This minds-on, hands-on activity begins with analysis and discussion questions that develop student understanding of homeostasis, negative feedback, and positive feedback. Next, students analyze examples of how breathing changes at high altitude and during vigorous exercise plus background information about cellular respiration and the circulatory and respiratory systems. Students use this information to develop a model of negative feedback regulation of blood levels of CO₂ and O₂. Then, they carry out an initial experimental test of their negative feedback model. A second, optional experiment collects quantitative data for a more rigorous test of their negative feedback model.

In a final optional section, each student group formulates a question or hypothesis concerning homeostasis and changes in breathing. Then they design a relevant experimental investigation, carry it out, and interpret the results. Information in these Teacher Preparation Notes can be used to facilitate student investigations of exercise, breath-holding, or the effects of CO₂ vs. O₂ levels.

We estimate that the introductory sections and first experiment can be completed in 1-2 50-minute class periods. The optional quantitative experiment will probably require another 1-2 50-minute periods. The optional final section will probably take two additional 50-minute periods. An alternative analysis and discussion activity on homeostasis, negative feedback and positive feedback is “Homeostasis, Negative Feedback, and Positive Feedback” (~50 minutes; http://serendipstudio.org/exchange/bioactivities/homeostasis).

Table of Contents
Learning Goals – pages 1-2
Supplies – page 3
Instructional Suggestions and Background Information
   General – page 3
   I. Homeostasis and Negative Feedback – pages 3-7
   II. Two Types of Feedback – pages 7-8
   III. Changes in Breathing Due To Negative Feedback Regulation of Blood Levels of O₂ and CO₂ – pages 8-11
   IV. Experiment 1 to Test Your Negative Feedback Model – pages 11-12
   V. Experiment 2 to Test Your Negative Feedback Model – 12-14
   VI. Further Investigations of Homeostasis and Changes in Breathing
      A. Changes in Blood Levels of O₂ vs. CO₂ – pages 14-15
      B. Holding Your Breath – page 15
      C. Physical Activity – pages 15-19
      D. Helping Students to Improve Experimental Design and Methods – page 19

Learning Goals
In accord with the Next Generation Science Standards:
• This activity helps students to prepare for the Performance Expectation:
   o HS-LS1-3. "Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis."

1 By Drs. Ingrid Waldron, Lori Spindler and Jennifer Doherty, Dept Biology, University of Pennsylvania, © 2018. We are grateful to Philadelphia area teachers for helpful input to improve this activity. These Teacher Preparation Notes and the related Student Handout are available at http://serendipstudio.org/sci_edu/waldron/#breath.

Students learn the following Disciplinary Core Idea:
- LS1.A "Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system."

Students engage in recommended Scientific Practices, including:
- "Developing and Using Models – Develop, revise, and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of the system."
- "Analyzing and Interpreting Data – Evaluate the impact of new data on a working explanation and/or model of a proposed process or system."
- "Constructing Explanations – Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena...".
- "Asking Questions – Ask questions that arise from examining models or a theory, to clarify and/or seek additional information and relationships."
- "Planning and Carrying out Investigations – Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence... decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly."

Students learn the Crosscutting Concept, "Stability and Change – Systems in dynamic equilibrium are stable due to a balance of feedback mechanisms."

Additional Specific Learning Goals
- Homeostasis refers to the maintenance of relatively constant internal conditions.
- Negative feedback occurs when a change in a regulated variable triggers a response which reverses the initial change and brings the regulated variable back to the set point. Negative feedback plays an important role in maintaining homeostasis. For example, negative feedback helps to maintain relatively constant internal body temperature.
- Positive feedback occurs when a change in a variable triggers a response which causes more change in the same direction. Positive feedback is useful when there is an advantage to making a rapid change. For example, positive feedback facilitates rapid formation of a platelet plug which helps to prevent excessive blood loss when a blood vessel is injured.
- Cells carry out cellular respiration to make ATP, and hydrolysis of ATP provides energy for many cellular processes. Cellular respiration requires O₂ and produces CO₂.
- The respiratory system and circulatory system work together to bring O₂ to cells all over the body and get rid of CO₂. When a person inhales, air with O₂ is brought into the lungs. O₂ diffuses from the air in the tiny air sacs of the lungs into the blood. The O₂-carrying blood is pumped by the heart to blood vessels near all the cells in the body. O₂ diffuses from the blood into the cells where O₂ is used in cellular respiration. CO₂ produced by cellular respiration moves through the blood to the lungs where it is exhaled.
- Negative feedback regulation of blood levels of CO₂ and O₂ helps to ensure that enough O₂ is delivered to meet the cells’ needs for cellular respiration and enough CO₂ is removed to prevent harmful effects.
- For a scientific investigation to yield accurate results, scientists need to begin by developing reliable, valid methods of measuring the variables in the investigation.
Supplies
For sections IV and V. Experiments to Test the Negative Feedback Model:
– one 8 gallon plastic garbage bag per student (13 gallon plastic garbage bags are much easier to obtain and can be used; students may need to breathe into the bag longer than 4 minutes or gather the bag to hold it over their mouth and nose partway down from the bag’s opening so the volume of air available for rebreathing is reduced.)
– some way of timing 4 minutes (experiment 1) and 8 consecutive 30-second intervals (experiment 2) for each group of four students

For section VI. Further Investigations of Breathing and Homeostasis, see pages 15-18.

