Objectives

1. Review the net process of respiration and the concept of energy metabolism.
2. Review factors that affect metabolic rate, including body size (Kleiber's Rule).
3. Estimate metabolic rate in beetle larvae using volumetric respirometry to measure the rate of oxygen consumption.
4. Compare total and mass-specific metabolic rates of two species that differ in body size.

Introduction

The capture, storage and utilization of usable energy by living organisms on earth involves two primary processes: photosynthesis and respiration. In photosynthesis, the energy of sunlight is used to drive the synthesis of carbohydrate (glucose, $C_6H_{12}O_6$) from carbon dioxide and water, as summarized by the following equation:

$$6CO_2 + 6H_2O + \text{light} \longrightarrow C_6H_{12}O_6 + 6O_2$$

This reaction does not happen directly, but rather as the net result of a number of enzyme-catalyzed steps within chloroplasts of plant cells. These steps form and use high energy compounds called ATP and NADPH that power the formation of carbohydrate from carbon dioxide and water.

Respiration undoes what was done by photosynthesis. Glucose combines with oxygen to give carbon dioxide and water:
Again, the equation represent the net result

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{heat}$$

of a sequence of several enzyme-catalyzed steps. These reactions include the metabolic pathways of glycolysis and respiration, and they form ATP and NADH.

The trouble with net equations is that they only show what you've got left at the end, and not all the fun you had getting there! In fact, essentially every energy-requiring process in the cell falls within this net equation. The ATP and NADH are used as fast as they are formed, to power active transport, movement, and synthesis. The energy becomes heat, (excepting any that is stored in new molecules by growth of the organism). Therefore, the net equation for respiration is the same as for combustion. Your cells release the same amount of heat energy from a gram of sugar as is released if you set it on fire.

Respiration occurs in most prokaryotes and in the mitochondria of eukaryotes. Both animals and plants have mitochondria and respire. Carbohydrates, lipids, amino acids and even nucleic acids can all be oxidized to provide energy via respiration.

Measurement of metabolic rate

The rate of energy use by an organism is called the **metabolic rate (MR)**. This important variable can be measured in several ways. **Heat production** is a direct measure of metabolic rate, because the energy used in metabolism is converted to heat. Accurate measurement of heat production requires sophisticated equipment. However, there are other indirect ways to infer metabolic rate.

Organisms that respire get essentially all of their metabolic energy from the process. Therefore, the **rate of oxygen consumption (RO_2,)** is directly proportional to metabolic rate. RO_2 can be measured and used to infer metabolic rate indirectly. The relationship between oxygen uptake and metabolic heat production has been measured empirically and is about **4.8 kcal/liter O_2**.

Many factors affect MR

Metabolic rate is not a constant. It is affected by several factors. For example, MR rises with body temperature. This is not surprising, because metabolism is a chemical process, and chemical reactions proceed faster at higher temperature. Usually the metabolic rate approximately doubles for each 10 ^{0}C increase in body temperature.

Exercise increases MR. A human in good physical condition can double MR during exercise. This increase is minor compared to some insects that can increase MR by a factor of 100 during flight. MR can also increase to help regulate body temperature. Mammals and birds exposed to cold increase MR, and therefore heat production, by shivering. This heat helps to keep body temperature constant.

Body size affects MR

Larger organisms have higher metabolic rate than smaller ones, if all other factors are equal. However, the relationship is not simple. You might expect an animal that is twice as large as another to use energy twice as fast. That is, you would expect metabolic rate to increase as a factor of body mass– double the mass, double the metabolic rate. However, that is not the case. In fact, MR is an exponential function of body mass, and the exponent is less than 1. In mammals, the relationship between total MR (kcal·day^{-1}) and body mass (W, kg) is:

1. Total MR = 70W$^{0.75}$

That means that the total MR less than doubles when mass doubles, so that the relationship between MR and body mass is a curve rather than a straight line (Figure 1). However, the relationship is a straight line if the scales are logarithmic (Figure 2).

