

## The five-minute prebreathe in evaluating carbon dioxide absorption in a closed-circuit rebreather: a randomized single-blind study

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### Abstract

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**Introduction:** Closed-circuit underwater rebreather apparatus (CCR) recycles expired gas through a carbon dioxide (CO<sub>2</sub>) 'scrubber'. Prior to diving, users perform a five-minute 'prebreathe' during which they self-check for symptoms of hypercapnia that might indicate a failure in the scrubber. There is doubt that this strategy is valid.

**Methods:** Thirty divers were block-randomized to breathe for five minutes on a circuit in two of the following three conditions: normal scrubber, partly-failed scrubber, and absent scrubber. Subjects were blind to trial allocation and instructed to terminate the prebreathe on suspicion of hypercapnia.

**Results:** Early termination was seen in 0/20, 2/20, and 15/20 of the normal, partly-failed, and absent absorber conditions, respectively. Subjects in the absent group experienced a steady, uncontrolled rise in inspired (P<sub>I</sub>CO<sub>2</sub>) and end-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>). Seven subjects exhibited little or no increase in minute volume yet reported dyspnoea at termination, suggesting a biochemically-mediated stimulus to terminate. This was consistent with results in the partly-failed condition (which resulted in a plateaued mean P<sub>I</sub>CO<sub>2</sub> near 20 mmHg), where a small increase in ventilation typically compensated for the inspired CO<sub>2</sub> increase. Consequently, mean P<sub>ET</sub>CO<sub>2</sub> did not change and in the absence of a hypercapnic biochemical stimulus, subjects were very insensitive to this condition.

**Conclusions:** While prebreathes are useful to evaluate other primary functions, the five-minute prebreathe is insensitive for CO<sub>2</sub> scrubber faults in a rebreather. Partly-failed conditions are dangerous because most will not be detected at the surface, even though they may become very important at depth.

### Key words

Scuba diving, rebreathers/closed circuit, carbon dioxide, hypercapnia, rebreathing, capnography, physiology

### Introduction

Closed-circuit rebreathers (CCRs) are popular in advanced recreational diving owing to advantages such as the minimization of gas consumption, especially during deep diving, and optimization of decompression. Rebreathers recycle expired gas around a circle circuit with one-way valves. Expired carbon dioxide (CO<sub>2</sub>) is removed as it passes through a 'scrubber' canister containing CO<sub>2</sub> absorbent that is most commonly soda lime (a mixture of sodium hydroxide and calcium hydroxide). Oxygen metabolised by the diver is replaced in the circuit to maintain a safe inspired partial pressure of oxygen (P<sub>I</sub>O<sub>2</sub>).

Rebreathers are more complex than open-circuit scuba equipment and more prone to operator errors.<sup>1</sup> Some of these relate to the CO<sub>2</sub> scrubber. The absorbent material has a finite capacity (approximately 12–15 L CO<sub>2</sub>·100 g<sup>-1</sup>) and must be changed regularly.<sup>2</sup> Errors include failing to replace the absorbent material in a timely manner, incorrect packing of the absorbent material into the scrubber canister, incorrect installation of the canister in the rebreather and, rarely, forgetting to install it entirely. Such errors may allow expired CO<sub>2</sub> to enter the inhaled gas which may in turn cause symptomatic hypercapnia (often referred to by

divers as CO<sub>2</sub> toxicity). There have been deaths during the use of rebreathers in which hypercapnia is thought to have contributed, one of which is comprehensively documented in the medical literature.<sup>3</sup> Hypercapnia also enhances the toxicity of oxygen<sup>4,5</sup> and the narcotic effect of nitrogen<sup>6</sup> breathed at higher partial pressures.

Most rebreather units do not measure inspired CO<sub>2</sub>, so most technical diver training agencies teach divers to conduct a five-minute 'prebreathe' as a means of checking scrubber function before entering the water. A prebreathe involves preparing the unit for diving, and then sitting quietly breathing on the circuit, ideally with the nose blocked. If the CO<sub>2</sub> scrubber is absent or faulty, the diver will re-inhale expired CO<sub>2</sub> and, in theory, should notice the early symptoms of hypercapnia such as dyspnoea and/or headache. The five-minute duration is assumed to be sufficiently long for early symptoms of hypercapnia to reliably manifest, but the validity of this practice has not been formally tested. Therefore, we measured the proportion of blinded subjects who could discern an absent or faulty CO<sub>2</sub> scrubber during a five-minute prebreathe test on a rebreather circuit. A secondary aim was to derive a physiological interpretation of the results.

## Methods

### TRIAL DESIGN AND PARTICIPANTS

This was a randomised, single-blind, controlled trial that took place at the Exercise Metabolism Laboratory, University of Auckland, in July 2014. The study protocol was approved by the University of Auckland Human Participants Ethics Committee (reference 012315).

The subjects were trained, certified and active adult divers. Preference was given to rebreather divers, but experienced open-circuit scuba divers were not excluded as they would be taught the same prebreathe technique and expected to use it if undertaking a rebreather training course. All subjects received a participant information sheet, a verbal briefing and provided written informed consent.

