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ORIGINAL ARTICLE

Effects of successive air and nitrox dives on human vascular function

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Abstract SCUBA diving is regularly associated with asymptomatic changes in cardiac, pulmonary and vascular function. The aim of this study was to evaluate the changes in vascular/endothelial function following SCUBA diving and to assess the potential difference between two breathing gases: air and nitrox 36 (36% oxygen and 64% nitrogen). Ten divers performed two 3-day diving series (no-decompression dive to 18 m with 47 min bottom time with air and nitrox, respectively), with 2 weeks pause in between. Arterial/endothelial function was assessed using SphygmoCor and flow-mediated dilation measurements, and concentration of nitrite before and after diving was determined in venous blood. Production of nitrogen bubbles post-dive was assessed by ultrasonic determination of venous gas bubble grade. Significantly higher bubbling was found after all air dives as compared to nitrox dives. Pulse wave velocity increased slightly (∼6%), significantly after both air and nitrox diving, indicating an increase in arterial stiffness. However, augmentation index became significantly more negative after diving indicating smaller wave reflection. There was a trend for post-dive reduction of FMD after air dives; however, only nitrox diving significantly reduced FMD. No significant differences in blood nitrite before and after the dives were found. We found that nitrox diving affects systemic/vascular function more profoundly than air diving by reducing FMD response, most likely due to higher oxygen load. Both air and nitrox dives increased arterial stiffness, but decreased wave reflection suggesting a decrease in peripheral resistance due to exercise during diving. These effects of nitrox and air diving were not followed by changes in plasma nitrite.

Keywords Diving · Flow-mediated dilation · Arterial stiffness · Nitrox · Blood nitrite

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Introduction

Decompression sickness (DCS) represents the most severe complication of SCUBA (self contained underwater breathing apparatus) diving. Although, in most cases, DCS is related to rapid ascent and subsequently inadequate decompression that results in rapid generation of nitrogen gas bubbles that can occlude vessels and induce ischemic damage, still some cases of DCS occur without evident violations of diving protocol. The most discussed culprit for DCS are gas bubbles that are generated on venous side of circulation and occasionally transfer to the arterial side. However, although a correlation between gas bubble abundance (bubble grade) and DCS was shown (Eftedal et al. 2007), still it appears that DCS is a multifactorial disease. Activation of complement and endothelial dysfunction were also implicated as possible factors underlying the

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development of DCS (Madden and Laden 2009; Ward et al. 1987). Even in the absence of DCS, SCUBA diving is associated with a number of asymptomatic changes that can be detected following a dive. They include generation of silent bubbles and alterations in cardiovascular function, i.e., decrease in ventricular contractility, increase in pulmonary arterial pressure, increased secretion of atrial natriuretic peptide and endothelial dysfunction; and pulmonary function, i.e., change in lung diffusing properties, reduction in spirometric parameters and accumulation of extravascular lung water (Dujic et al. 2006; Marabotti et al. 1999; Marinovic et al. 2009, 2010; Obad et al. 2007a).

In recent years there is an increasing trend of using technical gases such as nitrox (a mixture of oxygen and nitrogen) instead of air for recreational dives to extend diving time and accelerate decompression. However, despite the positive effects of nitrox on decreased nitrogen loading during compression that acts to decrease venous gas bubbling, diving with nitrox is associated with exposure to higher oxygen partial pressures than air diving. Also, a detailed comparison of physiological effects of air and nitrox dives is still lacking.

In the current study, we aimed to compare in detail the effects of air and nitrox dives using the same no-decompression diving profiles to 18 m of sea water. We assessed bubble production following dives and investigated in depth the vascular function by assessing flow-mediated dilation, pulse-wave velocity, wave reflection and endothelial NO production by measuring plasma nitrite concentration.

Methods

Study population

Ten male nonsmoking SCUBA divers were enrolled in the study. They were 40.3 ± 2.6 years old, with average height of 1.8 ± 0.1 m and 93.6 ± 11.1 kg weight. The study population comprised of highly trained divers in both air and technical gas (nitrox and trimix) diving. All divers completed the study and no one developed symptoms of DCS. All experimental procedures were conducted in accordance with the Declaration of Helsinki, and were approved by the Ethics Committee of the University of Split School of Medicine. Each method and potential risks were explained to the participants in detail and they gave their written informed consent before the experiment.