We've been talking about the total MR of an animal. Another way to think about

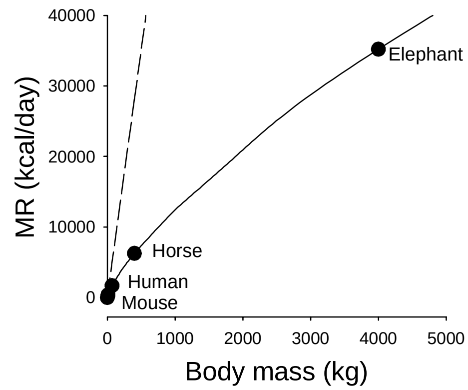

Figure 1. Relationship between total metabolic rate and body mass in mammals. The dotted line would apply if MR were a linear function of body mass. In fact, the slope of the line decreases with increasing body mass.

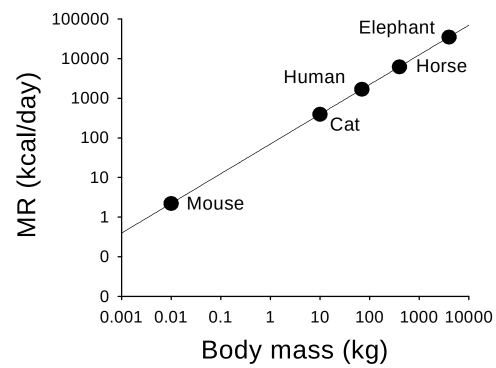

Figure 2. Same data as Figure 1. Note that both axes are log scales.

metabolic rate is the energy use per kilogram (per unit mass). This variable is called the **mass-specific MR.** It is the slope of the curve in Figure 1 (total MR divided by body mass). Interestingly, the mass-specific MR is smaller in larger animals. In fact, a gram of elephant consumes only about 4% as much energy as a gram of mouse! The relation between body mass and mass-specific MR in mammals is:

(2) mass-specific MR = 70W $^{-0.25}$

Units are kcal·day^{-1}·kg^{-1}. The exponent is negative, because mass-specific metabolic rate declines as mass increases. The relationship is a curve on linear axes (Figure 3) and a line on logarithmic axes (Figure 4).

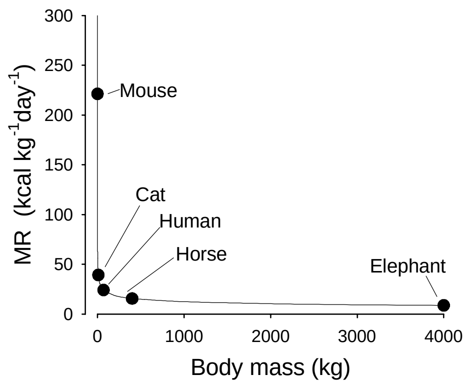

Figure 3. Relationship between mass-specific metabolic rate and body mass in mammals.

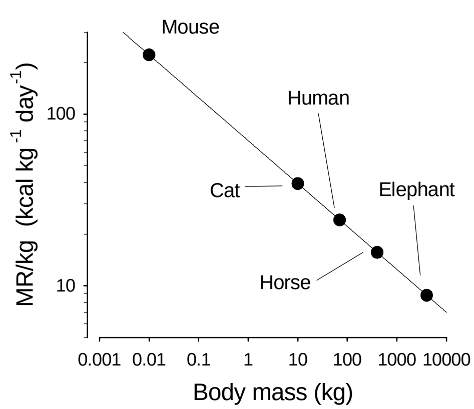

Figure 4. Relationship between mass-specific metabolic rate and body mass in mammals, on log axes. Same data as Figure 3.

Another factor affecting MR is taxon– different kinds of animals have different metabolic rates. For example, mammals have higher metabolic rates than reptiles or insects of similar body size. However, the slope of the line (the exponent) is very similar for nearly any group of organisms. We will be working with insects today. The equation for mass-specific MR of insects at 22^{o}C is:

(3) mass-specific MR = 30W $^{-0.25}$

On a log-log graph, the line for insects lies below, but parallel to, the line for mammals (Figure 5).

