Developing a Low Budget System to Mix, Store, and Deliver CO_2-rich Air for Human Research in the Small Liberal Arts College Setting

Justin R. Miller[1*], Denise R. Cook-Snyder[1,2*], Kirstyn Buchholz[1], Annika Evenhouse[1], Tim Nicosia[1], Amanda Grove[1,2], Theresa Regetz-Mularney[2], Emma Patschorke[2], Kelly Moench[2], Sophia Tajnai[1], Daniel P. Miller[2] , and Paul F. Martino[1]

[1]Department of Biology, [2]Department of Neuroscience, Carthage College, Kenosha, WI, USA
** These authors have contributed equally to this work.*

Abstract

Carbon dioxide (CO_2) levels are elevated on the International Space Station, and preliminary reports associate this increased CO_2 with headaches, changes in vision, and enhanced emotional experiences, including anxiety. As the duration of space travel extends, improved understanding of the physiologic effects of elevated CO_2 exposure is necessary to ensure the capabilities and safety of astronauts. However, studying human breathing has proven difficult at small liberal arts college due to cost and laboratory space constraints. Therefore, the goal of this project is to develop and validate a low budget system that can accurately and dependably mix, store, and deliver CO_2-rich air for human research in a liberal arts college setting. Our results provide user-friendly guidelines for building, validating, and maintaining such a system, and broad implementation of this system may increase the quantity and diversity of respiratory research relevant for space travel.

Introduction

Carbon dioxide (CO_2) is an important respiratory gas that proprtionally stimulates breathing in mammals as the percentage that is inhaled is increased. Humans have evolved with relatively constant and low atmospheric levels of CO_2 in the range of 0.04% of total atmospheric gases. It is well established that CO_2 levels are elevated well above 0.04% on the International Space Station due to difficulties in scrubbing the artificial atmosphere, and these levels have been highly associated with headaches (Law et al., 2014). Other physiological changes like impairments of memory, coordination, concentration, vision, and sleep, and enhanced emotional experiences have been reported, which could be the result of elevated CO_2 exposure (Stankovic et al., 2016; Watkins et al., 2017). These types of human physiologic homeostatic challenges are particularly concerning with extended space travel, including planned future travel to Mars. Indeed, long term emotional and cognitive performance, including coping with intense and prolonged stress, is an urgent concern for the success of such extended missions.

Our laboratory examines the relationship between the respiratory stress response to CO_2-rich air and vulnerability to stress (Bailey et al., 2005; Gladstone et al., 2005, Gorman et al., 2001). Clinical studies have demonstrated that a variety of factors make individuals more vulnerable to stress and anxiety disorders, including temperament characteristics and gender (Bailey et al., 2005). CO_2-rich air provides a reliable respiratory stressor (Forster et al., 1990; Gutting et al., 1991), thus allowing us to compare the stress response in vulnerable versus non-vulnerable individuals. Our institution is a small undergraduate liberal arts college, so our CO_2 system needs to be cost effective to build and maintain. Additionally, our system needs to be adaptable to classroom and research laboratory settings, including teaching undergraduates the fundamentals of respiratory physiology. Our main goal is to develop and validate a low budget system that can accurately and dependably mix, store, and deliver enhanced respiratory gases for human research in a liberal arts college setting. We believe the resulting system is readily transferable to other teaching and research institutions, offering easy-to-use equipment and methods that can ultimately increase the amount of respiratory research conducted relevant for space travel.

Materials and Methods

Gas Mixing and Containment: We acquired a Warren E. Collins, Inc. 13.5 liter respirometer/spirometer (catalogue #: P-1300) (Figure 1A and 1B). This respirometer/spirometer would need to be adapted to accurately and consistently mix respiratory gases and deliver those gases to college men and women. Both the inlet and outlet ports of the respirometer were modified by adding a one-inch PVC ball valve to allow for dependable opening and closing of the inlets. The valves were secured in place with plumber's putty. This putty is inexpensive, creates a tight seal, and is easily worked with and replaced.

