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The ins and outs of breath holding: simple demonstrations of complex respiratory physiology

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Skow RJ, Day TA, Fuller JE, Bruce CD, Steinback CD. The ins and outs of breath holding: simple demonstrations of complex respiratory physiology. *Adv Physiol Educ* 39: 223–231, 2015; doi:10.1152/advan.00030.2015.—The physiology of breath holding is complex, and voluntary breath-hold duration is affected by many factors, including practice, psychology, respiratory chemoreflexes, and lung stretch. In this activity, we outline a number of simple laboratory activities or classroom demonstrations that illustrate the complexity of the integrative physiology behind breath-hold duration. These activities require minimal equipment and are easily adapted to small-group demonstrations or a larger-group inquiry format where students can design a protocol and collect and analyze data from their classmates. Specifically, breath-hold duration is measured during a number of maneuvers, including after end expiration, end inspiration, voluntary prior hyperventilation, and inspired hyperoxia. Further activities illustrate the potential contribution of chemoreflexes through rebreathing and repeated rebreathing after a maximum breath hold. The outcome measures resulting from each intervention are easily visualized and plotted and can comprise a comprehensive data set to illustrate and discuss complex and integrated cardiorespiratory physiology.

oxygen; carbon dioxide; chemoreflexes; Hering-Breuer reflex; feedback loops

RESPIRATORY PHYSIOLOGY is a complex topic, which comprises both voluntary and involuntary components as well as underlying reflexes. However, breath holding is a simple and intuitive activity that can be used to highlight differing aspects of respiratory physiology and can be adapted for diverse educational and outreach settings, such as lectures, tutorials, and laboratories. Here, we outline a number of simple breath-hold demonstrations and interventions that target specific elements of respiratory control to lengthen and shorten breath-hold duration. We also incorporate tools and data, which can facilitate skills such as protocol design and implementation, data collection, analysis, and interpretation in small-group settings.

Background

Concepts associated with the control of breathing can effectively be taught with simple laboratory demonstrations. Breath holding can be used in a fun, interactive, and thought-provoking way to demonstrate many of the physiological concepts and principles underlying the control of breathing. The individual factors contributing to volitional breath-hold duration are relatively well known. However, understanding how these mechanisms interact to determine the break point of a breath hold remains a challenging area of integrative physiology (3, 12, 25).

The break point of a maximal breath hold is determined by the complex interactions of multiple factors including *1*) chemical (i.e., chemostimuli), *2*) mechanical (i.e., lung stretch), *3*) descending cortical "drive," and *4*) cognitive factors (e.g., volition, practice, and expectation; see Fig. 1) (2, 12). To better understand the limiting factors that determine breath-hold duration, it is important to understand the physiology involved in normal respiration.

The basic breathing rhythm and pattern is set through a number of interacting nuclei within the brain stem and pons (dorsal, pontine, and ventral respiratory groups) (for a review, see Ref. 22). Respiration is then coordinated through the various inputs impinging upon the respiratory controller. These inputs include descending drive from the cortex (7), chemoreceptors (13, 21), and pulmonary stretch receptors (e.g., Hering-Breuer reflex (see Ref. 16)), to name a few (see Fig. 1).

Central (brain stem) and peripheral (carotid bodies) respiratory chemoreceptors contribute to the maintenance of relatively stable blood gases through distinct but interacting chemoreflexes. The central chemoreceptors detect accumulating brain tissue P_{CO_2}/H^+ concentration, increasing respiration as CO_2 levels in brain tissue rise above a threshold (i.e., central chemoreflex). The peripheral chemoreceptors detect increases in arterial P_{CO_2}/H^+ concentration (reductions in arterial pH) and decreases in arterial Pa_{O_2}, increasing breathing in response to either stimuli through an O_2-CO_2 stimulus interaction (i.e., peripheral chemoreflex) (5, 8, 9, 18, 20, 23).

Mechanical factors that contribute to breathing include slow adapting pulmonary stretch receptors that fire in response to stretch or inflation of the lungs. When activated, these receptors send inhibitory signals to the respiratory centers in the brain stem to decrease the drive to breathe and inhibit inspiratory drive (e.g., Hering-Breuer reflex). This allows for relaxation and recoil of lungs and chest wall to occur, initiating expiration, protecting against overstretch (14, 16). A number of descending drives originating from higher brain centers also affect breathing. First, there is a naturally higher drive to breathe during wakefulness that is a result of cortical input into the respiratory controller. The removal of this drive to breathe during sleep or anesthesia reduces breathing and leaves its regulation solely reliant on the "chemical pilot" (i.e., chemoreflexes) (7). In addition, volition allows for voluntary alteration of the breathing pattern in response to various stressors (e.g, exercise, Valsalva maneuver, and breath holding). With high extreme volition an individual can maintain a closed airway even as descending drive starts to contract the respiratory muscles (e.g., involuntary diaphragmatic contractions). These

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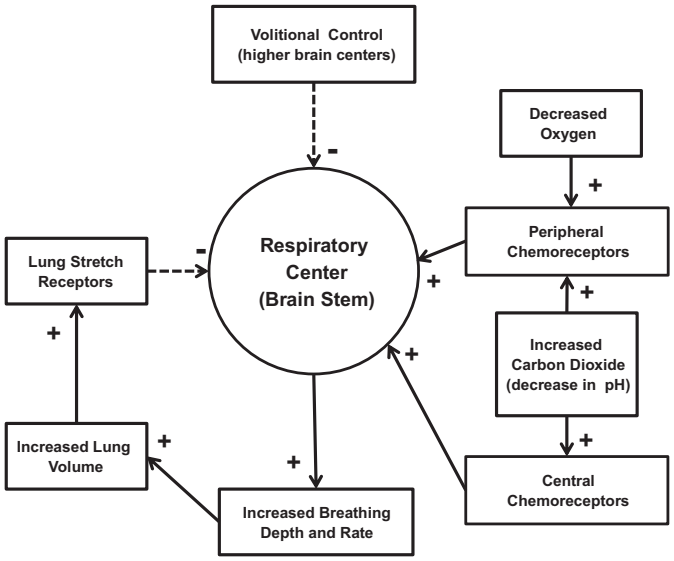