### EXPERIMENTAL CONDITIONS & RANDOMIZATION

Twenty prebreathe tests were conducted on a rebreather in each of the following experimental conditions: normal scrubber; partly-failed scrubber and absent scrubber as described in more detail below. To achieve sufficient numbers of trials in each condition, 30 blinded subjects were block randomised to prebreathe in two of the three scrubber conditions with a rest period of at least 20 minutes between the two experiments. Subjects relaxed between trials in the presence of study personnel to prevent them discussing their experience until the study was complete. For each subject, the order of conditions was constrained so that the condition likely to result in less CO<sub>2</sub> rebreathing was first. This constraint was concealed from the subjects and was necessary to prevent an obvious hypercapnia experience on the first prebreathe from biasing perceptions of scrubber condition on the second. Similarly, we concealed the block randomization pattern from subjects who were told, again to avoid biasing, that any combination of the two conditions was possible, including breathing on a normal circuit twice.

### EQUIPMENT CONFIGURATION

An Inspiration Evolution Plus rebreather (Ambient Pressure Diving, Helston, Cornwall) was assembled by the investigators for each prebreathe. The rebreather oxygen cylinder contained 100% oxygen and the diluent cylinder contained air. The rebreather oxygen controller was set to maintain a P<sub>I</sub>O<sub>2</sub> at 0.7 atm (71 kPa) throughout each experiment. This is a standard setting used by rebreather divers when at the surface.

Rebreather assembly followed the standard procedure described by the manufacturer with several exceptions. First, the CO<sub>2</sub> scrubber was configured according to the allocated condition. In the normal condition, the absorbent canister was installed as recommended, with the soda lime material replaced each day (after approximately 80 minutes

of a maximum recommended 180 minutes use). In the partly-failed condition, the scrubber canister was installed, but a known assembly error was intentionally committed: a sealing O-ring that directs all gas flow through the canister was omitted from the circuit, allowing some expired gas to bypass the scrubber. In the absent condition, the absorbent canister was completely omitted.

Second, a disposable anaesthetic circuit antibacterial filter (Covidien DAR, MA, USA) was incorporated into the mouthpiece of the rebreather circuit. The filter had a dual purpose. It served to mask any changes in the circuit breathing resistance resulting from the scrubber condition (particularly the absent condition) by imposing a fixed resistance at the mouth. In addition, replacement of the mouthpiece and filter for each subject allowed use of the same rebreather circuit for multiple subjects. In a supplementary experiment using simple manometry, we evaluated the efficacy of the filter in masking changes in circuit resistance related to the scrubber condition and its contribution to any increase in circuit resistance. With the filter present or absent, and with the rebreather configured as for each of the three experimental conditions, we measured peak inspiratory and expiratory pressures (cm H<sub>2</sub>O) at the mouthpiece with a respiratory pressure transducer (MLT844, AD Instruments, Dunedin) during sinusoidal mechanical ventilation (17050-2 Lung Simulator, VacuMed, Ventura, CA) over 1 minute (tidal volume V<sub>T</sub> 1.5 L; respiratory rate RR 10 breaths·min<sup>-1</sup>).

Third, a gas sampling line was attached to the dedicated port of the mouthpiece filter. This allowed continuous sampling for rapid response measurement of P<sub>I</sub>O<sub>2</sub> with a paramagnetic O<sub>2</sub> analyser (S-3A, AEI Technologies, Pittsburgh, PA), inspired CO<sub>2</sub> (P<sub>I</sub>CO<sub>2</sub>), and end-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) with an infrared CO<sub>2</sub> analyser (CD-3A, AEI Technologies, Pittsburgh, PA). A three-point calibration was performed at routine intervals for O<sub>2</sub> and CO<sub>2</sub> using reference gases spanning the measurement range. A pneumotachometer (MLT1000L, AD Instruments, Dunedin) was interposed in the exhale limb of the rebreather circuit for measurement of V<sub>T</sub>, RR and minute volume (V<sub>E</sub>). The device was calibrated prior to each trial and removed from the circuit at regular intervals for comparison with an external standard (3L Calibration Syringe, Hans Rudolph, Shawnee, KS). For safety, heart rate (HR) and oxygen saturation (SpO<sub>2</sub>) were monitored using a pulse oximeter (Rad-5, Masimo, Irvine, CA) with the audible signal silenced. All physiological parameters were sampled at 15 second intervals. The laboratory set-up is illustrated in Figure 1.

### EXPERIMENTAL PROCEDURE

Subjects were briefed in a standardised manner prior to their first prebreathe. They were reminded of the symptoms of hypercapnia, and it was emphasised that this was an experiment to determine whether the subjects could detect

**Figure 1**

Laboratory set-up; the subject breathes from the modified rebreather whilst seated facing away from the monitors and recording equipment



a scrubber problem if present; not to determine whether they could tolerate hypercapnia. Accordingly, the subjects were asked to terminate the prebreathe test as they would in a real-world scenario if they detected relevant symptoms.

Subjects donned the rebreather in the sitting position, and faced away from the monitoring equipment. The breathing circuit hoses were passed over the shoulders as in normal use. At commencement of the prebreathe period the mouthpiece was placed in the subject's mouth and the nose was occluded using a nose clip, which is recommended as best practice. Each prebreathe either continued for five minutes or was terminated by the subject if he or she discerned symptoms of hypercapnia. Subjects who terminated the prebreathe early were asked to describe their symptoms.

#### OUTCOMES

The primary outcome was a comparison of the proportion of subjects who detected symptoms of hypercapnia and terminated the prebreathe in each condition. A secondary aim was to interpret these results in the context of the physiological data ( $P_I\text{CO}_2$ ,  $P_{ET}\text{CO}_2$ ,  $V_T$ , RR, and  $V_E$ ).

