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## Effects of oxygen-enriched air on cognitive performance during SCUBA-diving – an open-water study

Anne-Kathrin Brebeck<sup>a</sup>, Andreas Deussen<sup>b</sup>, Henning Schmitz-Peiffer<sup>c</sup>, Ursula Range<sup>d</sup>, Costantino Balestra<sup>e</sup>, Sinclair Cleveland<sup>f</sup> and Jochen D. Schipke<sup>g</sup>

<sup>a</sup>Department of Ophthalmology, Universitätsklinikum Würzburg, Augenklinik und Poliklinik, Würzburg, Germany; <sup>b</sup>Institute of Physiology, Medical Faculty Carl Gustav Carus of TU Dresden, Dresden, Germany; <sup>c</sup>Institute of Neurology, Medical Faculty Carl Gustav Carus of TU Dresden, Dresden, Germany; <sup>d</sup>Institute of Medical Informatics and Biometrics, Medical Faculty Carl Gustav Carus of TU Dresden, Dresden, Germany; <sup>e</sup>Haute Ecole Paul-Henri Spaak, Environmental & Occupational Physiology Laboratory, Brussels, Belgium; <sup>f</sup>Institute of Neuro- and Sensory Physiology, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany; <sup>g</sup>Research Group Experimental Surgery, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

### ABSTRACT

**Background:** Nitrogen narcosis impairs cognitive function, a fact relevant during SCUBA-diving. Oxygen-enriched air (nitrox) became popular in recreational diving, while evidence of its advantages over air is limited. Aim: Compare effects of nitrox28 and air on two psychometric tests.

**Methods:** In this prospective, double-blind, open-water study, 108 advanced divers (38 females) were randomized to an air or a nitrox-group for a 60-min dive to 24 m salt water. Breathing gas effects on cognitive performance were assessed during the dive using a short- and long-term memory test and a number connection test.

**Results:** Nitrox28 divers made fewer mistakes only on the long-term memory test ( $p = 0.038$ ). Female divers remembered more items than male divers ( $p < 0.001$ ). There were no significant differences in the number connection test between the groups.

**Conclusion:** Likely owing to the comparatively low  $N_2$  reduction and the conservative dive, beneficial nitrox28 effects to diver performance were moderate but could contribute to diving safety.

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### KEYWORDS

Cognitive performance;  
memory; alertness;  
SCUBA-diving; nitrox

## Background

Nitrox has been the subject of much debate within the sport diving community, because potential toxic effects of the increased oxygen fraction remained unclear (Caney, Dietrich, & Hornsby, 2010). In spite of this, nitrox has become increasingly popular (Fock, Harris, & Slade, 2013; Gabel & Janoff, 1997). Increasing the oxygen proportion while decreasing the nitrogen ( $N_2$ ) proportion in the breathing gas likely reduces the risk of  $N_2$  narcosis and the formation of  $N_2$  bubbles that develop during ascent and after surfacing, and have been described to be responsible for inducing decompression sickness (Hobbs, 2008; Vann, Butler, Mitchell, & Moon, 2011).

**CONTACT** Jochen D. Schipke  [j.schipke@gmx.org](mailto:j.schipke@gmx.org)  Research Group Experimental Surgery, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

On the other hand, the partial pressure of oxygen ( $pO_2$ ) cannot be increased arbitrarily because of its potential toxic effects on the central nervous and the pulmonary system (Fock et al., 2013; Shykoff, 2005). The harmful  $N_2$ -effects occur at partial pressures considerably higher ( $>4.0$  bar) (Davis, Osborne, Baddeley, & Graham, 1972) than those at which harmful  $O_2$ -effects develop ( $>2.2$  bar) (Butler & Thalmann, 1986).

Exposure times for harmful  $N_2$ -effects to develop depend on the ambient pressure, for example, EEG changes became apparent within 6 min of breathing air at 9 bar (Pastena, Faralli, Mainardi, & Gagliardi, 2005).

