RAPID REPORT

A single air dive reduces arterial endothelial function in man

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During and after decompression from dives, gas bubbles are regularly observed in the right ventricular outflow tract. A number of studies have documented that these bubbles can lead to endothelial dysfunction in the pulmonary artery but no data exist on the effect of diving on arterial endothelial function. The present study investigated if diving or oxygen breathing would influence endothelial arterial function in man. A total of 21 divers participated in this study. Nine healthy experienced male divers with a mean age of 31 ± 5 years were compressed in a hyperbaric chamber to 280 kPa at a rate of 100 kPa min^{-1} breathing air and remaining at pressure for 80 min. The ascent rate during decompression was 9 kPa min^{-1} with a 7 min stop at 130 kPa (US Navy procedure). Another group of five experienced male divers (31 ± 6 years) breathed 60% oxygen (corresponding to the oxygen tension of air at 280 kPa) for 80 min. Before and after exposure, endothelial function was assessed in both groups as flow-mediated dilatation (FMD) by ultrasound in the brachial artery. The results were compared to data obtained from a group of seven healthy individuals of the same age who had never dived. The dive produced few vascular bubbles, but a significant arterial diameter increase from 4.5 ± 0.7 to 4.8 ± 0.8 mm (mean ± S.D.) and a significant reduction of FMD from 9.2 ± 6.9 to 5.0 ± 6.7% were observed as an indication of reduced endothelial function. In the group breathing oxygen, arterial diameter increased significantly from 4.4 ± 0.3 mm to 4.7 ± 0.3 mm, while FMD showed an insignificant decrease. Oxygen breathing did not decrease nitroglycerine-induced dilatation significantly. In the normal controls the arterial diameter and FMD were 4.1 ± 0.4 mm and 7.7 ± 0.2.8%, respectively. This study shows that diving can lead to acute arterial endothelial dysfunction in man and that oxygen breathing will increase arterial diameter after return to breathing air. Further studies are needed to determine if these mechanisms are involved in tissue injury following diving.

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During a dive where compressed gas is used, gas is taken up into tissues in proportion to the depth and duration of the dive. This excess gas has to be eliminated during pressure reduction (decompression) when returning to the surface. In many cases this will lead to gas coming out of solution, forming bubbles, which are the main cause of clinical symptoms of decompression sickness (DCS). Bubbles can be detected in the pulmonary circulation in the majority of decompressions from dives. Eckenhoff *et al.* (1990) showed in man that very low levels of supersaturation (the sum of partial pressures of gas in excess of environmental pressure) were sufficient to allow the growth of bubbles, as a long dive on air at 136 kPa was sufficient to form detectable bubbles in the venous system. These bubbles, when occurring without any acute clinical signs, have been termed 'silent bubbles' (Behnke, 1951) and have in general been assumed to have no negative effects. However, we have previously shown that vascular bubbles will lead to a reduction in endothelial function in the pulmonary artery (Nossum & Brubakk, 1999). Generally it has been assumed that venous bubbles will be trapped in the pulmonary circulation and will have no further effects on the arterial circulation. However, there is a statistical relationship between the occurrence of many bubbles in the pulmonary artery and central nervous decompression sickness (Nishi *et al.* 2003). It is generally

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assumed that a right to left shunt, as is possible in the presence of a persistent foramen ovale (PFO), is necessary for the bubbles to affect the arterial system. Several authors (Wilmshurst *et al.* 1989; Moon *et al.* 1989) have shown that there is a relationship between the occurrence of a PFO and the incidence of central nervous decompression sickness (DCS) and this could conceivably be linked to damage caused by vascular bubbles. Recently it has been shown that even light exercise will allow gas bubbles to move through the pulmonary circulation and enter the arterial circulation in individuals without any PFO (Eldridge *et al.* 2004).

This present study was designed to determine if a dive would induce a reduction in arterial endothelial function in divers and if any changes were related to venous bubble formation in the pulmonary artery.