#### POWER

We considered that 80% sensitivity for detection of a scrubber problem in the abnormal scrubber conditions would

indicate a potentially useful test. We anticipated that under the circumstances of the experiment, subjects might exhibit a high index of suspicion for  $\text{CO}_2$  scrubber problems, resulting in some false positives in the group breathing on a normal rebreather loop. Thus, allowing for a 30% false positive rate in the normal rebreather condition, we calculated that to demonstrate a statistically significant difference between terminations in the normal condition and in each abnormal condition where the test appeared useful (80% sensitivity) with 90% power and an alpha value of 0.05, we would need 20 subjects in each group.

#### STATISTICAL ANALYSIS

Descriptive data are presented as mean  $\pm$  standard deviation (SD) or median with ranges, as appropriate. The proportion of subjects terminating the prebreathe in each condition was calculated, and these were compared using a two-tail Fisher exact test (GraphPad Prism ver 6.01, San Diego, CA). The sensitivity, specificity and positive predictive values of the prebreathe were calculated.

#### Results

Baseline characteristics of the groups are described in Table 1.

#### PRIMARY OUTCOME

Twenty prebreathe tests were completed in each condition. The proportion of subjects terminating the prebreathe in each of the three conditions is shown in Table 2. The sensitivity of the prebreathe was 10% for the detection of a partly-failed scrubber, and 75% for detection of an absent scrubber. The specificity of the prebreathe was 100% as there were no false positives in the normal condition. The positive predictive value (PPV) was 100% (albeit in a high prevalence setting), indicating that all subjects who terminated because of perceived symptoms of  $\text{CO}_2$  toxicity were breathing on a loop with a faulty  $\text{CO}_2$  scrubber. The negative predictive value was 80% for an absent scrubber and 53% for a partly-failed scrubber.

The mean time to termination in the absent scrubber group was 3 minutes and 41 seconds (range 2 min 1 s to 4 min 52 s). Among the 18 subjects who terminated the prebreathe, the most frequently reported symptoms of hypercapnia were

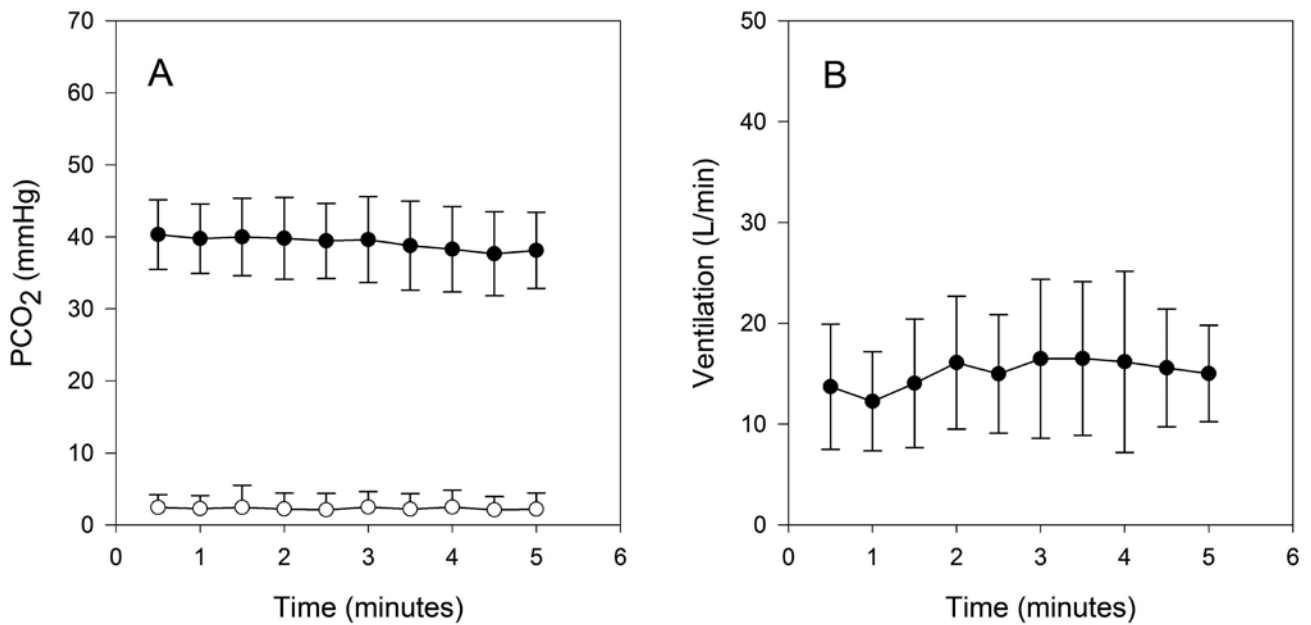
**Table 1**

Descriptive data for participants randomised to the three scrubber conditions;  $n = 20$  for all three conditions

	Normal scrubber	Partly-failed	Absent scrubber
Age (years) mean (SD)	42 (8)	44 (10)	42 (9)
Sex (M/F)	14/6	16/4	14/6
Body mass index ( $\text{kg}\cdot\text{m}^{-2}$ ) mean (SD)	28.6 (3.2)	27.7 (3.3)	28.4 (3.7)
Years of diving median (range)	18 (3–45)	14 (1–45)	15 (1–28)
Rebreather divers	15	12	13