Quite a few earlier studies comparing air with nitrox may have provided biased results, since they report on participants who were professional divers (Williamson, Clarke, & Edmonds, 1989), divers in a diving tower (Balestra, Lafère, & Germonpré, 2012), military divers (Dujic et al., 2005), or divers reaching much greater depths (40–45 m salt water; msw) (Hobbs, 2008) than recreational divers. Moreover, other studies rarely include females, thereby overlooking one-third of recreational divers. Importantly, the reactions of female divers may differ from male divers when breathing different gas mixtures. While breathing air in the laboratory, gender-dependent differences have been shown for both verbal and visuospatial working memory (Zilles et al., 2016).

This prospective, randomized, double-blind study aimed to investigate the effects of air or nitrox on cognitive performance in a heterogeneous group of recreational divers of both sexes under realistic environmental conditions. Based on the substantial literature on nitrogen narcosis (Davis et al., 1972), our hypothesis was that cognitive performance would be better preserved when breathing nitrox than breathing air.

As a matter of fact, gender differences with respect to cognitive performance became apparent during data analysis. Gender differences in the working memory (Zilles et al., 2016), in episodic memory, and in visual working memory have already been described at Normobaria (Pauls, Petermann, & Lepach, 2013). Other gender differences exist in the field of pharmacokinetics and pharmacodynamics of anaesthetic agents (Gear et al., 1996). Consequently, gender differences in the effect of breathing gases on memory and alertness became a secondary focus.

## Methods

### Volunteers

The study protocol was approved by the ethical committee of the Medical Faculty Carl Gustav Carus of TU Dresden (EK271092009). Divers were informed about the current study via an information brochure or the staff at a dive centre. Inclusion criteria were: age 18–70 years, valid medical fitness to dive, valid diver certification to dive to  $\leq 25$  m, German-speaking, and lack of prior knowledge of the cognitive test to be administered. Informed consent was obtained from all included volunteers. In total, 108 recreational divers participated in the study.

### Demographic data

The following personal data were collected: age, sex, height, body mass, smoking habits, and medication. In addition, diving history was documented in terms of number of dives, dives  $>25$  m salt water (msw), deepest dive, and years of active diving.

## Dive

The open-water dives were performed in the El Quadim Bay (Egypt). The water temperature was 28–29°C and the visibility always 10–20 m. The dives lasted on average  $62 \pm 5$  min to a maximum depth of  $24 \pm 1$  msw (means  $\pm$  standard deviation). All dive groups consisted of three participants: the investigator (AKB), one air diver, and one Nx28 diver (SUBEX air28®; 28% O<sub>2</sub>; 72% N<sub>2</sub>; Nx28). This particular nitrox blend was chosen for this study for two reasons: participants did not require additional certification and no O<sub>2</sub>-induced injury was to be expected at this level of O<sub>2</sub> enrichment. On the other hand, the relatively low N<sub>2</sub>-depletion would not strikingly reduce N<sub>2</sub>-related effects.

At predefined times, two cognitive tests were performed that had already been explained during the briefing for the dive plan. Each diver performed one dive only, on either air or nitrox. Only the compressor personnel were aware of the breathing gas that was contained in the specific tank. One volunteer could choose between a pair of tanks (12 or 15 l) that contained either air or nitrox. The other volunteer then chose his tank size from the other pair of tanks. Correct allocation was obtained after all tests had been completed, that is, at the end of the study.

## Psychometric testing

Cognitive performance was assessed by using a memory test and an alertness test. For completing the tests during the dive, the volunteers, after establishing negative buoyancy, knelt opposite to each other on the sea-floor (24 msw).

### Memory Tests

A visual memory test was used almost identical with a test used in a very similar study (Hobbs, Higham, & Kneller, 2014). Fifteen items were taken from lists provided from the German version of a verbal learn and memory test (Helmstaedter, Lendt, & Lux, 2001).

The items taken from two different lists varied from dive to dive to reduce the likelihood of prior knowledge of the items that were printed on a writing board and could be read within 30 s. Both subjects on a dive were shown the same test-board.