Dive protocol and timeline of measurements

Divers performed three consecutive dives with air and three consecutive dives with nitrox 36 (36% oxygen and 64% nitrogen) as breathing gases, with at least two weeks pause between the two series. All dives were no-decompression to 18 meters of sea water (msw) with 47 min bottom time. Water temperature was 20 ± 3°C at the surface and 16 ± 1°C at the bottom. Divers wore wet suits, a SCUBA apparatus and Galileo dive computer (Uwatec, Johnson Outdoors Inc., Racine, WI, USA). Dive profiles were downloaded from dive computers to a PC for analysis of the dive's depth and duration as well as the heart rate (HR). The divers restrained from exercise for 24 h before diving and during the dives they performed exercise at about 30% of maximum predicted heart rate according to age (HRmax).

After surfacing, the divers were transported to the onshore diving facility by boat and carefully examined by a physician specialist for any adverse effects of decompression. Before and after each dive, assessment of arterial/endothelial function was performed using SphygmoCor and flow-mediated dilation measurement. On the first and third dive of each diving series, venous blood samples were withdrawn before and immediately after the dives to measure the concentration of nitrite. At 20 and 40 min after resurfacing, venous bubble grade was assessed as described below.

Bubble grade assessment

Within 20 min post-dive, the subjects were placed in the supine position and a phase-array ultrasonic probe (1.5–3.3 MHz) was positioned to obtain a clear view of the right and left atria and ventricles. The transducer was connected to a Vivid q ultrasonic scanner (GE, Milwaukee, WI, USA) and echocardiographic recordings were stored for further analysis. Bubble grading was assessed by two observers. The monitoring was performed at 20 and 40 min after reaching the surface. The gas bubbles were observed as high intensity echoes in pulmonary artery and cardiac cavities and recorded at rest and after performing two coughs. Bubble grading was performed according to method described by Eftedal and Brubakk (1997). The grading system uses the following definition: 0, no bubbles; 1, occasional bubbles; 2, at least one bubble/4th heart cycle; 3, at least one bubble/cycle; 4, continuous bubbling; at least one bubble/cm^2 in all frames and 5, "white-out", individual bubbles cannot be seen.

Assessment of arterial pulse-wave velocity and augmentation index

SphygmoCor (Version 8.1; AtCor Medical Inc., Sydney, Australia) system was used for noninvasive assessment of wave reflection parameters based on the principle of applanation tonometry on radial artery using strain gauge

transducer placed at the tip of a pencil-type tonometer (Pauca et al. 2001). Sphygmocor calibration was performed using two measurements of blood pressure with a manual mercury sphygmomanometer. The estimate of the central (aortic) blood pressure waveform was derived from radial artery pressure recordings. From the central blood pressure waveform, augmentation index (AIx) was calculated based on the formula: AIx (%) = [($P2-P1$)/PP] × 100 using the pressure difference between initial systolic ($P1$) and reflected wave ($P2$) in relation to the pulse pressure (PP) (Baulmann et al. 2008). AIx corrected for the heart rate of 75 beats/min was also calculated (AIx_{corr}). Carotid–femoral pulse wave velocity (PWVc-f) was assessed simultaneously with ECG recording in two steps, with the first step being carotid pulse wave recording, and the second step recording of the femoral pulse wave. PWV was calculated from the pulse transit time and distance traveled by the pulse wave. For distance measurements a scale calibrated in centimeters was used. Mean value from two measurements that satisfied software quality control was used for further analysis of both PWV and AIx.

Flow-mediated dilation

Flow-mediated dilation (FMD) measurements were used to assess endothelial function before and after each dive (Raitakari and Celermajer 2000). FMD determines the arterial response to reactive hyperemia, an established measure of endothelium-dependant vasodilation mediated mainly by nitric oxide (NO) (Corretti et al. 2002; Joannides et al. 1995). The subjects were placed in a quiet room with temperature about 22°C and were resting for 15 min in a supine position before the measurements. Participants were tested at the same time of the day to account for diurnal variation in endothelial function. All participants were asked to refrain from caffeine for at least 12 h before testing. Measurements of brachial diameter were performed with 5.7–13.3 MHz linear transducer using Vivid q before and after a 5-min ischemia induced by the forearm cuff inflation as described previously (Obad et al. 2007a, b). To analyze the changes in brachial diameter, automated edge-detection software was used (Woodman et al. 2001).

