Effects of Successive Air and Trimix Dives on Human Cardiovascular Function

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ABSTRACT

MARINOVIC, J., M. LJUBKOVIC, A. OBAD, D. BAKOVIC, T. BRESKOVIC, and Z. DUJIC. *Med. Sci. Sports Exerc.*, Vol. 41, No. 12, pp. 2207–2212, 2009. **Introduction**: The use of trimix (a mixture of oxygen, helium, and nitrogen) has significantly increased among the diver population. However, data indicating how trimix dives at most common depths affect the cardiovascular function are sparse. The purpose of this study was to investigate the cardiovascular effects of trimix dives and compare them with air dives and to determine whether the repetition of dives in successive days affects their extent. **Methods**: Nine professional divers performed four dives in consecutive days where the dive depth was progressively increased to the maximum of 55 m. Divers used air in the first dive, nitrox 25 in the second, and trimix 20/30 in the third and fourth dives. Echocardiography was performed before and after each dive. **Results**: After each dive, a significantly decreased left ventricular ejection fraction and fractional shortening and an increased end-systolic volume without a change in end-diastolic volume were found, indicating a depressed systolic function of the left side of the heart. Assessment of the ratio between pulmonary artery acceleration time and right ventricular ejection time (used as an indicator of pulmonary artery pressure (PAP)) revealed an increase in PAP after all the dives. No physiologically relevant cumulative effects of the multiple dives or signs of acclimatization were found. **Conclusions**: The current study shows that the cardiovascular effects of trimix dives do not differ from those of the dives with compressed air. However, it suggests that even a very safe and conservative trimix diving profile exerts significant cardiovascular effects. **Key Words:** SCUBA, TECHNICAL DIVING, SYSTOLIC FUNCTION, PULMONARY ARTERY PRESSURE

Compressed air as the most commonly used breathing gas in scuba diving has well-known depth restrictions because of the risk for development of nitrogen narcosis and oxygen toxicity at greater depths. These limitations were substantially reduced, and the maximum safe depths were significantly increased with the introduction of gas mixtures of oxygen, helium, and nitrogen (trimix), which became a method of choice for dives deeper than 50–60 m of seawater (3). The popularization of diving in the last couple of decades resulted in a considerably increased number of recreational technical divers using trimix. Although the danger for developing decompression sickness (DCS) is lower after a trimix dive compared with after the air dive (21), decompression tables for the trimix dives are not completely validated, and there is no defined recompression protocol in case of a trimix dive-related DCS. Furthermore, scientific data on the impact of trimix dives on the human body are very sparse. The majority of research dealt with the effects of trimix on symptoms of high-pressure nervous syndrome during extreme dry dives in a hyperbaric chamber (1,2,19). No other human studies regarding the physiological effects of technical field dives with trimix have been reported in the scientific literature.

It has been well documented that diving with compressed air is associated with various physiological disturbances. Even after a single recreational dive, in addition to the asymptomatic venous gas bubbles that can be regularly seen in the right side of the heart and pulmonary artery, significant changes are found in the cardiovascular system. They include an increase in pulmonary artery pressure (PAP) (8), a right ventricular overload (15), a reduction in left ventricular contractile function (16), and arterial endothelial dysfunction (5), with some of these changes lasting up to 3 d (12). To date, no such investigations were performed for the trimix dives. Moreover, the majority of the studies examined the effects of a single dive, although repetitive dives in successive days are regularly performed by both professional and recreational divers. In contrast to a widespread notion that multiday diving increases the risk of DCS, some studies suggest that the risk of DCS might even decrease as a result of diving acclimatization (7,11). However, there are no data indicating how the multiple compressions and decompressions in consecutive days affect the cardiovascular function.

Therefore, the goal of the present study was to investigate the cardiovascular effects of trimix dives and

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compare them with the air dives. Moreover, we wanted to determine whether the repetition of dives in successive days affects the magnitude of these asymptomatic cardiovascular changes.