Short-term memory was tested immediately after removing the boards by listing all remembered items on a writing board with no time limit. Thirty minutes after the list was memorized, the divers knelt down in a quiet location (24 msw) and listed all remembered items with no time limit and without viewing the test-board again. An undemanding recreational dive served as distractor between both test times.

For each diver, short-term memory and long-term memory were both scored as: (1) number of correct items, (2) number of incorrect items. Incorrect items were responses that were given but were not contained in the original list.

### Alertness Test

The number connection test permits assessment of “cognitive processing speed” (Oswald & Roth, 2005). The test, among other medical areas, is used to assess the severity of encephalopathy (Adrover et al., 2012). One of the two test versions was performed once directly after the short-term memory test was completed. Printed numbers from 1–90 were connected up as quickly as possible using a pen. The numbers (font size 5 mm) were printed on a foil affixed to a board. The sequence of the numbers

was arbitrarily arranged with the exemption that the next higher number was located in the vicinity of the current number.

The diver started the test after a 5-s countdown; the number “1” was uncovered by the investigator’s hand. The test ended after number 90 was reached or after 4 min had elapsed. The diver’s attention was drawn to an error or “foul” (e.g. if he skipped a number) by a manual sign from the investigator on the last correct number. A “slip” was noted by the investigator if the diver himself had reset the pencil from an incorrect number to its predecessor. After completion of the test, fouls, slips, and performance time were noted on the diver’s board by the investigator.

## **Statistics**

MicroSoft Excel 2007 was used for calculations and graphs (Microsoft, Redmond, U.S.), and SPSS (PASW Statistics 18, IBM, New York, U.S.) was used to assess for statistical differences. According to the Kolmogorov–Smirnov test, many of the data were not normally distributed. Therefore, non-parametric methods (Mann–Whitney U-test, Wilcoxon test) were employed for comparison of two groups or points in time. Ordinal and nominal scaled characteristics were subjected to a contingency table analysis, evaluated with a  $\chi^2$ -test or Fisher’s exact test. Differences with  $p \leq 0.05$  were considered statistically significant. However, in accordance with a recent statement by the American Statistical Association (ASA), any threshold of the  $p$ -value is arbitrary. This statement is intended to steer research into a “post  $p < 0.05$  era” (Wasserstein & Lazar, 2016). Along with the ASA policy, we considered in this study  $p \leq 0.10$  to represent a strong trend towards differences, in case they were meaningful. Results are presented as median values with ranges unless otherwise stated.

## **Results**

### **Volunteers/groups**

108 experienced divers (females: 35%) from Germany, Austria, and Switzerland were recruited. Since there were no dropouts during the study, data could be analysed in full. No statistically significant socio-demographic differences, including the dive history, were found between the air-group and the nitrox-group, and the dive durations and maximum depths were also the same in the two groups (Table 1).

Although the divers with a history of decompression sickness (DCS) were evenly distributed between the two study groups, it remains unclear why the overall reported incidence was as high as 6.5%, whereas published data from large series show an incidence of only 0.1% (DAN, 2005).

Because the divers voluntarily took part in the study and thus were practising their hobby, motivation and commitment appeared to be high throughout.

### **Cognitive performance**

#### **Memory test**

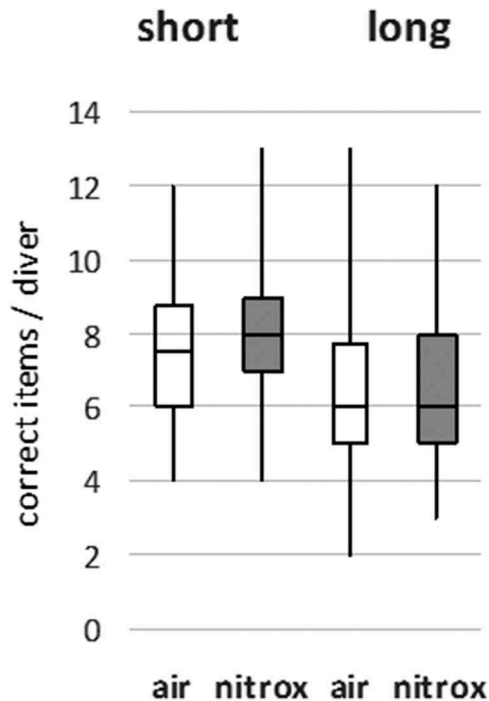
The number of correct items per diver did not differ statistically significantly between the air-group and the nitrox-group for either short-term (7.5 (4–12) vs. 8.0 (4–13);