Blood nitrite

Venous blood samples were withdrawn before and after first and third dive in each diving series (air and nitrox) and immediately centrifuged at 800 *g* for 10 min at 4°C. Heparinized plasma was transferred in an ice bath and within 60 min frozen at −80°C until analysis. Under these conditions, we did not observe any loss of nitrite in human blood (data not shown). Nitrite was measured in 100 µL plasma aliquots by gas chromatography–mass spectrometry (GC–MS) as their pentafluorobenzyl derivatives as described elsewhere (Tsikas 2000). Study samples were analyzed within several runs alongside quality control (QC) samples. Each nitrite measurement was performed in duplicate and the reported values represent their mean.

Statistical analysis

Data are given as mean ± standard deviation (SD). Normality of the distribution was confirmed for all parameters using Kolmogorov–Smirnov test. All the comparisons of parameters measured for a single dive (pre- and post-dive values) were performed using Student's *t* test for paired samples. In order to examine whether the parameters changed over the course of consecutive dives (potential cumulative effects), the ANOVA analysis for repeated measures with Bonferroni post-hoc analysis was used. Bubble grades are presented as median (25–75% quartile range) and were compared using nonparametric Friedman analysis of variance. In case of a significant difference, the Wilcoxon sign rank test was applied for the particular comparison. The limit of significance was set at $P < 0.05$. Analyses were done using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK, USA).

Results

Bubble grade

Bubble grade assessment at 20 and 40 min after the air and nitrox dives revealed no significant difference between bubble grades in three consecutive dives either with air or nitrox, and no signs of diving acclimatization during the three consecutive dives. Significantly higher bubbling was found after all air dives as compared to nitrox dives at 40 min post-dive ($P = 0.02$; Fig. 1). Also, there were seven arterializations of gas bubbles after air dives as compared to two arterializations after diving with nitrox.

Arterial stiffness

Indicators of arterial stiffness and wave reflections significantly changed following each dive (Table 1). The most direct measure of arterial stiffness, PWVc-f increased slightly (~6%) but significantly after both air and nitrox diving indicating an increase in arterial stiffness. However, AIx value decreased after each dive indicating a lesser wave reflection. No significant change in heart rate, systolic and diastolic blood pressure or pulse pressure was observed, although there was a tendency for a slight decrease in diastolic blood pressure following the dives. Moreover, no cumulative effect of consecutive dives and no

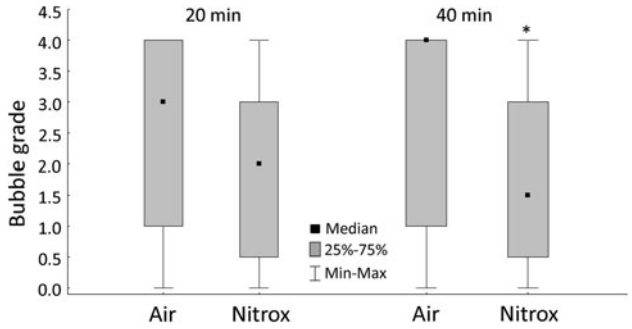

Fig. 1 Venous gas bubble grades after air and nitrox diving. Shown are the medians 25th and 75th quartiles (*shaded squared areas*) and ranges (*error bars*) of the venous bubble grades measured after the dives. *$P < 0.05$ versus air

Table 1 SphygmoCor parameters

	Air		Nitrox	
	Pre-dive	Post-dive	Pre-dive	Post-dive
AIx (%)				
Day 1	4.3 ± 7.6	−2.6 ± 11.7*	9.1 ± 5.0	0.6 ± 8.7*
Day 2	5.8 ± 7.5	0.6 ± 9.5*	8.8 ± 6.1	2.2 ± 7.6*
Day 3	7.7 ± 8.3	1.5 ± 8.4*	10.6 ± 4.8	1.0 ± 7.3*
AIx_{corr} (%)				
Day 1	−1.9 ± 5.5	−9.3 ± 8.7*	1.1 ± 3.6	−6.8 ± 5.5*
Day 2	−2.4 ± 5.7	6.1 ± 8.1*	2.0 ± 4.7	−6.2 ± 5.8*
Day 3	−0.1 ± 6.8	5.1 ± 7.1*	3.2 ± 3.9	−6.0 ± 4.9*
PWVc-f (m/s)				
Day 1	6.7 ± 0.6	7.0 ± 0.7*	6.3 ± 0.6	7.0 ± 0.4*
Day 2	6.4 ± 0.8	6.9 ± 0.8*	6.6 ± 0.4	7.1 ± 0.7*
Day 3	6.2 ± 0.9	6.7 ± 0.7*	6.5 ± 0.7	6.7 ± 0.6

