Measuring whole body inert gas wash-out

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Abstract
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Introduction: Quantifying inert gas wash-out is crucial to understanding the pathophysiology of decompression sickness. In this study, we developed a portable closed-circuit device for measuring inert gas wash-out and validated its precision and accuracy both with and without human subjects.
Methods: We developed an exhalate monitor with sensors for volume, temperature, water vapor and oxygen. Inert gas volume was extrapolated from these inputs using the ideal gas law. The device's ability to detect volume differences while connected to a breathing machine was analysed by injecting a given gas volume eight times. One hundred and seventy-two coupled before-and-after measurements were then compared with a paired *t*-test. Drift in measured inert gas volume during unlabored breathing was evaluated in three subjects at rest using multilevel linear regression. A quasi-experimental cross-over study with the same subjects was conducted to evaluate the device's ability to detect inert gas changes in relation to diving interventions and simulate power.
Results: The difference between the injected volume (1,996 ml) and the device's measured volume (1,986 ml) was -10 ml. The 95% confidence interval (CI) for the measured volume was 1,969 to 2,003 ml. Mean drift during a 43 min period of unlaboured breathing was -19 ml, (95% CI, -37 to -1). Our power simulation, based on a cross-over study design, determined a sample size of two subjects to detect a true mean difference of total inert gas wash-out volume of 100 ml.
Conclusions: We present a portable device with acceptable precision and accuracy to measure inert gas wash-out differences that may be physiologically relevant in the pathophysiology of decompression sickness.

Introduction

The ability to measure inert gas turnover is crucial to studying and understanding the pathophysiology of decompression sickness (DCS).[1] Physiologic interventions that alter cardiac output and/or tissue perfusion have been shown to affect inert gas turnover[2–7] and the risk of developing DCS.[7,8] However, the correlation between quantitative differences in inert gas turnover and the risk of DCS is not known. Measuring inert gas turnover can help us understand how to integrate physiological factors into mathematical decompression models and improve their predictive accuracy. Due to the technical complexity of the required equipment, the number of trials measuring inert gas turnover in the context of diving is limited.

To our knowledge, a very limited number of research groups have studied and published on techniques of measuring whole body inert gas wash-out and/or uptake.[2,9–12] More studies with continuous measurements of inert gas wash-out and/or uptake are needed to better understand gas dynamics and how they relate to the pathophysiology of DCS. These studies would benefit from devices able to continuously measure and statistically analyse changes in inert gas volumes over time.

The primary aim of this study was to present a portable closed-circuit device for quantifying inert gas wash-out over time and evaluate its performance both with and without human subjects. A secondary aim was to determine the required sample size to achieve statistical power for future cross-over studies.

Methods

The study was approved by the Swedish Ethical Review Authority (Dnr: 2020–06865) and all subjects provided informed, written consent to participate before the start of the study.

NITROGEN WASH-OUT

All measurements were performed at an ambient pressure of 101.3 kPa (1.0 atmosphere absolute [atm abs]). Nitrogen wash-out was detected as alterations in volume within a closed rebreathing system. To determine the proportion of volume changes attributable to nitrogen wash-out, adjustments based on the principles of the ideal gas law was used. The adjustments were based on the following sensors and system specifications; relative humidity and temperature in the total system volume, temperature in the carbon dioxide scrubber, total volume and changes in the counterlung volume. The volume conversion to standard temperature (V_{ST}) was performed for each section (hoses, scrubber and counter lung) in time increments as the temperature in the system and scrubber changed over the period of measurement according to equation 1 where T is the measured temperature in degrees Celsius and V_{AT} is the measured volume at ambient temperature.

$$V_{ST} = V_{AT}\left(\frac{273}{273+T}\right) \quad \text{Equation 1}$$

To further standardise the volume changes due to pressure difference and humidity a conversion to standard temperature and pressure, dry (STPD) was performed according to eq. 2 where P_B is the measured ambient pressure in kPa, P_{H2O} is the water vapour pressure for saturated gas at the ambient temperature in kPa, and RH is the measured relative humidity.

$$V_{STPD} = V_{ST}\left(\frac{P_B - P_{H2O} \cdot RH}{101.3\ kPa}\right) \quad \text{Equation 2}$$

The measured volume change was then multiplied with the fraction of nitrogen (1 – measured oxygen fraction) to determine nitrogen wash-out.