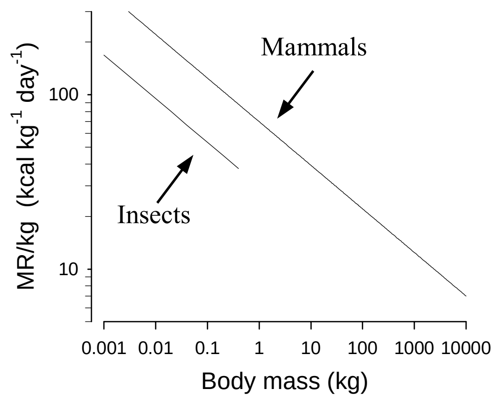

Figure 5. Mass-specific metabolic rate lines for mammals and for insects. Insects have lower MR at any given body mass, but lines have similar slope.

The remarkable fact that smaller organisms have higher mass-specific MR has puzzled biologists for a century. It was first articulated by Max Kleiber and is therefore often referred to as "Kleiber's rule" or the "mouse-elephant curve". Kleiber's rule has important implications in biology, including ecology, agriculture, and medicine. Some progress has been made recently in understanding why it exists. There is a good popular article from the New York Times linked to the lab page– you should read about it. Now let's see if we can test this relationship by measuring the metabolic rate of organisms of different sizes.

Materials and Methods

We will measure metabolic rate indirectly by measuring the rate of oxygen consumption. The method we'll use is called **volumetric respirometry** (Figure 6). When the larvae respire in the closed chamber, they remove O_2 gas and add CO_2 gas to the air. The volume of CO_2 produced is only slightly less than that of the O_2 consumed. However, if the CO_2 gas is chemically removed, the total volume of gas in the chamber will decrease equivalent to the O_2 consumed. This reduction of volume causes an equivalent volume of water to enter the pipette. The movement of water up the pipette can be recorded and used to deduce the rate of oxygen consumption and the metabolic rate.

The carbon dioxide gas is removed from the chamber with a solution of potassium hydroxide (KOH). CO_2 reacts to form potassium bicarbonate (K_2CO_3). This compound goes into solution so that its volume is negligible.

You will measure changes in volume to determine the amount of oxygen consumed. However, temperature and pressure also affect the volume of the gas, according to the ideal gas law ($PV = nRT$). Therefore, the respirometer is placed in a water bath to control temperature. A control respirometer, without animals, is placed alongside. Any changes in volume in the control respirometer are due to temperature or pressure change and are subtracted from the test results to correct them.

Procedures

1. Work in groups of 4. Discuss the principles involved among yourselves until you have a reasonable understanding of what is going on. Each group should have three respirometers and a water bath.

2. Your instructor will provide "regular" and "king" mealworms (*Tenebrio* and *Zoophobas*). Weigh out approximately 2 grams of each into two Petri dishes. Record the type, number and the exact mass of the organisms (it doesn't have to be exactly 2 g, but you need to know exactly what it is).