**Table 1.** Socio-demographic data and diving history of volunteers.

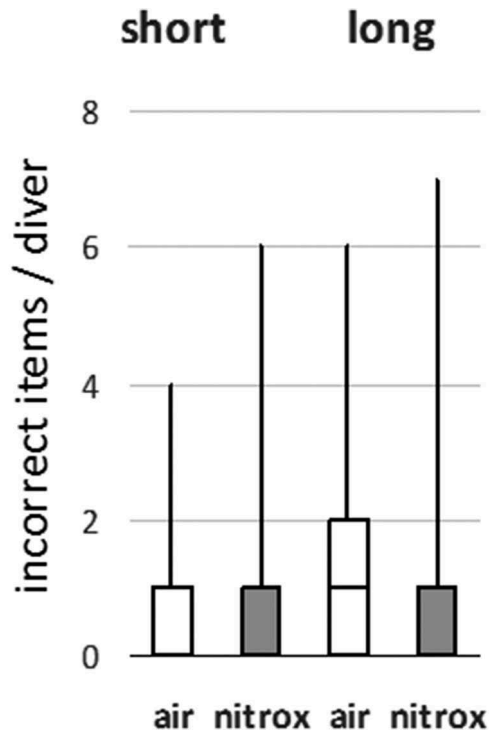
Groups	Air <i>n</i> = 54	Nitrox <i>n</i> = 54
Sex	20 ♀, 34 ♂	18 ♀, 36 ♂
Age [years]	42 (20–66)	42 (21–67)
BMI [kg/m <sup>2</sup> ]	24 (20–34)	24 (18–39)
Smoker	15 (4 ♀, 11 ♂)	20 (6 ♀, 14 ♂)
Number of dives	117 (15–6.000)	160 (14–7.000)
Dives >25 m	40 (2–3.000)	50 (3–3.000)
Years of active diving	7 (1–43)	5 (1–39)
Deepest dive [m]	42 (27–78)	40 (21–82)
Earlier DCS	6	6

No statistically significant differences were found between the air-group and the nitrox-group (U-test. Data: median (range)).

$p = 0.260$ ) or for long-term memory (6 (2–13) vs. 6 (3–12);  $p = 0.366$ ). Note that fewer correct items were retained in long-term memory than in short-term (Figure 1), but this was not statistically significant. The number of incorrect items per diver did not differ between gases in the short-term (0 (0–4) vs. 0 (0–6);  $p = 0.998$ ), but was significantly higher in air divers (1 (0–6)) than in nitrox-divers (1 (0–7)) for the long-term ( $p = 0.039$ ) (Figure 2). The number of divers who made mistakes increased by 22% in the air-group whereas it increased by only 7% in the nitrox-group. In detail, the number of incorrect words increased from 37 to 71 and from 38 to 52, respectively, ( $p = 0.078$ ) (Figure 3).



**Figure 1.** Visual Memory Test in Relation to Air and Nitrox after a Standardized Dive to 24 msw. Memory Was Assessed during the Dive Immediately after (=Short-Term) and after 30 min (=Long-Term) after Reading 15 Items from a Writing Board. The Number of Correctly Remembered Items per Diver Tended to Be Lower in the Long-Term Memory. Data are Presented as Median and Ranges.



**Figure 2.** The Number of Incorrectly Remembered Items per Diver Was Small. In the Long-Term, Nitrox Divers Listed Fewer Incorrect Items than Air Divers ( $*p < 0.05$  vs. Long-Term Air).

#### Memory test and age

Two groups were formed to analyse for age-dependency. Comparing divers  $<40$  years with those  $\geq 40$  years resulted in a significant difference, if correctly remembered short-term and long-term items were summed up. Younger divers remembered better than older divers. This result did not depend on the breathing gas (air:  $p < 0.010$  and nitrox:  $p < 0.001$ ).