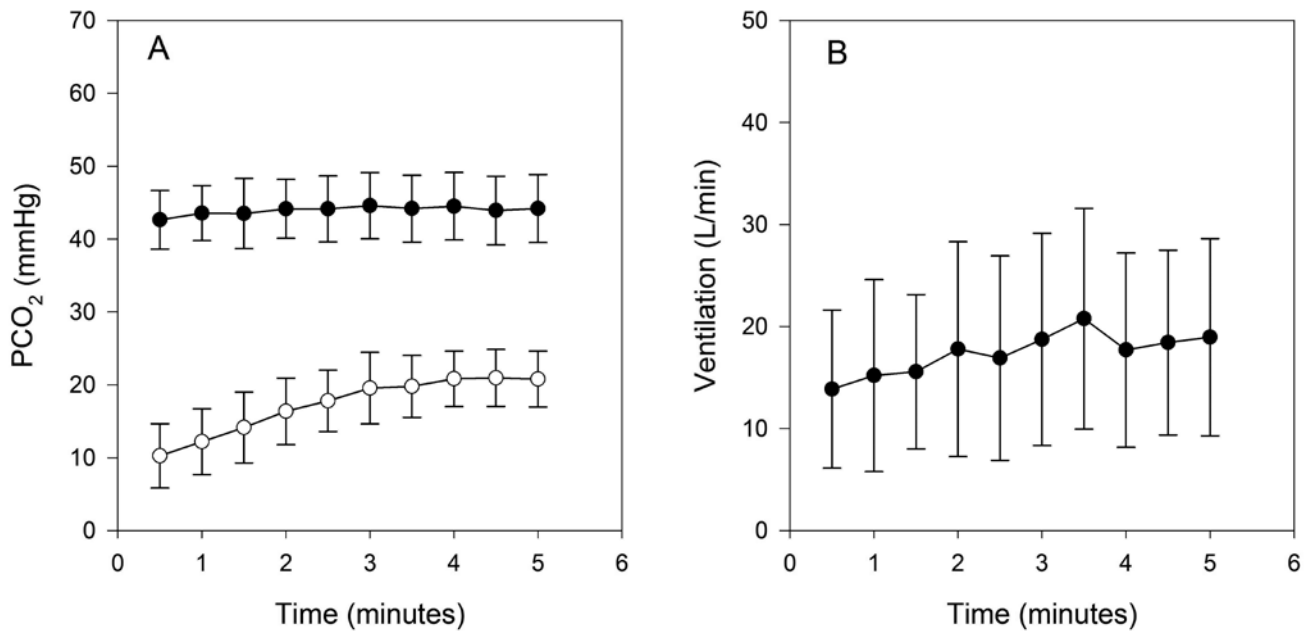
**Figure 2**

Normal scrubber condition (mean  $\pm$  SD); A – End-tidal (closed circles) and inspired (open circles) PCO<sub>2</sub>; B – minute ventilation during the course of a five-minute prebreathe; note in both cases the first reading was made 30 s after commencement of the prebreathe



**Figure 3**

Partly-failed scrubber condition (mean  $\pm$  SD); A – End-tidal (closed circles) and inspired (open circles) PCO<sub>2</sub>; B – minute ventilation during the course of a five-minute prebreathe; note in both cases the first reading was made 30 s after commencement of the prebreathe, therefore, these readings are not true baseline values; indicative baselines may be inferred from Figure 2 (normal scrubber condition)



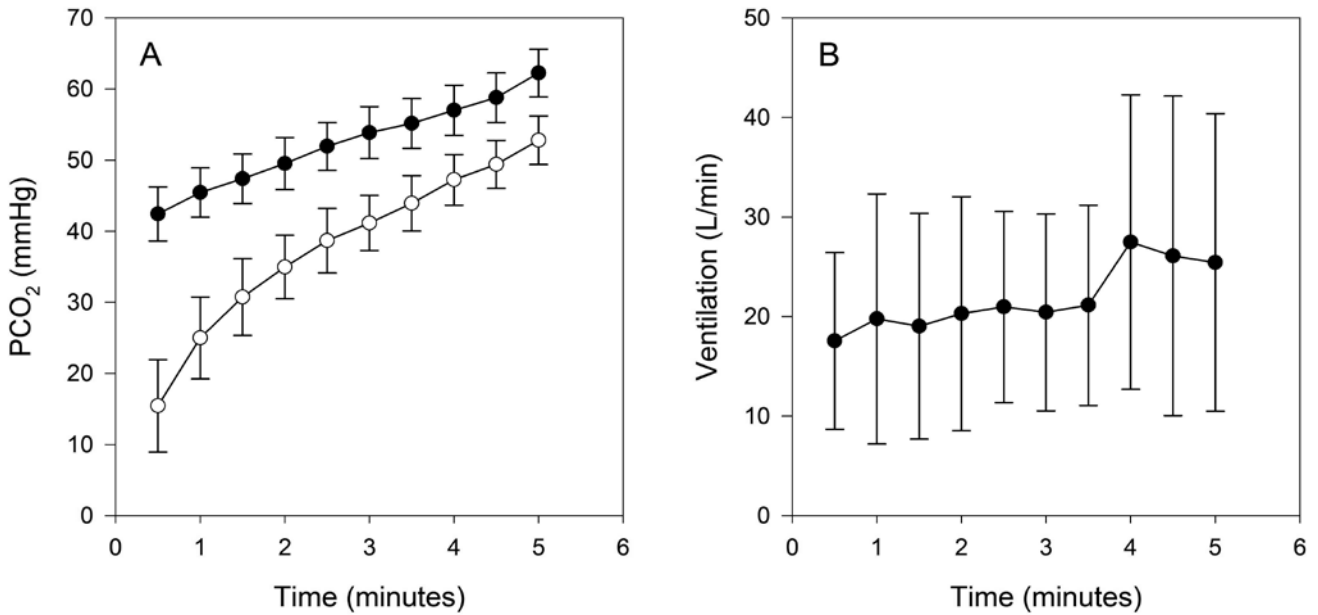
**Table 2**

Outcomes (numbers and proportion of subjects who terminated the prebreathe) for each of the three scrubber conditions; *P* values are for the comparison with the normal scrubber state