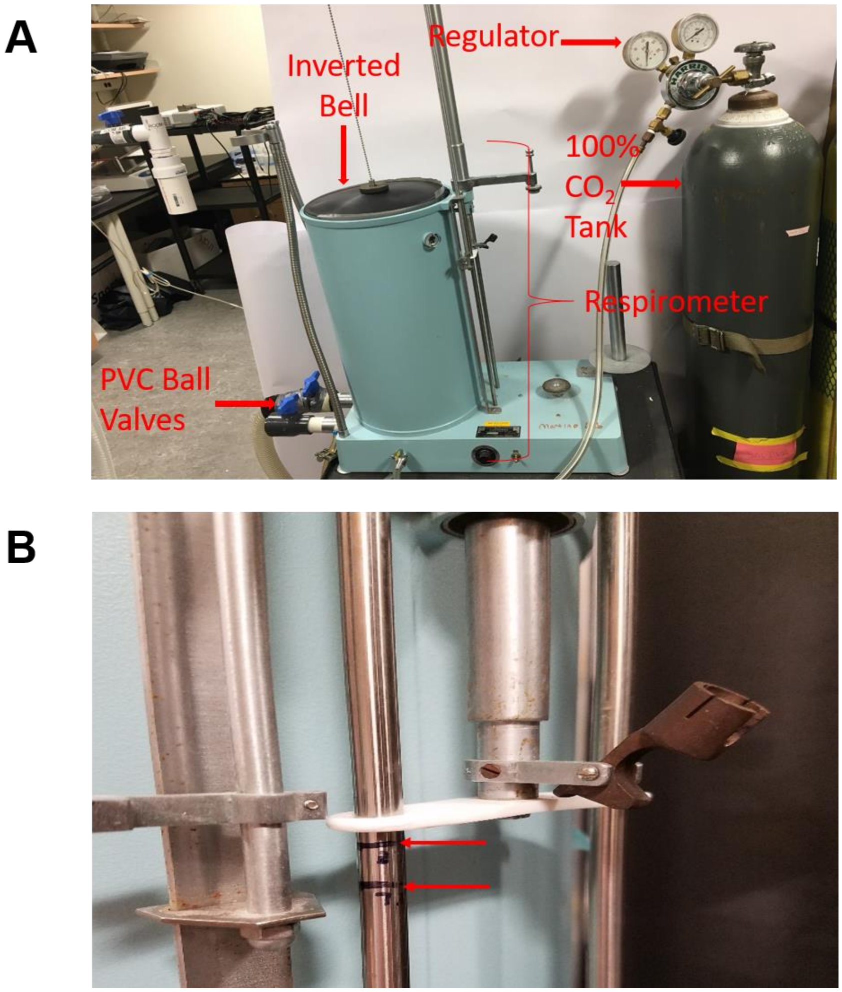

Figure 1: Modified Mixing Respirometer and a Medical Grade 100% CO_2 Tank. A. Customized 13.5 liter respirometer and a medical grade 100% CO_2 tank. Note in the lower left corner ball valves were added for reliable opening and closing of the inlet and outlet ports. The inverted bell and medical grade 100% CO_2 tank are labeled. B. Respirometer support rods with markings indicating the amount of medical grade 100% CO_2 gas to mix with room air to achieve 4.5% or 7% CO_2 gas (red arrows).

Additionally, we marked one of the two support rods on the respirometer to which the pulley system and the inverted bell are attached to accurately mix CO_2 and room air (Figure 1B). The inverted bell is the cylinder that sits in the middle of the respirometer and floats in the outer water chamber. When gas is added at a pressure of 20-25 psi (This delivery pressure is downregulated from the 100% CO_2 pressure of 900 psi, via a regulator.) to the inner chamber of the bell, the bell rises. The outer water chamber prevents the gas from escaping the inverted bell. Pictured in Fig. 1B are the marks that serve as an indicator of how much medical grade 100% CO_2 to add to the respirometer, before adding room air to complete the gas mixtures. To initially estimate the deflection of the inverted bell and therefore the sliding connector between the support rods and the bell, we used the simple ratio (Eq.1).

$$Eq. 1 \quad V_{Target}/V_{Total} = \%_{Target}/\%_{Total}$$

In terms of CO_2 we did not initially know the exact volumes of the 100% CO_2 gas to add to the respirometer, but we knew the total volume of the respirometer was 13.5 liters (Figure 1; Table 1, appended at the end of this manuscript). So armed with our equation (Eq. 1), we were able to very closely approximate how much CO_2 to add to the respirometer to achieve the gas mixtures of 4.5% or 7% CO_2 and 19.0% to 21% O_2, with the remaining balance being nitrogen, argon, and the other atmospheric gases. Once we mixed the gases using our calculated estimates, we were able to measure the true percentages of O_2 and CO_2 in the respirometer using the Oxigraf O_2CAP O_2 and CO_2 analyzer (Table 1), an O_2 and CO_2 measuring device. It is important to also check O_2 levels as very low levels of O_2 (10-17% O_2) will also elevate breathing and this would be a confounding effect.