Fig. 1. Schematic of factors affecting the control of breath-hold duration. Many factors contribute to changes in ventilation, some of which are voluntary (descending control from higher brain centers) and some of which are involuntary (i.e., changes in O_2 and CO_2 or lung stretch). These factors act on the respiratory centers in the brain stem and can increase or decrease ventilation accordingly. Increases in lung stretch will decrease the drive to breathe, whereas chemoreceptor stimulation (decreased O_2 or increased CO_2) will increase the drive to breathe. It is important to note that this is a simplified schematic of respiratory control, and there are many other factors that can affect ventilation.

responses can also exhibit a learning effect. For example, in breath holding, cognitive and psychomotor tasks, such as mental arithmetic or squeezing a ball, can divert attention away from the desire to breathe that is experienced during breath holding and increase its time (1). Additionally, successive breath holding attempts produce improvements in breath-hold time, suggesting a practice effect and habituation to the sensation of dyspnea (1).

Maneuvers that affect any of these mechanisms outlined above may improve breath-hold duration. Trained divers or other breath-holding specialists may optimize all of these contributing factors to greatly prolong breath-hold time. In this article, we outline a set of simple demonstrations, designed with the flexibility to be adopted as a complete laboratory or series of lecture or tutorial-based demonstrations. Importantly, we aim to characterize factors contributing to breath-hold duration. In this series of activities, students will perform a series of maximal breath-holding experiments to tease apart individual factors contributing to breath-hold duration.

Learning Objectives

After the completion of this activity, students should be able to do the following:

1. Explain the individual physiological mechanisms (and their relative importance) involved in the control of breath holding.

2. Explain the integrative physiology that allows for humans to voluntarily perform extreme breath holding.

3. Develop a hypothesis regarding integrative respiratory physiology and design an experiment to test it using human participants.

4. Safely collect and analyze data and draw appropriate conclusions.

5. Using the data, discuss the possible contributions of various physiological feedback loops that affect breath-hold duration.

6. Critique experimental design to improve future explorations.

Activity Level

Based on the availability of supplies and the simplicity of the procedures outlined here, this activity would be suitable for use in a variety of high school or undergraduate course settings. These demonstrations can be adapted to laboratory sessions, in-class tutorials, or in-class lectures. These activities have been designed for courses addressing integrative physiology and may be suitable for use in senior high school through to upper-year undergraduate university curricula.

Prerequisite Student Knowledge

Before doing this activity, students should have a basic understanding of the following:

1. Pulmonary structure and function (inspiratory muscles, pressure gradients, and lung volumes) as well as factors involved in the chemoreceptor control of breathing.

2. Basic reflex physiology, including various types of receptors (e.g., chemoreceptors and stretch receptors) and effectors (e.g., respiratory muscles), and how physiological feedback mechanisms work to maintain homeostasis.

3. The effects of changes in blood gases (high or low CO_2 and O_2) on ventilation.

In addition, students should know how to do the following:

1. Follow basic laboratory instructions and work efficiently in a team.

2. Record data in a data table.

3. If collecting a larger data set, students should be able to calculate basic descriptive statistics (e.g., mean and SD/SE) and plot graphs (e.g., bar graphs).

Time Required

A breath hold performed by untrained individuals can range from 30 s to 2 min. If students are performing these activities as part of a complete laboratory, our experience suggests that at least 2 h is required for the completion, recording of data, and discussion of experimental results. However, adoption of individual demonstrations included here for use in a classroom or tutorial session may be easily accommodated. A laboratory report or manuscript could also be assigned to develop literature review and data analysis skills.

METHODS

Equipment and Supplies

The following basic equipment is required per group for this activity (suggested 4–6 students/group):

One chair

One stopwatch

Nose clips (participant can hold their own nose if nose clips are unavailable)

One plastic bag (5–8 liters, easily obtained at a grocery store)

One record sheet or laboratory notebook (see Table 1 for an example)

Table 1. *Data collection table for breath-hold activities*

Part A: *demonstration of the chemoreflex control of breathing*

Activity 1: rebreathing from a closed circuit
Description: breathing in and out of a bag until the limit of tolerance is reached by participant or depth of breathing increases to the point where the bag is collapsed during inspiration. O_2 is decreasing and CO_2 is increasing with every breath.
Observations:

Part B: establishing a baseline breath-hold duration

Activity 2: end-inspiratory breath hold
Description: taking a full breath in and then performing a breath hold for as long as possible. Lung volume is maximized, and the air in the lungs is room air.
Hypothesis:
Breath-hold time:
Other observations:

Part C: *chemoreflex interventions*

Activity 3: end-inspiratory breath hold with prior hyperventilation
Description: hyperventilating for 30 s before an end-inspiratory breath hold. CO_2 levels in the blood are decreased.
Hypothesis:
Breath-hold time:
Other observations:
Activity 4: end-inspiratory breath hold with prior hyperoxia
Description: taking five breaths of 100% O_2 before an end-inspiratory breath hold. O_2 levels in the blood are increased.
Hypothesis:
Breath-hold time:
Other observations:
Activity 5: rebreathing followed by a breath hold
Description: rebreathing from a closed circuit for 1 min before completing an end-inspiratory breath hold. O_2 is decreased, and CO_2 is increased.
Hypothesis:
Breath-hold time:
Other observations:

Part D: assessing the role of lung stretch

Activity 6: end-expiratory breath hold
Description: taking a normal breath out and then completing a breath hold for as long as possible. Lung volume is minimized, and the air in the lungs is room air.
Hypothesis:
Breath hold time:
Other observations:
Activity 7: repeated rebreathing after a breath hold
Description: completing an end-inspiratory breath hold followed by two breaths from a closed circuit (bag) and then attempting to complete a second breath hold. This is repeated for three breath holds, if possible. Holding your breath increases CO_2 and decreases O_2; lung stretch receptors are activated while rebreathing.
Hypothesis:
Breath-hold time:
Other observations:

If available, the following optional equipment can be used to further document physiological responses:

Supplemental 100% O_2

One heart rate monitor

One finger pulse oximeter [oxyhemoglobin saturation (Sp_{O_2}), e.g., Nonin Onyx; can also be used to determine heart rate]

A capnograph (end-tidal CO_2 sensor, e.g., Masimo EMMA)

Ethical Approval for Working With Human Participants

Adopters of this activity are responsible for obtaining informed consent and/or ethics clearance to work with human participants at their home institution. In Canada, research activities must be cleared by a local Research Ethics Board and conform to the Tri-Council Policy Statement on research ethics (TCPS2), which is consistent with the Declaration of Helsinki. For a summary of the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings," please see www.the-aps.org/mm/Publications/Ethical-Policies/Animal-and-Human-Research. If this activity is used as a demonstration only, informed consent must still be obtained from participants. The sample traces included in this article (Figs. 2 and 3) were obtained in the laboratory of C. D. Steinback (Ethics Protocol ID 00048741) after written informed consent by participants.

Instructions

In groups of four to six, have one student participant sit comfortably in a chair and ensure that they are comfortable with holding their breath. Have a second student lead the demonstration by giving instructions and observing the participant. A third student can act as the timer, recording the duration of each breath hold to the nearest second. Additional students can record and/or use the additional optional equipment listed above, if available. Students can rotate through responsibilities as well as acting as a participant to obtain a complete data set. Students can work through each of the seven activities described below.

Part A: Demonstration of Chemoreflex Control of Breathing

Activity 1: rebreathing from a closed circuit. Rebreathing from a closed system will cause an increase in arterial CO_2 and decrease in arterial O_2 as a function of metabolic rate. In this observational activity, students will visualize the progressive increase in the rate and depth of breathing as the participant rebreathes from a bag. This is an important theoretical concept when discussing the chemical drive to breathe, which increases progressively throughout a breath hold. Students should be able to use this demonstration to develop a hypothesis regarding the outcomes (with respect to breath-hold duration) in subsequent activities. Figure 2 shows representative physio-