	Terminated	Not terminated	<i>P</i> -value
Normal	0	20	
Partly failed	2	18	0.487
Absent	15	5	< 0.0001

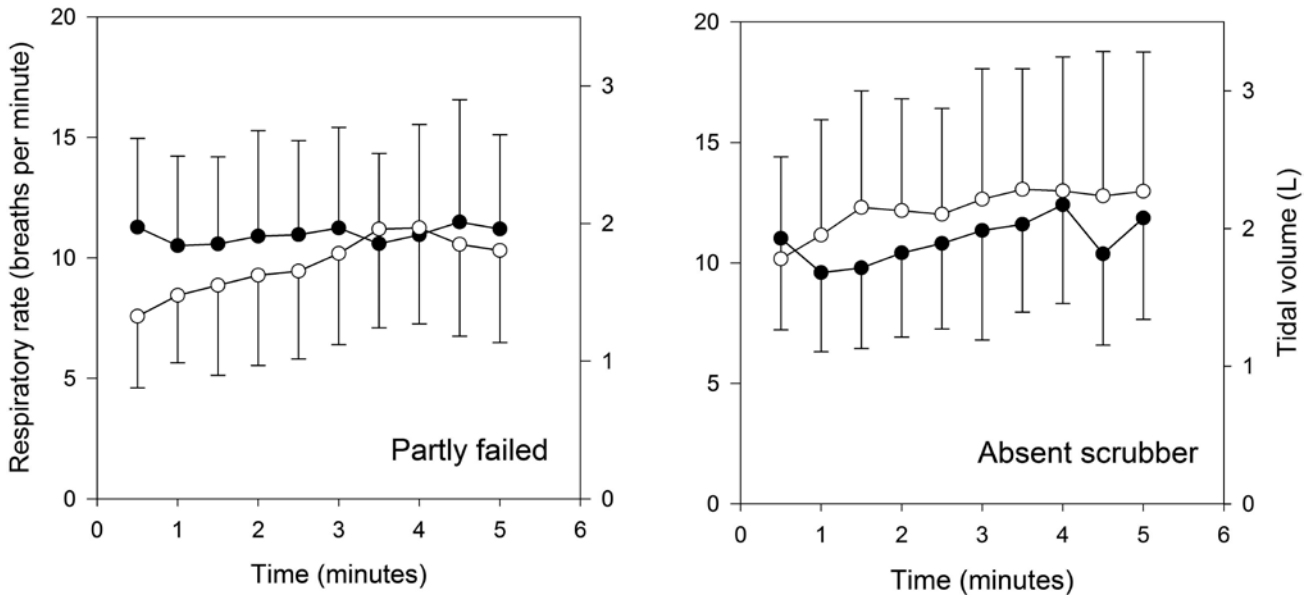
**Figure 4**

Absent scrubber condition (mean  $\pm$  SD); A – End-tidal (closed circles) and inspired (open circles) PCO<sub>2</sub> and B – minute ventilation during the course of a five-minute prebreathe. Note, in both cases the first reading was made 30 s after commencement of the prebreathe, therefore, these readings are not true baseline values, indicative baselines may be inferred from Figure 2 (normal scrubber condition)



**Figure 5**

Effect of the partly-failed and absent scrubber conditions on respiratory rate (closed circles) and tidal volume (open circles) (mean  $\pm$  SD) during the course of a five-minute prebreathe; respiratory rate remains relatively unchanged whilst tidal volume increases in both conditions; note in both cases the first reading was made 30 s after commencement of the prebreathe, therefore, these readings are not true baseline values