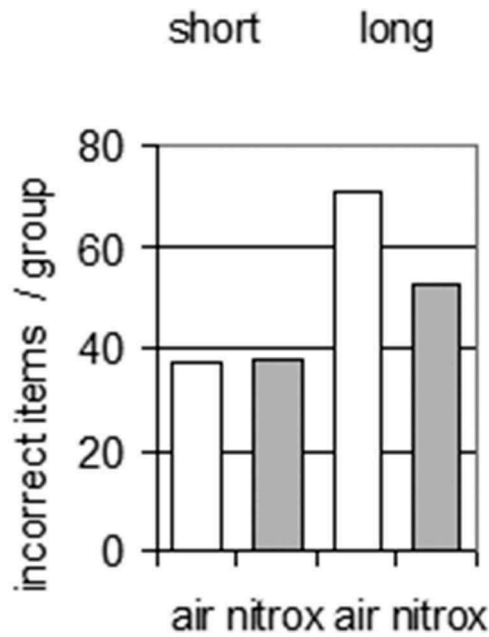
#### Memory test and gender.

In the short-term, females in the air-group correctly remembered 8 (5–12) items, those in the nitrox-group 9 (7–13) items ( $p = 0.403$ ), while males in both groups remembered 7 (4–11) items ( $p = 0.957$ ). The number of correct items retained in long-term memory was lower in both groups. Still, female air divers remembered 8 (3–13) and female nitrox divers 9 (4–12) items ( $p = 0.371$ ). Male divers remembered 5 (2–9) and 6 (3–10) items, respectively, ( $p = 0.324$ ).

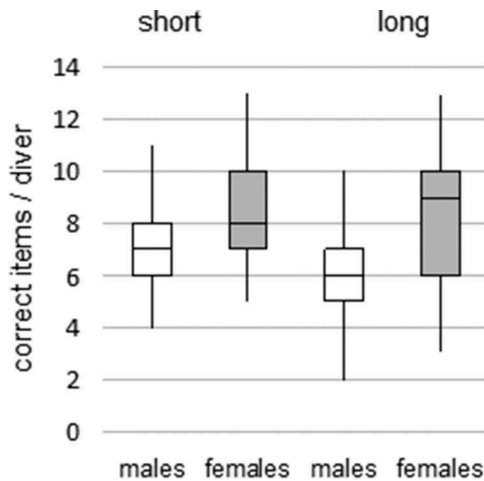
Pooling both breathing gases showed that females remembered significantly more items than males at short-term (8 (5–13) vs. 7 (4–11);  $p < 0.001$ ) and long-term (9 (3–12) vs. 6 (2–10);  $p < 0.001$ ) (Figure 4). There were no significant gender differences with respect to the number of incorrectly named items per diver short-term: 0 (0–6) vs. 0 (0–4); ( $p = 0.729$ ) and long-term: 0.5 (0–8) vs. 1 (0–8); ( $p = 0.204$ ). As for the entire study group, female air divers remembered fewer items than female nitrox divers (n.s).

#### Alertness test.

The alertness test took the same time for both groups (air:  $87.0 \pm 30.3$  s vs. nitrox:  $85.5 \pm 26.7$  s;  $p = 0.687$ ). The fouts within a group exhibited a strong trend for an



**Figure 3.** The Incorrectly Remembered Items per Group Did Not Exhibit Major Differences in Short-Term Memory (Air: 37 vs. Nitrox: 38). In the Long-Term, the Number of Incorrect Items Increased and a Breathing Gas-Dependent Difference Exhibited a Strong Trend (Air: 71 vs. Nitrox: 52;  $^+p = 0.078$ ). Columns Present Sums of Incorrect Items.