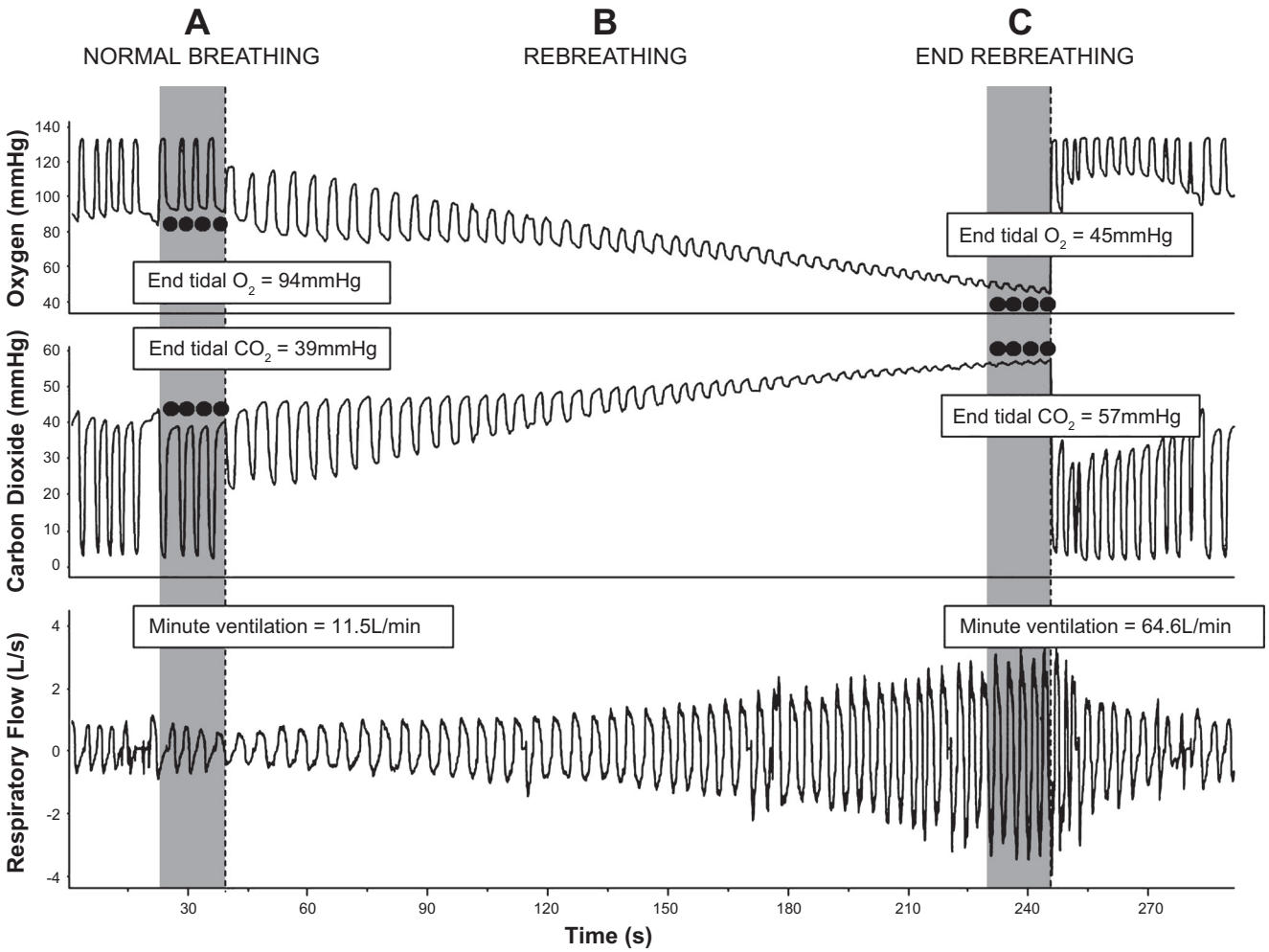

Fig. 2. An example of the changes in expired O_2, CO_2, and ventilation during rebreathing. During normal breathing (*A*; shaded region), end-tidal O_2 was 94 mmHg, end-tidal CO_2 was 39 mmHg, and minute ventilation was 11.5 l/min. The participant subsequently breathed in and out of a grocery produce bag (*B*) to gradually increase arterial CO_2 and decrease arterial O_2, triggering increases in ventilatory rate and depth (demonstrated in the respiratory flow channel). In this example, ~3.5 min of rebreathing decreased end-tidal O_2 to 45 mmHg, increased end-tidal CO_2 to 57 mHg, and increased minute ventilation to 64.6 l/min (*C*; shaded region). This protocol demonstrates the powerful increase in the drive to breathe during reduced O_2 or increased CO_2.

logical data from a typical rebreathe. In addition, students may be directed to primary literature discussing this topic in more detail (4, 6, 17, 26).

Directions. I: FILL THE BAG. With a nose clip in place, have the participant take a large breath of room air and then exhale into a previously empty plastic bag, closing the bag so that it stays full.

II: REBREATHE. Once the bag is full of expired air, have the participant resume normal breathing in and out of the closed bag. Have the time recorder start the stopwatch when the participant begins to rebreathe. The participant should continue to rebreathe until their depth of breathing causes the bag to collapse or until the participant reaches their limit of tolerance. The observer should terminate the test if the participant exhibits any signs or symptoms of discomfort or dizziness. In our experience, rebreathing should be limited to no longer than 2 min. Rebreathing for longer than 2 min may cause dizziness, with longer periods of rebreathing carrying an increased risk of syncope. If you are able to measure CO_2 using a capnograph, ensure to not exceed 50 mmHg of end-tidal CO_2. The duration of rebreathing should be recorded in the data collection sheet, along with any observations of changes in rate and depth of breathing. After the test, the participant should describe the sensation during rebreathing to the observers.

Part B: Establishing a Baseline Breath-Hold Duration (Control)

Activity 2: end-inspiratory breath hold. This baseline breath-holding activity establishes the typical breath-hold duration as a control and familiarizes the participant to the discomfort of breath holding and the type of protocol they will undergo. The investigators will need a stopwatch (and a finger pulse oximeter to track heart rate and Sp_{O_2}, if available).

Directions. I: BASELINE VALUES. Start by having the participant sitting comfortably, and have a timer and reader nearby. Record the resting heart rate and O_2 saturation, and if you have a capnograph and oximeter, also record the resting CO_2 and Sp_{O_2}.

II: PERFORM A BREATH HOLD FROM A MAXIMAL END INSPIRATION. Instruct the participant to take a full breath in and hold as long as they can. The time recorder should use a stopwatch to record the breath-hold duration in a data table (see Table 1 for an example). If you have an oximeter, have an observer call out and record the heart rate and O_2 saturation every 15 s for recording. If you have a capnograph, you can measure the CO_2 before starting and of the first expired breath after the break point, thus giving an indication of arterial CO_2 accumulation during the breath hold.

With all breath holds, the participant should be instructed not to "bear down" (i.e., no Valsalva maneuver) during the breath hold, and

the observer should watch for any signs of dizziness and note the time of onset of any involuntary diaphragmatic contractions should they occur.

Part C: Chemoreflex Interventions

Activity 3: end-inspiratory breath hold with prior hyperventilation. Hyperventilation (defined as an increase in alveolar ventilation in excess of metabolic demands) reduces the P_{CO_2} in the blood. Through the removal of CO_2, this activity is designed to identify the role of CO_2 in determining breath-hold time. Based on the results of *part A*, *activity 1*, of this laboratory, students should be able to hypothesize what effect prior hyperventilation would have on breath-hold duration. Students can use a capnograph to measure the end-tidal CO_2 before hyperventilating, after hyperventilation, and at the end of breath holding (i.e., break point).

Directions. I: HYPERVENTILATION. Instruct the participant to breathe deep and fast for 30 s. Extreme and prolonged hyperventilation can cause dizziness and lightheadedness, so the observer should be monitoring the participants for any signs of dizziness. Monitor end-tidal CO_2 if possible, and limit the hypocapnia to 25 Torr CO_2 to minimize the chances of dizziness and discomfort.