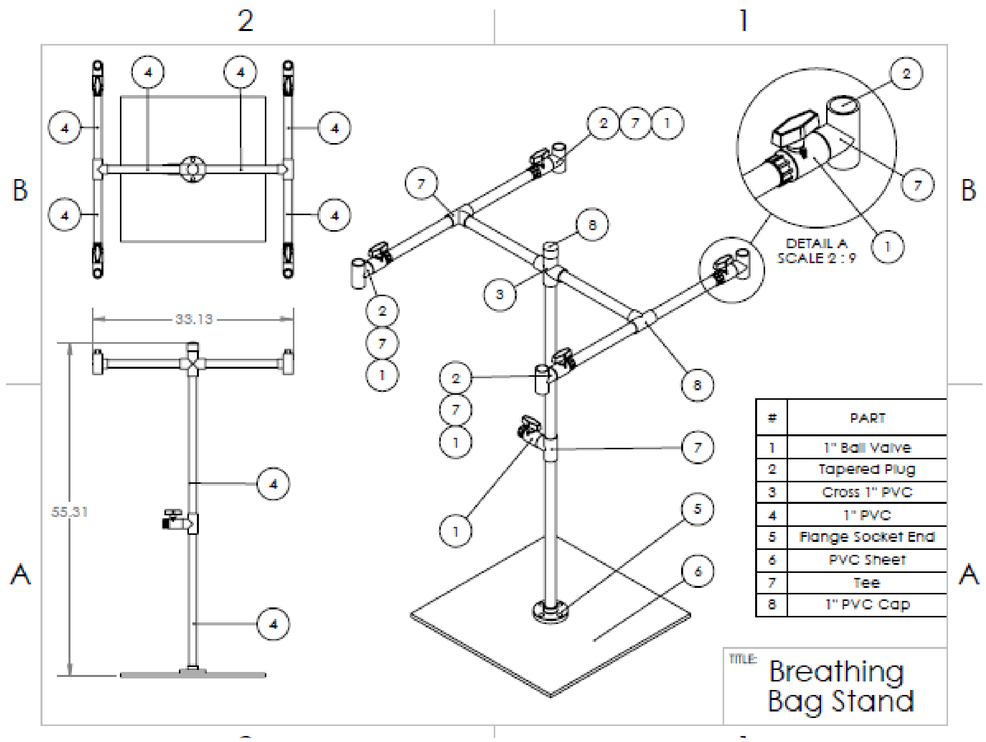

Figure 2: Breathing Bag Stand Schematic. This breathing bag stand is able to store 4 different levels of any atmospheric gas, including CO_2 when the appropriate respiratory balloons (not depicted) (Table 1) are attached.

Once the respirometer was completed and tested, the 4 bag breathing bag stand was built to store the mixed 4.5% or 7% CO_2 (Figure 2; Table 1). This system allows our laboratory to store the mixed gases for up to 1 hour without any change in the concentrations of gases that are stored in each bag. The bags are 300 gram weather balloons that have been used by several respiratory control labs. The stand is made of PVC pipes, PVC valves, stoppers, and bags. Note that the total estimated cost to build our system is $750.00, while a commercial system (WITT, MG 25 FLEX System, www.wittgas.com) cost including installation would be approximately $7,650.00

Gas Delivery and Monitoring: There are two well-established methods of transmitting elevated gases from tanks and hoses to study participants while simultaneously monitoring breathing. These methods are the older mouthpiece and nose clip (nose clip not shown) setup attached to a two-way non-rebreathing valve (Figure 3A) and the newer face mask system that covers both the mouth and the nose and is connected to a two-way non-rebreathing valve (Figure 3B). Note that these setups are mounted on a ring stand. The height of the mouthpiece and nose clip or the facemask can be adjusted to a height that is comfortable when the study participant is seated.