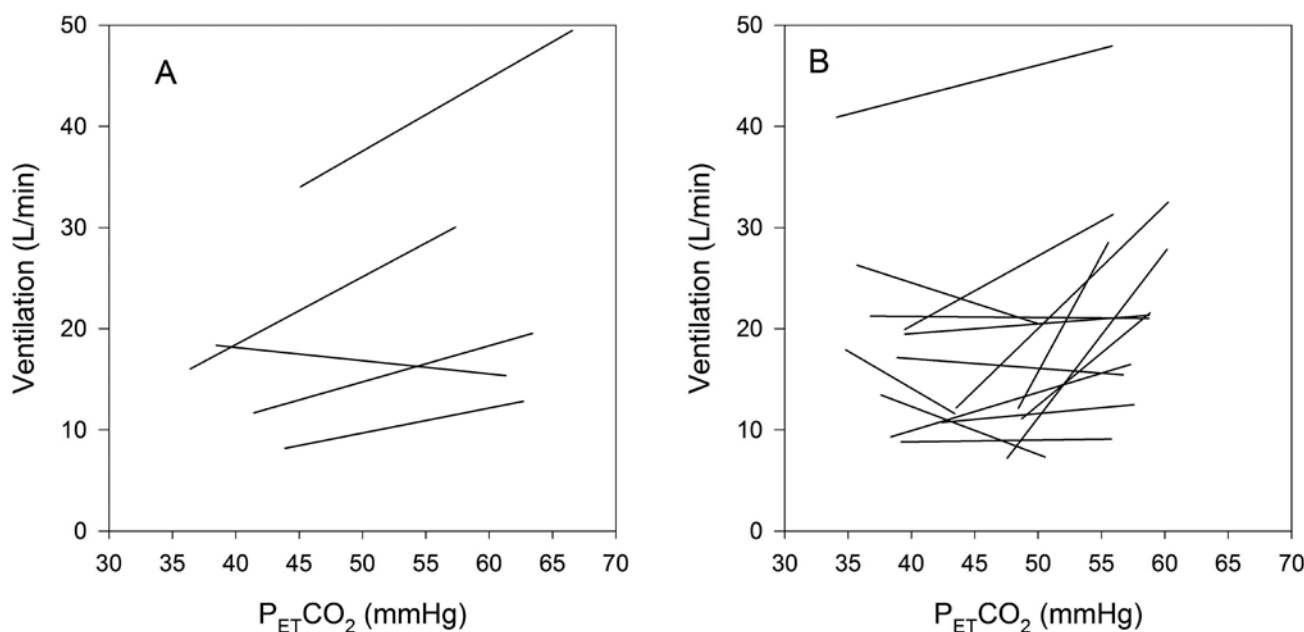


‘shortness of breath’ or ‘increased work of breathing’ (16 of the 18), followed by ‘dizziness’ or ‘light-headedness’ (3/18). Cognitive changes (3/18), anxiety (2/18), visual changes (1/18) and the perception of a ‘racing pulse’ (1/18) were also reported.

There were no significant differences between the subjects who were rebreather or open-circuit divers in relation to the primary outcome. For example, in the absent scrubber condition 9 of 13 rebreather divers versus 6 of 7 open-circuit divers terminated the prebreathe ( $P = 0.61$ ).

**Figure 6**

Subjects in the absent scrubber group, separated into A – those who completed the prebreathe, and B – those who terminated the prebreathe; each subject is represented by a straight line linking the  $P_{ET}CO_2$  and  $V_E$  pairs at the beginning and end of the prebreathe



**Table 3**

Peak inspiratory and expiratory pressures (cm H<sub>2</sub>O, mean (SD) shown) required for a breathing simulator to move a 1.5 L tidal volume around the rebreather circuit in the three scrubber conditions, and in the presence and absence of the mouthpiece filter; data represent the mean of 10 breaths measured over a 1-min period

Condition	Expiratory pressure		Inspiratory pressure	
Filter only	2.56	(0.03)	-2.42	(0.07)
Rebreather + normal scrubber	3.51	(0.02)	-4.33	(0.06)
Rebreather + partly failed	3.49	(0.04)	-4.14	(0.02)
Rebreather + absent scrubber	2.74	(0.01)	-3.24	(0.08)
Rebreather + filter + normal scrubber	4.67	(0.05)	-5.35	(0.07)
Rebreather + filter + partly failed	4.50	(0.05)	-5.31	(0.07)
Rebreather + filter + absent scrubber	4.21	(0.09)	-5.06	(0.05)
Δ normal vs. absent scrubber, no filter	0.77	-1.09		
Δ normal vs. absent scrubber, with filter	0.46	-0.29		

**EFFECT OF THE THREE CONDITIONS ON PHYSIOLOGICAL PARAMETERS**

The effects of the three experimental conditions on  $P_I CO_2$ ,  $P_{ET} CO_2$  and ventilation during the five-minute prebreathe period are shown in Figures 2 to 4. In the normal scrubber condition (Figure 2) these parameters did not change significantly throughout the prebreathe. A low  $P_I CO_2$  (< 5 mmHg), which did not change, was detected in this condition.

In the partly-failed condition, the mean  $P_I CO_2$  rose immediately and by three or four minutes into the prebreathe had plateaued near 20 mm Hg (Figure 3A). Despite this, the mean  $P_{ET} CO_2$  did not change due to a small compensatory increase in mean ventilation (Figure 3B) achieved

predominantly by an increase in  $V_T$  (Figure 5).

In the absent scrubber condition, the mean  $P_I CO_2$  and  $P_{ET} CO_2$  rose inexorably (Figure 4A) despite an increase in mean  $V_E$  (Figure 4B); the latter once again explained primarily due to an increase in  $V_T$  rather than respiratory rate (Figure 5). There was, however, marked variability among individuals in the ventilation response to rising  $P_{ET} CO_2$  (Figure 6). Some individuals tolerated increases in  $P_{ET} CO_2$  to higher than 50 mm Hg with no change or even a decrease in  $V_E$ , whilst others quickly increased  $V_E$  to levels around 40–50 L·min<sup>-1</sup> very early as the  $P_{ET} CO_2$  began to rise.

These observations still applied when subjects were separated into those who terminated (Figure 6B) and those who did not (Figure 6A), and into rebreather divers and open-

circuit divers (data not presented). The reported symptoms precipitating termination were often inconsistent with the obvious physiological responses. For example, all seven subjects who terminated despite no significant increase ( $\leq 2 \text{ L}\cdot\text{min}^{-1}$ ), no change, or even a decrease in  $V_E$  still cited dyspnoea as a precipitating symptom. Heart rate did not rise as the  $P_{ET}\text{CO}_2$  increased in this group (including the subject who perceived a “ *racing heart* ”); the mean ( $\pm$  SD) heart rate at minutes 1 to 5, being  $73 \pm 11$ ,  $73 \pm 9$ ,  $74 \pm 10$ ,  $76 \pm 12$  and  $72 \pm 12 \text{ beats}\cdot\text{min}^{-1}$  respectively.