METHODS

Study population. The study enrolled nine divers aged 35.9 ± 8.7 yr (range = 24–49 yr), with an average body mass index of 25.0 ± 2.7 $kg \cdot m^{-2}$ (range = 22.4–27.9 $kg \cdot m^{-2}$) and height of 1.83 ± 0.04 m (range = 1.75–1.89 m). Spirometric measurements indicated mean forced vital capacity at 113.2 ± 13.7% and forced expiratory flow in the first second at 106.2 ± 17.2% of predicted values. All participants were professional divers with considerable diving experience (several thousand hours of air diving), and three of the divers had experience in diving with trimix and nitrox gas mixtures. Previously, during their professional career, two of the divers experienced a mild form of DCS (skin and joint symptoms) that were not treated in the decompression chamber. Two of the participants were smokers (15–20 cigarettes per day).

At the time of the study, the divers showed no signs of acute or chronic illness. 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.

Location of the study and dive protocols. The diving site was in the relative vicinity of the field laboratory, and divers were transported to the site by a powerboat during a 30-min ride. The location of the site was chosen because it allowed dives of suitable depth and duration. The outside temperature varied between 24 and 26°C, and the bottom sea temperature was 15°C for all the dives (ranged from 14 to 16°C). In all the performed dives, four divers were equipped with dry suits, whereas five used wet suits.

The study was performed while the divers were participating in the technical diving course for nitrox and trimix. During this time, they performed four dives in four consecutive days, where the dive depth was progressively increased to the maximum of 55 m, and the diving profiles and the breathing gas mixtures were predetermined by the course requirements. In the first dive, air was used as the breathing gas. In the second dive, nitrox 25 (mixture of 25% oxygen and 75% nitrogen) was used, and in the last two dives, the breathing gas was trimix 20/30 (a mix of 20% oxygen, 30% helium, and 50% nitrogen). All the dives included a 2-min decompression stop at the depth of 21 m, where a switch to nitrox 50 (mixture containing 50% O_2 and 50% nitrogen) was made. The exact diving profiles are depicted in Figure 1.

Timeline of measurements. Before each dive, measurements of blood pressure, body weight, and echocardiographic parameters were performed in all divers. After completion of the diving protocol, these measurements were taken again 60 min upon resurfacing. All the dives throughout the study were performed at approximately the same time of the day (morning).

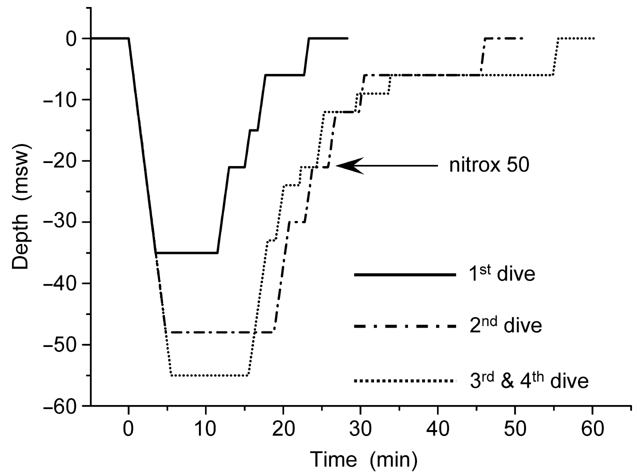

FIGURE 1—Shown are the profiles of the performed dives. *Arrow* indicates the depth (21 m) where the divers changed their breathing gas to nitrox 50.

Echocardiographic monitoring and venous gas bubbles detection. The subjects were placed in the supine position and an echocardiographic investigation with a phase array probe (1.5–3.3 MHz) using Vivid 3 Expert ultrasonic scanner (GE, Milwaukee, WI) was conducted. All echocardiographic investigations were performed by an experienced cardiologist.

Venous gas bubbles were seen as high-intensity echoes in the right side of the heart and the pulmonary artery after diving at rest and after two coughs. Images were graded and transferred into a linear scale as previously described (8).