3. Your lab instructor will provide screened capsules with KOH. Place one of these in each respirometer to absorb CO_2.

4. Add the organisms to the test respirometers (but not the control) and then

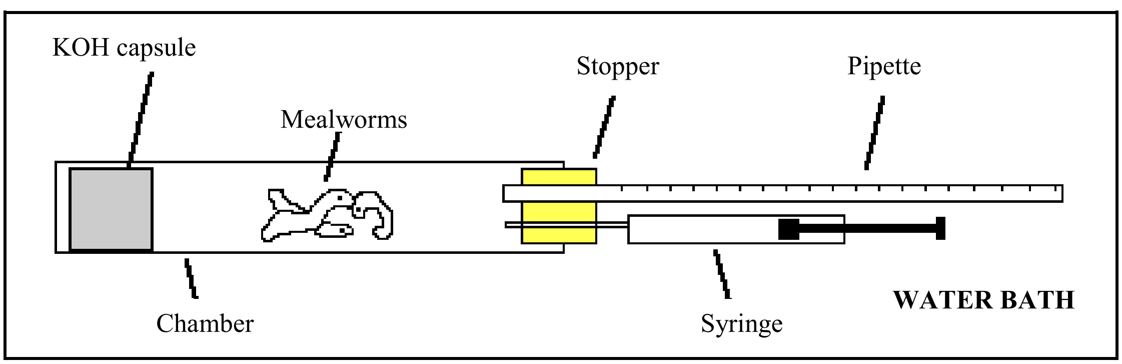

Figure 6. Respirometer for measuring oxygen consumption of mealworms. The assembly is immersed in a water bath for temperature control. As the volume in the chamber decreases, water enters the pipette and its advance is measured to mark the change in volume over time. The syringe is used to add air to the system and adjust the internal volume.

carefully insert the volumetric assembly in each. Moisten the stoppers first so that the fit is airtight.

5. Fill the syringes halfway with air (i.e., retract the plunger halfway).

6. Place the respirometers in the water bath but do not yet immerse the ends of the pipettes. Wait for 10 minutes so that the temperature of the respirometers can equilibrate with the bath.

7. After 10 minutes, immerse the pipette tips. Water will enter the tips (because of the small increase in pressure compressing the gas inside) and then begin moving slowly inward because of oxygen consumption.

8. Start with the meniscus of the test units near zero.* Adjust the meniscus position, if necessary, by injecting or withdrawing air using the syringe. The control should be set near 0.5 ml (near the middle of the scale). Record time to the second and record the position of the meniscus in all of the respirometers.

9. Wait for the meniscus in the slowest test respirometer to move at least 0.5 ml* and then record the time and the volume of all the respirometers again.

10. After the measurement, push air into the test respirometers with the syringes to reset the meniscus near zero.* It should not be necessary to adjust the control. Note the time and the positions.

11. Repeat steps 9-10 three times. Basically you are determining how long it takes each group of worms to consume a measured amount of oxygen. These measurements will be used to determine RO_2. The control will show whether any volume change is taking place due to other factors, such as temperature. change. Any change in the control volume will be subtracted from the change in the test respirometer volume.

* It is not necessary to adjust to exactly zero– only to record the position exactly. Likewise, the change can be greater or somewhat less than 0.5 ml—just measure it accurately.

Table 1– Data sheet for recording respirometry experiment.

Type	**Mass (grams)**	**Number**
Regular	________	________
King	________	________

Time	**Position of meniscus** A (regular)	B (kings)	C (control)
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______
______	______	______	______

Assignment:

1. Calculate rates of oxygen consumption as follows:

   $RO_2 = (V_{initial} - V_{final}) / (T_{final} - T_{initial})$

   where V is volume in ml and T is time in minutes. Divide the time in seconds by 60 so that you get time as a decimal number– for example, if the elapsed time was 10 minutes and 30 seconds, that is (10*60)+30 = 630 seconds, and 630/60 = 10.5 minutes). Units of RO_2 should be ml/minute.

2. The amount of "oxygen consumption" in the control should be small and might be positive or negative. If there was any RO_2 in the control, subtract this from the test RO_2's.

3. You should have 3 calculated measurements of RO_2 for each of the two test groups of worms, and should have corrected them, if necessary, for any change in the control.

We're not done yet, because our standard equation for mass-specific metabolic rate, to which we wish to compare our results, is in units of kcal • kg^{-1} • day^{-1}. Recall that metabolic heat production is about 4.8 kcal per liter of oxygen (page 43). There is an explanation of how to deal with these conversions below:

<u>Using conversion factors</u>

We often need to convert measurements from one unit to another using a conversion factor. For example, the factor for conversion between grams and ounces is 0.03527 ounces/gram. How do you know whether to divide or multiply by the conversion factor? Prepare an equation that cancels all the units except the units that you want for the answer.

