Experiment AMe-1: Small Animal Respiratory Exchange Ratio (RER)

Background

There are two main sources of energy available for animal metabolism: carbohydrates (CHO) and fats. These molecules are broken down, or catabolized, into carbon dioxide, water, and energy. However, the oxidation of fats requires more oxygen than the oxidation of carbohydrates.

The oxidation of a molecule of carbohydrate is expressed by the following equation:

\[ 6 \text{O}_2 + C_6\text{H}_{12}\text{O}_6 = 6 \text{CO}_2 + 6 \text{H}_2\text{O} + 38 \text{ATP} \]

As shown in this equation, 6 molecules of carbon dioxide are produced for every 6 molecules of oxygen consumed during the oxidation of carbohydrates, a ratio of 1.0.

The oxidation of a molecule of fatty acid is expressed by this equation:

\[ 23 \text{O}_2 + C_{16}\text{H}_{32}\text{O}_2 = 16 \text{CO}_2 + 16 \text{H}_2\text{O} + 129 \text{ATP} \]

As shown in this equation, 16 molecules of CO\(_2\) are produced for every 23 molecules of O\(_2\) consumed during the oxidation of fatty acids, a ratio of 0.7.

The energy requirements of the animal are met with a mixture of energy derived from carbohydrates and fats. The activity being performed determines the proportion of carbohydrates and fats being utilized. At rest, a body derives about 40% of its energy from carbohydrates and 60% from fats. As the intensity of activity increases, the demand for energy increases, and a greater proportion of this demand is met by the oxidation of carbohydrates because the catabolism of fat is too slow to supply the amount of energy required.

As the ratio of energy supplied by fats and carbohydrates shifts during changes in activity, the ratio of carbon dioxide produced to oxygen consumed also shifts. The ratio of carbon dioxide produced to oxygen consumed during cellular metabolism can be measured by determining the changes in the concentrations of oxygen and carbon dioxide in the air that passes into and out of the organism being studied.

Homeothermic or endothermic (warm-blooded) animals and poikilothermic or ectothermic (cold-blooded) animals have a metabolic rate related to body mass at a slope of 0.75. These organisms will show very different metabolic rates based on the way they maintain their body temperatures. Endotherms have a constant body temperature and maintain this elevated temperature by endogenous heat production. They tend to have a high VO\(_2\), high heat production, low thermal conductivity (good insulation) and a high metabolism - up to 5 times the metabolism of ectotherms. Ectotherms or poikilotherms base their internal body temperature on the thermal condition outside their bodies. Their body temperatures are high in warm environments, but low in cool environments. They adjust body temperature by means other than heat production and heat loss - thus having a lower overall metabolism than endotherms. Ectothermic animals tend to have a low VO\(_2\), low heat production and are poorly insulated (Figure AMe-1-B1).
Figure AMe-1-B1: Chart showing the way body temperature fluctuates in ecto- and endothermic organisms.

In these animals, the amounts of oxygen consumed and carbon dioxide produced are measured using an oxygen/carbon dioxide gas analyzer connected to a small animal chamber. The gas analyzer measures the concentration of oxygen consumed and carbon dioxide produced by the organism in the chamber. When the concentrations and volumes are brought together in a series of equations, the volume of oxygen consumed per minute, known as VO$_2$, and the volume of carbon dioxide produced per minute, known as VCO$_2$, are determined. The ratio of VCO$_2$/VO$_2$ is the Respiratory Exchange Ratio (RER), which can be used to determine the proportion of carbohydrates and fats utilized, and the energy expended per liter of oxygen consumed, during an activity (Table AMe-1-B1).

The fat and carbohydrate percentages utilized during an activity are determined using the following equations:

\[
\frac{(1.00-\text{RER})/(1.00-0.70)) \times 100}{100\% - \%\text{Fat utilized}} = \%\text{CHO utilized}
\]

The energy expended during an activity is calculated from the RER and the volume of oxygen consumed. For example, if the RER is 0.90, the energy expended is 4.92 kcal/liter O$_2$.

If 2.5 liters of oxygen are consumed per minute for 20 minutes, a total of 246 kcal are expended during the activity:

\[
(2.5\text{LO}_2/\text{minute})(20\text{min})(4.92\text{kcal/liter O}_2) = 246\text{kcal}
\]
At less intense activity levels, the rates of energy expenditure and RER values are lower. To expend the same amount of energy at a less intense level of activity, the duration of activity must be longer. For example, if the RER is 0.80, the energy expended is 4.80 kcal/liter O_2. If 1.7 liters of oxygen are consumed per minute, 8.16 kcal are expended per minute:

\[(1.7\text{LO}_2/\text{minute})(4.80\text{kcal/liter O}_2) = 8.16\text{kcal/min}\]

To expend 246 kcal at a rate 8.16 kcal/min would require 30 minutes, 9 seconds:

\[246 \text{kcal}/(8.16 \text{kcal/min}) = 30.15 \text{ minutes}.\]