The closed circuit (see Figure 1) consisted of a mouthpiece, a modified soda-lime scrubber to remove carbon dioxide (Inspiration Tempstik scrubber, AP Valves, Cornwall UK), an ASVPOD for oxygen injection (device manufactured by Poseidon Diving Systems AB, Gothenborg Sweden with oxygen sensing and dosage, and temperature sensing), a counterlung (ISMIX counter bellows, Interspiro AB, Täby, Sweden) with a volume sensor and a CPOD (Poseidon Diving Systems AB, Gothenburg, Sweden) for oxygen, temperature and water vapour sensing. The oxygen fraction was regulated at a setpoint of 21% by the two Poseidon PODs via a computerised algorithm for sensing and dosage.

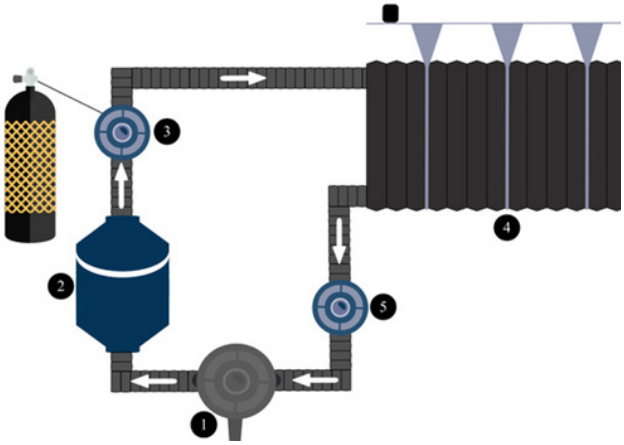
DESIGN AND SUBJECTS

Experimental measurements for evaluation of volume detection

To evaluate the device's sensors, software, and mechanical setup, we utilised a breathing machine and known gas flows.

Figure 1

Closed-circuit device for measuring inert gas wash-out volume; 1 – mouthpiece; 2 – soda-lime CO_2 scrubber; 3 – ASVPOD , with PO_2 sensing and dosage, and temperature sensing; 4 – counterlung with a position sensor for volume sensing; 5 – CPOD with PH_2O, temperature and PO_2 sensing



The following experiment presents a case in which the closed circuit was connected to a breathing machine (Life Support Equipment Test Facility, Ansti Test Systems Ltd, Fareham UK) with a fixed respiratory rate of 15 breaths per minute and a tidal volume of 700 ml. Paired volume registrations were analysed before and after injections of 1,996 ml, STPD air.

Human measurement for evaluation of potential drift and a quasi-experimental crossover study

Three subjects meeting the Swedish Armed Forces physical standards for diving were recruited. All were male, non-smoking and between 35–43 years old (Table 1).

To evaluate the drift the three subjects underwent one control measurement each. During these measurements they remained at rest in a supine position.

A quasi-experimental crossover study was conducted with four distinct diving interventions, with nitrogen wash-out measurements immediately after each dive. The four interventions were: 18 metres of sea water (msw) with bottom time of 50 minutes performed either dry or immersed (18 msw / 50 min, dry/immersed) and 39 msw with bottom time of 10 min performed either dry or immersed (39 msw / 10 min, dry/immersed).

All dives were performed in a hyperbaric chamber (HAUX 2300) at the naval base in Karlskrona, Sweden. During the dry dives, the subjects remained either lying down or seated at rest. The immersed dives occurred in water at 10 (± 1)°C with the subjects wearing undergarments, wet gloves, and dry suits; and snorkeling while engaged in low intensity finning. Compression and decompression were completed in accordance with US Navy Diving Table revision 7 at 23 msw·min^{-1} and 9 msw·min^{-1} respectively.