II: PERFORM A BREATH HOLD FROM A MAXIMAL END INSPIRATION. After 30 s of hyperventilation, have the participant perform a maximal inspiration (similar to *part B*, *activity 2*) and then instruct them to perform a maximal breath hold. If a pulse oximeter is available, the participant should be stopped from holding their breath if Sp_{O_2} reaches 85%. If a pulse oximeter is not available, the breath hold should be stopped at 3 min.

Activity 4: end-inspiratory breath hold with prior hyperoxia. In much the same way that hyperventilation removes CO_2 as a chemostimuli and increases the duration of a breath hold, breathing 100% O_2 (hyperoxia) increases the capacity for breath-hold duration by prolonging the time before peripheral chemoreceptors are stimulated by hypoxia/hypercapnia during breath hold. If available, supplemental O_2 can be used to perform this demonstration. Based on the results of *part A*, *activity 1*, of this laboratory, students should be able to hypothesize what effect breathing 100% O_2 would have on breath-hold duration. Students can use a pulse oximeter if they wish to have a measure of starting Sp_{O_2} (in %) before and during breath holding.

Directions. I: PREBREATHE 100% O_2. Fill a bag with 100% O_2 and instruct the participant to breathe 100% O_2 from a bag for five normal breaths. After the fifth breath, instruct the participant to start a breath hold after a full inspiration, as described above.

II: PERFORM A BREATH-HOLD FROM A MAXIMAL END INSPIRATION. Have the participant perform a maximal inspiration (similar to *part B*, *activity 2*).

Activity 5: breath hold after rebreathing. Rebreathing from a closed circuit (e.g., *part A*, *activity 1*) does not allow metabolically derived CO_2 to be cleared from the blood or for atmospheric O_2 to enter the blood. Thus, the accumulation of CO_2 stimulates central and peripheral chemoreceptors and the reduction in O_2 also stimulates peripheral chemoreceptors. In the opposite way that *activities 3* and *4* prolong breath-hold duration, performing a breath hold after 60 s of rebreathing will demonstrate the role that chemoreceptor activation plays in reducing breath-hold duration.

Directions. I: REBREATHE. Instruct the participant to rebreathe from a closed circuit in a similar fashion to *part A*, *activity 1*, for 60 s or until chemoreflex activation is apparent through increased in rate and depth of breathing.

II: PERFORM A BREATH-HOLD FROM A MAXIMAL END-INSPIRATION. After 60 s of rebreathing, have the participant perform a maximal inspiration breath hold for as long as possible (similar to *part B*, *activity 2*).

Part D: Assessing the Role of Lung Stretch

Activity 6: end-expiratory breath hold. Maximal inspiration activates slow-adapting pulmonary stretch receptors, preventing overinflation by initiating expiration and/or reducing the drive to breathe. Expiration reduces lung stretch and the activity of these receptors. This activity is designed to illustrate the role of lung stretch in regulating breath-hold duration.

Directions. I: PERFORM A BREATH HOLD FROM A NORMAL END EXPIRATION. At the end of a normal expiration, have the participant perform a maximal breath hold. The recorder should time the breath hold and record the duration of the breath hold on the data collection sheet for comparison with other breath-hold durations.

Activity 7: repeated rebreathing after breath holding. After *activities 1–6* of this laboratory, students should have an understanding of individual mechanisms that may influence breath-holding duration (e.g., levels of O_2 and CO_2; lung stretch). Before this activity, students should be encouraged to hypothesize which contributing factor, chemical drive or lung stretch inhibition, is more potent with respect to influencing breath-hold duration. As classically described by Fowler (11), by interspersing successive breath holds with periods of rebreathing, this activity is designed to demonstrate the powerful influence of lung stretch during the act of breathing on breath-holding duration, even in the face of increases in blood gas chemostimuli.

Directions. I: INITIAL BREATH HOLD AFTER MAXIMAL END INSPIRATION. Instruct the participant to take a full breath in and hold it as long as they can.

II: REBREATHING. Once the participant can no longer hold their breath, have them breathe out into a previously empty bag, rebreathing for two breaths. They should then be encouraged to inspire the full volume of gas from the same bag and attempt to hold their breath again. Repeat this process of intermittent breath hold and rebreathing until the participant can no longer hold their breath or until they have held their breath three times (for a representative tracing, see Fig. 3). If the participant is feeling dizzy or experiencing any sign of dizziness as noted by the observer, this protocol should be stopped immediately.

Troubleshooting

There is limited equipment required for this simple laboratory demonstration. Short of making sure that the disposable bags have no leaks, and that the participant makes a complete seal on the bag, very little technical difficulty is expected. Make sure the stopwatch, pulse oximeter, and capnograph have new batteries. In some cases, participants may require a number of practice trials to follow directions accurately. It is often helpful to give the participants 5 min between trials to recover before beginning another breath hold.

Safety Considerations

Students with any of the following conditions/states should not serve as the participants: known cardiovascular disease (e.g., diagnosed hypertension or cardiac arrhythmias), known respiratory disease (e.g., asthma), or if they are a regular smoker.

Breath holding for prolonged time can cause dizziness, lightheadedness, and possible fainting. If at any time during any breath hold the participant feels dizzy, they should begin breathing again immediately. One person should be observing the participant during every breath hold and watching for signs of dizziness. The signs of dizziness (and fainting) include face flushing, sweating, shaking, or loss of balance. If any of these signs are observed, the participant should be instructed to breathe again. The participant should perform all breath holds in the seated position, and the observer should also be standing close enough to support them should they need it. Make sure the participant is always observed, and communicate with the participant during and after each exercise.