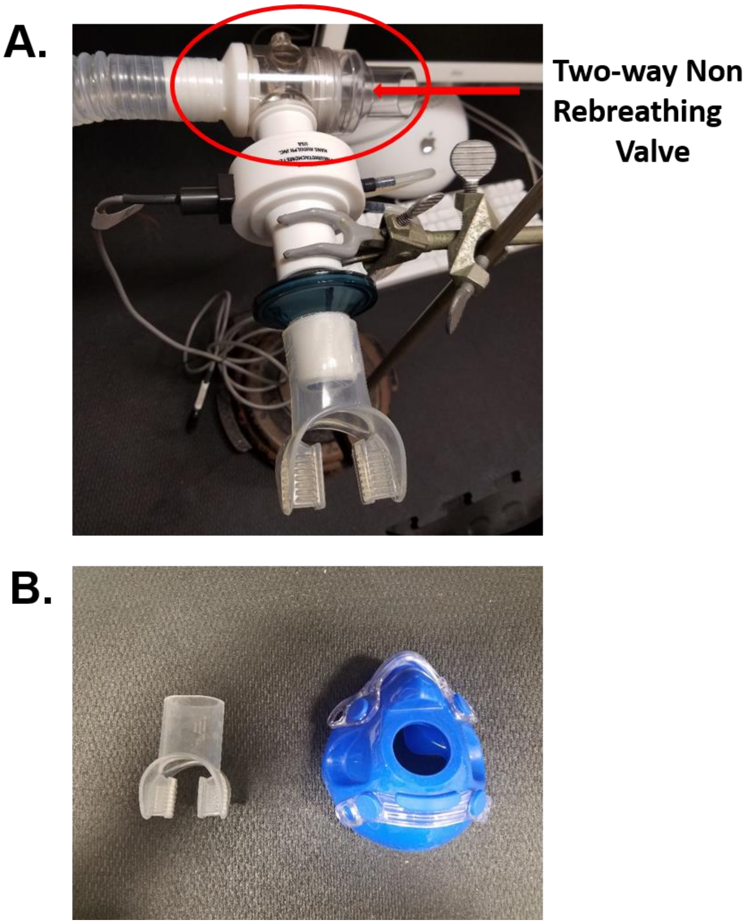

Figure 3: Two-way Non-Rebreathing Valve and Breathing Setups. A. Two-way non-rebreathing valve attached to a ring stand and equipped mouthpiece. B. Comparison of the face mask and mouthpiece. The valve was attached directly to the port in the facemask instead of being mounted on the ring stand.

Using these two setups, our next goal was to determine which method would work best to monitor breathing of human participants at rest and during enhanced CO_2. In order to address this goal, our lab performed several small pilot studies approved by the Carthage IRB Committee. In the first study, Carthage undergraduate students were recruited and randomly assigned to breathe through the mouthpiece and nose clip or the face mask (n = 4) to measure eupneic breathing. Each subject was instructed to breathe room air normally for three consecutive five minute trials for a total of fifteen minutes. The rationale for separating the consecutive control breathing periods into five minute bins was to determine the ideal control period to use before beginning the enhanced CO_2 challenge. Airflow (minute ventilation (V_E)) in liters per minute, breathing frequency (f_b) (breaths/minute), and tidal volume (V_T) (liters) were all measured with a Bio Pac MP36 airflow transducer. Each participant was tested twice once with the mouthpiece and nose clip, and once with the facemask, and each test was separated by a week. The order of testing was alternated for every other subject, as to avoid a learning effect. In a parallel study (Figure 4 and Figure 6), Carthage undergraduate students were recruited and randomly assigned to breathe using the mouthpiece and nose clip or using the facemask (n = 4) similar to the previously mentioned pilot study. Each participant was tested twice once with the mouthpiece and nose clip, and once with the facemask, and each test was separated by a week. The order of testing was alternated for every other subject, as to avoid a learning effect. As described previously, each subject was instructed to breathe room air normally for fifteen minutes, then in this pilot study participants were additionally exposed to four minutes of 4.5% or 7% CO_2 and then allowed to recover from CO_2 for four minutes. The study design is depicted in figure 4.

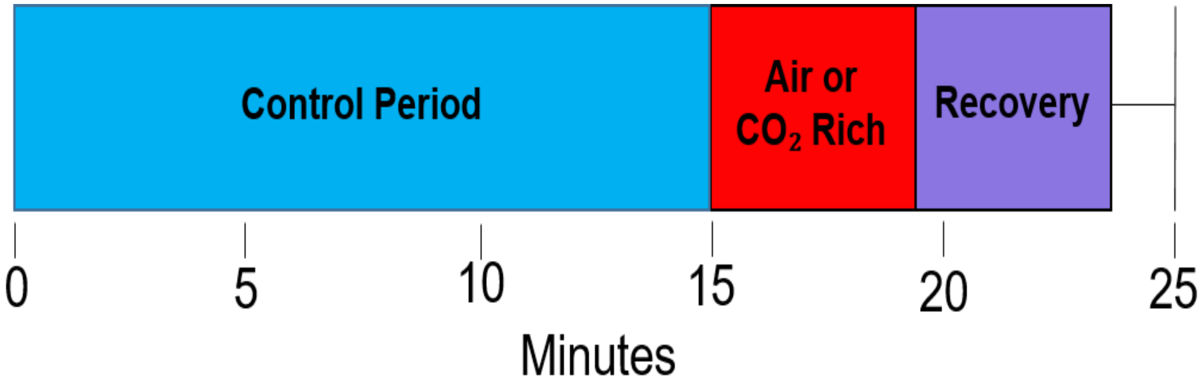

Figure 4: This is the experimental timeline for the second pilot study comparing breathing through the mouth piece and nose clip setup versus breathing through a mask both during control (room air inhalation) and during 4.5% or 7% CO_2 inhalation (n = 4). The control room air period is 15 minutes, followed by 4 minutes of breathing either 4.5% or 7% CO_2, followed by a 4 minute recovery period breathing room air.