Shown are pre-dive and post-dive measurements of augmentation index (AIx), augmentation index corrected for heart rate (AIx_{corr}) and carotid-to-femoral pulse wave velocity (PWVc-f)

* $P < 0.05$ pre-dive versus post-dive

difference in effects on these parameters between air and nitrox dives were observed.

Flow-mediated dilation

Results of FMD assessment are shown in Fig. 2. Although there was a trend for reduction of FMD after air dives, only nitrox diving significantly reduced FMD after all dives. There was no difference in brachial artery basal diameter and pre-dive FMD values between consecutive dives (data not shown).

Blood nitrite

No significant differences in blood nitrite concentrations were found before and after dives (Fig. 3).

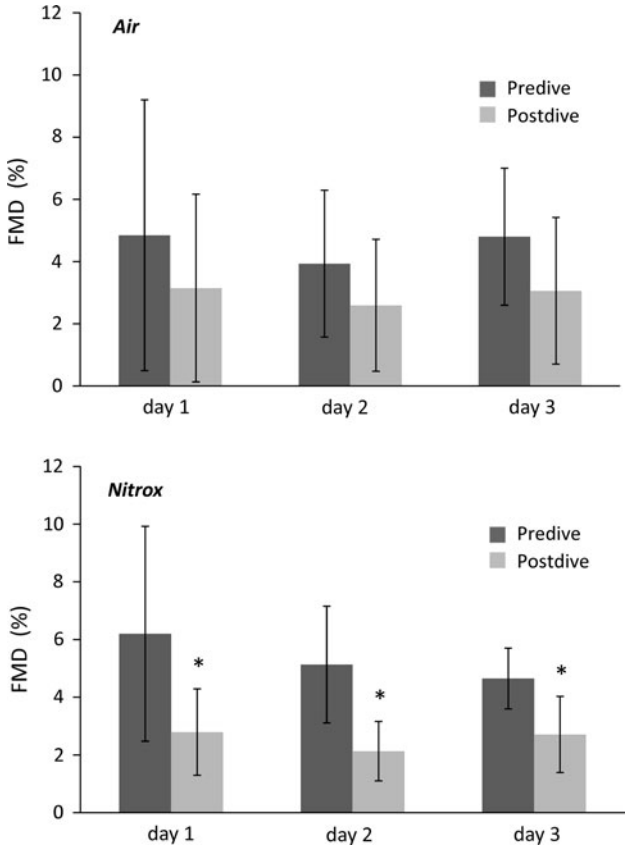

Fig. 2 Flow-mediated dilation before and after dives. Shown are FMD values for air and nitrox dives on days 1, 2 and 3 (*$P < 0.05$ pre-dive vs. post-dive)

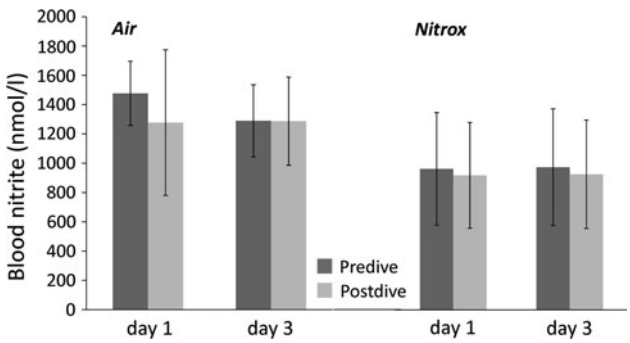

Fig. 3 Blood nitrite before and after dives. Shown are blood nitrite measured pre- and post-dive on days 1 and 3 of consecutive air and nitrox diving, respectively

Discussion

In the current study, we compared in detail acute effects of air and nitrox multiday diving on vascular/endothelial function. We found that SCUBA diving with both inhaled gases induced mild alterations of (macro)vascular function, namely an increase in aortic stiffness, while nitrox dives

had more consistent negative effect on endothelial function, indicated by reduced FMD despite significantly lower gas bubbles load compared to air.

