Chapter 14

Exercise Physiology: the Response of Metabolic Rate to Physical Activity

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Introduction

This two-week lab exercise is used near the end of a 10-week course in animal physiology for second-year biology majors at the University of Chicago. Similar versions of the lab have been used in small introductory courses for non-majors at the University of Chicago and in an upper-level physiology course for biology majors at the University of California, Berkeley. The goals of this lab are to

- Help students relate physical concepts of energy, work, and power to metabolic rate and human physical activity
- Illustrate the connection between cellular processes of respiration and muscle contraction and systems-level processes of gas exchange and external work
- Provide a variety of possible investigations to allow students to be creative and follow their own interests in conceiving an experiment
- Develop students' abilities to collaborate in developing hypotheses, taking physiological measurements, analyzing data, and communicating results.

The first 3-hour lab period is devoted to learning to take physiological measurements, trying out the exercise equipment, and designing the independent investigation. Each group of 3-4 students designs an experiment based on one of the suggested questions or on one of their own. A one-page proposal is submitted to the instructor by each group a few days later so that any needed supplies can be obtained and the instructor can provide feedback before the experiment is performed. The second lab period is devoted to data collection and analysis. Students use a spreadsheet for calculations and statistical software to summarize data and to test hypotheses. The group submits a short lab report that includes their results and analysis. Each student individually writes a formal paper on either this lab or another investigative lab performed earlier. At the end of the semester, each group prepares an oral presentation on one of these labs for the class symposium.

This lab employs metabolic rate as the primary variable to measure the response of the human body to physical activity. Researchers in exercise physiology typically study metabolic rate using sophisticated computer-driven open spirometry systems that continuously measure oxygen
consumption and carbon dioxide production, calculate the parameters of interest, and graph them in real time (Brooks et al. 2000). For use in undergraduate instructional labs, these systems are generally too costly (ca. $20,000 US per station) and too “black-box,” with little student involvement in measurements or perception of what is being measured. We chose instead to use an older, open spirometry technique in which expired air is collected for a set time in a Douglas bag, which is processed later to measure oxygen content, carbon dioxide content, temperature, and volume. This batch processing allows three to six student groups to share one set of gas analyzers and volume meters, thus saving substantially on the cost. Air collection in a Douglas bag (actually a 120-liter meteorological balloon) dramatically illustrates the difference in minute volume between rest and exercise. The procedure requires good teamwork to collect and process the gas samples efficiently. The number of data points that can be obtained with the Douglas bag system is less than with an automated system, but students still obtain sufficient replication of several treatments to analyze statistically.

This lab evokes students’ curiosity about how their bodies work and gives them the freedom to pursue novel lines of inquiry. Depending on the time and equipment available, the lab can incorporate various other techniques, such as electromyography, electrocardiography, pulse oximetry, and measurement of lung volumes, to expand the range of possible investigations.

### Materials

#### For Each Group (3-4 students)
- Treadmill or cycle ergometer
- Wireless heart rate monitor
- Two-way non-rebreathing valve, assembled, with support that fits over head
- Large rubber band to hold valve onto support
- 20” spiral ribbed hose to carry air from two-way valve to Douglas bag
- Rubber meteorological balloon attached to 1 1/4” PVC ball valve with a hose clamp
- 60-ml syringe with small stopcock attached (no needle)
- Computer with Excel and StatView software
- Noseclip
- Stopwatch

#### For the Laboratory
- One or two dry gas meters, with 5” spiral ribbed hose on inlet port and hypodermic needle inserted in middle of upper hose end
- Digital thermometer with wire probe inserted into outlet port of dry gas meter and taped in place
- One or two oxygen and carbon dioxide analyzers
- Dessicator containing gas drier tubes of Drierite dessicant
- Spray bottle of tap water for dampening shirts beneath chest electrodes
- Cylinder of nitrogen gas to calibrate oxygen analyzer
- Cylinder of 5% CO₂, 20% O₂, and 75% N₂ to calibrate O₂ and CO₂ analyzers
- Ankle (cuff) weights, 2-lb, 5-lb., 10-lb.

### Notes for the Instructor

#### Sources of Materials

**Gas collection**

A two-way non-rebreathing valve is needed to allow the subject to inhale room air while directing exhaled air into the Douglas bag. We use the Hans Rudolph (7200 Wyandotte, Kansas City, MO 64114) medium valve (model 2600, $142.00 US) with a (optional) head support (model 2726, $264.00 US). One of us (TFC) has used the type of valve provided with the breath volume set
(CE-69-2646) from Carolina Biological Supply, though these tend to leak some air during slow breathing. These valves can be obtained in larger quantities from Hubbard Scientific (PO Box 2121, Fort Collins, CO 80522) for about $10 US each. We obtained 120-liter latex meteorological balloons (#22631, $81 US each), 1 1/2" I.D. plastic spiral tubing (#22263), rubber tubing ends (#22254), and tubing cement (#22977) from Collins Medical (220 Wood Road, Braintree, MA 02184). The Douglas bags were attached with a hose clamp to the outside of a 1 1/4" I.D. PVC ball valve available at hardware and plumbing supply stores. When shopping for ball valves, look for models in which the outside of each end is round rather than multifaceted to allow a good seal with the bag. The Collins rubber tubing ends friction-fit nicely on the outside of the non-rebreathing valve and the inside of the PVC ball valve. If the gas analyzer requires a constant flow of the sample air through the sensor, then a brass tube can be inserted into a hole drilled in the PVC ball valve to serve as a sampling port. A flexible tube and a syringe stopcock attached to the sampling port then allow the air from the Douglas bag to be pumped through the gas analyzer.