Results

Validation of the Modified Gas Mixing Respirometer and Breathing Bag Stand

After 10 trials of gas mixing to achieve a mixture of 4.5% CO_2, we calculated means of 4.19 ± 0.26% CO_2 and 19.91% ± 0.17% O_2. After 10 trials of gas mixing to achieve a mixture of 7% CO_2, we calculated a mean of 7.17% ± 0.66% CO_2 and 19.14% ± 0.28% O_2. This validated that indeed our lab can accurately mix CO_2-rich air. We re-measured the gas percentages in the gas bags after 1 hour and each time the percentages were identical to when the bags were initially filled. At this point in the process we moved on to compare and evaluate the face mask versus the mouthpiece and nose clip for delivering this CO_2-rich air to college age adults.

Comparison of the Mouthpiece and Nose Clip Setup Versus the Facemask Setup During Eupneic Breathing

Additionally, we analyzed the effects of two different forms of airflow delivery systems (facemask versus mouthpiece and nose clip) on eupneic breathing over three consecutive five minute trials. Figure 5A-C depicts eupneic f_b, V_T, and V_E over the three 5 minute trials with the facemask. Breathing was analyzed during each 5 minute room air breathing trial and it was determined that during the last 5 minute eupneic breathing trial that our measured breathing variables were close to the established reference limits of f_b of 8-16 breaths/minute, V_T of 0.5-0.9 liters, and a V_E of 5-14 liters/min (Widmaier, et al., 2008). We measured a f_b of 16.10 ± 1.63 breaths per minute, a V_T of 0.55 ± 0.06 liters, and a V_E of 7.45 ± 0.70 liters per minute with the facemask. Similarly, Figure 5D-F depicts the breathing measurements using the mouthpiece and nose clip. These panels show a f_b of 14.95 ± 2.18, a V_T of 0.61 ± 0.09 liters, and a V_E of 8.34 ± 1.65 liters per minute with the mouthpiece and nose clip. Additionally, to further investigate the potential differences of subjects breathing through a mask versus breathing through a mouthpiece we performed an ANOVA statistical test. A 2x3 within subjects ANOVA comparing apparatus (mask vs. mouthpiece) across 15 minutes of room air exposure in blocks of 5 minutes was performed for f_b, V_T, and V_E. For f_b, we found a significant main effect of time, $F(2,6) = 20.474$, $p = 0.002$, indicating that f_b decreased significantly across the 15 minutes of mask and mouthpiece room air breathing. V_E showed a