Instructional Suggestions and Background Information
In the Student Handout, numbers in bold indicate questions for the students to answer and
➢ indicates a step in the experiment for the students to do.

For the analysis and discussion questions, you can maximize student participation and learning, by having your students work individually, in pairs, or in small groups to complete groups of related questions and then having a class discussion after each group of related questions. In each discussion, you can probe student thinking and help them develop a sound understanding of the concepts and information covered before moving on to the next group of related questions.

A key is available upon request to Ingrid Waldron (iwaldron@upenn.edu). The following paragraphs provide additional instructional suggestions and background information – some for inclusion in your class discussions and some to provide you with relevant background that may be useful for your understanding and/or for responding to student questions.

I. Homeostasis and Negative Feedback
For question 1, you may want to encourage your students to consider the effects of temperature on the structure and function of molecules (e.g. optimum temperature for enzyme function).

For question 2, you may want to discuss how physical activity, including shivering, results in increased production of heat. All types of energy conversion are inefficient result in the production of heat. During cellular respiration, only about 50% of the energy in nutrient molecules is transferred to ATP and the other 50% is converted to heat. During ATP expenditure by cells, another 25% of the energy derived from food becomes heat. Internal friction during physical activity contributes to additional heat production. During muscle activity, only about 20-25% of the chemical energy expended is captured in the kinetic energy of muscle contraction and the rest of the energy is converted to heat.3

The paragraph below question 2 and the flowchart in question 3 refers to a control center for temperature regulation. Temperature regulation depends on several interconnected regions within the hypothalamus, with at least one region that stimulates responses that warm the body and another that stimulates responses that cool the body.

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3 In mammals, negative feedback regulation maintains a relatively high body temperature which allows mammals to move rapidly even when environmental temperatures are low. This type of thermoregulation depends on a relatively high metabolic rate which requires a high caloric intake. Mammals and birds are homeotherms, in contrast with most other types of animals which are poikilotherms (core body temperature generally varies with the environmental temperature).
The Student Handout gives the set point for normal body temperature as 37°C = 98.6°F. As shown in this figure, this is a good estimate for rectal temperatures. However, oral temperatures in healthy individuals tend to be lower, with a mean value of 36.4°C. It should also be mentioned that body temperature varies by time of day, with lower temperatures in the early morning and higher temperatures in the late afternoon or early evening.

The results from 20 studies with strong or fairly strong evidence of normal oral, rectal and tympanic temperature (°C) in adult men and women are presented. Temperature is obtainable as mean value (bold lines), 1st and 3rd quartiles (unfilled bars) and range (thin lines). (From Sund-Levander et al, 2002, Scand. J. Caring Sci. 16:122-8)

The figure in question 3 of the Student Handout and the figure below illustrate several important points about negative feedback regulation of internal body temperature.

- Negative feedback maintains body temperature within a narrow range by changing other aspects of body physiology (sweating, shivering, blood flow to the skin). These changes persist until body temperature is restored to the set point range and then the sweating or shivering and the changes in blood flow are turned off.
- The key stimulus for these changes is the discrepancy between the set point temperature and the actual body temperature.
- Negative feedback often operates via more than one type of physiological response. In addition to the responses shown in the figure below, behavioral responses such as putting on a sweater or moving out of the sun contribute to negative feedback regulation of body temperature. 4
- Your body temperature depends on the balance between the amount of heat generated by your body’s metabolism (influenced e.g. by shivering and exercise) and the amount of heat lost to or gained from the environment (influenced e.g. by sweating and changes in circulation).

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4 Students analyze the role of changes in blood flow to the skin in “Homeostasis, Negative Feedback, and Positive Feedback” (http://serendipstudio.org/exchange/bioactivities/homeostasis). Students may ask about goosebumps when a person is cold. In other mammals with dense fur this response traps a layer of air that helps to insulate the skin surface from the environment and thus reduces heat loss. In humans, this response is ineffective because the hair on our skin is not thick enough to trap an insulating layer of air.
You may want to ask students one or more of these additional questions to further their understanding of negative feedback regulation of temperature:

- Is negative feedback regulation of body temperature similar for heat from the environment and for heat generated by the body during physical activity? (Students have presumably experienced sweating while at rest in a hot environment and while engaged in vigorous physical activity in a cool environment. This should help them to realize that negative feedback responses to increased body temperature are similar whether the increase is due to environmental heat or physical activity.)
- Why should you drink more water if you are exercising in a hot environment?
- Why is a person’s temperature more likely to get dangerously high if he or she is exercising in a hot, humid environment? (In a humid environment, sweat does not evaporate as well, so the cooling effect of sweating is reduced.)