Gas analyzers

We use a Servomex (J&M Instrument Co. Inc., 2224 Industrial Drive, Unit A, Highland, IN) analyzer (model #1450, $8,000 US) with the oxygen and carbon dioxide cells mounted in series so that both gases can be read from a single 50-ml air sample pushed through manually from a syringe. Most other gas analyzers require a steady flow of gas through the sensor cell, which makes the sample processing a little more cumbersome. The measurement of carbon dioxide can be eliminated to reduce complexity or cost, as calculated values of oxygen consumption and metabolic rate are not greatly affected by changes in respiratory quotient. A less-expensive oxygen analyzer ($400 US) and carbon dioxide analyzer ($1200 US) are available from Qubit Systems, Inc. (4000 Bath Road, 2nd Floor, Kingston, Ontario, Canada · K7M 4Y4).

Volume Measurement:

We use a Harvard Apparatus (84 October Hill Rd., Holliston, MA 01746) dry gas meter (# AH 50-6164) available for $1,645 US. One of us (TFC) has used a similar Parkinson-Cowan gas meter but we are unaware of any current manufacturer.

Exercise Equipment:

The Monark Testing Ergometer, (model 828E, $1049 US), has long been the standard mechanical ergometer for research and has the advantage over newer electronic devices of easy calibration and sturdiness. Commercial treadmills at $5,000 US or more are ideal, but we have had good experience with some of the better home models, including the Pacemaster Pro Plus ($1,700 US). We use Polar wireless heart rate monitors (Pacer model, $69.00 US) available at many exercise equipment stores or from their web page (www.polarheartratemonitors.com).

Preparation and Cleanup

Removing water vapor

Most gas analyzers require the sample to be free of water vapor. We use a 12 cm length of flexible 1/2" I.D. tubing filled with Drierite indicating desiccant, with a tuft of glass wool and a syringe stopcock at each end. This is mounted on the gas analyzer inlet and changed by the instructor when half of the Drierite is saturated.

Care of Douglas bags

At end of lab, instructors or students detach bags from the PVC ball valves and turn them inside out to dry. When dirty or after 8-10 uses, the bag and the spiral plastic tubing are washed in mild detergent. Small holes in bags can be patched with tape, rubber cement, or bicycle-tube repair kits. To store the bags for more than 2 weeks, add talcum powder (1 teaspoon) to the dry bag and
shake to coat inside to prevent the rubber from sticking to itself. Before the next use, the bags must be turned inside out and thoroughly shaken outdoors to remove powder.

Two-Way Valves and Mouthpieces

These are cleaned and disinfected after every use. Completely disassemble the valves, wash them by hand in mild dishwashing detergent, and rinse with tap water. Then soak all parts in glutaraldehyde disinfectant (Cidex or Glutarex) for 15 minutes and rinse in tap water before reassembling.

Safety Issues

Because this lab involves physical activity and physiological measurements on humans, it may need to be approved by the institutional review board at your school that authorizes all experimentation on human subjects. (We obtained approval for our protocol for some years, but later were told by our board that we were exempt.) Our principal safety concerns are with overexertion by sedentary persons or by those with medical problems and with disease transmission by shared mouthpieces and valves. We encourage students who do not exercise regularly to choose less strenuous activities for their investigations. The guideline of keeping the heart rate below 75% of its theoretical maximum to keep metabolism aerobic helps avoid this problem. Completely disassembling the valves and soaking all the parts and mouthpieces in a glutaraldehyde disinfectant after washing can prevent disease transmission.

The open-ended nature of the independent investigation creates the possibility of an unanticipated safety hazard in a non-standard procedure proposed by students. The lab instructor must review all student proposals with this concern foremost and consult with a qualified faculty member or supervisor about any unusual proposals. Careful supervision of the students during lab is necessary to prevent high-risk behaviors and enforce mature, careful treatment of experimental subjects.

Teaching Suggestions

When demonstrating the procedure to students, foster teamwork by pointing out the roles of each group member. The recorder can time the trials, tell the subject when to do what, and record data in the notebook as it is taken. The air collector should hold the Douglas bag and hose, taking care to support its full weight, and turn the valve on and off as directed by the recorder. A fourth group member could monitor the heart rate or enter the data into a spreadsheet. To get reliable, consistent, data, the subject must be free to focus on doing the appropriate activity without distractions, i.e., the group should treat the subject well. He or she should not be worrying about the timing, trying to hold up the Douglas bag, or be stressed in any way. When a trial is finished, someone should assist the subject with removing the apparatus, including holding a paper towel under the mouthpiece to catch the possibly embarrassing but inevitable drool.

Errors to Anticipate:

1. If students attach the Douglas bag hose to the inlet port of the two-way non-rebreathing valve rather than the outlet port, the subject will be trying to inhale from the empty bag. This is a 'self-correcting' problem, but it helps if the valve is assembled so that the outlet port is always on the right.
2. Students are sometimes confused about operating the stopcock on the syringe, which leads them to direct their precious air sample out into the room rather than through the gas analyzer.
3. When vigorously squeezing air from the Douglas bag through the dry gas meter, it is easy to dislodge the PVC ball valve from the hose on the meter. Have one student hold the valve and hose together while
another other squeezes the bag. The “squeezer” can stop partway through to remove a sample with the syringe, or a third student can take the sample during the squeezing.

4. To detect problems with sample handling or calibration of the gas analyzer, students should “spot check” the values they record from the gas analyzer by noting whether the %O\(_2\) and %CO\(_2\) sum to about 20, the approximate percentage of O\(_2\) and CO\(_2\) in the atmosphere.

5. Even if gases have been analyzed properly, it is not uncommon in a resting trial to see a respiratory quotient greater than one, meaning that more CO\(_2\) is added to the air than O\(_2\) is removed. This is due not to some bizarre metabolic pathway but rather to an artifact of hyperventilation. If a student breathes more deeply or rapidly than “needed” during the trial, this hyperventilation drives off more CO\(_2\) from bicarbonate in the plasma. To prevent hyperventilation, the subject should make an effort before inserting the mouthpiece to become aware of her natural rhythm and depth of breathing at rest, and then consciously maintain this during the trial. Hyperventilation is less of a problem during exercise trials as long as the subject has had some time to become accustomed to wearing the apparatus.