Assessment of left ventricular function. Two-dimensional echocardiographic studies were performed using a standard examination protocol. The apical four-chamber view that displays all four cardiac chambers, as well as the ventricular and atrial septa, was identified initially by palpation of the cardiac apex with the patient in the left lateral decubitus position. The ultrasound probe was positioned in the parasternal long axis to visualize structures of the left side of the heart, primarily the inferoposterior wall and interventricular septum, and two-dimensionally guided M-mode echocardiography was then performed. The cross-sectional axis of the left ventricle (LV) at the papillary muscle tip level was measured. Measurements of LV septal, posterior wall, and cavity dimensions (LV interventricular septum (IVS) and LV posterior wall (LVPW)) were performed at the end-diastolic and end-systolic periods. Three consecutive cardiac cycles were measured, and the average values were obtained. All the measurements were made according to the American Society of Echocardiography (17).

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TABLE 1. Bubble grade and bubble count recorded 60 min after each dive.

	Bubble Grade		Bubble Count	
	60 min	60-min Cough	60 min	60-min Cough
First dive	0 (0–3)	0 (0–3)	0.3 ± 0.4	0.4 ± 0.5
Second dive	2 (0–4)	3 (0–4)	0.9 ± 1.6	2.5 ± 2.4*
Third dive	2 (0–4)	3 (0–4)	1.4 ± 2.1	2.5 ± 2.4*
Fourth dive	1 (0–3)	2 (0–3)	0.3 ± 0.4	0.3 ± 0.4

Bubble count is expressed as the number of bubbles per square centimeter. Values are presented as median (range) for bubble grade and mean ± SD for bubble count. Differences between days were compared using repeated-measures ANOVA with Bonferroni *post hoc* test.
* $P < 0.05$.

From two-dimensional and M-mode measurements, the following parameters were derived: LVIDd and LVIDs (left ventricular internal diameter in diastole and systole, respectively), endocardial fractional shortening (FS) and stroke volume (SV). FS was calculated on the basis of the following formula: FS (%) = 100[(LVIDd − LVIDs)/LVIDd]. SV was derived from diastolic and systolic LV volumes using the formula of Teichholz et al. (18): SV (mL) = EDV − ESV, where EDV is end-diastolic volume and ESV is end-systolic volume. This value was multiplied by HR to obtain a value for cardiac output (CO; $L \cdot min^{-1}$). Ejection fraction was calculated from the following, EF (%) = 100[(EDV − ESV)/EDV], and was used as an index of cardiac systolic function.

Assessment of pulmonary arterial pressure. Transthoracic echocardiography was used to estimate mean PAP before and after the dive. The transducer was pointed to the outflow tract of the right side of the heart, with pulse wave Doppler sample volume positioned at the level of the pulmonary valve annulus. The pulmonary artery flow velocity was recorded during cessation of breathing. Doppler measurements were averaged during three consecutive cardiac cycles, and pulmonary artery AccT (acceleration time (ms); represents the time interval between the onset and peak of pulmonary flow velocity) and RVET (right ventricular ejection time (ms), representing the time interval from the onset to termination of the systolic pulmonary flow velocity) were measured. AccT/RVET ratio was calculated for each cardiac cycle, and on the basis of the previously shown good relationship between AccT/RVET and invasively measured PAP and pulmonary vascular resistance, it was used as an index of the mean PAP (13,20).

Statistical analysis. Data are given as the mean ± SD. Normality of the distribution was confirmed for all parameters using the Kolmogorov–Smirnov test. All the comparisons of parameters measured for a single dive (predive and postdive values) were performed using Student's *t*-test for paired samples. To examine whether the parameters changed over the different dives (potential cumulative effects of the consecutive dives), repeated-measures ANOVA with Bonferroni *post hoc* analysis was used. A *post hoc* power analysis was performed with 80% statistical power to determine the extent of the minimal changes in the baseline (predive) values that we could detect with $P = 0.05$ as the borderline significance level. All analyses were done using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK).

RESULTS

All nine divers successfully completed all of the diving protocols, and nobody developed any symptoms of DCS. After each dive, the body weight decreased slightly but significantly, although no differences in the extent of weight loss or the cumulative weight loss were found between the dives (data not shown). Measurements of blood pressure revealed that the systolic blood pressure significantly decreased after the fourth dive and the diastolic pressure increased after the third dive (data not shown). Mean arterial pressure was not affected in any of the days.