In recent years, nitrox diving is becoming more popular among divers due to lower nitrogen load, increased oxygen concentration and resulting shorter decompression. Also, anecdotally, large number of divers report that nitrox dives are associated with less fatigue after diving as compared to air dives. In the current study, we used identical no-decompression diving profiles for both nitrox and air diving and, as expected, found less venous gas bubbling following nitrox dives compared to air. Also, as in our recent studies, we found crossing of gas bubbles to systemic arterial side. These arterializations occurred more often after air dives as compared to nitrox (seven vs. two arterializations, respectively), which could be explained by the higher overall bubble load after air diving (Ljubkovic et al. 2010, 2011). However, despite higher gas bubbling after air dives, nitrox diving had greater impact on endothelial function, as evidenced by significantly reduced FMD response after nitrox diving. Aortic stiffness and wave reflections have been shown to be rather good predictors of cardiovascular disease (Laurent et al. 2006). Carotid-to-femoral pulse wave velocity (PWVc-f), generally accepted as the most reliable noninvasive measure of arterial (aortic) stiffness, was increased after all dives indicating an increase in aortic stiffness after diving. This increase in PWVc-f was relatively small averaging at ~0.4 m/s [changes >1 m/s are considered as clinically relevant to the assessment of cardiovascular risk (Lantelme et al. 2002)], however, it was consistently observed. Aortic stiffness is a parameter that depends on both structural and functional vessel properties: amount of collagen and elastin in arterial wall, mass and tone of arterial wall smooth muscle, distending pressure and endothelial function (Oliver and Webb 2003). In the current study, an acute, post-dive rise in central arterial stiffness is most likely mediated by the acute increase in arterial muscle tone, since other functional parameters (distending pressure) were not significantly changed after diving. On the other hand, assessment of AIx, another, more indirect indicator of arterial stiffness, revealed greater negativity of AIx after each dive suggesting a reduced wave reflection to the aorta and consequently reduced systolic load on the heart immediately following the dive. AIx is a complex parameter that is, besides arterial stiffness, affected by other cardiovascular parameters such as heart rate and peripheral resistance (Kelly et al. 2001; Wilkinson et al. 2000). An increase in arterial stiffness would increase the value of AIx. This discrepancy between increased PWV showing increased arterial stiffness and more negative AIx showing reduced wave reflection could be explained by the decreased post-dive peripheral resistance, which would reduce the wave reflection and consequently decrease AIx. Possible effects of heart rate on AIx were avoided using AIx corrected for heart rate—AIx_{corr}. Since our subjects performed exercise during their dives (at approximately 30% HRmax), we suggest that the observed significant decrease in AIx post-dive is due to peripheral exercise-induced vasodilation of resistance vessels that damps the amplitude of the reflected wave and results in reduction of AIx. Similar observation and explanation were given in a study by Vlachopoulos et al. (2010) where AIx was significantly decreased in marathon runners after race. In conclusion, it seems that, although slightly increased aortic stiffness after both air and nitrox dives (as suggested by the increase PWVc-f) acts to increase the systolic load of the left ventricle, exercise during the dives, through vasodilation of arterioles and reduction in total peripheral resistance, reduced the amplitude of the reflected wave and consequently opposed this increase in systolic cardiac load.

A change in arterial stiffness post-dive could have resulted from the effects of immersion. Boussuges et al. (2009) reported that prolonged thermoneutral immersion significantly increased carotid-to-pedal PWV. Also, exposure to cold during diving with resulting sympathetic activation could also cause this increase in arterial stiffness (Boutouyrie et al. 1994; Edwards et al. 2006). Increased aortic muscle tone could also be partly mediated through the increased oxidative stress in SCUBA diving. Oxidative stress is known to be one of the major scavengers of NO, reducing its bioavailability (Landmesser et al. 2006). However, although nitrox dives should be associated with greater oxidative stress compared to air dives, there was no difference in their effects on PWVc-f. On the other hand, air dives were associated with greater production of venous gas bubbles, some of which also arterialized (crossed from venous to arterial systemic side). Arterialized gas bubbles could have directly affected the systemic arterial endothelium, and nonarterialized bubbles could have, through endothelial microparticles, indirectly affected the endothelium and caused an increased tone of large arteries. Indeed, a rise in endothelial microparticles expressing vascular cell adhesion molecule-1 (VCAM-1) was found following simulated air, but not oxygen SCUBA dives (Vince et al. 2009).