The figure below shows a general model of negative feedback and applies this model to one aspect of body temperature regulation. The Student Handout discusses the control and effector components of temperature regulation, but not the sensor component. There are temperature receptors in the skin, abdominal organs, and brain (hypothalamus and elsewhere in the CNS).
Before your students answer question 5, you may want to show a brief video on homeostasis, negative feedback and temperature regulation (available at https://www.khanacademy.org/partner-content/mit-k12/mit-k12-biology/v/homeostasis).

As discussed on page 2 of the Student Handout, negative feedback regulation does not imply having a constant temperature at all times. You can change the set point on the thermostat in a home and, similarly, physiological responses can change your body's set point for temperature regulation. For example, when you have an infection, the phagocytic cells that defend against bacteria and viruses send a chemical signal to the temperature control center in the brain. This chemical signal increases the set point for temperature regulation, so you develop a fever. When the set point for body temperature is increased above the normal body temperature, then a normal body temperature may result in shivering and feeling chills. The fever helps your immune system fight the infection since the increase in temperature generally increases the immune response and decreases growth of many infectious microorganisms.

During exercise, body temperature tends to increase because the increased energy expenditure (up to 15-fold above resting levels) results in increased heat production which may exceed the ability of the body to get rid of heat. Usually, this results in fluctuation of body temperature within an acceptable range (see figure on the right below). In this case, the rise in temperature is

5 The same chemical signal also triggers a tired, achy feeling so you want to rest, which helps your body mobilize resources to fight the infection.

6 "Brain damage from a fever generally will not occur unless the fever is over 107.6°F (42°C). Untreated fevers caused by infection will seldom go over 105°F (40.6°C) unless the child is overdressed or in a hot place." (https://medlineplus.gov/ency/article/003090.htm) However, a fever may indicate a serious infection that should be treated medically.

7 Physiological problems may result if a person exercises vigorously when it is very hot and humid so that heat loss is decreased (e.g., due to reduced radiation of heat from the body and sweat dripping from the body without evaporating). Fluid loss through sweating, together with peripheral vasodilation to facilitate heat loss, can result in reduced blood pressure and heat exhaustion. Heat exhaustion can be protective if it prevents continued exertion when the body is unable to give off enough heat. If excessive exertion continues in a hot and humid environment, this can result in heat stroke and even death. If the body stops sweating to conserve fluids and the cardiovascular system directs blood away from the body surface in order to maintain needed blood flow to the brain and other vital
not due to a change in set point, but instead is due to inability of the negative feedback mechanisms to cope with the amount of temperature stress.

II. Two Types of Feedback

The term feedback is appropriate since, in both cases, an initial change in a variable stimulates a response in the same variable. Negative feedback reverses the direction or sign of the change, while positive feedback augments the initial change.

Positive feedback is useful when there is an advantage to a rapid transition between two states, e.g., from blood flowing freely in a blood vessel to formation of a platelet plug in an injured blood vessel.\(^8\) Positive feedback in platelet plug formation contributes to homeostasis by preventing excessive loss of blood and thus conserving fluid and helping to maintain blood pressure. The platelet plug is reinforced by the formation of a blood clot (see figure on the last page of these Teacher Preparation Notes).

The figure below provides additional information about positive feedback in the formation of a platelet plug. Undamaged endothelial cells in the lining of the blood vessels secrete chemical signals that inhibit platelet aggregation and blood clot formation, so the platelet plug and blood clot are limited to the location where the endothelium has been damaged.

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body organs, this can result in escalating body temperature. If internal core temperature reaches 106°F (41°C) most people suffer convulsions, and if internal core temperature exceeds 110°F (43.3°C) neuron malfunction and irreversible damage to proteins are likely to prove fatal. Additional information is available in "Heat and exercise: Keeping cool in hot weather" (http://www.mayoclinic.com/health/exercise/HQ00316).

\(^8\) Another example of positive feedback occurs during childbirth (see http://www.johnwiley.net.au/highered/interactions/media/Foundations/content/Foundations/homeo4a/bot.htm). This positive feedback helps to speed up the transition from a fetus in the uterus receiving oxygen via the placenta to a baby that has been born and is breathing on his or her own). Of course, positive feedback is not the only way that the body achieves rapid change; for example, neural control of muscles or secretory organs can also produce rapid responses.
After question 9, you may want to ask your students these questions.