Table 1
Characteristics of participants

Subject	Age (years)	Weight (kg)	Height (cm)	Training (min·week^{-1})	Resting heart rate (beats·min^{-1})	Body fat (%)	Body mass index (kg·m^{-2})
1	37	90.3	185	200	45	16.4	26.1
2	43	75.1	180	100	60	15.3	23.2
3	35	85.3	185	500	65	12.5	24.9

To decrease inter- and intraindividual variance of respiration volumes, all nitrogen volume measurements were performed with the subject resting in a supine position. The volume in the system was measured at the end of each expiration as end-expiratory volume tends to be more reliable (less variance) relative to end-inspiratory volume.[13] The zero setpoint was defined as the median of the first ten extracted data points.

STATISTICAL ANALYSES

The statistical methods were devised through a collaborative effort involving two biostatisticians.

Experimental measurements for evaluation of volume detection

The device's ability to detect known injected gas volumes was analysed with a paired *t*-test. Measurements before were paired with measurements after gas injections of known volumes. The difference between the injected and the measured volumes indicates the accuracy of the device. The 95% confidence interval (CI) of the measured volume is an indication of the precision.

Human measurement for evaluation of potential drift and a quasi-experimental crossover study

End-expiratory plots with grouped linear regressions were used to visualise the control measurements in relation to the measurements of nitrogen wash-out after each intervention. We used time periods 0–15 minutes and 33–43 minutes, allowing us to observe both the difference in nitrogen wash-out flow rates (greater in the beginning) and total volume difference which was more distinct in the end of the wash-out period.

Inert gas volume drift during control measurements with human subjects was analysed with a multilevel linear regression model. The grouped linear regression line (with random individual intercepts) from the end expiratory plots were compared with the expected zero-line. The drift was expressed as the model's estimate which reflects the mean nitrogen volume difference from zero (ml), and the model's time factor which reflects the mean nitrogen flow rate difference from zero (ml·min^{-1}).

The device's ability to detect inert gas wash-out volume differences in a crossover study with three subjects was analysed with a multilevel linear regression model (random individual intercepts). The model's estimate for the period 33–43 min (when the wash-out curves flatten) was used to detect mean differences in total inert gas wash-out volumes. The model's time factor was used to analyse the flow rate differences (ml·min^{-1}), within a given period.

The secondary aim was analysed using Monte Carlo based simulations using the multilevel linear regression models and the data obtained from the dive profile of 18 msw / 50 min, performed immersed and dry, during the interval 33–43 minutes. The required sample size was estimated to detect a given inert gas volume difference with 80% power and a false positive rate (alpha) of 0.05, to inform the design of future experimental cross-over studies using the device.

Results

EXPERIMENTAL MEASUREMENTS FOR EVALUATION OF VOLUME DETECTION

The difference between the injected volume (1,996 ml) and the measured volume (1,986 ml) was -10 ml. The 95% CI for the measured volume was 1,969–2,003 ml. In total eight injections of 1,996 ml and 11–34 paired before-and-after measurements (a total of 172 paired volume registrations) were analysed.

Human measurement for evaluating potential drift and a quasi-experimental crossover study

During one of three control measurements a gas leak was discovered. This was assumed to be constant and was corrected for in the data processing. The three individual control measurements (0–43 min) for drift analyses showed a mean nitrogen volume difference of -19.24 ml (95% CI -37.15 to -1.49) and a flow rate difference of -0.23 ml·min^{-1} (95% CI -0.93 to 0.56) (Table 2).

The quasi-experimental crossover study could significantly detect mean nitrogen wash-out volume differences between the different interventions. The mean differences in inert gas wash-out (total volumes and flow rates) are shown in Table 3 and the end-expiratory plots with grouped linear regression lines during the wash-out periods 0–15 min

Table 2
Control measurements with three subjects for drift analyses with mean nitrogen (N_2) volume and flow rate differences in relation to the expected zero; CI – confidence interval