**Calculation of Metabolic Parameters**

To expedite the calculation of parameters such as minute volume, oxygen consumption, and metabolic rate, students are given an Excel template file (available from the authors) containing most of the equations (Fig. 1). When students enter their raw data in the shaded cells, the parameters in the unshaded cells are calculated automatically. Students later add additional rows to record or calculate other variables appropriate for their investigations. Two spreadsheets are provided for use with the treadmill and the ergometer, respectively. Equations and conversion factors for these calculations were obtained from McArdle et al. (1996).

![Figure 1. Excel spreadsheet used by students to calculate metabolic parameters.](http://www.zoo.utoronto.ca/able/proc/contents.htm)

**Communicating the Results of the Experiment**

Because every group performs a different, often novel experiment, this is an ideal lab exercise to use as the topic of a formal paper and oral presentation. At the ABLE workshop, participants were provided with a handout used at the University of Chicago on writing the research paper. One of us (TFC) expanded this material to create a web site to use in a mammalian physiology course at the University of California, Berkeley. These pages are now posted on the ABLE web site (http://www.zoo.utoronto.ca/able/proc/contents.htm), linked to the table of contents for this Proceedings volume. At Berkeley, students submit a draft of the paper to a mock journal, *Physiological Advances and Retreats*, following specific instructions for authors patterned after a real journal. The lab instructor, acting as a journal editor, sends the draft out anonymously to two
students in the same lab section. These peer reviewers follow specific guidelines to write a formal review, which is forwarded anonymously to the author. The author submits a final draft, after which all drafts and reviews are graded by the instructor.

At both Berkeley and Chicago, students prepare oral presentations with PowerPoint slide shows to report to the lab section on their investigations. This symposium is run like a contributed paper session at a scientific meeting, complete with a program listing titles and abstracts. Typically, each student in the group presents a section of the talk to allow all to participate. The diversity of projects prevents the presentations from being repetitive and allows students to learn from each other's investigations.

Examples of Student Creativity

Many students prefer to come up with a novel question that pertains to their personal experience or interest rather than picking one of the investigation ideas suggested by the instructor. We encourage such creativity and try to come up with the necessary supplemental materials as long as the proposed experiment poses no undue hazard. Here are some of the creative exercises performed by our students:

1. What is the cost of being a knight? Students researched the approximate weight of a suit of armor and used cuff weights attached to torso, arms, and legs to simulate the energetic requirements of walking in a suit of armor.

2. How does shoe type affect the cost of locomotion? Students walked or ran in running shoes, hiking boots, high-heeled women's dress shoes, or no shoes and measured the energetic cost per kilometer.

3. What good are joints? Students restrained the flexibility of various joints used in walking through the ample application of duct tape and splints made of cardboard or wood and measured metabolic rate while walking on a treadmill.

4. What is the cost of skipping? Although it was difficult to 'skip' on a treadmill AND collect exhaled breath, they succeeded in determining that skipping on a downhill incline was more efficient than walking downhill.

5. How does environmental temperature affect heart rate and metabolic rate? Students measured resting heart rate and metabolic rate on a subject in a walk-in cooler and freezer and while overly clothed in the lab.

6. How does skin temperature change with exercise? A liquid crystal temperature band (Carolina Biological Supply, CE-69-5676, $11.50 US) can be attached to various body parts during exercise.

7. What triggers the dive reflex? Many mammals exhibit a diving reflex, including a reduction in heart rate, reduced cardiac output, and reduced blood flow to the periphery. Students who submerge their heads in a dishpan of water and observe a substantial drop in heart rate. By varying water temperature, using a snorkel while submerged, and holding their breath out of the water, they’ve been able to tease apart the relative influences of several factors on this response. This exercise does not actually use the oxygen consumption measurements, but the heart rate response can be supplemented with measurements of blood pressure with a sphygmomanometer and functional oxygen saturation of arterial hemoglobin with a pulse oximeter.

8. How does anatomical dead space affect breathing? Anatomical dead space refers to the volume of air contained in the non-gas-exchanging parts of the respiratory system (mouth, bronchi, etc.), which is essentially wasted ventilation with each breath. In some diseased lungs (and to a lesser extent in healthy lungs), some alveoli are excessively ventilated, or receive inadequate blood flow. This mismatch is termed ventilation-perfusion inequality, and could be thought of as wasted ventilation. Although it is difficult to experimentally manipulate the ventilation-perfusion inequality, students have varied the anatomical dead space by adding tubes of different lengths between the mouthpiece and the non-
rebreathing valve. This affects % oxygen and carbon dioxide in the expired air as well as minute volume, and maximum VO₂.

Student Outline

Cautions!

- At least one person in your lab group will serve as the experimental subject. If you volunteer, then you should wear shoes and loose-fitting clothing suitable for exercising indoors (e.g., t-shirt, shorts, cotton socks, running shoes). The exercise may include walking and running on a treadmill and riding a stationary bike.
- If you are pregnant or have any medical condition, such as heart disease, asthma, or a leg injury, that might preclude strenuous exercise in the form of walking, running, and pedaling a bicycle, please allow another member of your group to volunteer to be the experimental subject.
- If at any time during your exercise trials, you find the exercise abnormally difficult or if you feel physical discomfort, discontinue the activity.