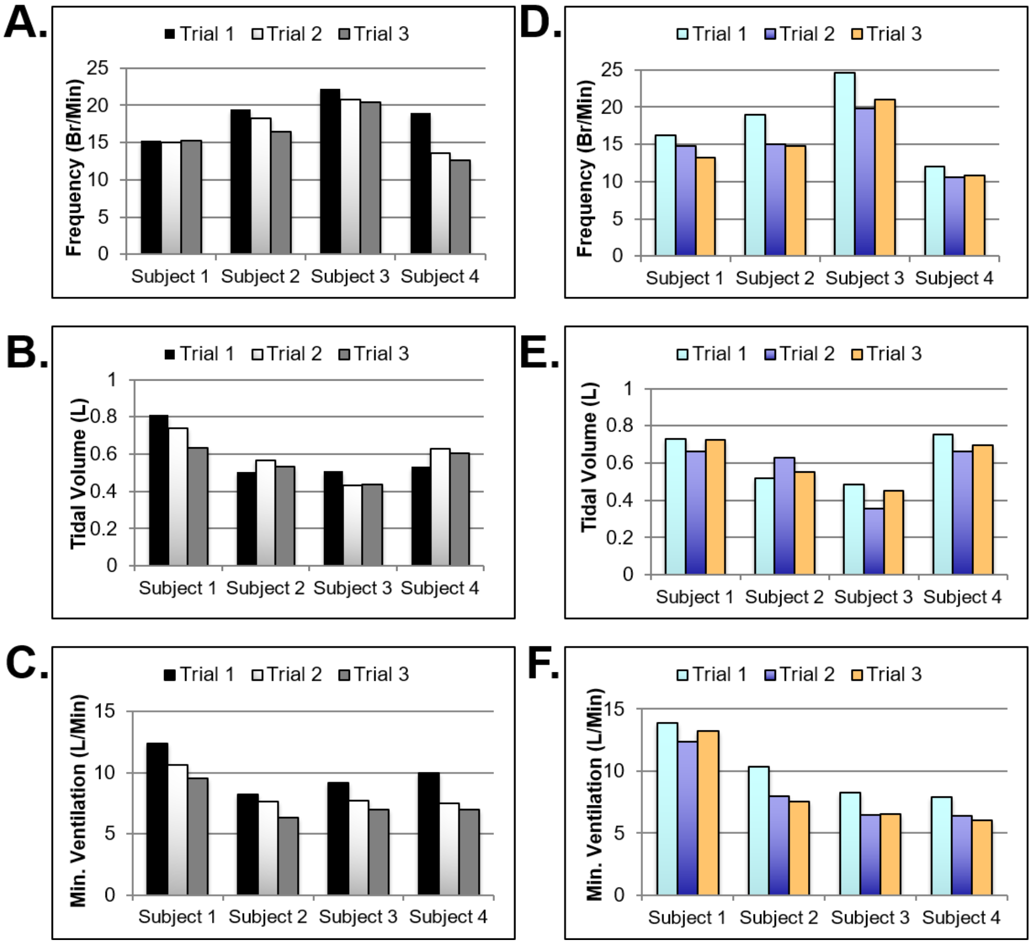

Figure 5: The respiratory output measured using the facemask setup or mouthpiece and nose clip setup is comparable after a 15 minute eupneic breathing acclimatization period. Each trial represents a consecutive five-minute period. In A, B, and C is shown breathing frequency (f_b) , tidal volume (V_T), and minute ventilation (V_E) , respectively, with subjects breathing through the face mask. In D, E, and F is shown breathing frequency, tidal volume, and minute ventilation, respectively, with subjects breathing through the mouthpiece.

similar significant reduction, F(2,6) = 148.792, p = 0.000008. For f_b and V_E there were no other significant main effects or interaction, indicating that these measures were reduced regardless of apparatus used for breathing. For V_T, there were no significant main effects of interactions. These data indicate that under room air eupneic breathing conditions, the face mask and the mouthpiece and nose clip setup allow similar breathing frequency, tidal volume, and minute ventilation recordings to be made, and there is a significant reduction in all 3 breathing parameters regardless of the breathing apparatus the participant is connected to, to measure breathing.

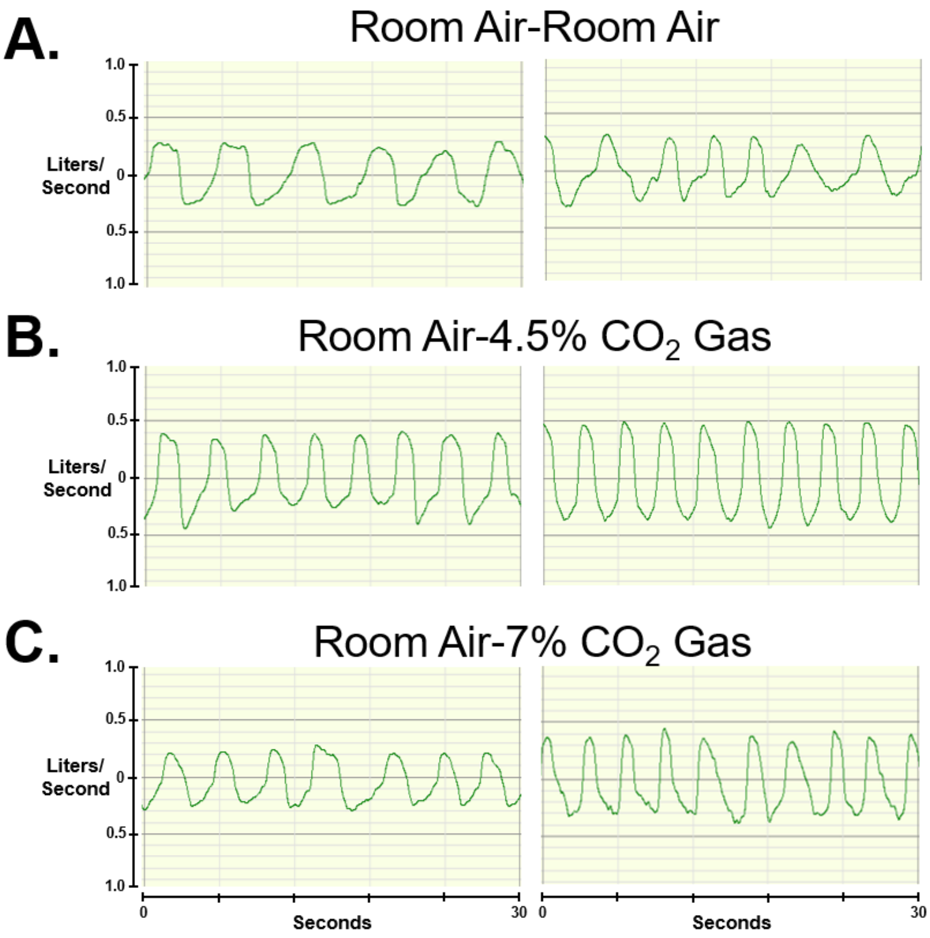

Figure 6: Representative Examples of Respiratory Flow During Room Air and CO_2 Gas Breathing Using the Mouthpiece and Nose Clip Setup. Upward deflection indicates expiratory flow and downward deflection indicates inspiratory flow. Panels on the left depict control period of room air breathing, and panels on the right depict breathing through the gas delivery apparatus A. Room air, B. 4.5% CO_2 gas, C. 7% CO_2 gas. The y-axes represent flow rates of liters/second while the x-axes represent 30 seconds in time. To estimate the breathing rate per minute, one would count the breaths depicted by the figure and multiply by 2.