Time period	Mean N_2 volume difference ml (95% CI)	Mean N_2 volume flow rate difference ml·min^{-1} (95% CI)
Drift (33–43 min)	-73.91 (-105.46 to -39.11)	2.46 (-3.29 to 7.98)
Drift (0–43 min)	-19.24 (-37.15 to -1.49)	-0.23 (-0.93 to 0.56)
Drift (0–15 min)	-40.25 (-68.39 to -11.02)	0.68 (-2.11 to 3.92)

Table 3
Mean differences of inert gas wash-out total volumes and flow rates; CI – confidence interval

Intervention	Mean nitrogen total volume difference (33–43 min) ml (95% CI)	Mean nitrogen volume flow rate differences (0–15 min) ml·min^{-1} (95% CI)
Dry, 18 msw / 50 min vs controls	409 (356 to 458)	20 (15 to 26)
Dry, 39 msw / 10 min vs controls	542 (487 to 596)	32 (27 to 37)
Immersed, 18 msw / 50 min vs controls	660 (600 to 715)	32 (27 to 37)
Immersed, 39 msw / 10 min vs controls	719 (664 to 774)	28 (22 to 35)
Immersed 18 msw / 50 min vs dry 18 msw / 50 min	264 (215 to 309)	12 (7 to 18)
Immersed 39 msw / 10 min vs dry 39 msw / 10 min	104 (36 to 172)	-5 (-13 to 2)

Table 4
Power simulations regarding detection of mean inert-gas wash-out volume differences; *n* – participants required for 80% power (alpha = 0.05)

Effect size (ml)	*n*
400	2
300	2
200	2
100	2
50	4

and 33–43 min are shown for the different dive profiles in Figures 2 and 3. All measurements were recorded between 5–48 minutes (with 5 minutes after surfacing set as zero for all recordings) after surfacing for all interventions. One out of three recordings for the profile 39 msw / 10 min immersed, was only possible between 0–13 minutes because of battery problems.

The dives at 18 msw / 50 min immersed had an increased mean nitrogen wash-out volume of 264 ml (95% CI 215 to 309 ml) compared to the dry dives, and the mean flow rate during the first 15 min was 12 ml·min^{-1} (95% CI 7 to 18 ml) higher. The dives at 39 msw / 10 min immersed had an increased mean nitrogen wash-out volume of 104 ml (95% CI 36 to 172 ml) compared to the dry dives and the flow rate during the first 15 min was 5 ml·min^{-1} (95% CI -13 to 2) lower.

Our power simulation, based on this cross-over study design, found a sample size of two subjects would be able to detect a mean difference of total inert gas volume of 100 ml with 80% power and a false positive rate (alpha) of 0.05 (see Table 4).

Discussion

MAIN FINDINGS

We present a portable closed-circuit device for quantifying inert gas wash-out volumes following decompression. Performance without and with human subjects demonstrated acceptable precision and accuracy to measure relevant differences. With this device, a sample size of two is sufficient to detect a mean difference of 100 ml. The device is easy to transport and suitable for use in the field.

Figure 2

End-expiratory plots with grouped linear regression lines for the three subjects during nitrogen wash-out period 0–15 min and 33–43 min, for the dive profile 18 msw / 50 min and control measurements; a – control measurements versus measurements 0–15 min after a dry dive; b – controls measurements versus measurements 33–43 min after a dry dive; c – control measurements versus measurements 0–15 min after an immersed dive; d – control measurements versus measurements 33–43 min after an immersed dive; e – measurements 0–15 min after a dry dive versus measurements 0–15 min after an immersed dive; f – measurements 33–43 min after a dry dive versus measurements 33–43 min after an immersed dive

