunissen et al. 2013a; Lambrechts et al. 2013c).

The absence of detectable bubbles in the VD group failed to preserve the microcirculatory function. This points to the differences in the adaptive mechanisms in these two vascular territories, since endothelium-independent vasodilation was preserved in brachial artery but not in cutaneous microcirculation following the "vibrations–dive" protocol. The absence of microbubbles in the microcirculation cannot be totally ruled out in our protocol, and the response to SNP was still impaired post-dive in this group although PMPs and thrombin generation (which are more likely to be linked with VGE) remained unchanged, suggesting that the impairment of microvessels is independent of circulating bubbles. Whether it results from increased ROS production during the dive has never been tested before, but is an attractive hypothesis. Recently, Wang and colleagues showed that the damage of cultured endothelial cells during simulated air dives results from the generation of ROS due to both increased hydrostatic pressure and hyperoxia (Wang et al. 2015). Since we previously showed that hyperbaric hyperoxia did not modify the response of skin blood flow to ACh or to SNP in divers (Lambrechts et al. 2013c), we can hypothesize that microcirculatory vessels could be more sensitive to the effect of hydrostatic pressure alone. However, this hypothesis needs further investigation.

In conclusion, the vibration preconditioning allowed us to limit the formation of bubbles during decompression, which in turn prevented the impairment of main conductance arteries but not microvessels. This suggests that the main conductance arteries are altered by circulating bubbles, besides hyperoxia and/or hydrostatic pressure, probably through activation of the coagulation cascade and increased ROS production, whereas microcirculation might be impaired by hydrostatic pressure but not VGE. It also suggests that if vascular dysfunction is involved in the occurrence of decompression sickness, not only VGE but other factors (including markers of the coagulation cascade and oxidative stress) should also be considered to assess the risk of DCS. Moreover, whether microvascular dysfunction could be involved in the risk of decompression sickness, as well as the link between oxidative stress, hydrostatic pressure and microvascular dysfunction, requires further investigation in the future.

Acknowledgements This work is part of the PHYPODE European network. This study was supported by the European Commission under the FP7-PEOPLE-2010-ITN program (Grant Agreement No. 264816).

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