Overview

By measuring how quickly a person consumes oxygen and produces carbon dioxide, we can infer the rate of energy expenditure and the mix of fats and carbohydrates metabolized. In this lab, you will use this technique on a member of your group to investigate some aspect of the energetics of locomotion and the physiological responses to varying levels and types of exercise. You could use a bicycle ergometer to measure the power output of your leg muscles at various combinations of pedaling rate and resistance. This power output divided by the rate of energy expenditure gives an estimate of the efficiency of your muscles. Another option is to use a treadmill with variable speed and inclination to examine the energetic costs of different gaits, of different slopes, and of carrying added weights. You may come up with other kinds of questions to investigate with this equipment as well. In the first lab period, you will learn to collect and analyze data on gas exchange and heart rate as you obtain some preliminary data on your experimental subject. By the end of this period, your group should have discussed with your instructor an idea for your independent investigation and started your proposal, which is due a few days later. After revising your experimental design, you have a second lab period to complete data collection and analysis.

Background

When you eat food, its sugars, fats, and amino acids from its proteins serve a variety of energy-requiring tasks within your body before its stored energy is released ultimately as heat, and its carbon and hydrogen are combined with oxygen and released as water and carbon dioxide.

Energy

Like most animals, we obtain nearly all the energy we need from the food we ingest. The chemical energy stored in the bonds of these nutrients is made available for a variety of energy-requiring tasks within our cells. Of the food that we eat, some cannot be digested and absorbed, and is lost in our feces (Figure 2). The nutrients that are absorbed in our intestine, including amino acids, carbohydrates, and fats, may be stored for later use or used immediately to perform various tasks. Biological tasks that require energy include (1) the mechanical work of contracting muscles (e.g. contraction of the diaphragm to draw air into our lungs), (2) chemical synthesis of molecules needed by our cells (e.g. structural proteins, enzymes), and (3) transport of molecules across membranes against concentration gradients. Many of these tasks can be considered “internal work” needed for maintenance and growth of our body. However, some of the food energy is used to do work outside our bodies such as lifting objects and running, what we might consider “external work” (these definitions of internal and external work used in your textbook do not correspond to the physicist's definition of work, hence the quotation marks).
Energy is most typically defined as the capacity to do work and is measured in the same units as work. Recall that work is expressed with the S.I. unit the joule (equivalent to a newton meter), and can be represented by the relationship

\[ Work = Force \times Distance \]
The unit of energy more commonly seen in nutritional and metabolic studies is the calorie, which equals 4.2 joules. One potential source of confusion is the use in the nutrition literature and on food packaging of the Calorie (with a capital "C"!) to represent 1000 calories (with a lower case "c"!). To avoid this problem, we will consistently use the term kilocalorie (kcal) whenever we mean 1000 calories.

Each form of energy, including chemical, mechanical, electrical, and heat, can be converted to each other form, but with one peculiar asymmetry. Heat, the kinetic energy of the random motion of molecules, is the least generally usable form of energy and the form to which all other forms tend to degrade. According to the second law of thermodynamics, each form can be converted entirely to heat energy, but no form of energy can be transferred to another form without at least part of it ending up as heat. For instance, the chemical energy in coal can be transferred to electrical energy in a generating plant. But even in a modern generator, only about 35% of the energy released by the burning of coal ends up as electrical energy. The other 65% is degraded to heat, which, from the point of view of the utility, is a waste product. You may use that electricity to run a blender, where the motor converts part of the energy to mechanical energy of the blade and releases the rest as heat. The motion imparted by the blade to your milkshake is eventually transferred to the molecular level and becomes heat. Thus, all of the chemical energy originally contained in the coal is eventually degraded to heat. Living organisms operate under the same thermodynamic principles as the power plant and blender, but suffer the additional constraint of the temperature sensitivity of biological materials. We cannot literally burn our fuels at the extremely high temperatures of a power plant to improve the efficiency of transfer.

What do these characteristics of energy mean for organisms? Every transfer of energy in our bodies, even from one chemical form to another, will entail some "loss" of that energy as heat. Ultimately all of the chemical energy we ingest will end up as heat somewhere. That heat may be useful for maintaining our body temperature in a cold environment or it may be a harmful waste product to be disposed of quickly if we are exercising hard on a hot day. In either case, the heat dissipated in energy transfer represents a loss of energy available to perform work. One option for your investigation is to find out just how efficiently your body can convert stored chemical energy into mechanical energy available to do external work in locomotion.

Metabolic Rate

An organism's rate of energy expenditure, or metabolic rate, provides a useful index of its activity level. Just as energy shares the units of work, metabolic rate shares the units of power. Power is the rate of doing work, or

\[ \text{Power} = \frac{\text{Work}}{\text{Time}} \]

The S.I. unit is the watt (equivalent to a joule sec). Other commonly used units include kcal/min (1 watt = 0.0143 kcal/min) and horsepower (1 hp = 746 watts = 10.67 kcal/min). Physiologists who use oxygen consumption as a measure of metabolic rate often express metabolic rate as mL O₂/min or L O₂/min rather than converting oxygen measurements into energetic units.

Metabolic rate can be assessed either directly by measuring the energy released by an organism or by various indirect means:

- **Direct Calorimetry:** In principle, the simplest way to measure metabolic rate is to take advantage of the fact that all energy consumed by an organism ends up as heat. By enclosing an organism inside a thermally insulated chamber (calorimeter) and monitoring the temperature rise inside, one can measure directly the rate of energy expenditure. While this method is simple in theory, chambers large enough for humans are very expensive and do not allow the freedom of movement necessary to study many physical activities. When other, less direct methods of calorimetry are developed, they are tested against direct calorimeters to verify their accuracy.

- **Indirect Calorimetry:** The preferred alternative to direct calorimetry is to use oxygen consumption as a proxy for heat production. The amount of energy released is proportional to the amount of oxygen consumed in cellular respiration. There are two critical assumptions that must be met for this method to be accurate. First, the organism must use primarily aerobic respiration. This occurs normally in humans at rest and during moderate exercise. However, in heavier exercise, humans use anaerobic metabolic pathways such as glycolysis and fermentation, which provide useful energy in the form of ATP without consuming oxygen. If anaerobic pathways are used, oxygen consumption underestimates the true metabolic rate. In order to avoid this problem, a subject in average physical
condition should not exceed 75% of the maximum heart rate. The maximum heart rate can be roughly estimated by subtracting the subject’s age from 220 (e.g., max. heart rate for a 30 year-old is 220 - 30 = 190). Trained endurance athletes can exercise at higher heart rates without using substantial anaerobic metabolism.