What would go wrong if your body used positive feedback to regulate body temperature? For example, what would happen if a person sweated when temperature decreased?

After question 9, you may want to help students recognize the generality of the principles analyzed by discussing the Crosscutting Concept, "Stability and Change – Systems in dynamic equilibrium are stable due to a balance of feedback mechanisms."

III. Changes in Breathing Due To Negative Feedback Regulation of Blood Levels of O₂ and CO₂

You may want to use this long section title to discuss with your students how negative feedback involves changing some things (e.g. breathing rate and depth) in order to keep something else nearly constant (e.g. blood levels of O₂ and CO₂). Negative feedback regulation of blood levels of O₂ and CO₂ is needed to preserve health. O₂ is needed for cellular respiration to provide ATP; muscle cells can substitute lactic acid fermentation for cellular respiration for a while, but neurons have little or no ability to carry out lactic acid fermentation. Therefore, the brain is particularly sensitive to low O₂. If the brain is deprived of O₂ for a few minutes, parts of the brain can be permanently damaged. If oxygen deprivation continues, the person can become “brain-dead”. Inadequate O₂ for your body’s cells can also cause strain on your heart. Excess CO₂ can result in excess acidity which can disrupt the function of many proteins. Excess CO₂ can cause muscle spasms, racing heart, disorientation, and even death.

In this section, students develop a model of negative feedback regulation of blood levels of O₂ and CO₂, based on:

- examples of how breathing changes at high altitude and during vigorous exercise
- background information about cellular respiration, breathing and circulation.

As altitude increases and the concentration of O₂ in air decreases, rate and depth of breathing increase. To help your students think about question 10, you may want to challenge them to describe what would happen to blood levels of O₂ if the rate and depth of breathing was the same at high altitude as it had been at sea level.
If the transition to high altitude is rapid, a person is likely to experience acute mountain sickness; one major reason is that the increased breathing in response to low \( \text{O}_2 \) removes \( \text{CO}_2 \) faster than it is produced by cellular respiration and, as \( \text{CO}_2 \) levels fall, alkalosis develops. (\( \text{CO}_2 \) dissolved in the water of the blood is a major source of acidity: \( \text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \))

Over the long term, acclimatization to living at high altitudes results in several adaptive changes:
- increased red blood cell production so the blood carries more \( \text{O}_2 \) per mL
- increased number of capillaries within the tissues so \( \text{O}_2 \) has a shorter distance to diffuse to reach the cells
- more mitochondria in cells so available \( \text{O}_2 \) is used more efficiently
- kidneys retain more \( \text{H}^+ \).

If your students are not familiar with cellular respiration and ATP, you may want to introduce these topics with the analysis and discussion activity, "How do biological organisms use energy?" (available at http://serendipstudio.org/exchange/bioactivities/energy).

In response to question 12 students are expected to recognize that vigorous exercise uses more ATP which is generated by higher rates of cellular respiration which requires more \( \text{O}_2 \). Increased breathing is also needed to get rid of the increased \( \text{CO}_2 \) produced by increased cellular respiration. As shown in the figure below, both rate and depth of breathing increase as exercise intensity increases. This change in breathing helps to maintain homeostasis by providing more \( \text{O}_2 \) and removing more \( \text{CO}_2 \) to meet the demands of increased cellular respiration in the muscles and heart. For further discussion of the regulation of breathing during and after exercise, see pages 16-17.

![Respiratory system response to exercise](https://thoracickey.com/respiratory-system-response-to-exercise-in-health/)

If your students are not familiar with how people breathe, you may want to provide some additional explanation. During inhalation, the lung is expanded by contraction of the diaphragm and certain rib muscles; as shown in the figure below, the diaphragm pulls downward as the muscle shortens. The expansion of the lungs reduces the pressure inside the lungs below the air pressure in the surrounding environment and air moves into the lungs. During exhalation, the diaphragm and rib muscles relax and the elasticity of the lungs causes the lungs to get smaller.
This increases the air pressure inside the lungs above the external air pressure so air moves out of the lungs. Thus, quiet breathing is due to the alternation between contraction of breathing muscles which results in inhalation and relaxation of breathing muscles which results in exhalation. This rhythmic pattern of contraction and relaxation of the breathing muscles is due to a rhythmic pattern of stimulation that originates in the medulla in the brainstem. In deep breathing, contraction of certain rib muscles contributes to exhalation. A simple animation showing inhalation and exhalation is available at [http://www.smm.org/heart/lungs/breathing.htm](http://www.smm.org/heart/lungs/breathing.htm).