Of particular note, activities that include rebreathing or hyperventilation are more likely to result in lightheadedness or dizziness, and

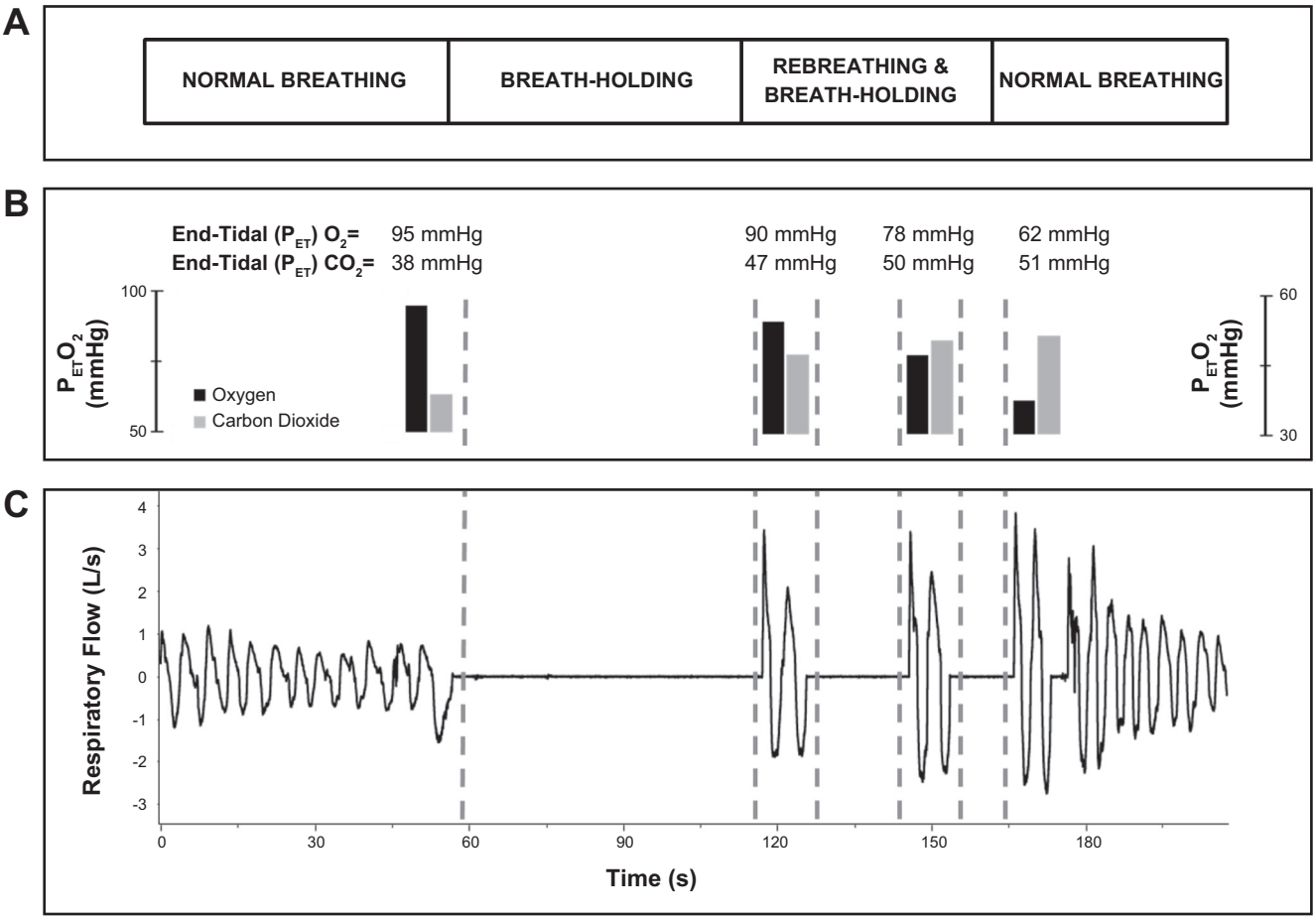

Fig. 3. Sample repeated breath hold and rebreathing tracing. *A*: repeated rebreathe protocol. After a period normal breathing, the participant holds their breath. At the end of the first breath hold, the participant breathes in and out of a bag twice and holds their breath again. This procedure is repeated twice. *B*: changes in the pressure of end-tidal O_2 ($P_{ET_{O_2}}$; solid bars) and end-tidal CO_2 ($P_{ET_{CO_2}}$; shaded bars). During normal breathing, end-tidal O_2 is 95 mmHg and end-tidal CO_2 is 38 mmHg. The end-tidal gases immediately after the first breath hold are 90 and 47 mmHg of O_2 and CO_2, respectively; 78 and 50 mmHg of O_2 and CO_2, respectively, after the second breath hold; and 62 and 51 mmHg of O_2 and CO_2, respectively, after the third breath hold. *C*: respiratory flow tracing (from spirometry) demonstrating the breathing pattern during the repeated rebreathing protocol. This protocol demonstrates the importance of lung stretch in depressing the drive to breathe, evident by the ability to hold one's breath despite decreasing O_2 and increasing CO_2 levels during the breath-holds.

extra precautions should be taken to eliminate the risks of fainting. The hyperventilation duration should not exceed 60 s before the breath-hold test in *part C*, *activity 3*. In addition, if you are able to measure CO_2 (e.g., an EMMA capnograph or AD Instruments gas analyzer), limit the level of hypocapnia during the hyperventilation period to a minimum of 25 Torr end-tidal P_{CO_2}. Hyperventilation-induced hypocapnia causes cerebral vasoconstriction, causing some individuals to feel transient dizziness. Interestingly, the fastest way to increase CO_2 and ameliorate these symptoms is to perform a breath hold to retain metabolically derived CO_2.