Methods

Study population

Nine male divers aged 31 ± 5 years participated in the diving study. Their mean body mass index (BMI) was $26 \pm 3\,\mathrm{kg\,m^{-2}}$. The subjects were all experienced divers with a mean of 726 h hours of diving (range 50–2000). None had experienced DCS in the past. At the time of the study all had a valid medical certificate for diving. An additional group of five divers aged 31 ± 6 years with a BMI of $28 \pm 2\,\mathrm{kg\,m^{-2}}$ represented the oxygen breathing group, while a group of seven healthy subjects who never had dived served as controls. Their mean age was 31 ± 4 years and their body mass index was $25 \pm 3\,\mathrm{kg\,m^{-2}}$, not significantly different from the two other groups. All experimental procedures were conducted in accordance with the Declaration of Helsinki, and were approved by Research Ethics Committee of the University of Split School of Medicine. Potential risks were explained to all subjects in detail and they gave written informed consent before the experiment. None of the participants of the study used any drugs, and food intake, alcohol and smoking were prohibited for 6 h prior to the test. All protocols were performed in the morning.

The nine divers were compressed in a hyperbaric chamber (Brodosplit, Split, Croatia) to 280 kPa at a rate of $100\,\mathrm{kPa\,min^{-1}}$ breathing air; they remained at pressure for 80 min. They were then decompressed at a rate of $90\,\mathrm{kPa\,min^{-1}}$ to 130 kPa, where they remained at rest for 7 min before they were decompressed to the surface pressure (100 kPa) at the same rate (United States Navy air decompression procedure). This shallow, long air dive has been shown to reproducibly produce a significant number of bubbles (Dujic *et al.* 2004). Following the dive, subjects were placed in the left supine position and an echocardiographic investigation with a phase array probe (1.5–3.3 MHz) using a Vivid 3 Expert ultrasonic scanner (GE, Milwaukee, USA) was performed by an experienced cardiologist (DD). High quality images were obtained in all subjects and gas bubbles were seen as high intensity echoes in the right heart and the pulmonary artery. Monitoring was performed every 20 min for 80 min after reaching surface pressure. Images were graded as previously described (Eftedal & Brubakk, 1997). This grading system has been used extensively in several animal species as well as in man. It has also been demonstrated that the grading system for Doppler (Spencer, 1976) coincides with that used for images (Brubakk & Eftedal, 2001). The grading system is non-linear when compared to the actual number of bubbles in the pulmonary artery. The bubble grades were converted into number of bubbles per square centimetre as previously described (Nishi *et al.* 2003). The number of bubbles were determined at each of the measurement times and then integrated to give an average bubble number for the whole observation period. The oxygen breathing group breathed a 60% oxygen–nitrogen mixture for 80 min at surface pressure (100 kPa).

Endothelial function

Endothelial function was determined according to the method of Raitakari & Celermajer, 2000) in all subjects participating in the study. This method determines the arterial response to reactive hyperaemia, flow-mediated dilation (FMD) (Corretti *et al.* 2002).

The subjects were placed in a quiet room with temperature about 20°C and were rested for 15 min on the bench in a supine position before measurement. Measurements were then performed with a 5.7–13.3 MHz linear transducer using a Vivid 3 Expert ultrasonic scanner (GE). Brachial artery diameter was measured from longitudinal images with lumen–intima interface visualized on both (anterior and posterior) walls. Images were acquired using ECG gating during acquisition, using the onset of R wave to identify end diastole. When the images were chosen for analysis, the boundaries for diameter measurement were identified manually with an electronic caliper. Pulsed Doppler measurements for measuring blood flow velocity were performed with the sample volume placed in mid-artery. The position of the transducer was marked to ensure the same position of the transducer for all measurements. Once the basal measurements were obtained, arterial occlusion was created by inflating a cuff placed on the forearm to 240 mmHg for 5 min. After 5 min inflation the cuff was deflated producing a brief high-flow state resulting in artery dilatation due to increased shear stress. Flow and diameter were measured during the first 15 s and then at the end of the first, second, third, fourth and fifth minute. Subjects were then rested for 10 min to get back to baseline diameter. We did not use nitroglycerine to assess endothelial-independent dilatation in the divers as we have previously shown that endogenous nitric oxide

