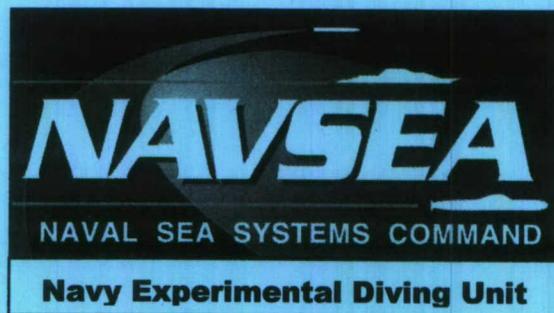


**Navy Experimental Diving Unit
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Panama City, FL 32407-7015**

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**PULMONARY OXYGEN TOXICITY WITH EXERCISE:
SINGLE MK 25 REBREATHING DIVES
OR SPLIT 6-HOUR EXPOSURES**



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INTRODUCTION

Respiratory system stresses that may result in symptoms or pulmonary function changes begin with those of immersion, translocation of blood from the legs and abdomen into the lungs, and hydrostatic pressure differences from the mouth to the submerged chest. Exercise demands both increased ventilation, meaning increased respiratory pressure swings, and increased pulmonary blood flow. Elevated oxygen partial pressure (PO_2) adds possible local effects of oxygen on lung tissue and systemic toxic effects throughout the body. Previous work at Navy Experimental Diving Unit (NEDU) measured the effects of elevated PO_2 (1.3 to 1.4 atmospheres [atm]) during resting immersion¹⁻⁴ or immersion with exercise^{5,6} when gas was delivered underwater with the MK 20 underwater breathing apparatus (UBA).

Any UBA provides resistance to airflow, both inspiratory and expiratory, and may include elastic and additional hydrostatic loading. The MK 25 (Draeger LAR V) 100% oxygen rebreather has a chest-mounted rebreathing bag (counter lung), which generates a positive pressure at the mouth of a prone diver, and an axial-flow scrubber to remove carbon dioxide (CO_2). In some of the experiments reported here, the effects of respiratory loading of the MK 25 UBA were compared to those of the MK 20 open circuit UBA during exercise. We studied pulmonary function after single four-hour dives with exercise and compared them to results after similar dives using the MK 20 UBA. We also studied pulmonary effects of two and four days with split six-hour oxygen exposures including exercise: on two consecutive days, two three-hour MK 25 dives were separated by four-hour surface intervals, with an overnight interval of 14 hours; similar resting dives had appeared to be similar in effect to single four-hour resting dives.⁴ Later, two three-hour MK 20 dives with exercise were separated by four-hour surface intervals on two consecutive days, and the two days were repeated after a three-day break.

METHODS

General

Dives were conducted in the 15-foot deep freshwater test pool at NEDU. Protocols had been reviewed and approved by the NEDU Institutional Review Board, and divers gave written informed consent.

For all dives, water temperature was 84 ± 5 °F (29 ± 3 °C). Four waterproof cycle ergometers (Collins; Louisville, CO) were mounted to mimic a swimming configuration, with the centers of the shoulder supports 12 ± 2 inches (30 ± 5 cm) above the axis of the pedals, and 3.5 feet (1.1 m) above the pool bottom. A diver on an ergometer had his face under about 11 feet (3.4 m) of water and his chest only slightly deeper, for 1.3 atm

PO₂ if the gas was 100% oxygen. Sitting or lying on the bottom, a diver at rest was under about 14 feet (4.3 m) of water, for 1.4 atm PO₂ with 100% oxygen. Pairs of divers sharing an ergometer alternated 30 min of cycling with 30 min of rest. Divers, who wore Polar heart rate monitors (Polar USA; Lake Success, NY), were asked to give hand signals of their heart rates every five minutes during their exercise phases, and the loads on the ergometer brakes were adjusted to maintain divers' heart rates at 105 ± 5 beats per minute. This heart rate is considered to represent moderate exercise for sustained periods.⁷