Venous gas bubbles were detected 60 min after each dive with and without cough, with a significantly higher number of bubbles after the second and third dives compared with the first and fourth dives (60-min cough; Table 1).

Assessment of the left ventricular systolic function (Table 2) revealed a dive-induced decrease of the CO after the second and fourth dives. Also, HR was slightly decreased after diving, whereby statistical significance was reached only for the first dive. Moreover, the EF and FS were significantly reduced after each dive. Assessment of

TABLE 2. Cardiac volumes and function in divers before and after each dive.

	Before Dive	After Dive
CO ($L \cdot min^{-1}$)		
First dive	5.7 ± 1.0	5.2 ± 0.9 (0.093)
Second dive	6.3 ± 0.9	5.7 ± 1.0* (0.028)
Third dive	5.6 ± 0.9	5.4 ± 0.8 (0.39)
Fourth dive	6.0 ± 0.9	5.4 ± 0.8* (0.038)
HR (bpm)		
First dive	67.2 ± 9.3	62.3 ± 11.8* (0.024)
Second dive	73.7 ± 10.8	69.0 ± 12.4 (0.126)
Third dive	67.9 ± 4.7	67.4 ± 8.8 (0.826)
Fourth dive	71.4 ± 9.6	67.3 ± 9.7 (0.124)
SV (mL)		
First dive	85.6 ± 16.7	83.9 ± 13.7 (0.38)
Second dive	86.3 ± 14.6	84.1 ± 12.8 (0.13)
Third dive	82.8 ± 13.3	79.6 ± 14.1 (0.36)
Fourth dive	84.0 ± 14.4	81.2 ± 13.9 (0.16)
EF (%)		
First dive	67.8 ± 3.3	64.0 ± 4.3* (0.04)
Second dive	67.5 ± 2.5	64.4 ± 3.4* (0.001)
Third dive	67.4 ± 2.5	62.2 ± 5.4* (0.009)
Fourth dive	68.4 ± 1.5	63.4 ± 3.1* (0.009)
FS (%)		
First dive	38.1 ± 2.6	35.1 ± 3.1* (0.003)
Second dive	37.7 ± 2.0	35.2 ± 2.4* (0.0003)
Third dive	37.8 ± 2.1	33.8 ± 3.9* (0.004)
Fourth dive	38.5 ± 1.3	34.0 ± 2.6* (0.0004)
ESV (mL)		
First dive	40.4 ± 9.3	47.1 ± 11.3* (0.02)
Second dive	41.9 ± 7.8	46.6 ± 7.8* (0.02)
Third dive	40.1 ± 7.3	48.1 ± 8.3* (0.0005)
Fourth dive	38.7 ± 5.8	46.4 ± 6.0* (0.0009)
EDV (mL)		
First dive	126.0 ± 24.1	131.2 ± 22.1 (0.17)
Second dive	128.0 ± 20.3	130.7 ± 18.3 (0.13)
Third dive	122.0 ± 19.5	127.6 ± 17.3 (0.15)
Fourth dive	122.7 ± 19.8	127.7 ± 18.4 (0.07)

Shown are mean values ± SD. *P* values of predive versus postdive comparisons are shown in parentheses.
* $P < 0.05$.

TABLE 3. Pulmonary artery function in divers before and after each dive.

	Before Dive	After Dive
AccT (ms)		
First dive	163.0 ± 30.1	142.5 ± 21.0* (0.006)
Second dive	157.9 ± 30.7	126.8 ± 26.0* (0.006)
Third dive	158.5 ± 18.8	129.8 ± 19.3* (0.0007)
Fourth dive	147.2 ± 17.5	126.9 ± 18.0* (0.005)
RVET (ms)		
First dive	358.4 ± 44.8	349.8 ± 30.6 (0.639)
Second dive	337.8 ± 30.8	329.9 ± 26.6 (0.296)
Third dive	345.9 ± 39.4	340.0 ± 32.2 (0.618)
Fourth dive	334.2 ± 38.5	333.6 ± 31.1 (0.965)
AccT/RVET		
First dive	0.46 ± 0.08	0.41 ± 0.07 (0.056)
Second dive	0.45 ± 0.06	0.38 ± 0.08* (0.019)
Third dive	0.46 ± 0.06	0.38 ± 0.07* (0.0001)
Fourth dive	0.45 ± 0.07	0.38 ± 0.04* (0.019)

Shown are mean values ± SD. *P* values of predive versus postdive comparisons are shown in parentheses.