Table 1. Bubble grade following air dive to 280 kPa for 80 min

Diver	Bubble grade 20 min	Bubble grade 40 min	Bubble grade 60 min	Bubble grade 80 min	Bubbles per cm^2 (integrated)
1	0	0	1	1	0.025
2	0	0	0	0	0
3	0	0	1	0	0.0125
4	1	1	1	1	0.05
5	0	0	0	0	0
6	0	0	0	0	0
7	0	1	1	0	0.025
8	0	0	0	0	0
9	0	0	0	0	0
Median/mean	0	0	0	0	0.025 ± 0.04

may influence the degree of bubble formation (Wisloff *et al.* 2003, 2004). The data were saved on tape (TDK SVHS Xp PRO) on a video recorder (Sony SVHS Hi-Fi SVO 9500 MDP). Measurements were performed before the dive and approximately 30 min after surfacing from the dive as well as before and after oxygen breathing.

The accuracy of the method was tested by having the investigator perform multiple measurements of diameter and FMD in five healthy non-divers on two separate days.

Flow mediated dilatation (FMD) was calculated as the percentage increase in brachial artery diameter from the resting state to maximal dilatation. Blood flow was calculated from the mean velocity measurements and the vessel diameter, assuming that the vessel was circular.

The oxygen breathing group underwent the same investigation for FMD before and after exposure. Furthermore, in this group, the endothelium-independent dilatation was determined by giving 1 mg nitroglycerine sublingually. The seven individuals in the control group were studied once using the same procedure as above.

Differences in diameter and FMD before and after the dive and oxygen breathing, respectively, were calculated as percentage changes.

Statistical analysis

Data are given as the mean ± standard deviation (S.D.) or as mean and 95% confidence intervals. Differences in arterial diameter and response to hyperaemia were determined using a one-sided Student's *t* test for paired samples. Differences between the diver and the control groups were compared using an unpaired *t* test. The limit of significance was set at $P = 0.05$.

Results

Surprisingly, following the dive, few bubbles were detected (Table 1). In no case could bubbles be seen in the left ventricle, indicating that no large PFO was present. In the diving group the basal diameter of the brachial artery increased from 4.5 ± 0.7 to 4.8 ± 0.8 mm. FMD decreased from 9.2 ± 6.9 to 5.0 ± 6.7%. Figure 1 shows the percentage difference in FMD and brachial artery diameter before and after the dive; both differences are significant. FMD was 1.4 ± 3.0% in the divers where bubbles were detected and 7.8 ± 6.5% in the divers where no bubbles were seen; this difference is not significant ($P = 0.09$).

Blood flow at rest did not change significantly following the dive. Following release of arterial occlusion, there was an immediate increase in blood velocity, while the arterial diameter slowly increased for 4–5 min following release. The mean increase in diameter pre-dive was 5.2% 1 min after release and 9.2% after 5 min; the values after the dive were 4.9 and 6%, respectively. There were no significant differences in maximum blood flow before and after the dive (312 ± 136 and 314 ± 117 ml min^{-1}, respectively), nor were there any significant changes in heart rate.

The changes in brachial artery diameter and FMD after oxygen breathing can be seen in Fig. 2. The basal diameter increased from 4.4 ± 0.3 to 4.7 ± 0.3 mm ($P = 0.005$); this increase is not significantly different from what was observed after the dive (7.9 *versus* 7.9%). FMD decreased from 6.4 ± 2.7 to 4.7 ± 4.2%; this decrease was not significant, and smaller than the decrease observed following the dive (32 *versus* 63%). The maximum dilatation following nitroglycerine decreased slightly from 11.9 ± 4.5 to 8.9 ± 2.2% after oxygen breathing; this difference was not significant.

In the control group the arterial diameter was 4.1 ± 0.4 mm and the FMD was 7.7 ± 2.8%. There was no significant differences in FMD between the divers and the control group before exposure. The arterial diameter was not significantly smaller than that observed in the divers pre-dive; however, after the dive or oxygen breathing the diameter was significantly larger in the experimental groups ($P = 0.03$). The diameter increase in controls was 6.7% 1 min after release of arterial occlusion and 5.3% after 5 min.