Before MK 25 divers entered the water, they used the standard procedure for the MK 25 to purge nitrogen from their breathing circuits.⁸ Briefly, with the oxygen supply off, divers inhaled from their mouthpieces and exhaled through their noses until the rebreathing bag was empty. They then opened the oxygen supply valve and added oxygen until the bag was full. Again, they inhaled oxygen from the bag and exhaled the mixed nitrogen and oxygen from their lungs into the atmosphere until the bag was empty, when they refilled the bag and began to breathe on the closed-circuit loop. Once the purge was complete, we used a manual syringe to draw a gas sample from the breathing circuit through a small port added at the exit from the scrubber and used a portable oxygen analyzer with a B1 micro fuel cell (Teledyne Analytical Instruments; City of Industry, CA) to read gas fraction. If the fraction was less than 0.9, the diver repeated the purge maneuver, and we sampled again. We also sampled the gas in the system when the divers surfaced.

Divers in the four-hour experiments surfaced after two hours for a five-minute air break to drink and eat, if they desired to do so. Before they continued their dives, they purged the system again to at least 90% oxygen, as above.

For all dive series, pulmonary function — specifically, flow-volume loops and single breath diffusing capacity of the lung for carbon monoxide (D_LCO) — was measured (CPL, Ferraris Medical; Louisville, CO) during the week before the dives (baseline), within two hours after divers surfaced, and for two days after the dive series was complete. At baseline, on surfacing, and on the first day after the dives, blood samples were taken and hemoglobin and carboxyhemoglobin concentrations measured (CO-oximeter, Instrumentation Laboratory; Lexington, MA) for correction of diffusing capacity values;⁹ the value from the first day after diving was used also on the second day after diving. D_LCO measurements were also adjusted to avoid analyzer transients.¹⁰ Additional flow-volume loops were measured in the morning before diving, and, when applicable, before diving after the four-hour surface interval. Subjects were asked about respiratory symptoms — specifically, cough, inspiratory burning, chest tightness, or shortness of breath — at each pulmonary function measurement session.

We checked for changes in forced vital capacity (FVC), forced expired volume in 1 second (FEV₁), average forced expired flow between 25% and 75% of expired volume (FEF₂₅₋₇₅), maximum forced expired flow (FEF_{max}), and D_LCO. A value was considered

to be depressed if it fell below the lower 95% confidence interval (CI) for day-to-day variation as measured at NEDU in nondiving subjects — namely, FVC, -7.7%; FEV₁, -8.4%; FEF₂₅₋₇₅, -16.8%; FEF_{max}, -17%; and D_LCO, -14.2%.¹

Single Four-hour Exercise Dives

Twenty-three U.S. Navy divers, described in Table 1 below, participated.

Two Days: Three-hour Dive, Four-hour Surface Interval, Three-hour Dive

Sixteen U.S. Navy divers (Table 2) began the series, and thirteen completed it. This series included physical performance testing, described below, on each day between dives and on the first follow-up day.

Four days: Three-hour Dive, Four-hour Surface Interval, Three-hour Dive

Seven U.S. Navy divers (Table 3) began the series, and three completed it. This dive series was similar to the two-day series, but the two days of diving were repeated after a three-day interval. Because of subject discomfort in the previous series, the MK 20 open circuit UBA was used with its full face mask instead of the MK 25. Physical performance was measured at baseline, between dives on each dive day, and on the first day following each set of dives. When blood samples were collected (see **METHODS, General**, above), additional samples were drawn for measuring antioxidant potential in plasma, as described below in “*Antioxidant Assay*.”

Physical Performance Testing

After previous dive series with PO₂ = 1.3 atm, many divers reported fatigue that they felt was disproportionate to the effort expended during the dives. During the series of two three-hour dives per day we attempted to quantify fatigue through measurements of physical performance. Using a testing battery developed at NEDU,¹¹ we tested pull-ups, stair stepping, hand grip, and shooting skills. Measurements were planned for baseline, between dives, and on the day following each multiple-dive dive series. Because of equipment problems, some measurements could not be made, as is noted with the results.

PULL-UPS

The subject completed as many pull-ups as possible, and a chin-activated switch on the pull-up bar recorded each.

STAIR STEPPING

Stair stepping was timed for one minute. The subject, who wore a 45 lb backpack, stepped up two 10” steel steps, placed his second foot on the top, then stepped down

the steps and placed his second foot on the floor for the count of one. A photocell and light beam on the first step provided the counts.