* $P < 0.05$.

AccT, time interval between the onset and peak of pulmonary flow velocity; RVET, time interval from the onset to termination of the systolic pulmonary flow velocity.

ESV and EDV showed a significant increase in ESV after the dive, with no change in EDV, indicating a reduced left ventricular systolic function.

As shown in Table 3, pulmonary artery AccT was significantly shortened after all dives, and no significant differences between predive and postdive RVET were found. The resulting AccT/RVET ratios were consequently significantly reduced in all but the first dive, indicating an increase in the mean pulmonary arterial pressure.

No acute cumulative effect of the multiple dives for any of the measured parameters was found, whereby the baseline (predive) did not change from the first to the fourth dive (Tables 1–3).

DISCUSSION

The major finding of this study is that field dives with trimix of oxygen, helium, and nitrogen result in a decreased systolic function of the LV and increased pulmonary arterial pressure. The direction or the extent of these changes did not differ from the air dives. Moreover, repetition of the dives in successive days did not significantly affect the magnitude of these outcomes (neither cumulative effects nor acclimatization).

The principal goal of this study was to identify the cardiovascular effects of the field scuba dives with trimix and compare them with the effects of the dives with the compressed air. A group of experienced divers performed four nonidentical dives in the successive days, with the first being a dive with air, the second with nitrox 25, and last two dives with trimix as the breathing gas. The depths were progressively increased with the resulting differences in decompression profiles (Fig. 1). All the dives were rather short, which, together with the use of nitrox 50 at decompression, had the goal of decreasing the nitrogen load and increasing the efficiency of its elimination from blood and tissues thus shortening decompression. Our measurements of the venous gas bubble load showed that the number of bubbles in the RV was significantly higher after the second (nitrox 25) and third (trimix 20/30) dives compared with the other two dives. The low number of bubbles in the first dive can be attributed to the smaller depth and shorter dive duration. Interestingly, the bubble load detected after the fourth dive (trimix 20/30), although it had an identical profile as the third dive, was surprisingly low. A possible explanation might be the high variability in the individual response from dive to dive (from our previous experience, the identical dives performed by the same person often result in a different number of bubbles). An additional speculation might be the adaptation to the nitrogen load due to the process of diving acclimatization, although additional studies are required to attribute the found differences to this mechanism.

Monitoring of more general parameters such as blood pressure and body weight revealed that the divers lost on average 1 kg with each dive, most likely due to dehydration. Although the hematocrit level was not measured, the body weight would return to predive values by the next day, confirming that it was caused by the loss of water. A drop in systolic pressure could also be explained by the dehydration (as well as a depressible effect of the dive on the systolic function of the LV) (16). Similar effects of the fluid loss were also described in a recent study by Gempp et al. (9). On the other hand, the observed slight increase in diastolic blood pressure could possibly result from the increased peripheral arterial resistance due to the cold-induced increase in sympathetic output.

Echocardiographic assessment of the left ventricular function showed that all of the dives, irrespective of their depth, duration, and the used breathing gas, decreased the systolic performance indicated by reduced EF and FS and increased ESV. There were no differences in the extent of these changes between different dives, and comparison of the predive baseline values revealed that there was no physiologically significant cumulative effect of the repetitive dives. This might suggest that these changes are not influenced by the used breathing gas, maximum depth, or multiple dives. However, because the divers performed four dives using different gas mixtures and diving profiles, we cannot exclude the possibility that, for example, the smaller cardiovascular changes caused by diving with trimix were offset by the larger cardiovascular effects of greater depth, thereby resulting in no difference compared with the air dive. To precisely discriminate between the effects of gas mixture and diving depth and duration, more detailed investigations are necessary in the future. The depressible effect of the air dives on the left ventricular function was described previously (8,10,15,16). In our recent study, the depressible effect of a single air dive lasted for at least 24 h because cardiac parameters did not return to the baseline the next day (16). However, in the current study, the ventricular function did return to the baseline. A possible explanation for such a discrepancy might be that the divers in this study were experienced professionals that are already adjusted to

the stress induced by diving (because of the training-induced effect or genetic predisposition), whereas the subjects in our previous studies were recreational divers who dive less frequently. In addition, the diving stress was different in the two studies, with differences in the bottom times, decompression profiles, and breathing gases. However, the mechanism responsible for these changes is still unknown. In animal models, the decrease in CO after diving was related to reduced venous return and increased afterload of the RV and/or LV (because of increased pulmonary and systemic vascular resistance, respectively) (4,6,22).