#### MANOMETRY EXPERIMENT

Peak inspiratory and expiratory pressures recorded at the mouthpiece with the filter present and absent in each of the three experimental conditions are shown in Table 3. As anticipated, the difference in pressures between the full scrubber and absent scrubber condition was reduced (and therefore less likely to be apparent to subjects) when the filter was in place.

#### Discussion

Rebreathers are complex devices with many failure points and potential user errors. Errors in preparation, assembly or installation of the  $\text{CO}_2$  scrubber may result in  $\text{CO}_2$  rebreathing and hypercapnia. Hypercapnic events, in turn, may potentiate oxygen toxicity or precipitate other fatal accidents. As a screen to detect such errors, most divers are taught to conduct a five-minute ‘prebreathe’ on the assembled rebreather circuit prior to diving.

The validity of this prebreathe strategy has been questioned. A small, non-peer-reviewed study reported that none of 14 subjects terminated a five-minute prebreathe on a rebreather with no scrubber canister installed.<sup>7</sup> Ventilation parameters were not reported, and it is not clear how the subjects were briefed. It is therefore difficult to compare the results to those we report here. Nevertheless, our study also indicates that the prebreathe strategy is insensitive to failure of the  $\text{CO}_2$  scrubber.

Most importantly, we exposed the partly-failed group to a known assembly error that allowed a fraction of the expired gas to bypass the  $\text{CO}_2$  scrubber canister, resulting in a  $P_I\text{CO}_2$  that rose to approximately 20 mmHg over several minutes. Despite this, 18 of 20 subjects did not terminate the prebreathe in this condition. Other errors or problems encountered in the real world may result in more (or less) inspired  $\text{CO}_2$  than in this partly-failed scenario, and these would be correspondingly more (or less) likely to be detected by a prebreathe. However, since a quarter of our subjects did not terminate even when allocated to the worst possible  $\text{CO}_2$  rebreathing scenario (complete omission of the  $\text{CO}_2$  scrubber canister) the prebreathe must be considered an insensitive test over the entire range of errors leading to partial failure.

An interesting physiological consideration in interpreting

these results is “*what causes subjects to terminate a prebreathe?*” Although our study was not designed specifically to answer this question we made some relevant observations. Our data suggest that an increase in ventilation is not a prerequisite for subjects to perceive dyspnoea (Figures 4B and 6). Virtually all terminating subjects, including those whose ventilation did not increase, cited shortness of breath as one of the precipitating symptoms. Thus, it is possible that in at least some subjects termination is driven biochemically; that is, by symptoms (including the perception of dyspnoea) mediated by an increasing arterial  $P_a\text{CO}_2$ , rather than by perception of an actual increase in ventilation. This may help to explain the very poor sensitivity of the prebreathe in the partly-failed condition. In that setting (Figure 2), a relatively small increase in ventilation, certainly below a threshold noticeable to the vast majority of our subjects, was sufficient to compensate for a  $P_I\text{CO}_2$  that plateaued near 20 mm Hg. This prevented the  $P_{ET}\text{CO}_2$  from increasing, and therefore the subjects in the partly-failed group were not exposed to the same biochemical stimulus (an increasing  $P_a\text{CO}_2$ ) which seems likely to have driven termination in the absent scrubber group.

The ability to maintain normocapnia during a surface prebreathe despite partial scrubber failure should not be interpreted to indicate that minor degrees of bypass are benign. Indeed, as has been mentioned previously, commission of the assembly error we used to produce a repeatable partly-failed condition is widely reported among divers, and (anecdotally) has led to hypercapnia-induced incidents. This apparent inconsistency whereby the same partly-failed condition causes hypercapnia during diving but not during a prebreathe can be explained by the derangement of respiratory control that occurs during a dive.

Static lung loads, external resistance to gas flow, and increased respired gas density all contribute to an increase in the work of breathing during a dive.<sup>8</sup> It has been known for decades that in some divers this increased work causes hypoventilation and  $\text{CO}_2$  retention, even in the absence of an increased  $P_I\text{CO}_2$ .<sup>9</sup> This tendency has been characterized as a propensity for the respiratory controller to sacrifice tight  $\text{CO}_2$  homeostasis in order to avoid performing the respiratory work that homeostasis would require.<sup>10</sup> There is evidence that the presence of inhaled  $\text{CO}_2$  during exercise and respiratory loading further blunts respiratory drive,<sup>11,12</sup> paradoxically (in the present context), at the very time that responsiveness is crucial to safety.

Not surprisingly, others have reported that a  $P_I\text{CO}_2$  similar to that in our partly-failed condition is dangerous when combined with exercise and external breathing resistance similar to that imposed by a rebreather apparatus. In an experiment aiming to investigate maximum acceptable  $\text{CO}_2$  breakthrough levels in rebreather circuits, a 2%  $\text{C}_I\text{O}_2$  (15 mmHg) combined with relevant levels of resistance, exercise, and oxygen breathing caused dangerous levels of  $\text{CO}_2$  retention with poor awareness in many of the subjects.<sup>13</sup>

It was concluded that, for diving safety when using typical underwater breathing apparatus,  $P_{iCO_2}$  must be maintained as close to zero as possible. Thus, we reiterate the point that divers should not assume partial scrubber failure and  $CO_2$  rebreathing at levels similar to those measured in our study are benign simply because our subjects maintained a normal  $P_{ET}CO_2$  in this condition. It is notable that we detected a very small amount of inhaled  $CO_2$  (~ 3 mmHg) even in the normal scrubber condition (Figure 2). This could have been due to dead space in the mouthpiece and/or filter, trivial incompetency in the mouthpiece non-return valves, a very low level of  $CO_2$  bypass at the scrubber or a combination of these factors. Since we only studied one rebreather, we do not know whether this is a generalized phenomenon.