HAND GRIP

Hand grip and endurance were tested one hand at a time. The subject squeezed a calibrated grip device in a series of three brief (0.5 to 1 s) maximum efforts, and the average of the three peak forces was considered maximum grip strength. He then held between 45% and 55% of that force for as long as he could; the system logged all time above 45% maximum grip and ended the test when the grip dropped below 30% maximum.

SHOOTING

Subjects used a shooting system with a specially modified weapon (Model 747 carbine, 5.56 mm) that fired pneumatically, without ammunition. Compressed air release simulated recoil. A pseudorandom sequence of reflective targets was presented to the subject over two minutes, and the system recorded the number of shots fired and number of targets hit. Score was the total for all targets, and the score for each target was $2 \cdot (1 - 0.3 \cdot \text{number of misses})$ to reflect both speed and accuracy.

Antioxidant Assay

A colorimetric assay (kit TA 01, Oxford Biomedical; Oxford, MI) was used to assess total aqueous antioxidant potential in samples of plasma. The assay evaluated the concentration of Cu^+ reduced by antioxidants from Cu^{++} when plasma samples were added to the test solution. Cu^+ , when combined with bathocuproine, forms a stable yellow-colored complex with an absorption maximum between 480 and 490 nm. Uric acid, a reducing agent (antioxidant), is used for the standard curve, and results can be expressed in uric acid equivalents.¹² A Biotech ELx800 absorbance reader (BioTech; Winooski, VT) was used to read the microassay plates.

Blood samples were collected with heparin to prevent clotting and spun in a refrigerated (4 °C) centrifuge for 12 minutes. The plasma supernatant was frozen at -70 °C for later processing. When all samples had been gathered, they were allowed to come to room temperature, were diluted with the kit's buffer solution, and were read at 490 nm for background absorption. Copper solution was added, the plates were incubated for three minutes at room temperature, the stop solution was added, and the plates were read again. The absorptions were compared with the standard curve.

Table 1.
Subject characteristics, four-hour MK 25 exercise dives

n = 23	Median	Range
Age (years)	37	26 – 46
Height (inches)	71	62 – 75
Weight (lb)	190	140 – 255

Smokers Never: 14 Occasional: 2 Former: 7
 # With respiratory allergies Current: 2 Other seasons: 7
 # Using anti-inflammatory medication: 2

Table 2.
Subject characteristics, paired three-hour MK 25 exercise dives with four-hour surface intervals, two days

n = 16	Median	Range
Age (years)	35	20 – 50
Height (inches)	70.5	68 – 73
Weight (lb)	183	160 – 215

Smokers Never: 11 Occasional: 2 Former: 3
 # With respiratory allergies Current: 0 Other seasons: 2
 # Using anti-inflammatory medication: 2

Table 3.
Subject characteristics, paired three-hour MK 25 exercise dives with four-hour surface intervals, four days

n = 7	Median	Range
Age (years)	36	28 – 39
Height (inches)	69	68 – 72
Weight (lb)	198	175 – 235

Smokers Never: 1 Occasional: 1 Former: 5
 # With respiratory allergies Current: 0 Other seasons: 3
 # Using anti-inflammatory medication: 1

RESULTS

Single Four-hour Exercise Dives, MK 25

Of the 23 subjects, none showed changes in pulmonary function variables when measured after surfacing, but four subjects (23%) had values outside the normal variation on the following day: three in flow-volume parameters (13% incidence), and one in D_LCO (4% incidence). Four reported mild respiratory symptoms, and one reported moderately severe symptoms. Additionally, four subjects complained of fatigue after these dives. On the day after diving, one subject had FEF_{25-75} depressed by 21%, with the value still 17% low on Day +2; one had both FEV_1 and FEF_{25-75} low, by 10.3% and 17%, respectively; one had FEV_1 decreased by 11.6%; and one had D_LCO down by 18%. For comparison, after four-hour exercise dives with open circuit UBAs, 5% of the subjects had either flow-volume or D_LCO changes (90% binomial CI, 0.9%–15%), and 23% reported symptoms (90% CI, 12%–36%).⁵

Two Days with Three-hour Dive, Four-hour Surface Interval, Three-hour Dive, MK 25

Three divers who began this series were forced to withdraw: one because he was involved in a motor vehicle accident in the evening after his first day of diving, one because he could not tolerate the MK 25, and one because he had a presyncopal episode during the performance battery testing after his first dive. We consider only the third of these to be dive-related, and then only tangentially: a substantial effort to do pull-ups coupled with dehydration from the three-hour immersion that included ninety minutes of exercise in moderately warm water caused problems for this aerobically trained individual (resting heart rate <50 beats/min). He recovered quickly with rest and rehydration.