Comparison of the Mouthpiece and Nose Clip Setup Versus the Facemask Setup for Delivering CO_2-rich Air

No participants were able to tolerate 4.5% CO_2 or 7% CO_2 for more than about approximately 1 minute when wearing the facemask, but in contrast, participants breathing through the mouthpiece and nose clip were able to tolerate those inspired levels of CO_2 for the full four minute protocol. Separately, we tested for the maximal duration of 4.5% or 7% CO_2 breathing in a subset of participants, who were able to tolerate 20-35 minutes of 4.5% CO_2 and 10-15 minutes of 7% CO_2 breathing when wearing the mouthpiece and nose clip. Figure 6 depicts breathing tracings from participants using the mouthpiece and nose clip to breathe room air, followed tracings of breathing 4.5% CO_2 or 7% CO_2. As previously stated, fifteen minutes was chosen as the length of the room air control period, because of previously mentioned data that suggests that it takes about that amount of time to have a person relaxed enough to measure true eupnea (quiet breathing at rest). In Figure 6A, the left panel depicts room air breathing straight from an open ended hose sitting in the laboratory without a gas bag during the control period (eupnea), while the right panel depicts the room air breathing after switching to a room air filled gas bag. Our results suggest no change in the f_b, V_T, and V_E from room air breathing with no gas bag 12.12 ± 1.25 to 12.78 ± 1.58 breaths per minute, 0.56 ± 0.12 to 0.61 ± 0.12 liters, and from 7.11 ± 1.54 to 7.80 ± 1.67 liters/min to breathing room air from a gas bag, respectively. This demonstrates that switching from room air with no gas bag to a room air filled gas bag does not affect the breathing rate or tidal volume. In Figure 6B, we see the difference of breathing during the control period to breathing 4.5% CO_2, where f_b increased from 14.15 ± 1.7 to 17.13 ± 1.31 breaths per minute, and V_T increased from 0.62 ± 0.08 to 0.84 ± 0.07 liters, and V_E from 8.77 ± 1.24 to 14.38 ± 2.42, respectively. Finally, in Figure 6C, there is a greater increase in breathing from room air control breathing to breathing 7% CO_2, where the f_b increased from 14.01 ± 1.0 to 21.54 ± 1.31 breaths per minute, the V_T increased from 0.59 ± 0.06 to 1.1 ± 0.15 liters, and V_E increased from 8.27 ± 1.89 to 23.69 ± 2.56 liters/min, respectively.

Discussion

Our main goal of this study was to develop and validate a low budget system that can accurately and dependably mix, store, and deliver CO_2-rich air for human research in a liberal arts college setting. Our process for this study was to 1) build a low cost CO_2 mixing and containment system, 2) validate that our system could accurately mix, deliver, and measure ventilation during different levels of CO_2-rich air breathing, and 3) compare mouthpiece and nose clip setup versus face mask airflow delivery system . We demonstrated that we could build a CO_2 mixing, storage, and delivery system. We also demonstrated that our CO_2-rich breathing increased breathing proportionately from 0.04% ambient (room air) CO_2 to 4.5% CO_2, and then again up to 7% CO_2. Similar proportional increases in breathing have been demonstrated by other laboratories (Forster et al., 1990; Gutting et al., 1991). Overall, these results serve as important validation that our system using the mouthpiece and nose clip setup is accurately delivering enhanced CO_2, and inducing respiratory stress in a predictable and proportional manner.