The second critical assumption is that the subject has reached a steady state in which oxygen uptake exactly balances energy expenditure. In the first few minutes after starting exercise, anaerobic pathways are used while the oxygen transport system and aerobic metabolic pathways gear up. When exercise ceases, there is a period of oxygen uptake in excess of current energy expenditures. For this reason, oxygen uptake measurements should be taken after an organism has been exercising at a constant rate for at least several minutes. Only under this steady-state condition is the rate of oxygen uptake a good indicator of metabolic rate.

In order for you to meet these assumptions, you should (1) exercise at a rate that does not push your heart rate over 75% of its theoretical maximum (to minimize anaerobic metabolism), and (2) maintain a constant rate of exercise for 3-4 minutes before gas collection as well as during gas collection (to assure a steady state condition). However, you may prefer to violate these conditions if you wish to look at physiological responses to more strenuous exercise (violating assumption 1) or to examine what happens during the transition from rest to exercise (violating assumption 2). This is fine as long as you don’t claim your oxygen consumption to represent total metabolic rate.

To measure oxygen uptake, you will collect your expired air for a period of two or more minutes in a bag, called a Douglas bag. By dividing the total volume collected by the number of minutes, you obtain the minute volume expired, \( \dot{V}_E \), in liters per minute. You will also measure the concentration of oxygen and carbon dioxide in the expired air and use this information to calculate your rate of oxygen uptake, \( \dot{V}_{O_2} \), and rate of carbon dioxide production, \( \dot{V}_{CO_2} \).

The Respiratory Quotient: The correspondence of oxygen uptake and energy expenditure varies somewhat depending on the nutrient that is being metabolized by our cells. This is because carbohydrates, fats, and proteins differ in the amounts of oxygen needed to oxidize their carbon and hydrogen atoms. We cannot determine the amounts of each nutrient metabolized by simply analyzing the diet, since interconversion of the nutrients occurs constantly in our bodies (e.g. sugar \( \longrightarrow \) fat, etc.). However, by measuring carbon dioxide production, \( \dot{V}_{CO_2} \), along with oxygen consumption, \( \dot{V}_{O_2} \), we can estimate the amount of each nutrient utilized. To do this, we begin by calculating the ratio of carbon dioxide produced to oxygen consumed; this ratio is called the respiratory quotient, \( RQ \).

\[
RQ = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}
\]

Actually, when these measurements are taken from expired air rather than from the metabolizing cells directly, this is called the respiratory exchange ratio. Under steady-state conditions of exercise, the respiratory exchange ratio is a very good estimate of the respiratory quotient. You might want to consider how and why the respiratory exchange ratio might deviate from the respiratory quotient under various conditions, such as hyperventilation.

To understand why the respiratory quotient varies with the type of nutrient oxidized, consider the balanced chemical equations for complete oxidative respiration. Carbohydrates, of the general formula \((CH_2O)_n\), oxidize completely to carbon dioxide and water as shown below for glucose:

\[
C_6H_{12}O_6 + 6 O_2 \longrightarrow 6 CO_2 + 6 H_2O
\]
Since one molecule of O₂ gas occupies the same volume as one molecule of CO₂ gas, the RQ for carbohydrates is

\[
RQ = \frac{6 \text{ volumes of CO}_2}{6 \text{ volumes of O}_2} = 1.0
\]

The oxidation of a fat, such as tripalmitin, requires that considerably more oxygen be added than carbon dioxide is released:

\[
2 \text{C}_{51}\text{H}_{98}\text{O}_6 + 145 \text{O}_2 \rightarrow 102 \text{CO}_2 + 98 \text{H}_2\text{O}
\]

In this case the RQ is

\[
RQ = \frac{102 \text{ volumes of CO}_2}{145 \text{ volumes of O}_2} = 0.703
\]

The RQs for various fats differ slightly, but the average is 0.71. For proteins, the RQ calculation is complicated by the use of some of the carbons and oxygens to combine with nitrogen to form the waste product, urea, but the RQ for proteins turns out to be intermediate between that of carbohydrates and fats, averaging 0.82. In order to determine very precisely the mix of carbohydrates, fats, and proteins metabolized during a given period, it is necessary to measure urinary nitrogen output as well as RQ. However, the amount of protein metabolized during exercise is minimal provided the person has neither engaged in a prolonged fast nor consumed an excess amount of protein in the last meal. In this lab exercise we will ignore the contribution of protein to metabolism and use the RQ to estimate the amounts of fats and carbohydrates utilized. An RQ near 1.0 suggests that carbohydrates are the primary energy source utilized, whereas an RQ of to 0.71 indicates the exclusive use of fats. Intermediate values of RQ indicate that a mixture of fats and carbohydrates is being metabolized. The exact percentages of kilocalories derived from each source can be calculated and is listed in a table, below, in the section on calculations.

To calculate the metabolic rate, \( V_{O_2} \) can be converted from rate of oxygen consumption to rate of energy expenditure by use of the respiratory quotient and the known caloric values of carbohydrates (4.1 kcal per gram) and fats (9.3 kcal per gram).

**Efficiency**

Efficiency is generally an output of something divided by an input. It is a dimensionless index (expressed as a percentage), so the units of the numerator and denominator must be identical. For instance, a coal-fired power plant might have an efficiency of 30%, meaning that for every 100 watts of coal burned, 30 watts of electricity are produced. If you wish to know how efficiently your muscles convert the energy of nutrients into energy that actually accomplishes work, then the input would be metabolic rate (in kcal/min) and the output would be the power output measured on the ergometer (in kcal/min).