The average UBA oxygen fraction after three hours of diving was 0.83, standard deviation was 0.12, the minimum measured was 0.60, and the maximum was 0.95.

Of the 13 divers who completed two consecutive days with two three-hour dives separated by a four-hour surface interval, six reported mild symptoms at some time (46%, 90% CI: 22%–1%), with one diver reporting some moderate inspiratory burning (Table 4). Three divers had mild symptoms after each dive but had no changes in pulmonary function: one reported symptoms in the evening of each dive day, one after his first dive only, and one after his second dive only. We lost three sets of flow-volume measurements on the second day of diving when a valve leak in one of the measurement systems went undetected until divers were in the water on their second dive, and one baseline D_LCO because the sampling line was cracked. However, six divers, only one of whom also reported respiratory symptoms, showed decreases in pulmonary function — two during and four at the end of or after the dive series. Details are shown in Table 4.

Table 4.

Respiratory symptoms and decreases in pulmonary function variables, two "3-4-3" dives

Subject	Day 1 #1	Day 1 SI	Day 1 #2	Night	Day 2 #1	Day 2 SI	Day 2 #2	Day +1
1		FVC -7.8%					c	
2							FEV ₁ -9.4% FEF ₂₅₋₇₅ -19.6%	
3	i				FEF _{max} -19.8%			
4								FEV ₁ -8.8%
5							D _L CO -18%	
6				f			FVC -12.8% FEV ₁ -15.9% FEF ₂₅₋₇₅ -25.8% D _L CO -16.2%	
7				t				c, t, f
8		s,t	s,t			s,t	s,t	
9				i				
10		i	i		t	i	t	t (Fever, infection)
11-13	No signs or symptoms							

"c" represents cough; "i," inspiratory burning; "f," fatigue; "s," shortness of breath; and "t," chest tightness. Roman face represents a mild symptom, and **t**, a moderate one.

Physical Performance Testing

We were unable to use the shooting data because the gun for the laser shooting system had an intermittent fault during these tests. Postdive data for four subjects were not collected until dive Day +4. One subject injured his back, and one became ill and withdrew from testing on the final day.

Among the seven subjects for whom comparisons could be made, two had become available to dive without having done baseline physical performance measurements. We therefore compared dive day and postdive results in all seven — as well as baseline, dive day, and postdive results in five subjects. Analysis of variance showed no differences in the number of stair steps or pull-ups completed at different sessions, although three subjects completed physical training including pull-ups before coming for testing on Day +1. Among the five subjects with baseline measurements, right and left hand (RH and LH) grip strength showed marginal changes or less with day (RH: $p < 0.09$; LH: $p = 0.14$), even though the differences from baseline to the postdiving day were distinct: RH, 137 to 119 lb; LH, 131 to 114 lb. However, in the seven subjects average RH maximum grip strength differed across days ($p < 0.02$), with that on Day +1 lower than that on either dive day: Dive Day 1, 130 lb; Dive Day 2, 133 lb; postdive day, 118 lb. Average RH force-time product was also differed with day ($p < 0.04$): Dive Day 1, 8300 lb·s; Dive Day 2, 8600 lb·s; postdive day, 5100 lb·s. LH maximum grip was also decreased on Day +1: Dive Day 1, 124 lb; Dive Day 2, 132 lb; postdive day, 115 lb ($p < 0.04$). But LH force-time product was only marginally decreased: Dive Day 1, 6400 lb·s; Dive Day 2, 8100 lb·s; postdive day, 5075 lb·s ($p < 0.1$).

Four Days with Three-hour Dive, Four-hour Surface Interval, Three-hour Dive

Of the four divers who dropped out of this series, one was forced to withdraw with severe middle ear absorption syndrome (“Draeger ear”) after the first day of diving, one with muscle twitches during the second dive on the second day, one with nausea early during his fifth dive, and one in distress with pulmonary symptoms in the final hour of his eighth dive. The nausea was not dive related; it continued throughout the day while the subject was breathing room air.