The figure below provides additional information about the structure of the respiratory system. If your students are familiar with the terms alveolus/alveoli, you may want to use these terms to replace the terms air sac/air sacs on page 4 of the Student Handout. If students inquire why you need lungs instead of using your skin as a gas exchange surface, you can explain that gas exchange surfaces must be moist and also the total surface area of the alveoli is greater than the surface area of your skin; this explains why frogs can use their skin as a major gas exchange surface, but not humans.
You may want to ask your students why we need to have both a respiratory system and a circulatory system. The respiratory system is needed to bring air with O\textsubscript{2} into the air sacs of the lungs where O\textsubscript{2} can diffuse into the blood in the tiny blood vessels that surround these air sacs. The circulatory system is needed to carry O\textsubscript{2} from the lungs to the cells throughout the body. Similarly, the circulatory system is needed to carry CO\textsubscript{2} from the body cells to the lungs where the respiratory system can move stale air with excess CO\textsubscript{2} out of the body. As you discuss the role of the circulatory system, you may want to mention to your students that the circulatory system has multiple additional important functions such as transport of hormones, food molecules (e.g. glucose), heat, and antibodies and white blood cells to fight infection.

In question 14, students develop a model that describes how changes in breathing contribute to negative feedback regulation of blood levels of O\textsubscript{2} and CO\textsubscript{2}. You may want to ask the students to apply this model to the situations described in questions 10 and 12 (going to a high altitude and vigorous physical activity).

IV. Experiment 1 to Test your Negative Feedback Model
In this section, students carry out an experiment to test the model they developed in question 14 which describes how changes in breathing contribute to negative feedback regulation of blood levels of O\textsubscript{2} and CO\textsubscript{2}. Changes in the amount of air breathed into the lungs per minute (pulmonary ventilation in milliliters per minute) can result from changes in breathing rate (breaths per minute) and/or changes in depth of breathing (tidal volume in milliliters per breath). By simple algebra:

\[
\text{Pulmonary Ventilation} = \text{Breathing Rate} \times \text{Tidal Volume}
\]

Student responses to question 14 may refer to increased breathing without considering whether increased breathing is due to increases in breathing rate or depth of breathing. Question 16c
introduces this distinction, which will be important for section V, so you may want to encourage students to make predictions specifically about changes in breathing rate and changes in depth of breathing.

You may need to reassure your students that the warnings about the hazards of suffocation that they have seen on plastic bags refer to infants. There is no risk that middle school, high school or college students will suffocate during this experiment! However, a student who has a serious respiratory or heart problem probably should not be a subject in the experiments. It may be advisable for a participating student with asthma to keep his/her inhaler close at hand for use if needed.

The video available at [https://www.youtube.com/watch?v=l60LE0M0bk8&feature=youtu.be](https://www.youtube.com/watch?v=l60LE0M0bk8&feature=youtu.be) demonstrates how to prepare the bag and breathe into the bag. You will want to view this video and then either demonstrate the procedure for your students or have them watch this video. Helpful advice includes:

- To begin, the bag should be opened completely and swished through the air to fill it.
- Gather the top of the bag in your hands and use a finger to poke a hole just big enough for your nose and mouth. Make sure to have a tight seal between the bag and your face, so no air is leaking in and out of the bag.
- Maintain a tight seal throughout the entire test interval.

Most students will notice a change in their breathing, typically after 2-3 minutes of breathing into the bag. A subjective feeling of discomfort is most immediately noticeable, but each student should also try to notice whether his or her breathing becomes more rapid and/or deeper.

V. Experiment 2 to Test Your Negative Feedback Model

You may want to introduce the section on “Developing Your Experimental Procedures” (page 6 of the Student Handout) by emphasizing that scientists need to develop reliable and valid methods of measurement in order to get accurate results. Reliable methods produce the same, consistent results on different repetitions of the same experiment. Valid methods produce results that accurately reflect the variable the scientist is trying to measure. The development of reliable and valid methods is a major component of scientific research.

For this section, helpful advice includes:

- Each subject should stand while breathing into the bag to make it easier for observers to see the whole bag to evaluate the breathing rate and depth and also to standardize posture for different subjects.
- To evaluate the rate and depth of breathing, students should look for a crease in the bag and watch how it changes. For depth of breathing, the unit of measurement is arbitrary – essentially the minimum noticeable increase or decrease. Instructions for students to develop this unit of measurement are provided on page 6 of the Student Handout.

Results of the experiment vary for different subjects (and even for the same subject in repeated trials). One reason for this variation is that breathing is highly subject to voluntary control. Trends may differ because of distractions in the environment, emotional influences, or other

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9 A second demo video which shows prolonged breathing into the bag is available at [https://www.youtube.com/watch?v=UjzBRiX1Jpc&feature=youtu.be](https://www.youtube.com/watch?v=UjzBRiX1Jpc&feature=youtu.be).
types of brain activity that may influence breathing. These issues are discussed in questions 20 and 25.