When measurement of diameter and FMD was performed repeatedly on separate days in five healthy individuals, the mean percentage intraobserver variability in diameter was 0.3 (95% CI 0.3) and 8.0 (95% CI 4.6) in FMD.

Discussion

The main finding in this study is an increase in brachial artery diameter and a reduction in arterial endothelial function following a single air dive that produced very few gas bubbles. Although it cannot be excluded that small gas bubbles can have been missed, direct observation of decompression bubbles indicates that they are in the range of 100–200 μm (Gersh *et al.* 1944; Grulke *et al.* 1973), a size that is easily detectable by ultrasound. Furthermore, no large PFO and left ventricular bubbles could be seen, and thus the presence of a significant number of gas bubbles in the arterial circulation is not very likely.

In the divers breathing 160 kPa oxygen for 80 min, there was a significant increase in arterial diameter with a concomitant non-significant reduction in FMD. As a non-significant decrease in dilatation following nitroglycerine was also seen, it is tempting to suggest that increased oxygen tensions influence smooth muscle function and that this effect is present even after oxygen breathing is stopped.

The diameter increases observed in this study are considerable. It is possible that the percentage reduction in FMD is partly caused by the increase in diameter. The two are probably linked, and both reduced dilatation and increased diameter are associated with increased risk of cardiovascular disease (Celermajer *et al.* 1994). In a large study, Kuvin *et al.* (2001) found FMD of 10.5% in normal controls and of 6.3% in patients with significant atherosclerosis. These are values similar to what was observed in the divers before and after the dive (9 and 5%, respectively). The divers in the present study had a brachial diameter and FMD before the dive similar to that seen in normal individuals and in the present control group.

FMD is most probably mediated by nitric oxide (NO) produced by the endothelial cells, as the response can be nearly completely abolished by L-NMMA (Mullen *et al.* 2001). This is also supported by other studies (Meredith *et al.* 1996). Following cigarette smoking, an intervention that is known to impair NO function, the diameter decreased by approximately 10% both at rest and following ischaemia (Stoner *et al.* 2004). We do not know the mechanism for the increase in basal diameter seen in this study. However, it is known that hyperoxia leads to vasoconstriction and that this may act as a trigger for an increased NO production (Demchenko *et al.* 2000). Increased NO production has been seen following hyperbaric oxygen breathing, but the increase was small when 100 kPa oxygen was used (Thom *et al.* 2003). Furthermore, oxygen breathing induces NO production in pulmonary endothelial cells (Cucchiaro *et al.* 1999), but seems to have little effect on endothelial cells from the systematic circulation (Whorton *et al.* 1997). We do, however, have preliminary data indicating that NO production is increased at 650 kPa breathing air, corresponding to a partial pressure of oxygen of 103 kPa (authors' unpublished observation, 2005).

As a degree of bubble formation occurred following this dive, the question remains if this will have an effect on arterial endothelial function. There was a considerably larger reduction in FMD in the divers with bubbles than in those without (7.8 *versus* 1.4%), although this difference was not significant ($P = 0.09$). We have shown in the rabbit that few bubbles will lead to endothelial dysfunction in the pulmonary artery between 1 and 6 h after exposure (Nossum *et al.* 2002). If we assume that endothelium in the venous circulation is activated following a dive, studies have shown that activated endothelium will produce endothelial microparticles and those particles can initiate endothelial dysfunction at remote sites (Brodsky *et al.* 2004). Endothelial microparticles have a size of a few micrometres and could possibly pass the lung filter and enter the arterial system. Thus, it is conceivable that changes in arterial endo thelial function can occur without direct contact with the bubbles.