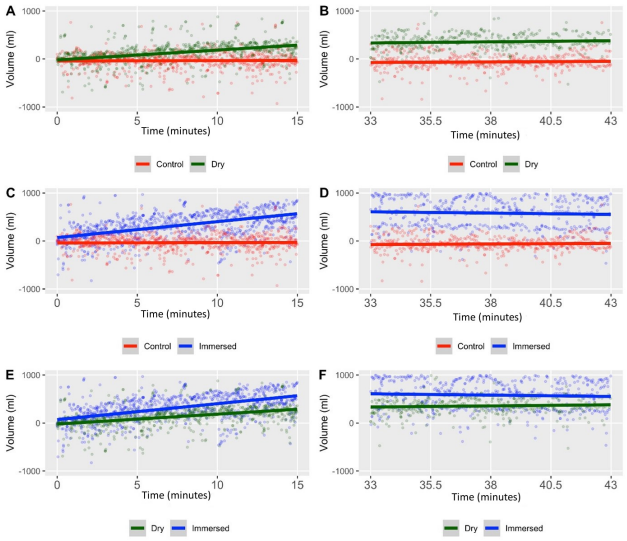
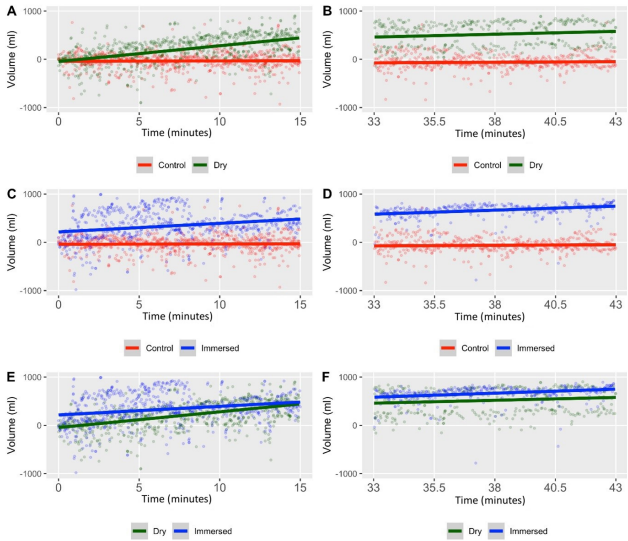


Figure 3

End-expiratory plots with grouped linear regression lines for the three subjects during nitrogen wash-out period 0–15 min and 33–43 min, for the dive profile 39 msw / 10 min and control measurements; a – control measurements versus measurements 0–15 min after a dry dive; b – control measurements versus measurements 33–43 min after a dry dive; c – control measurements versus measurements 0–15 min after an immersed dive; d – control measurements versus measurements 33–43 min after an immersed dive; e – measurements 0–15 min after a dry dive versus measurements 0–15 min after an immersed dive; f – measurements 33–43 min after a dry dive versus measurements 33–43 min after an immersed dive



The evaluation without human subjects demonstrated that the device detected 1,996 ml as 1,986 ml (95% CI 1,969–2,003). This accuracy (a discrepancy of only 10 ml) exceeds the requirements needed to detect significant differences in gas wash-out.

The quasi cross-over study revealed significant differences in nitrogen wash-out volumes between both control versus interventions and interventions versus interventions. The difference between dry dives and immersed dives after the profile 18 m / 50 min, was significant for both total wash-out volume and the wash-out flow rate for the first 15 minutes.

IMPLICATIONS

Our small quasi cross-over study, analysed with multilevel linear regression, suggests a sample size of two subjects could be sufficient to detect mean inert gas wash-out differences after immersed versus dry dives and between different dive profiles. However, these findings are related to the device's observational error and may not account for differences between our study subjects and the general population.

In future studies with larger sample sizes our device together with a multilevel linear regression model could probably detect effects related to divers' anthropometry.

Since earlier studies have shown significant changes in DCS risk after cold versus warm immersed decompression[8] and dry versus warm immersed pre-denitrogenation[7] we argue that inert gas volume differences in our quasi cross-over study may play a role in the pathogenesis of DCS. Equal volume differences have also been observed in cold versus warm exposures and negative pressure breathing during denitrogenation at 1 atm abs.[4,5]

STRENGTHS AND LIMITATIONS

A strength of the device is its ability to extract data points that correspond to end exhalation and to continuously calculate STPD volume changes of inert gas. This methodology generates a data point for every breath, a more frequent data collection interval than achieved in prior studies.[3,9,12] The intra- and inter-individual variance at the end of a normal passive exhalation (functional residual capacity) has been shown to be more stable compared to other spirometrically defined points.[13] All extracted data points were analysed with a multilevel linear regression model which minimises bias from breathing instructions or manual selection of data points.