Symptoms and changes in pulmonary function tests are described in full for the seven subjects in Table 5. Statistical tests have little validity for a set of three subjects. Although a sample size of five is still very small, we can compare the first dive set with the previous series; two of five subjects had symptoms, and two of five showed pulmonary function changes, a result corresponding fairly closely to six of thirteen for either symptoms or changes in pulmonary function in the two-day series. The combined numbers, eight of eighteen, yield 44% with either respiratory symptoms or changes in pulmonary function — 90% CI from 24% to 66%.

Table 5.

Pulmonary symptoms and decreases in pulmonary function variables, two sets of two "3-4-3" dives with 3 days between sets.

Subject	Day 1	Night	Day 2	Break	Day 3	Night	Day 4	Post
1				Gun -28% RH grip -6% LH grip +2%		f		FVC -10.5% FEV ₁ -9.6% Gun -49% e
2			FEF ₂₅₋₇₅ -17.2%	Gun -28% RH grip -10 % LH grip +7 %				Gun -51%
3	No signs or symptoms			Gun -4 % RH grip +6 % LH grip -11 %	No signs or symptoms			Gun -7%
4	f, t		f, t	Gun +23 % RH grip -4 % LH grip -14 %	f, t	t	t (end)	<u>t</u>
5	<u>f</u>		FEF ₂₅₋₇₅ -18.1% i, t	Gun -2% RH grip -15% LH grip -14 %	FEF ₂₅₋₇₅ -18.6% (end)			
6	c		(end)					
7	FEF ₂₅₋₇₅ -17.5%	<u>Draeger</u> <u>ear</u> (end)						

"c" represents cough; "e," exercise intolerance; "i," inspiratory burning; "f," fatigue; "s," shortness of breath; and "t," chest tightness. Roman face represents a mild symptom; **bold**, a moderate one; bold and underlined, a moderately severe symptom; and *italic bold underlined*, a severe one.

Physical Performance Testing

Performance indices did not differ statistically from baseline to the day after the first set of dives in the five subjects who completed that part of the test, with some subjects showing modest increases in indices to offset the small decreases seen in others. Because only three subjects completed eight dives, we can say little about overall effects. For those three subjects, average changes were RH grip, +4.6 lb (4%); RH endurance time, +1.5 s (1.3%); LH grip, +1.3 lb (1%); LH endurance time, -4.8 s (-13%); pull-ups, 0; stair steps, +4.2 (18%); and shooting score, -33 (-36%). The individual changes in shooting scores were -51%, -7%, and -49%. The increase in stair step numbers is in doubt, because we discovered that the photocell counter had registered some double counts.

Performance measures and self-reported fatigue and exercise intolerance (Table 5) were not related in this small sample. It is worth noting that the reported exercise intolerance was during a six-mile run four days after the last dive; endurance but not short-term performance may have been impaired, or reduced exercise tolerance may have been a secondary effect with latency longer than the testing period.