As part of developing this system, we needed to choose the facemask or the mouthpiece and nose clip setup to both deliver the mixed elevated CO_2 to the participants, and to also monitor the breathing of these participants. During room air eupneic breathing both the facemask setup and the mouthpiece and nose clip setup were equally accurate and within the expected physiological range. During experiments at 4.5% and 7% CO_2, we were not able to accurately measure breathing with the facemask, due to participants reporting discomfort. The longest we were able to record any breathing under elevated CO_2 conditions was approximately 1 minute. In addition, the breathing signals that we were able to record were erratic and could not be analyzed by the Bio Pac software. It is probable that during quiet resting breathing where the flow rate of air is low, the added constriction of the facial muscles by the mask is not problematic. During increased breathing with CO_2-rich air, however, the compression of the facial muscles and the mouth, by the facemask make it difficult to breathe and ultimately so uncomfortable that the participants could not tolerate the elevated breathing during CO_2 exposure. Alternatively, the increased stress of CO_2 may trigger an anxiety reaction, so the discomfort with the face mask is perceived and not due to the mask itself. Ultimately, it is unclear exactly why participants could not tolerate breathing enhanced CO_2 through a mask. This however is beyond the scope of this current study and requires future research to elucidate. The more traditional mouth piece and nose clip setup presented no problems with delivering or recording breathing during the inhalation of 4.5% and 7% CO_2. Therefore, the mouthpiece and nose clip is more effective for delivering and measuring ventilation while breathing CO2-rich air. Ultimately, these findings suggest that at a small liberal arts college with limited research funds and laboratory space, an affordable, handmade, accurate, and reproducible CO_2 mixing and delivery system can be built, validated, used, and maintained. It is conceivable that such a system could be duplicated at other primarily undergraduate institutions, and that undergraduates can use such a system in the classroom and the research laboratory. Also, it is possible that such a system could be used to test human and animal responses to not only multiple levels of CO_2-rich air, but also different levels of hypoxia (low oxygen) (12, 13, 14% O_2, etc.) to study the effects of humans at altitude. This system could be custom made to fit many sizes of experimental laboratory setups and even be made small enough to be portable. It is clear that a low budget, easy to build and maintain system such as the one described has great potential to be able to study the effects of extended exposure to CO_2 during spaceflight without the interference of the confound of less gravity. The ability to separate the effects of elevated ambient CO_2 might give us further insight into the true effects of gases versus gravity during extended space flight.

Acknowledgements

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Table 1: This is a table that includes all the parts, part numbers, manufacturers' and distributors' number of parts used to construct this CO_2 system.

Items	**Unit**	**Number of Units**	**Manufacturer's #/Model #**	**Manufacturer**
CO2 Mixing System				
Respirometer (Tissot Spirometer) 13.5 Liters	each	1		Warren E. Collins, Inc.
High Pressure PVC Clear Tubing, I.D. 3/4", O.D. 1-1/8"	each	1	52385k768	McMaster Carr
CO2 Delivery System				
Homemade PVC Breathing Bag Stand with 4 Bags	each	1		D.I.Y., Paul Martino, Justin Miller, and Emma Patschorke
Homemade Breathing Bag Stand with 4 Bags, Parts				
Low Pressure PVC and CPVC Ball Valves 1 inch	each	5	4876k23	McMaster Carr
Tapered Plugs Large End 11/4 inch Small End 1 inch	Package of 10	1	6448k104	McMaster Carr
Cross Dark Gray PVC Unthreaded 1 inch	each	1	4881k324	McMaster Carr
Thick Wall Dark Gray PVC Unthreaded OD 1 inch ID 1/2 inch	10 feet	1	48855k13	McMaster Carr
Flange Unthreaded Female Socket End	each	1	4881k214	McMaster Carr
Dark Gray Chemical Resistant PVC Sheet 1/2 inch x 24 inch x 24 inch	each	1	8747k151	McMaster Carr
Tees Female Unthreaded 1 inch	each	8	4880k43	McMaster Carr
Cap Female Socket End 1 inch	each	1	4881k53	McMaster Carr
Weather Balloon, 300 grams Natural	each	4	8237	Scientific Sales, Inc.
Face Systems', Parts				
Breathing Valve	each	1	112079	Hans Rudolph
Nose Clip	each	1	112571	Hans Rudolph
Medium Bite Mouth Piece	each	1	602073	Hans Rudolph
Large Bite Mouth Piece	each	1	602070	Hans Rudolph
7700 Series V2 Mask Oro-Nasal Bilevel with AAV and No Vents Medium	each	1	113501	Hans Rudolph
Other				
100% Medical Grade CO2	each	1		Praxair
CO2 Regulator	each	1		Praxair
Clean-Bor Tubing 35 mm O.D. length 108"	each	2	1011-108	Hans Rudolph
CO2 Analysis System				
Oxygen and CO2 Analyzer	each	1	O2CAP	Oxigraf
Respiratory Data Collection and Analysis System				
MP150 Systems for Windows	**each**	1	MP150WSW	BioPac Systems Inc.
Respiratory amplifier (RSP) C-series	**each**	1	RSP 100C	BioPac Systems Inc.
Respiratory Effort Xdcr, TP	**each**	1	TSD 201	BioPac Systems Inc.
Diff Amp Module C series	**each**	1	DA 100C	BioPac Systems Inc.
Heated Pneumotach Transducer	**each**	1	TSD 137H	BioPac Systems Inc.