Even if bubble formation and its effect on the endothelium may be a likely candidate for causing the reduction in FMD, other mechanisms may also be

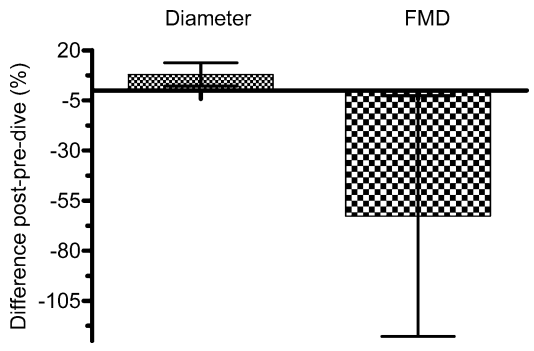

Figure 1. Difference in Brachial artery diameter and flow-mediated dilatation (FMD) before and after the dive
The data are the mean and 95% confidence intervals.

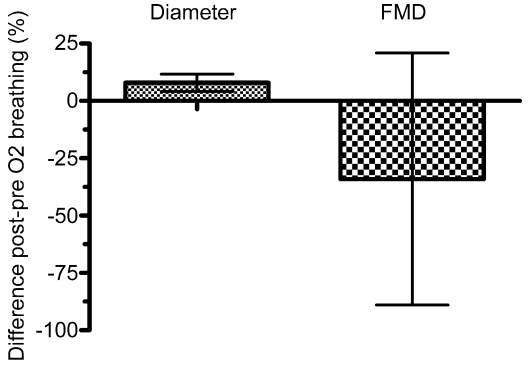

Figure 2. Difference in Brachial artery diameter and flow mediated diatation (FMD) before and after breathing 60% O_2
The data are the mean and 95% confidence intervals.

involved. Following sympathetic stimulation, there is significant reduction in FMD (Hijmering *et al.* 2002). Many factors related to the dive may cause sympathetic stimulation; the present study demonstrates that hyperoxia also may play a role.

This study was not designed to elucidate the possible mechanism of the increase in basal diameter and the reduction in FMD observed. In normal individuals the maximum diameter is usually found 1 min after the release of the occluding cuff (Celermajer *et al.* 1994; Mullen *et al.* 2001). This was also found in our study in the control subjects. In the divers, even before the dive, the increase was much more gradual, with maximum vasodilatation seen at the end of the observation period of 5 min. We do not know the importance of this, but note that some of the divers clearly had reduced endothelial function even before the dive and some of them even showed a reduction in diameter following release of the cuff (divers 1 and 5).

The dive procedure followed was similar to the one used in a previous study (Dujic *et al.* 2004) and there was no significant difference in age and body size between the two diver groups. In that previous study considerable bubble formation was seen, with a median bubble grade of 3 and an integrated bubble load over the observation period of 0.98 bubbles cm^{-2}. Compared to the mean bubble load of 0.0125 bubbles cm^{-2} seen in the present study, this demonstrates a significant individual variability causing a difference in bubble formation by a factor of 100 between the two studies.

In order to distinguish between endothelium-mediated vasodilatation and direct smooth muscle reaction, it is usual to give nitroglycerine, which will lead to vasodilatation even in the absence of endothelium (Corretti *et al.* 2002). This was not done in the individuals performing the dive in this study, as we have shown in several studies that NO may influence bubble formation (Wisloff *et al.* 2004; Wisloff *et al.* 2003). Based on the changes seen in the divers breathing oxygen in this study, it cannot be excluded that some of the reduction in FMD was caused by a reduction in smooth muscle function. However, a pilot study in five divers showed normal response to administration of nitroglycerine, indicating that smooth muscle function remains intact following a dive and that the reduced FMD following a dive is mainly endothelium dependent.

This is a study in a few individuals and the results therefore have to be viewed with caution. There has for many years been considerable controversy about the long-term effect of diving on the organism and some authors have claimed that diving will lead to permanent CNS changes (Todnem *et al.* 1990). However, while it is well acknowledged that decompression accidents can lead to CNS changes, no changes have been conclusively documented in divers who have not had any such accidents (Dutka, 2003). The present study shows that diving, even with minimum bubble formation, may impair endothelial function. However, regeneration of injured endothelial cells occurs and may prevent permanent injury (Dimmeler & Zeiher, 2004). Further studies are needed to determine the importance of these findings as a possible mechanism for long-term changes.

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