One way to measure power, or rate of doing work, is with a cycle ergometer. It works by measuring two parameters of your efforts to pedal it: (1) the resistance force (quantified by the deflection of a weighted pendulum attached to a friction belt that rubs on the flywheel), and (2) the pedaling rate (measured in revolutions per minute, rpm). You calculate the power output by multiplying the velocity of pedaling by the resistance force. These two constituents of power can be varied independently, and have different implications for muscle physiology. The resistance is
proportional to the force your muscles must develop, whereas the pedaling rate is proportional to the velocity of shortening of your muscles.

Don’t confuse the cycle ergometer with a bicycle. There is no way to measure your speed or distance with the ergometer; it measures power instead. However, it can help you to answer questions about the efficiency of your muscles and about the relative merits of pedaling rapidly against a light load vs. pedaling slowly against a heavy load.

One further consideration: what metabolic costs do you want to include in your efficiency calculations? If you use the metabolic rate measured while pedaling as your denominator, then you are including the costs of many other bodily functions (digestion, excretion, biosynthesis, etc.) besides the contraction of skeletal muscles involved in pedaling. Depending on your goals and questions, this may or may not be desirable. Physiologists use several methods to calculate efficiency, including:

1. Gross Efficiency = (power output / metabolic rate) x 100
2. Net Efficiency = P \(/ (MR_{pedaling} - MR_{resting})\) x 100
3. Work Efficiency = P \(/ (MR_{pedaling with load} - MR_{pedaling with no load})\) x 100
4. Delta Efficiency = \(\left[(P_2 - P_1)/(MR_2-MR_1)\right]\) x 100

How can you measure work rate or power when you are walking or running? This is trickier than it might seem at first. Recall that power is defined as a velocity moved against a force. When you walk or run on the level, most of your effort is directed at moving your body vertically against the force of gravity as your center of mass moves up and down with each step. Yet our measure of velocity is normally horizontal, a direction that faces little opposing force (assuming you’re not walking into a strong wind). Therefore the most obvious measures of walking, distance or speed, don’t give us a useful measure of work performed, meaning it is virtually impossible (meaning, don’t even try) to do the calculations for efficiency when walking horizontally on a treadmill. Keep this in mind when designing your experiment. One way to circumvent this problem is to measure the vertical component of velocity as you ascend stairs or walk on an inclined treadmill. In this case, you are moving at a known velocity vertically against the force of gravity.

Cost of Locomotion

For many physiological questions, the energetic cost of locomotion is of more interest than the work rate or efficiency of that locomotion. This cost can be expressed as energy used per distance (e.g., kcal/km) or oxygen used per distance (e.g., Liters O2/km) by dividing the metabolic rate by the velocity of motion. The cost of locomotion can be converted to a mass-specific basis by dividing the cost by the person’s mass. As in the case of efficiency calculations, you should think carefully about the metabolic costs you want to include or exclude from your calculations.

The Oxygen Delivery System

As metabolic rate increases with exercise, the demand for more oxygen and the need to dispose of excess carbon dioxide lead to physiological changes in the gas exchange and gas transport mechanisms. In addition to studying variation in metabolic rate, you could examine the strategies used by the respiratory and circulatory systems to take up, deliver, and dispose of more gases.

The rise in minute volume, \(\dot{V}_E\), with increased exercise could be accomplished by increasing the volume of each breath, the tidal volume, increasing the number of breaths per minute, the breath frequency, or by some combination of the two. By counting breaths as you collect the expired air in the Douglas bag, you can calculate tidal volume by using the relationship
Another option is to examine the relationship of heart rate to metabolic rate for various activities.

**The Investigation**

The equipment provided in lab will allow you to measure your metabolic rate, breath frequency, tidal volume, and heart rate while resting, pedaling the cycle ergometer, or walking or running on the treadmill. The treadmill can be used on the level or inclined to simulate walking uphill or downhill. In addition, weights are available that can be carried in a backpack or strapped to the waist, legs, or arms. The methods presented above provide various ways of measuring efficiency and the cost of locomotion. Given these facilities and techniques, there are many questions you could address, including those listed below.

**Possible Questions:**

1. How efficiently do a person's muscles convert chemical energy into kinetic energy available to do work?
2. Does efficiency vary with the rate of work (power output) of the muscles?
3. Does efficiency vary with the velocity of shortening of the muscles?
4. Does efficiency vary with the load on the muscles?
5. If power output is held constant, does efficiency vary with load or velocity of shortening of the muscles?
6. How does the energetic cost of running vary with speed?
7. How does the energetic cost of walking vary with speed?
8. How do the costs of walking and running compare at various speeds? Are there certain speeds and gaits that minimize energetic costs of locomotion?
9. What is the energetic cost of carrying extra weight and how does it vary with the amount of weight carried? (Weights are available that can be carried in a pack or worn around waist or any part of leg or arm.)
10. Does the energetic cost of carrying extra weight vary according to where on the body it is carried?
11. How does the energetic cost of walking vary with the steepness of a hill?
12. How does heart rate vary with metabolic rate? Does the type of activity affect heart rate?
13. How do breath frequency and tidal volume vary with metabolic rate?
14. Does the cost of walking or running vary with stride length or frequency? Is there an optimal stride length or frequency at each speed?
15. Does the relationship between speed and the length and frequency of strides vary among individuals? Is it related to height, leg length, or weight?