Antioxidant Assay

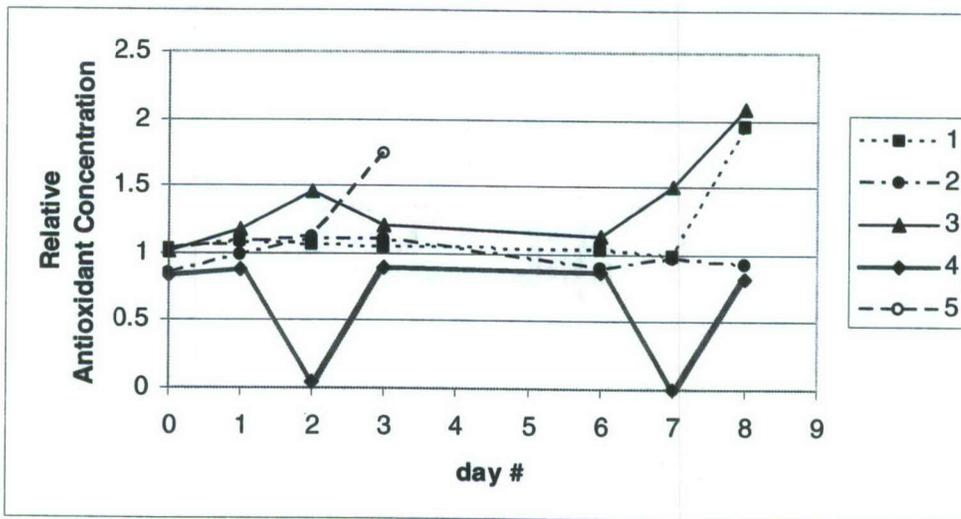


Figure 1. Antioxidant concentrations relative to 1 millimolar uric acid as functions of time. Legend shows subject numbers as given in Table 5. Day 0 is baseline; days 1, 2, 6, and 7 are diving days (samples collected after two dives).

The initial antioxidant concentrations in plasma were not notably different among subjects, but responses to dives were very different. Concentrations decreased to negligible levels in one diver after each of his second of two consecutive dive days. Concentrations after the dive series approximately doubled in two divers. One diver showed no appreciable change.

DISCUSSION

The MK 25 UBA, purged to at least 90% oxygen at the start, on average contained 83% oxygen at the end of a three-hour dive. Tissue nitrogen washout into breathing gas is most rapid when the driving pressure is highest. Thus, these divers most likely experienced the dilution of breathing gas early in the dives and breathed about 1.2 atm oxygen (range: 0.8 atm to 1.3 atm) while underwater.

The effects of single dives with exercise appear similar for MK 25 rebreather UBAs and MK 20 open circuit UBAs. The MK 25's increased impediment to breathing relative to the MK 20's may be counteracted by the lower PO_2 , but it is likely that the effect of either is too small to detect in a sample of 23 divers. Furthermore, divers accommodate to the differences in loading — for example, by adjusting posture and cycling with heads low to minimize the static load from the chest-mounted counter lung. For four-hour dives with exercise, MK 25 and MK 20 UBAs with 100% oxygen are equivalent from a pulmonary standpoint: dives with either UBA provoke respiratory symptoms in 23% of participants and cause mild changes in pulmonary function in 5% to 13% of divers.

With the MK 25 UBA, dives three to four hours in length were very taxing. Divers reported substantial mouthpiece or face mask discomfort. Additionally, the onset of exercise, if abrupt, was difficult because the volume of the rebreathing bag limited the initial breaths. These difficulties multiplied during the series of two three-hour dives per day for two days, dives that many people were reluctant to consider.

It was clear that few if any divers would consent to dive for a total of six hours per day on four days with the MK 25. Because results from single MK 25 dives with exercise showed such dives to be not significantly different from similar dives with the MK 20, we elected to change the UBA for the series of four dives. We conducted what was to have been the first of two sets dives, but with a substantial attrition. That, coupled with participants' comments that continuing as long as they had was substantially difficult, led us to terminate the series of dives.

The "3-4-3" scenario with exercise is not tenable for repeated exercise diving. Two days of these dives caused respiratory symptoms in 44% of participants and changes in pulmonary function variables in 44% of divers, and these two groups did not generally overlap. Only five of 21 subjects to start completed two days of diving with neither

symptoms nor signs. Two of the subjects without problems after two days of diving were in the four-day series, and one of them showed decreases in pulmonary function variables on the day after his fourth dive (Table 5). The other subject had no symptoms or signs throughout the testing period.

The physical performance battery did not quantify the fatigue reported by our divers, perhaps because it tests specific muscular fatigue in the short term. Results of total antioxidant power are too few to be enlightening, but they highlight the interindividual differences in response to oxygen dives.

CONCLUSIONS

The use of the MK 25 rebreather UBA rather than the MK 20 open circuit UBA does not increase the incidence of pulmonary oxygen toxicity. As we have reported elsewhere, underwater exercise does have an important effect, however — an effect that seems to consist primarily of increasing the time to recovery after a dive. Thus, a dive day consisting of three hours diving with exercise, a four-hour surface interval, and another three hours of diving with exercise is not sustainable if the routine is repeated after 16 hours overnight. Otherwise, it appears that results with the MK 20 and exercise apply directly to dives with the MK 25 and exercise, and vice versa.

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