Due to the variability in results, you should collect individual data from all the subjects in your class and calculate class averages for the number of breaths and depth of breathing. You will need to take into account the methodological issues that arise if one or more subjects fail to complete the entire four minutes of breathing into the bag. As question 19a indicates, this can lead to erroneous conclusions unless you adopt one or more of these alternative approaches.

- Use only the data for the 30 second intervals when all subjects were breathing into the bag.
- Calculate averages for only the subjects who completed all four minutes of breathing into the bag.

The best approach will depend on the specific pattern of how many subjects stopped breathing into the bag and when they stopped. You may want to use a combined approach, e.g. averaging the data for each of the first seven 30-second intervals for the subjects who have complete data for 3.5 minutes.

While you are collecting the data from the student groups and calculating the class averages, students should write answers to questions 19-20.

In our experience, most subjects show a clear trend to increased depth of breathing, but changes in the rate of breathing are inconsistent both within and between subjects. These observations are in accord with scientific research results which show that increased CO$_2$ is associated with more consistent increases in depth of breathing and smaller, inconsistent increases in rate of breathing. You may want to relate these findings to the observation that deeper breathing is more efficient than more rapid breathing as a way to increase intake of O$_2$ and release of CO$_2$. To understand the reason for this, consider what happens when you begin to inhale. The first air to enter the air sacs in the lungs is air that was just exhaled into the bronchioles, bronchi, trachea, pharynx, mouth and nose (see figure on page 10). This recently exhaled air has low O$_2$ and high CO$_2$, so it is less useful than fresh air for gas exchange in the air sacs of the lungs. A very shallow breath will bring only this recently exhaled air into the air sacs. A deeper breath will bring proportionately more fresh air with high O$_2$ and low CO$_2$ into the air sacs; this will increase diffusion of O$_2$ into the blood and diffusion of CO$_2$ out of the blood.

An example of greater changes in depth of breathing than in rate of breathing is shown in the figure below, which presents results for one subject who was re-breathing into a “grocery produce bag”.

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10 This same research (e.g. Journal of Applied Physiology 47:192-6) has found breathing rates of 16-20 breaths/min. for adults breathing room air at rest (vs. 12 breaths/min. generally reported in textbooks and on the web).
If your results are similar to our findings, discussion of questions 22-24 will provide the opportunity to talk about the importance of testing even predictions that seem eminently reasonable. Failure of experimental results to confirm a prediction can lead to new insights and an improved hypothesis – in this case, the recognition that negative feedback regulation of blood levels of O₂ and CO₂ affects primarily depth of breathing, with less effect on breathing rate. This result makes sense biologically, since increased depth of breathing is a more efficient way of improving gas exchange.

It will be helpful to include a discussion of methodological issues that should be considered in interpreting the results, including:

- individual variability and the importance of sample size for assessing overall trends
- sources of error and changes in method that could improve the accuracy of experimental results
- the ambiguity of interpreting an experiment where both the amount of O₂ and the amount of CO₂ changed simultaneously
- how confident they are in their interpretations

VI. Further Investigations of Homeostasis and Changes in Breathing
Sections A-C below suggest several topics that student groups may want to explore and also provide some methodological suggestions. Section D provides some suggestions for helping students improve their experimental design and methods.

A. Changes in Blood Levels of O₂ vs. CO₂
The experiment in section IV demonstrates the importance of increased CO₂ and/or decreased O₂ in stimulating increased breathing to maintain homeostasis. However, this experiment does not allow us to distinguish the relative importance of changes in levels of CO₂ vs. O₂. Students can
use the following procedure to compare the effects of decreased O₂ combined with increased CO₂ vs. the effects of decreased O₂ levels with relatively little increase in CO₂ levels.

Repeat the experiment described on page 6 of the Student Handout with your group members as subjects. Repeat this experiment as originally described and while breathing into a plastic bag that has KOH to absorb CO₂. **Be very cautious in handling KOH since it is caustic. Use gloves and avoid inhalation of KOH fumes. Also, safe disposal procedures should be observed (https://pubchem.ncbi.nlm.nih.gov/compound/potassium_hydroxide).** To prepare the bag with KOH:

- Put a piece of filter paper in the bottom of a finger bowl, and use a spatula to put approximately 6-7 pieces of KOH in the finger bowl.
- Moisten the filter paper with a few scattered drops of water (KOH has to be moist in order to absorb CO₂).
- Cut a piece of cheesecloth a few layers thick and big enough to surround the finger bowl; use a rubber band to close the cheesecloth over the finger bowl.
- Place the finger bowl in an 8 gallon plastic bag which has been filled with air.