**Materials and Methods**

*Using the Heart Rate Monitor*

Attach the transmitter to the electrodes on the chest strap by snapping it on. The two snaps serve as electrical contacts between the two plastic electrodes and the transmitter. Fasten the chest strap over your shirt around the lower part of your rib cage with the electrodes centered on the front of your chest. The shirt material must be wetted to assure electrical contact with your skin. The electrical activity of your heart, picked up by the electrodes, is sent by the transmitter via a radio signal to a watch-like receiving unit fastened to the handlebar of the exercise equipment you are using. The receiver should begin displaying a pulse when you approach within a few feet. If not, press the small button on the left of the receiving unit to turn it on. If you aren't getting a pulse rate (the little heart symbol should show a parenthesis with each beat), then check to make sure the electrodes are damp and haven't slipped down too low on your chest.
If you intend to use oxygen consumption as a measure of total metabolic rate, you must be exercising in an aerobic steady-state condition. If you work too hard, then some of the ATP used may be generated by anaerobic glycolysis rather than aerobic respiration, in which case oxygen consumption will underestimate your true metabolic rate. To avoid this problem, you should keep your heart rate below 75% of its maximum. Calculate your theoretical maximum heart rate by subtracting your age from 220 (e.g., max. heart rate for a 30 year-old is 220 - 30 = 190). When you choose exercise rates on the bicycle ergometer or treadmill, make sure your heart rate does not go above 75% of this theoretical maximum if you want an accurate estimate of metabolic rate.

**Measuring Metabolic Rate**

A. **Preparing for the gas collection:**

1. Obtain a two-way valve, an acrylic device with three ports, and a slender vial protruding from a central chamber. Attach a clean mouthpiece to the center white port. Attach a spiral-reinforced hose to the transparent port. Hold the valve by the slender transparent vial (which functions as a saliva trap) and bite down on the mouthpiece. When you inhale through the mouthpiece, fresh room air enters through a one-way valve in the white port on the left. When you exhale, the expired air enters the hose through another one-way valve.

2. Obtain a Douglas bag attached to a large valve. Open the valve and empty the bag of air by rolling it up.

3. Begin the exercise activity (or resting if measuring resting metabolic rate) and breathe as naturally as possible into the mouthpiece without the Douglas bag attached. Try not to hyperventilate or control the frequency or depth of your breathing. Continue this activity for at least four minutes at the same pace while your classmates monitor your pulse. This period of continuous activity at a constant pace should allow you to achieve a steady state where the oxygen consumption is exactly meeting the metabolic demands of current activity. Your pulse and breath rate should be constant before you begin collecting the expired air. If you wish, you may begin the four-minute period without the mouthpiece inserted as long as you insert it at least one minute before the bag is attached.

B. **Collecting the gas:**

In most trials you will collect the expired air for exactly two minutes, although for the resting trial you should use a longer interval, say three minutes, to get enough air to measure accurately.

4. To begin collecting the gas, one member of the group inserts the free end of the hose into the open valve on the Douglas bag and continues to hold the bag so that it can fill freely and does not burden the subject. The other student measures the elapsed time with a stopwatch and records the pulse rate. The experimental subject should be free to concentrate on maintaining a constant exercise rate and relaxed breathing.

5. At the end of the collection interval, remove the hose from the Douglas bag and simultaneously close the valve to trap the air. The experimental subject should slow down the rate of exercise gradually, especially if exercising hard. The closed Douglas bag is now ready to be carried over to the volume and gas analysis stations.

C. **Analyzing the gas:**

6. At the dry gas meter, attach the Douglas bag valve via a short length of hose to the inlet port of the meter. Press the reset button to zero the reading on the digital display.

7. Attach a 50-mL sampling syringe to the sampling needle in the hose and open the valve on the syringe.

8. Open the valve on the Douglas bag and gently squeeze the bag to force the air through the meter.

9. As you continue to squeeze air out of the bag, collect a 50-mL sample of the air in the syringe. Once the syringe is full, close its valve and remove the syringe for later gas analysis. As the bag gets close to empty, roll it up to force the remaining air out.
10. As the Douglas bag empties, monitor the temperature of the exhaust air on the electronic thermocouple thermometer (the sensor is in the outlet port of the gas meter). When the bag is empty, record the total volume collected and the maximum temperature of the air and disconnect the bag from the hose.

11. At the gas analyzer, attach your sample syringe to the tube of blue-colored Drierite on the inlet port of the analyzer. Open the valve on the syringe and expel the air sample gently and steadily into the gas analyzer. When the reading has stabilized, record the % oxygen and % carbon dioxide from the displays. The purpose of the Drierite is to remove all water vapor from your gas sample before it enters the analyzer.

**Operating the Cycle Ergometer**

Adjust the seat on the ergometer to a comfortable height (knee slightly bent at lowest pedal position). For each work rate, you will need to choose a combination of pedal revolutions per minute (rpm) and a resistance setting. As you pedal, you can view the pedal rpm on the digital display. The resistance, in kiloponds, is visible through a window on the panel. This unconventional unit, the kilopond (kp), is chosen for convenience such that the power output, in watts, is equal to the resistance, in kp, times the pedaling rate, in rpm. This is analogous to calculating power as a force times a velocity. Note that you can achieve a given power output at a variety of combinations of resistance and pedaling speed (e.g., 100 watts can be achieved by pedaling 100 rpm against a resistance of 1 kp, 67 rpm against 1.5 kp, or 50 rpm against 2 kp). The resistance to pedaling is adjusted with the blue wheel at the bottom of the panel. Turn this wheel while you are pedaling at a constant rpm until the desired resistance is displayed in the window (and by the pendulum on the side of the machine). The power reading measures the rate of work to deflect the pendulum on the side of the machine. In addition to this power, you are also fighting some friction of internal bearings and the chain drive. To get a more accurate measurement of total power output, increase the calculated power by 9% to reflect these frictional losses. When you have finished a trial, pedal more slowly, or lower the resistance to “cool down” for a few minutes before stopping.