For example, let's work out the following problem: If a car travels 57 miles per hour for 23 minutes, how far has it traveled? We know that the answer should be in units of distance, which in this case is miles. But multiplying minutes by miles/h leaves us with units of min x miles/h, which is not what we want. To get our answer in miles we must include another conversion, 1 hour/60 min. Note that all of the units cancel out, except for miles.

$$\frac{57 \text{ miles}}{1 \text{ h}} \times \frac{1 \text{ h}}{60 \text{ min}} \times 23 \text{ min} = 21.9 \text{ miles}$$

Sometimes units can be complex, such as the units of mass-specific metabolism, which are kcal per kg body mass per day. There are alternative ways to write such complex units. You might see them written as: "kcal/kg/day" (which is incorrect) or "kcal/(kg•day)" which is better. However, the most versatile way to write complex units is with exponents. These units would be "kcal•kg^{-1}•day^{-1}". A number with an exponent of –1 is the inverse of that number, so "kg^{-1}" means "1/kg". This notation seems odd at first, but it becomes more useful as the units get more complex.

Let's work out the units conversion of RO_2 in ml•min^{-1} to total MR, in kcal•day^{-1}. There are 3 different conversion factors that must be applied to RO_2.

1) Original units of RO_2= ml•min^{-1}
2) ml to liters: multiply by 0.001 L•ml^{-1}.
3) liters of oxygen to kcal of heat: multiply by 4.8 kcal•L^{-1}.
4) minutes to days: multiply by 1440 minutes•day^{-1}.

String all of these together and cancel to demonstrate that you get appropriate units, then multiply it out to determine the overall conversion factor– a single number that you will multiply by RO_2 to make the conversion to units of kcal•day^{-1}.

Next, convert total MR to mass-specific MR, using the mass (W) of each group of mealworms as follows:

1) Convert W, the mass of the mealworms, from g to kg by multipling by 0.001 kg•g^{-1}
2) Divide total RO_2 by W (or multiply by W^{-1}). Units should now be comparable to the those in the standard equations (kcal•kg^{-1}•day^{-1}).

Table 2. Record the indicated data and the results of your calculations of RO_2 and MR below. There is a similar table on the next page that you will hand in as your assignment next week.

	Regular mealworms (*Tenebrio*)	**King mealworms (*Zoophobas*)**
Total mass of group	________	________
Number of individuals	________	________
Individual body mass	________	________
RO_2 (ml/min) The results of the three trials for each test group and the average of all trials.	1. ________ 2. ________ 3. ________ Mean: ________	1. ________ 2. ________ 3. ________ Mean: ________
Factor for converting RO_2 to MR (kcal/day)	________	________
Mean total MR (kcal/day)	________	________
Mean mass-specific MR (kcal•kg^{-1}•day^{-1})	________	________
Predicted mass-specific MR for insects of this body mass– (calculate using equation 3).	________	________

Respiration Lab Assignment

Name______________________________

Names of other group members____

	Regular mealworms (*Tenebrio*)	King mealworms (*Zoophobas*)
Total mass of group	________________________	________________________
Number of individuals	________________________	________________________
Individual body mass	________________________	________________________
RO_2 (ml/min) The results of the three trials for each test group and the average of all trials	1.________________________ 2.________________________ 3.________________________ Mean: ________________________	1.________________________ 2.________________________ 3.________________________ Mean: ________________________
Factor for converting RO_2 to MR (kcal/day)	________________________	________________________
Mean total MR (Kcal/day)	________________________	________________________
Mean mass-specific MR	________________________	________________________
Predicted MR for insects of this body mass (from equation 3)	________________________	________________________

Questions (type answers on separate page):

1. Based on equation 3, how would you predict the mass-specific MR of *Zoophobas* should compare with that of *Tenebrio*? Be quantitative– by what factor should they differ? Do your measured metabolic rates fit the prediction? Explain.

2. What other factors might affect metabolic rate in this experimental situation besides Kleiber's rule?

3. From the news article linked to the Bio 121 lab page: describe three other processes or characteristics that change disproportionately with body size, as MR does.