**B. Holding Your Breath**
Humans have considerable voluntary control over their breathing, as evidenced when you hold your breath, talk, sing, or play a wind instrument. We can temporarily override the negative feedback regulation of blood levels of O₂ and CO₂, but if O₂ gets too low and/or CO₂ gets too high a person will involuntarily start to breathe, thus restoring homeostasis. To explore the contribution of negative feedback regulation of blood levels of O₂ and CO₂ to the maximum time a person is able to hold his or her breath, students can measure the maximum duration of breath-holding after a period of breathing room air vs. after breathing into the bag (as in the class experiment). To get reliable data on duration of breath-holding:

- Each subject should take a deep breath, keep his or her mouth closed and hold his or her nose, avoid even minor diaphragm contractions, and focus on holding his or her breath as long as possible.
- For each experimental condition, it is best to use the average of three breath-holding times separated by 10 minute intervals.

You may also want to encourage students to notice the characteristics of their breathing immediately after they have held their breath as long as they can.

Additional suggestions for student experiments are available in “The ins and outs of breath-holding: simple demonstrations of complex respiratory physiology” (https://www.physiology.org/doi/abs/10.1152/advan.00030.2015?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed). One caution concerns their suggestion to assess the effects of hyperventilation on duration of subsequent breath-holding; there is a risk of losing consciousness if a person hyperventilates a lot before breath-holding. Fortunately, it is practically impossible to hold your breath until unconsciousness after normal breathing.

**C. Physical Activity**
Through personal experience and section III of the Student Handout, students will be familiar with the need for increased breathing during strenuous exercise in order to maintain homeostasis. Students are often tempted to suggest experiments that will simply replicate this phenomenon, which is already well known to them. Scientists do need to replicate previous findings to ensure their reliability, but the observation that rate and depth of breathing increase during and
immediately after vigorous physical activity is already well-established. Therefore, we suggest that you encourage students to develop an experiment that can expand their understanding beyond what they already know and provide new information about changes in breathing due to exercise. Appropriate questions might include:

- Is the change in breathing greater for aerobic exercise (e.g. jogging in place) vs. strength training (e.g. using resistance bands) or yoga?\(^{11}\)
- Does breathing rate double if a person exercises twice as fast (e.g. doubling the number of jumping jacks in a minute) or twice as hard (e.g. two resistance bands instead of one for strength training exercise)?
- Are the changes in rate of breathing similar to the changes in depth of breathing? Does this vary, depending on the type and intensity of exercise?
- How long does it take for breathing rate and/or depth to return to resting levels after different types and durations of exercise?

Feasible methods for measuring breathing rate and depth generally do not work well when a person’s head is moving, so students will probably need to compare breathing rate and depth before each subject begins exercising vs. breathing rate and depth right after the end of exercise and at intervals during recovery. The following paragraphs suggest two possible ways that your students can measure rate and depth of breathing before and after exercise. Alternatively, each student can assess his or her own breathing rate and depth before and after exercise. If you develop any improvement for either of the methods described below or a good alternative method for measuring the rate and depth of breathing, please let me know (iwaldron@upenn.edu). Thank you!

One low-tech, effective method for detecting each breath uses a small piece of facial tissue taped to the subject’s nose so the tissue hangs over the edge of one nostril (see figure below). A piece of tissue the shape and size shown works well for measuring the rate and depth of breathing. This method only works if you keep your mouth closed and breathe through your nose. If you want to use this method, you may find it helpful to use the page of templates provided on the next to the last page of these Teacher Preparation Notes. Groups which are using this method will also need a pair of scissors, a facial tissue, and a length of sensitive skin medical tape.

\(^{11}\) You may want to have available resistance bands and/or instructions for and pictures of yoga poses in case students want to include exercise of this type in their experiment.
An alternative approach to measuring breathing rate and depth is to use a 2 inch length of ¾ inch PVC pipe with a metallic streamer taped so that it flops over one edge. (PVC pipe diameter refers to the internal diameter, not the external diameter.) We have used a metallic streamer ~¼ inch wide, with a total length of ~3 ¾ inches; we obtained the streamers from a "foil fringe garland" purchased at a party store.

To prepare the 2 inch lengths of PVC pipe, we first used a hack saw to cut the needed number of pieces of pipe and then smoothed the edges of the ends using an X-Acto knife or a single-edge razor blade in a holder. For sanitary reasons, you will need one piece of pipe for each student in your largest class.

To disinfect the pieces of pipe for use in another class, we recommend the following procedure:

- Wash your hands with soap and water for at least 30 seconds. Rinse and then dry with a paper towel.
- Remove the streamers and tape and scrub the inside and outside of each PVC tube using a brush or pipe cleaner and soap and water until the tube is clean.
- Shake extra water off the tubes. Soak the tubes in 70% isopropyl alcohol for 5 minutes or in bleach (5 mL of 6% bleach in 8 ounces of water) for 3 minutes or microwave the tubes for 5 minutes.
- Rinse the tubes. Place the tubes on a clean surface to dry.
- Be sure to wash your hands with soap and water for at least 30 seconds before handling the dry tubes to store them in a plastic bag.
Any student who has been excused from physical education may need to be excused from participating as a subject in the experiments in this section. You may want a student with asthma to keep his/her inhaler close at hand for use if needed. Students should be advised to wear appropriate clothing and footwear for physical activity.