RESULTS AND DISCUSSION

Expected Results

Students should collect their own data (recorded in Table 1). After a review of basic underlying physiology and factors that affect breath-hold duration (e.g., Fig. 1), students should be able to hypothesize how breath-hold duration will be affected during each activity. Expected results from each activity are shown in Figs. 2–4. Specifically, expected breath-hold durations for *activities 2–6* are shown in Fig. 4 (mean data collected from 20 participants). Expected results are briefly outlined below.

Activity 1: rebreathing from a closed circuit (2 min). Given the increase in metabolically derived CO_2 and a reduction in arterial O_2, experimenters will observe an increase in the rate and depth of breathing. See Fig. 2 for a representative tracing. This demonstration illustrates the chemoreflex control of breathing.

Activity 2: end-inspiratory breath hold. This is the "control" demonstration that other breath-hold durations can be compared with. This activity will result in an intermediate breath-hold duration.

Activity 3: end-inspiratory breath hold with prior hyperventilation. Given the reduction in arterial CO_2 that results from prior hyperventilation, breath-hold duration should be longer than the control breath hold, as it will take longer for the threshold for chemoreceptor activation to be reached.

Activity 4: end-inspiratory breath hold with prior hyperoxia. Similar to *activity 3* above, a breath hold after prior hyperoxia will result in a longer breath-hold duration than the control.

Activity 5: breath hold after rebreathing. Due to the accumulation of chemostimuli (i.e., high CO_2 and low O_2), per-

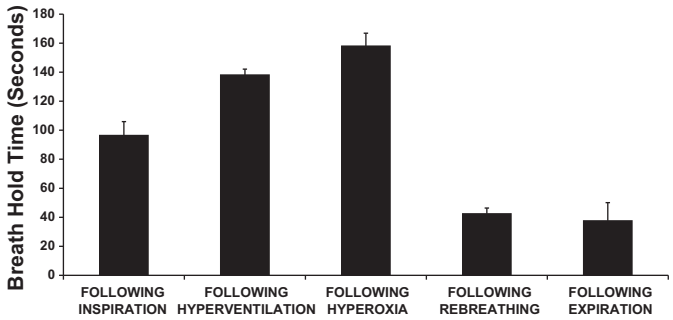

Fig. 4. Data summary of sample breath-hold duration data. Representative data for mean breath-hold times (n = 20; error bars represent SE) were collected in the laboratory of C. D. Steinback. Using the breath hold after the inspiration time (96 s) as the control, hyperventilation increased breath-hold duration to 138 s by lowering the arterial P_{CO_2} before the start of the breath hold. Hyperoxia increased breath-hold time to 157 s by increasing the arterial P_{O_2} before the start of the breath hold. Conversely, rebreathing for 1 min before the start of a breath hold decreased the time from 96 to 42 s by increasing the P_{CO_2} and decreasing the P_{O_2} before the start of the breath hold. Each of these demonstrates the role of chemoreceptor pathways in the control of breathing. The role of the lung stretch receptors is shown by comparing the breath hold after inspiration (control; 96 s) to the breath hold after expiration (37 s).

forming a breath hold after 60 s of rebreathing will result in a shorter duration than the control.

Activity 6: end-expiratory breath hold. Due to both the lack of inhibitory lung stretch and a smaller lung volume reservoir to mix atmospheric air with arterial blood, this breath-hold duration will likely be the shortest compared with the control.

Activity 7: repeated rebreathing after breath holding. Despite the fact that chemostimuli are not being relieved after the first breath hold, the act of breathing itself relieves the sensation of dyspnea, allowing the participant to perform another breath hold. Each successive breath hold will become shorter in duration as chemostimuli continue to accumulate.

Discussion/Misconceptions

There are a number of misconceptions that can be addressed with these demonstrations. First, it is common for people to assume that breath-hold duration is dictated primarily by lowered O_2 levels. That is to say, that O_2 is reduced during a breath hold, and the chemoreceptor drive to breath is responsible for the sensation of dyspnea and contributes to break point. If you were to observe Sp_{O_2} using a pulse oximeter in *part B*, *activity 2*, you would observe that although P_{O_2} is likely reduced, Sp_{O_2} is maintained at high levels, even after a few minutes of breath holding. Indeed, to activate the peripheral chemoreceptors in the absence of any changes in CO_2, one would need to reduce arterial O_2 to ~60 mmHg. If you have a capnograph available, measuring the CO_2 of the first breath after break point after the breath hold would have demonstrated higher levels of CO_2 (hypercapnia). These two observations illustrate that CO_2 accumulation acting on central and peripheral chemoreceptors makes larger contributions to the urge to breathe compared with the relatively minor reduction in O_2 during short-duration breath hold. The rebreathing test (*part A*, *activity 1*), which could also be performed in a background of 100% O_2, illustrates the powerful urge to breathe driven by increasing CO_2 levels. Similarly, the breath-hold duration after prior hyperventilation where hypocapnia is induced, also illustrates the relationship between CO_2 and breath-hold duration, albeit in the opposite direction.