**Operating the Treadmill**

1. Stand on the two metal footrests that flank the belt while starting the treadmill. Do not stand on the belt until it has reached a slow walking speed (to avoid damage to the motor).
2. Fasten the clip on the end of the key cord to your clothing and insert the magnetic key into the recess on the control panel. This turns the power on. The magnetic key is a safety feature that will turn off the treadmill if you fall or pull on the cord. Please use this to stop the treadmill only in an emergency, as it causes excessive wear.
3. Set the time to 30 minutes by pressing the MINx10 button.
4. Set the speed to the desired level (in mph) by pressing the FASTER button.
5. While standing on the metal footrests, press START/STOP to start the belt moving.
6. Once the belt has reached a slow walking speed, step on the belt and walk to maintain your position. The belt gradually speeds up to the set speed; you can tell that the set speed has been reached when the decimal point in the speed readout stops blinking.
7. You can adjust the speed at any time by pressing the FASTER and SLOWER buttons.
8. To stop the treadmill, press START/STOP.

**Calculations**

Most of the calculations will be performed using a spreadsheet. After you enter the raw data you collect in each trial, most of the values shown below will be calculated automatically. You should read this section to understand what the variables mean and how they are obtained from the raw data. Additional calculations will be necessary to determine other variables of interest, depending on your experiment, such as efficiency, cost of locomotion, etc. To calculate these, you may enter the appropriate equations directly into the spreadsheet.

**Minute Volume, $V_{E}$**

Measuring gases by volume is problematic. If your sample warms up, the volume increases. Increase the pressure, and it shrinks in volume. Dry it out (remove water vapor), and the volume decreases. To get around this problem when comparing gas volumes measured under different conditions, physiologists convert
volumes measured under ambient conditions, called ATPS (Ambient Temperature, ambient Pressure, Saturated with water) to standard conditions, STPD (Standard Temperature, 0°C, Pressure 760 mm Hg, Dry). We will assume that our volumes were measured at standard atmospheric pressure, ignoring slight variations caused by weather patterns. To obtain $\dot{V}_{E, STPD}$ corrected for temperature and dryness, multiply your measured minute volume, $\dot{V}_{E, ATPS}$, by the factor in the table below corresponding to the ambient temperature that you measured at the dry gas meter.

$$\dot{V}_{E, STPD} = \dot{V}_{E, ATPS} \times \text{Correction Factor}$$

### Table 1. Conversion factors for standardizing measured air volumes to standard conditions.

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<th>Correction Factor</th>
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### Calculating Oxygen Consumption, $\dot{V}_{O_2}$

Oxygen consumption represents the difference in oxygen concentration of the inspired air and expired air multiplied by the total volume of air breathed. However, the total volume inspired is often more than the volume expired, even after correcting for temperature, pressure, and dryness, because more oxygen is removed by the lungs than carbon dioxide is released (if the respiratory quotient is less than 1.0). To correct for this discrepancy, we incorporate a term into the equation for oxygen consumption to account for the different concentrations of nitrogen in the inspired and expired air. (Nitrogen, an inert gas in respiration, increases in concentration in the expired air only because not all of the oxygen molecules lost have been replaced by carbon dioxide.)

$$\dot{V}_{O_2} = \dot{V}_E \left[ \frac{\% N_{2E}}{\% N_{2I}} \times \% O_{2I} \right] - \% O_{2E}$$

For inspired air: $\% O_{2I} = 20.93\%$

$\% N_{2I} = 79.04\%$

For expired air: $\% O_{2E}$ was measured with the gas analyzer

$\% N_{2E} = 100\% - \% O_{2E} - \% CO_{2E}$

$\% CO_{2E}$ was measured with the gas analyzer

The rate of oxygen consumption, in liters per minute, then simplifies to

$$\dot{V}_E \left[ (100 - \% O_{2E} - \% CO_{2E}) \times 0.265 \right] - \% O_{2E}$$
Use this equation to calculate $\dot{V}_{O_2}$.

**Calculating Carbon Dioxide Production, $\dot{V}_{CO_2}$**

Carbon dioxide production can be obtained by multiplying the % CO$_2$ you measured in the expired air by the minute volume of expired air (corrected to STPD). We can safely ignore the tiny amount of carbon dioxide in the inspired air (0.03%), which is below the level of detection of our gas analyzer. The equation, then, is

$$\dot{V}_{CO_2} = \frac{\dot{V}_E \times \%CO_2E}{100}$$

**Using Respiratory Quotient, RQ, to Calculate Metabolic Rate**

The respiratory quotient is the ratio of carbon dioxide production to oxygen consumption:

$$RQ = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}$$

This index allows us to convert the $\dot{V}_{O_2}$ to energy units and estimate the relative contributions of fat and carbohydrate to the measured metabolic rate using Table 2.

To convert $\dot{V}_{O_2}$ to metabolic rate (MR), or energy expenditure in kilocalories per minute, multiply $\dot{V}_{O_2}$ by the conversion factor listed under "kcal/liter O$_2$" for the RQ you calculated.

$$MR = \dot{V}_{O_2} \times \text{(kcal/liter O$_2$)}$$

To estimate the amounts of carbohydrate and fat metabolized during a given period of activity, use the appropriate conversion factors from the last two columns.

- **Carbohydrate used** = $\dot{V}_{O_2} \times \text{(time in minutes)} \times \text{(grams CH}_2\text{O per liter O}_2)$
- **Fat used** = $\dot{V}_{O_2} \times \text{(time in minutes)} \times \text{(grams fat per liter O}_2)$
Table 2. Respiratory Quotient (RQ) and conversion factors for determining energy expenditure per minute and the amount of carbohydrate or fat metabolized during exercise.

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Acknowledgements

We gratefully acknowledge the Howard Hughes Medical Institute for their financial support in developing the exercise and equipping the laboratory. We also thank Dr. Roz Potter, University of Chicago, for her assistance and logistical support during the ABLE conference.

Literature cited

Appendix A

Further Reading