A number of subjects exposed to the absent scrubber condition failed to increase or actually decreased ventilation as  $P_{ET}CO_2$  increased (Figure 6). Although this is at odds with classical descriptions of the  $P_{ET}CO_2/\dot{V}_E$  response,<sup>14,15</sup> substantial variability in the ventilation response to rising  $P_{ET}CO_2$  has been reported previously in both non-divers and divers.<sup>14,16-18</sup> There is some evidence that divers are more prone to abnormal responses and that diving itself conditions participants to become 'CO<sub>2</sub> retainers'.<sup>19</sup> The subjects in our study were relatively experienced divers. Moreover, some aspects of our experimental conditions may have been contributory. For example, the rebreather used in our study would have imposed greater external breathing resistance than the low resistance respiratory measurement equipment typically used in studies of  $CO_2$  response, and greater external resistance may dampen the ventilatory response to inhaled  $CO_2$  as discussed earlier.<sup>11,12</sup> In addition, to be consistent with usual diving practice, the subjects breathed a high fraction of inspired oxygen (70%), and elevated inspired oxygen may make a further contribution to dampening the  $CO_2$  response.<sup>16</sup>

There are several limitations to our study. First, subjects performed the prebreathe in a laboratory environment that does not faithfully simulate the distracting conditions on a dive deck before a dive. We attempted to lessen any impact of the laboratory setting by maintaining lively conversation among investigators (without directly involving the subjects) throughout each prebreathe trial.

Second, unlike a real world scenario in which there would be a low expectation of problems, and although blinded, our subjects knew there was a substantial chance of being randomised to breathe on a loop with a scrubber fault. It was reassuring that despite this, there were no false positives among 20 subjects when there was a normal scrubber in place. Nevertheless, the experiment almost certainly promoted vigilance and our results arguably represent a best possible case for prebreathe sensitivity (see also the fourth point below).

Third, due to difficulties in recruiting 60 subjects for the study, we block-randomised 30 subjects to two of three

scrubber conditions and imposed a concealed manipulation on the order of those two conditions such that the condition least likely to result in hypercapnia was tested first in all participants. This required the subjects to undertake two prebreathes at least 20 minutes apart. Since the groups had some subjects in common, they are not entirely independent. We also considered the possibility of one exposure to inhaled  $CO_2$  somehow affecting the physiological response to a second administered in close succession, but others have shown that this does not happen.<sup>14</sup>

Fourth, the use of an antibacterial filter did impose a small increase in the manometric pressures required to move a fixed gas volume around the circuit (Table 3). This could have contributed to an increased tendency to retain  $CO_2$ , but given subjects in the normal condition (and even the partly-failed condition) maintained a normal  $P_{ET}CO_2$ , there is little evidence to suggest a prominent effect in that regard. The small increase in breathing resistance imposed by the filter may also have increased sensitivity of the prebreathe to the fault conditions by increasing the likelihood of dyspnoea as the  $P_{ET}CO_2$  increased. Thus, we reiterate that our results arguably represent a best possible case for prebreathe sensitivity.

Finally, the investigators were not blinded to scrubber condition. It is therefore possible that the primary outcome could have been influenced by subtle differences in the way we interacted with the subjects. However, there was little opportunity for this. Once the prebreathe started, no attempts were made to ask the subjects questions or engage them in any conversation. Discussion about the state of the rebreather or the outcomes for other subjects were explicitly avoided during experimental runs.

We also believe the study has several strengths. First, it is the only study known to address this issue with blinded subjects and careful physiological monitoring. Second, the fact that none of 20 subjects terminated when breathing with a normal scrubber suggests that expectation of problems was not excessively high, blinding was effective, and the slight increase in resistance associated with use of the antibacterial filter did not substantially increase perceptions of hypercapnic symptoms. Third, the study incorporated a repeatable partly-failed condition arising from an assembly error known to have occurred many times in real-world diving. The implications for translation of study findings to the diving community are obvious. Finally, all 30 volunteers attended the study sessions and completed their allocated experimental trials. There were no drop outs resulting in missing data.

## Conclusions

The five-minute prebreathe is an insensitive test for  $CO_2$  scrubber function in a diving rebreather, even when the scrubber canister is absent. A prebreathe is nevertheless recommended for purposes such as checking the function



of the oxygen addition system before entering the water, but a duration less than five minutes should be adequate for that purpose. Arguably the most important secondary finding of our study is that partial scrubber failure in a rebreather is a particularly insidious fault if divers rely on a prebreathe to detect it. By modestly increasing ventilation, subjects typically maintain normocapnia during a surface prebreathe in this condition, resulting in a false negative that is dangerous because normocapnia is much less likely to be maintained during the dive itself. These findings raise concerns around methods for testing and monitoring safe CO<sub>2</sub> elimination in rebreather circuits. Several manufacturers offer CO<sub>2</sub> analyzers in the inhale limb of the rebreather circuit as an option, but these are not yet mainstream features. We recommend that rebreather training courses emphasize the importance of correct packing and installation of CO<sub>2</sub> scrubber canisters. There is mounting evidence that divers are poor at recognizing the early symptoms of hypercapnia (during both prebreathes and diving) and strategies for avoidance of hypercapnia should be prioritized.

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