In this study, four of the divers were using dry suits and five were using wet suits. A comparison of the dive-induced cardiovascular changes between the divers using different types of suits revealed no differences in the first three dives. However, in the fourth dive, the divers wearing wet suits had a smaller dive-induced decrease in the FS, SV, and EF compared with those wearing dry suits (data not shown). Therefore, it seems that the depressible effect on the left systolic function was less in divers with wet suits. This could be due to the multitude of factors with one of them being a greater sympathetic activation due to cold in divers wearing wet suits. However, because this dive was identical to the third dive where no differences were found, this discrepancy could be only a random variability. No other effects of the type of the suit on other parameters (body weight change or bubble grade) were found.

Determination of pulmonary artery AccT/RVET ratio revealed a consistent increase in PAP after all the dives that was similar as shown in our previous studies (8,16). There were no significant differences in the magnitude of the effects between the dives using air, nitrox, or trimix, indicating that these changes in pulmonary vasculature will occur regardless of the breathing gas used. The mechanism of the increase in PAP is still unknown, whereby one possibility could be the damage of the pulmonary endothelium by the venous gas bubbles lodged in the pulmonary circulation (15). Although in our previous studies (8,16) we found no correlation between the number of bubbles and the change in the AccT/RVET ratio (divers with no detectable bubbles had still a significant decrease in AccT/RVET), the bubbles of a size below the level of detectability (20–30 μm) might still be present and cause endothelial damage resulting in the release of vasoactive humoral factors (14). Furthermore, oxidative stress due to exposure of divers to hyperoxia during the dives could be an additional mechanism of the pulmonary endothelial damage resulting in changes in PAP. However, the extent of changes in PAP found in this study, where inhaled PO_2 (1.55 bar) was close to the allowed maximum and approaching the level of oxygen toxicity, was not different from that in our previous studies where PO_2 was lower (0.84 bar) (16). This suggests that oxidative stress during the dive is not responsible for these effects. An additional line of evidence is the finding that administration of antioxidants did not affect the degree of the dive-induced increase in PAP (16). An investigation of other factors (nitric oxide, humoral factors, immersion, and hypoxia) that are potentially responsible for the changes in PAP is warranted in the future.

One limitation of this study is the small number of subjects, making it necessary to view the obtained results with caution. However, the repeated measurements (predive and postdive) in each participant mean that each diver was his own control, thus facilitating detection of any potential changes in the measured parameters. The diving profiles and the used breathing gas mixture varied from day to day, as determined by the technical diving course program. Therefore, we are unable to draw conclusions regarding the mechanism responsible for the observed changes and we cannot discriminate between the effects of the gas mixture, diving depth, and duration. However, the fact that deeper and longer trimix dives did not induce larger cardiovascular effects compared with the air dive might possibly suggest that trimix dives would induce less cardiovascular effects compared with the air dives of the same depth and duration. This will be addressed in our future studies. The absence of change in any of the predive parameters in successive days suggests that there are no acute cumulative effects of the multiple dives. The power analysis revealed that, with 80% statistical power, we were able to detect the within-days baseline changes that are larger than 3.7% (in case of EF variable) to 15.2% (in case of AccT/RVET variable). Therefore, we likely had the sufficient power to detect the physiologically relevant changes in the predive parameters. However, some smaller changes that are below our level of detection cannot be excluded.

Lastly, this study suggests that even very safe and conservative trimix diving profiles have significant asymptomatic effects on the cardiovascular function, and future studies will be necessary to address the extent of these changes after more demanding diving protocols.

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