One limitation of our evaluation on human subjects is a small sample size that is not representative of the general population. It is possible that other subjects have greater variance of end-expiratory plots, smaller nitrogen volume

differences or problems breathing through the mouthpiece. Another limitation is the manual adjustment that needed to be done to correct for a gas leak during one of the control measurements. This adjustment may not have affected precision, but likely had an effect on accuracy. A third limitation is the counterlung of the device, which had a tendency to catch when gliding in the suspension device. This may have had an effect on the device's ability to provide exact data points. A fourth limitation is that our statistical model needs to define appropriate time periods to analyse the differences in total nitrogen wash-out volumes and flow rates, respectively.

Conclusions

We present a portable device with acceptable precision and accuracy to measure inert gas wash-out differences that may be physiologically relevant in the pathophysiology of decompression sickness.

When using a cross-over study design, our power simulations estimated that a sample size of two subjects may be sufficient to detect physiologically relevant differences of inert gas wash-out volumes.

References

1 Brubakk AO, Neuman TS, editors. Bennett and Elliott's physiology and medicine of diving. 5th ed. Edinburgh: Saunders; 2003.
2 Balldin UI. Effects of ambient temperature and body position on tissue nitrogen elimination in man. Aerosp Med. 1973;44:365–70. PMID: 4694843.
3 Balldin UI, Lundgren CE. Effects of immersion with the head above water on tissue nitrogen elimination in man. Aerosp Med. 1972;43:1101–8. PMID: 5076612.
4 Pendergast DR, Senf CJ, Fletcher MC, Lundgren CE. Effects of ambient temperature on nitrogen uptake and elimination in humans. Undersea Hyperb Med. 2015;42:85–94. PMID: 26094308.
5 Lundgren CE, Eckhardt LG, Senf CJ, Bowdwin MR, Pendergast DR. Negative pressure breathing increases cardiac output and nitrogen elimination in seated subjects. Undersea Hyperb Med. 2013;40:403–10. PMID: 24224284.
6 Pendergast DR, Senf C, Lundgren CE. Is the rate of whole-body nitrogen elimination influenced by exercise? Undersea Hyperb Med. 2012;39:595–604. PMID: 22400450.
7 Balldin UI. The preventative effect of denitrogenation during warm water immersion on decompression and decompression sickness in man. First Annual Scientific Meeting of the European Undersea Biomedical Society. Försvarsmedicin, Stockholm, 1973:239–43.
8 Gerth WR, Ruterbusch VL, Long ET. The influence of thermal exposure on diver susceptibility to decompression sickness. NEDU Technical Report NEDU TR 06-07. Panama City (FL): Navy Experimental Diving Unit; 2007
9 Anderson D, Nagasawa G, Norfleet W, Olszowka A, Lundgren C. O_2 pressures between 0.12 and 2.5 atm abs, circulatory function, and N_2 elimination. Undersea Biomed Res. 1991;18:279–92. PMID: 1887516.
10 Dick AP, Vann RD, Mebane GY, Feezor MD. Decompression induced nitrogen elimination. Undersea Biomed Res. 1984;11:369–80. PMID: 6535313.
11 Sundblad P, Franberg O, Siebenmann C, Gennser M. Measuring uptake and elimination of nitrogen in humans at different ambient pressures. Aerosp Med Hum Perform. 2016;87:1045–50. doi: 10.3357/AMHP.4680.2016. PMID: 28323592.
12 Kindwall EP, Baz A, Lightfoot EN, Lanphier EH, Seireg A. Nitrogen elimination in man during decompression. Undersea Biomed Res. 1975;2:285–97. PMID: 1226586.
13 Johnson BD, Weisman IM, Zeballos RJ, Beck KC. Emerging concepts in the evaluation of ventilatory limitation during exercise: the exercise tidal flow-volume loop. Chest. 1999;116:488–503. doi: 10.1378/chest.116.2.488. PMID: 10453881.

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