**Figure 4.** Visual Memory Test in Relation to Gender after a Standardized Dive to 24 msw. Memory Was Assessed during the Dive Immediately after (=Short-Term) and after 30 min (=Long-Term) after Reading 15 Items from a Writing Board. In the Short and in the Long-Term, the Female Divers Remembered More Items per Diver Correctly than the Male Divers ( $p < 0.001$ ).

advantage for nitrox (15 vs. 9;  $p = 0.068$ ; 40%). The slips within a group exhibited no notable differences (22 vs. 19;  $p = 0.755$ ). Nor were any gender-dependent differences found.



## Discussion

Any advantages for recreational divers of using nitrox instead of air are uncertain. In this prospective, randomized, double-blind, open-water study the four main findings were (1) air-breathing divers made more errors in recall in the long-term memory test. (2) A strong trend suggests that the number of divers who made mistakes increased from short-term to long-term by 22% in the air-group, whereas it increased by only 7% in the nitrox-group. (3) In the alertness test, the nitrox-group showed a trend for fewer fouls (40%) compared with the air-group. (4) In the memory test, female divers recalled more items than males in both the short and long-term.

Likely, no cognitive tests were developed for underwater use. For “dry” use, the verbal learn and memory test had been developed. Because verbal communication between recreational divers is unfeasible, a visual test was employed using items from the verbal test (VLMT; Helmstaedter et al., 2001). An almost identical test had been used before in a very similar study (Hobbs et al., 2014). As a matter of fact, both visual and verbal tests have been used in previous studies (Palta, Schneider, Biessels, Touradji, & Hill-Briggs, 2014), one of which reported that recall for lists of objects was better after showing the physical objects than when the names of the objects were solely spoken (Kirkpatrick, 1894).

Likewise, the alertness test was not developed for underwater use, which might have impaired performance. However, the aquatic environment (e.g. visibility, posture) was the same for all participants and would have affected them to the same extent.

From a statistical point of view, it could be regarded as a limitation that the divers did not act as their own controls. In the context of bubble formation, this is all the more regrettable, if one remembers the large variation in susceptibility to bubble formation (Carturan et al., 2002; Cialoni, Pieri, Balestra, & Marroni, 2014). However, single-subject studies have their own limitations: among others, carry-over effects and order effects (Kazdin, 1982). Because a completely randomized design – in terms of data analysis and convenience – is probably the simplest experimental design (Stat Trek 2016), we undertook a randomized design with 54 pairs of divers, one diver breathing air and the other breathing nitrox28 on identical dives.

When recruiting volunteers’ representative of recreational divers, we strove for a heterogeneous group with respect to age, sex, fitness level, BMI, and diving experience. However, these factors will increase the biological noise and make the achievement of statistically significant differences less likely. Given this heterogeneity, great care was taken to ensure randomization to the control group and experimental groups.

Cognitive performance determines one’s behaviour in an unknown environment. If performance is restricted, strategies for dealing with a danger may be impaired or even disabled (Williamson et al., 1989). Increased  $pN_2$  may impair cognitive abilities such as executive functions, intellectual performance, short-term memory, alertness, and reaction times to optical and acoustical signals, particularly at  $pN_2$ -values  $>4.0$  bar (Balestra et al., 2012; Williamson et al., 1989).

Nevertheless, narcotic effects can also be demonstrated at shallower depths (Hobbs et al., 2014). As with the reaction towards alcohol (Vann et al., 2011), there is no all-or-nothing reaction, but adverse effects do increase continuously (Lowry, 2002; Petri, 2003).

A recent study of 41 sports divers breathing air (depths: 33–42 m seawater; msw) showed that free recall was significantly reduced compared to shallow water (1–11

msw). Interestingly, the divers could accurately judge the decrease in their memory performance, which implies that metacognition was well preserved (Hobbs et al., 2014).

In another recent study with 20 sports divers, memory performance was significantly worse in deep water (31–36 msw) compared with shallow water (2–6 msw), and this impairment did not differ significantly between air and nitrox30 (Hobbs, 2014). Only in few other studies nitrox was not superior to air with respect to nitrogen narcosis. The narcotic effects did not depend on breathing air or nitrox (Linnarsson et al., 1990), and another small study even reports worse psychomotor performance when breathing nitrox rather than air (Frankenhaeuser, Graff-Lonnevig, & Hesser, 1963).