As indicated earlier, in the current study FMD measurements revealed that nitrox diving is associated with significantly decreased post-dive FMD, while air diving, despite the tendency to decrease FMD, did not have significant effect. This was somewhat surprise finding, since in all our previous studies, we observed a significant FMD attenuation after air dives (Brubakk et al. 2005; Obad et al. 2007a, b; 2010). Possible explanation for this discrepancy is that the maximum depth of dives from the current study is almost half the depth from air dives in the previous studies, resulting in lower level of oxygen loading. Since oxidative

stress is one of the major mechanisms whereby SCUBA diving reduces bioavailability of NO in conduit vessels (indicated by reduced FMD) (Obad et al. 2007a) the level of oxidative stress in air diving to 18 msw was possibly not severe enough to cause depletion of NO that is sufficient to induce significant functional changes of shear-induced vasodilation. A line of evidence supporting this finding is that nitrox 36 dives to 18 msw, with higher level of oxidative load, significantly attenuated FMD post-dive. Also, antioxidants were shown to reverse negative diving-induced effects on FMD (Obad et al. 2007a).

Concentration of nitrite (NO_2^-) in the blood/plasma is used as an indicator of NO (Grau et al. 2007). In the current study, we assessed plasma nitrite concentration before and after both air and nitrox dives and found no difference between pre- and post-dive values, suggesting no significant difference in NO levels. This finding of unchanged nitrite pre-dive versus post-dive levels in both nitrox and air seems to contradict the finding of reduced FMD after nitrox diving, which suggests that NO production is reduced following nitrox dives. However, it is important to note that FMD is used to assess vasoreactive response to increased shear that is partially dependent upon NO production, while in our measurements we assessed NO production (through nitrite measurement) under basal conditions. Indeed, it was shown that that there is a good correlation between blood nitrite and reactive post-ischemic hyperemia measured by impedance plethysmography, whereby the blood was withdrawn every 10 s during the reactive hyperemia itself (Schwarz et al. 2011). Our finding of unchanged NO/nitrite could be explained by the fact that in our subjects we observed both vasoconstrictive response of large (conduit) arteries, as indicated by increased PWVc-f, and vasodilative response of small (resistance) arteries, as indicated by the decreased AIx after each dive. The two opposing responses of systemic vasculature in NO production might have offset each other and resulted in no significant change in post-dive nitrite concentrations. Another explanation could be that the changes in nitrite concentration were of such a small extent and varied greatly among the individuals that potential differences could not be reliably detected in this number of subjects.

It is important to note that the current study does have certain limitations. First, it was performed in limited number of subjects, making it necessary to view the obtained results with caution. However, the repetition of measurements (pre-dive and post-dive) in each participant means that each diver was his own control, thus facilitating detection of any potential changes in the measured parameters. Second, we did not normalize our FMD results to shear rate due to limitations of the software for FMD analysis and its partial incompatibility with our ultrasound device, which resulted in our inability to measure flow and estimate shear stress.

Conclusions

In conclusion, our results indicate that after both air and nitrox diving there was a small vasoconstrictive response of large (conduit) arteries, as indicated by increased PWVc-f, and vasodilative response of small (resistance) arteries, as indicated by the decreased AIx after each dive. Increased arterial stiffness of large conduit arteries such as aorta (evidenced by increase in PWV) could be due to the effects of immersion, cold and sympathetic stimulation, as well as oxidative stress (in nitrox diving) or bubble-induced endothelial microparticles (after air dives). All of these factors could affect arterial compliance; but the extent of influence of each of these factors could not be determined based on the data from the current study. A decrease in AIx is most likely due to peripheral vasodilation resulting from physical activity that dilates resistance arteries in working skeletal muscles. On the other hand, a reactive response of conduit arteries to increased shear stress (as assessed by FMD), was attenuated following nitrox dives, but not air dives. These effects were observed despite the significantly lower number of gas bubbles following nitrox dives as compared to air, suggesting that higher oxygen load during nitrox dives are responsible for the observed endothelial dysfunction.

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