This figure shows the typical time trend of changes in breathing during and after moderate exercise. Minute volume is the volume of air breathed in a minute = breathing rate x volume per breath.

Notice that breathing increases rapidly when exercise begins, with a substantial increase within seconds. Breathing remains elevated for a while after exercise; this brings in the extra oxygen needed to metabolize the lactic acid that accumulates during vigorous exercise.

In the Student Handout the increase in breathing during exercise is presented in the context of discussion of negative feedback regulation of blood levels of O₂ and CO₂. Although negative feedback regulation probably contributes to fine-tuning the change in breathing during exercise, multiple lines of evidence indicate that this negative feedback is not the primary cause of increased breathing during exercise. For example, during exercise, blood levels of O₂ and CO₂ generally show only small and inconsistent changes from the levels observed at rest; these small and inconsistent changes in blood levels of O₂ and CO₂ are in sharp contrast to the substantial increases in breathing rate and depth during many types of exercise.

A broad range of evidence indicate that multiple mechanisms contribute to the increase in breathing during exercise.

- Available evidence indicates that the motor areas of the cerebral cortex simultaneously stimulate the motor neurons of the exercising muscles and the respiratory neurons in the medulla. The direct input from motor areas to the respiratory center is a major reason for the very rapid increase in breathing at the beginning of exercise. This feedforward mechanisms illustrates how homeostasis can result from other mechanisms besides negative feedback.
- Sensory receptors that respond to joint and muscle movement provide input that stimulates increased breathing during exercise. (This response can also be observed during passive movement of a person's limbs).
- During moderate exercise, sensory receptors that respond to blood levels of H⁺, O₂, CO₂, and epinephrine, as well as body temperature, may influence breathing.
- During intense exercise, anaerobic fermentation results in the production of lactic acid which reduces pH which can help to stimulate increased breathing during and after exercise.¹²

¹² Information about lactic acid production and energy metabolism during exercise is presented in the analysis and discussion activity, "How do muscles get the energy they need for athletic activity?" (available at http://serendipstudio.org/exchange/bioactivities/energyathlete).
These findings illustrate the important principle that even when experimental results are compatible with a hypothesis (e.g. the hypothesis that increased breathing rate and depth during exercise is due to negative feedback), it is important to consider possible alternative interpretations before concluding that the results support the hypothesis. The multiple reinforcing mechanisms that contribute to the regulation of breathing are typical of the redundancy observed in many biological regulatory systems.

Students will probably notice that the heart beats faster and stronger during exercise. During exercise the total amount of blood pumped per minute can increase as much as fourfold in an untrained person and eightfold in a trained athlete. Most of the increase in amount of blood pumped per minute goes to the active muscles; at rest ~20% of blood flow goes to skeletal muscles, whereas during vigorous exercise ~90% of blood flow goes to skeletal muscles. You may want to link student observations of faster and stronger heartbeats to the discussion in Section III of how the respiratory and circulatory systems cooperate to provide the O₂ needed for cellular respiration and remove the CO₂ produced by cellular respiration.

D. Helping Students to Improve Experimental Design and Methods

Before students answer question 26b, you may want to have them discuss basic methodological points such as:

- the importance of changing only the variable they are testing and controlling other variables in their experimental design
- the importance of standardizing their experimental methods, including their methods for recording changes in breathing
- the importance of replication (e.g., having each member of the group participate as a subject).

This is a good opportunity to improve your students’ abilities to plan and carry out an investigation, “…: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly.”

We have found it useful to check that each data sheet corresponds to the student group’s experimental design and clearly specifies what observations will be recorded.

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Templates for possible use in exercise experiments (see pages 14-15)

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1. **Injury.** A blood vessel is severed. Blood and blood components (e.g., erythrocytes, white blood cells, etc.) are leaking out of the breaks.

2. **Vascular spasm.** The smooth muscle in the vessel wall contracts near the injury point, reducing blood loss.

3. **Platelet plug formation.** Platelets are activated by chemicals released from the injury site and by contact with underlying collagen. The platelets become spiked and stick to each other and the wound site.

   - Initial platelets are activated by chemicals released from the injured cells and by contact with broken collagen.
   - Bound platelets release chemicals that activate and attract other platelets.

4. **Coagulation.** In coagulation, fibrinogen is converted to fibrin (see part b), which forms a mesh that traps more platelets and erythrocytes, producing a clot.

   - Platelets move toward source of chemical signals and bind. Platelet plug grows in size.
   - Fibrin strands secure platelets and erythrocytes, effectively plugging the break.

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(a) The general steps of clotting

(b) Fibrin synthesis cascade