When comparing the contradictory results of these latter three studies with the present study with respect to nitrox effects, differences in the experimental design become significant. In the study by Hobbs, divers were not randomized to the breathing gas, used nitrox30, and dove to depths equivalent to ambient pressures of 4.1–4.6 bar (Hobbs, 2014). The preliminary study by Linnarsson and colleagues has been published only as an abstract, included 10 divers, and provides no information on the nitrox mixtures used (Linnarsson et al., 1990). The other small study by Frankenhaeuser and colleagues using different nitrox blends included 12 participants and reports that nitrogen pressures up to 3.9 bar had but slight effects on objective performance (Frankenhaeuser et al., 1963), which is in poor harmony with the literature stating narcotic N<sub>2</sub>-effects for pN<sub>2</sub>-values at around 4.0 bar (Balestra et al., 2012; Davis et al., 1972; Rostain & Lavoute, 2011).

To possibly resolve the conflicting results, effects of excess oxygen are discussed that exert a potentiating effect on the narcotic nitrogen action at high pressure (Frankenhaeuser et al., 1963; Hesser, Fagraeus, & Adolfson, 1978). Later studies are in concert with such narcotic O<sub>2</sub>-effects at increased pressures (Fowler, Ackles, & Porlier, 1985). Thus, it may well be that the different findings of Hobbs (2014) and us are owing to different pO<sub>2</sub>-values in both studies, which amount to between 1.23–1.38 bar and only to 0.98 bar, respectively.

An age-dependent decline in memory is extensively described (Hoyer & Verhaeghen, 2006). In Germany, divers ≥40 years are regarded as “older” and are therefore required to visit a diving physician for their “fit-to-dive” certification each year, rather than every 2 years. Two groups were formed on this basis. The age-related analysis showed that divers <40 years memorized significantly more short-term plus long-term items than the divers ≥40 years. Interestingly, this result was almost identical in both breathing gas groups, that is, narcotic N<sub>2</sub>-effects did not affect the result.

Gender-dependent differences in cognitive performance are known (Pauls et al., 2013; Zilles et al., 2016). Before we began this study, it was unclear whether enough female divers would participate to make a statistical analysis of gender differences feasible. Only after about 30% of the participants were females, data analysis demonstrated that female divers performed better than male divers on the memory tests, when the breathing gases data were pooled for analysis. Because cognition-related studies of female divers are sparse, it appeared appropriate to report our data.

The female divers were less experienced than the male divers and may have been more highly motivated to perform the dive as properly as possible. Also, the female divers were an average of 6 years younger than the male divers. Within this young adult

age, highest gender differences in non-visuospatial abilities with an advantage for women have been reported (Pauls et al., 2013). Our findings suggest that gender differences in diver performance may merit investigation.

## Conclusion

Increasing the oxygen fraction reasonably improved cognitive competence during a dive at 24 msw due to the reduced narcotic effects of the nitrogen fraction. The higher level of visual performance of female divers was unexpected. In light of the sparse data on female divers, it is worth reporting.

It is a strength of this study that it presents data on a rather large group of recreational divers representing the large community of non-professional and non-military divers. In addition, gender differences with respect to cognitive performance (memory) are described. A weakness of the study is that, for safety reasons, only a moderately O<sub>2</sub>-enriched nitrox was used and not a gas mixture that is more frequently used in diving centres, for example, Nx32 or Nx36. It is obvious that the results of this study are limited to the diving conditions as the dive profile, water conditions, and physical labour. On the other hand, diving experience might also have an impact on the outcome. Finally, younger divers, that is, children and adolescents, were not included in the study. Future research should be carried out to further investigate cognition-related gender differences. Moreover, possible differences should also be investigated in the laboratory to better understand effects of immersion and ambient pressure. After all, practical consequences are simple. If during a dive, correctly remembering details or immediately reacting to adverse events is of vital importance, the usage of oxygen-enriched breathing gas will improve diving safety. Still, great emphasis must be placed on the toxic effects of oxygen.

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## Disclosure statement

No potential conflict of interest was reported by the author.

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