Second, even if someone is aware of the role of chemoreceptors in respiratory control, these explanations may also be partly misleading with respect to breath-hold duration, as they are an incomplete explanation of the factors that affect breath-hold duration and break point. As the activity in *part D*, *activity 7*, illustrates, increases in chemostimuli may not be as important as the absence of the physical act of breathing (i.e., lung stretch) in driving the urge to breathe. In this activity, despite the fact that blood gases are not corrected by the act of breathing, the participant is still able to breath hold for more time after a few respiratory cycles. This confirms that there are other factors at play during the respiratory cycle (e.g., transient lung stretch), independent of blood gas levels.

As such, students should be encouraged to identify multiple mechanisms at play during breath holding at varied lung volumes. In particular, it is important to note that inspiration (i.e., larger lung volume) may activate lung stretch receptors but also increases the amount of O_2 available in the lungs, which can diffuse into the blood and increases the "sink" into which CO_2 can diffuse as it leaves the lungs. Conversely, expiration decreases the volume of O_2 in the lungs and reduces the sink into which CO_2 can enter (27). In addition, students may be directed to primary literature discussing this topic in more detail (10).

Evaluation of Student Work

Students should collect data for each activity listed above on multiple participants (if time permits) or pool the data from each individual group. Students can then plot the mean data of the breath-hold duration from each activity and present the data in bar graphs (similar to Fig. 4) with SDs using any data analysis and graphing software program (e.g., Microsoft Excel). In this way, data can be compared between trials, and the variability present in any population of participants can be illustrated. If the course includes statistical analysis, have students perform either paired t-tests (when comparing any one breath-hold duration with the control activity) or use repeated-measures ANOVAs to compare the data across all activities, using an appropriate post hoc test for pair-wise comparisons.

Critical Thinking Questions

Question 1. Explain how arterial blood gas composition changes when performing a breath hold at rest and the effects on drive to breathe of both CO_2 and O_2. Include references where necessary.

ANSWER. When a breath hold is performed at rest, arterial O_2 levels begin to drop and arterial CO_2 begins to rise (pH levels drop) as a function of metabolism (15, 19). The rise in CO_2 and drop in pH stimulate both peripheral and central chemoreceptors, and the decrease in O_2 (if reduced significantly) stimulates the peripheral chemoreceptors. The chemoreceptors then relay this information to the medullary respiratory center of the brain stem, eliciting an increase in the drive to breathe. During voluntary breath holding, you may observe involuntary respiratory movements (diaphragmatic contractions) when this occurs.

Question 2. Draw a feedback loop of the chemical control of breath holding.

ANSWER. Draw-in sequence: *1*) homeostasis, *2*) breath hold, *3*) consecutive increase in arterial CO_2, decrease in pH, and decreases in arterial O_2 concentrations, *4*) increase in chemoreceptor activity, *5*) convergence of afferent sensory data at the medullary respiratory center, *6*) increase drive to breathe, *7*) break point or end of breath hold, and *8*) back to homeostasis. See Fig. 1 for components to include.

Question 3. Provide an explanation as to why a breath hold after a full inspiration produces longer breath-hold duration than a breath hold after expiration. Use results collected from the activities you performed and relevant references to support your answer.

ANSWER. During a maximal inspiratory breath hold, you activate the pulmonary stretch receptors, which send signals to the brain to decrease the drive to breathe (10, 24). If you hold your breath after an expiration, you will have decreased the stimulus to the stretch receptors, making the drive to breathe more prominent sooner. Additionally, the amount of available O_2 is changed with changing lung volumes. Larger lung volumes allow for a greater volume of gas to help dilute the increase in metabolically derived CO_2 levels.

Question 4. Identify and explain one technique not demonstrated in the aforementioned activities that may decrease breath-holding time. Justify your answer by proposing the mechanism involved and use the results collected from this laboratory to support your answer.

ANSWER. Breath-holding time can be increased/decreased by manipulating one of the factors involved in the control of breath-holding (shown in Fig. 1). Any technique that *1*) decreases O_2, *2*) increases CO_2, or *3*) decreases lung volume/lung stretch will suffice. For example, if a volunteer were to exercise for 1 min before holding their breath (maximal inspiration), they would have increased their metabolism thereby increasing the rate at which O_2 is consumed and CO_2 is produced. This would decrease breath-hold time by activating both central and peripheral chemoreceptors earlier.

Inquiry Applications

These simple breath-hold activities can be a valuable tool for undergraduate students to apply their knowledge of physiology in an integrative setting. To increase the inquiry level, the instructor can allow students to decide what activities are to be included before the breath hold (e.g., full inspiration, expiration, and exercise), what level of body position or activity (e.g., lying, sitting, standing, eyes open or closed, or walking on a treadmill), what students direct their attention to during the breath hold (e.g., environmental distraction), and what additional equipment and measurements to make (e.g., Sp_{O_2} or end-tidal CO_2).

Students can use the experiments from this laboratory exercise to further explore and understand extreme sports, environments, and pathophysiological conditions that compromise respiratory physiology. Examples of topics that students can study include free diving, breath-hold records, the mammalian diving reflex, synchronized swimming, underwater hockey, high-altitude physiology, sleep apnea, and chronic obstructive pulmonary disease. To do this, they must search the published scientific literature using PubMed (www.pubmed.com) and read, summarize, and reference published articles on their chosen topic. Within these reports, students are required to compare the physiology of their selected topic to healthy normal individuals breathing room air at one atmosphere at sea level.

Wider Educational Applications

This activity can be adapted to laboratory, classroom, or tutorial settings, both in small and large groups, to lower-level to higher-level students of physiology. In addition, these activities can be used in public outreach activities (e.g., high school and public talks) to engage nonspecialists in the importance and relevance of understanding the physiology of everyday life.

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