### Table AMe-1-B1: Respiratory Exchange Ratio (RER) as a Function of the Proportions of Energy Sources.

<table>
<thead>
<tr>
<th>RER</th>
<th>Energy kcal/liter O_2</th>
<th>% Energy from CHO</th>
<th>% Energy from Fats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.70</td>
<td>4.69</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.75</td>
<td>4.74</td>
<td>15.6</td>
<td>84.4</td>
</tr>
<tr>
<td>0.80</td>
<td>4.80</td>
<td>33.4</td>
<td>66.6</td>
</tr>
<tr>
<td>0.85</td>
<td>4.86</td>
<td>50.7</td>
<td>49.3</td>
</tr>
<tr>
<td>0.90</td>
<td>4.92</td>
<td>67.5</td>
<td>32.5</td>
</tr>
<tr>
<td>0.95</td>
<td>4.99</td>
<td>84.0</td>
<td>16.0</td>
</tr>
<tr>
<td>1.00</td>
<td>5.05</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

In this experiment, students will measure the expired CO₂ and consumed O₂ values, RER, and proportion of fat and carbohydrates utilized while an endotherm (mouse or rat) is in the small animal chamber. They will repeat the experiment with an ectothermic organism (frog, snake or lizard) and make a comparison between the RER values of these two types of animals. Students will then be able to make an accurate assessment of the metabolic capability of these organisms. These measurements will be performed quickly and easily using an iWorx gas analyzer.

As an additional calculation, students can adjust the values for CO₂ and O₂ using Body Weight Normalization and Effective Mass Correction. CO₂ and O₂ values are normalized with respect to the animal’s body weight and corrected according to an effective mass value. Using a value of 1.0 will eliminate any effective mass correction, if desired.
Experiment AMe-1: Small Animal Respiratory Exchange Ratio (RER)

Equipment Required
PC or Mac Computer
iWorx IXTA, USB cable, and power supply
Small Animal Chamber
Gas sample tubing
iWire-GA CO$_2$/O$_2$ gas analyzer with filter
A-CAL-200 Calibration Kit
Barometer and thermometer
Mouse or Rat
Snake, Frog or Medium sized Lizard
Balance or scale

Setup the IXTA and iWire-GA
1. Connect the iWire-GA to the iWire1 port on the front of the IXTA, and plug it into the wall using the power supply.
2. Plug the IXTA into the wall and, using the USB cable, to the computer.
 NOTE: The iWire-GA must be plugged into the IXTA prior to turning both machines on.
3. Turn on the IXTA and the iWire-GA.
4. Click on LabScribe
5. Click Settings → Animal Metabolism → SmallAnimalRER-iWireGA
6. Once the settings file has been loaded, click the Experiment button on the toolbar to open any of the following documents:
   • Appendix
   • Background
   • Labs
   • Setup (opens automatically)

Setup the Metabolic Cart
1. Locate the small animal chamber in the iWorx kit (Figure AMe-1-S1).
2. Locate iWire-GA gas analyzer, the gas analyzer power supply, 2 inlet filters, room air sampling tube, and a 6ft long Nafion sampling tubing in the iWorx kit.
3. Position the gas analyzer on the desktop, so that the analyzer can be connected to the data recording unit and the small animal chamber at the same time.

4. Make sure to connect the iWire-GA to the iWire 1 port on the front of the IXTA. Turn both units on after connecting this cable.

5. On the iWire-GA, place one filter on the “Room Air” port, place a second filter on the “Sample In” port. Attach the braided end of the Nafion sampling tube to the filter on the “Sample In” port. (Figure AMe-1-S1).

6. Place the other end of the Nafion sampling tube on the gas sampling port near the outlet of the small animal chamber.

7. Connect the Room Air filter port to the tubing with the black leuer lock, and connect the other end to the inlet port of the small animal chamber.

Figure AMe-1-S1: The front panel of both the IXTA and the iWire-GA showing the connections to and from the small animal chamber with the mouse inside.
Calibrating the iWire-GA Gas Analyzer

*Note:* Warm up the gas analyzer for at least 15 minutes prior to use. Make sure the calibration gas tank is located close to the gas analyzer.

Prior to use users must perform a two-point calibration of the oxygen and carbon dioxide sensors. The recommended gas mixtures include:

- 12% O\(_2\) and 5% CO\(_2\) with the balance of the mixture being N\(_2\); or,
- 16% O\(_2\) and 4% CO\(_2\) with the balance of the mixture being N\(_2\).

Room air can provide both the high concentration of O\(_2\) at 20.90%, and the low concentration of CO\(_2\), 0.04%.

This procedure will calibrate both the O\(_2\) and CO\(_2\) channels.

Connect the gas sample tubing of the A-CAL-200 Calibration Kit (Figure-AMe-1-S2) to the Luer-Lock connector on the output of the regulator.

![Regulator (A-50-11946)](image.png)

*Figure Ame-1-S2: Calibration Kit (A-CAL-200).*

**Record the Voltage Outputs of the Gas Sensors**

1. Turn on the gas analyzer for at least 15 minutes before performing a calibration.
2. Prepare the equipment that will deliver any gas samples, other than room air, to the iWire-GA:
   - Clamp and secure any gas cylinders that will used to provide gas samples near the gas analyzer.
   - Attach the regulator to the gas cylinder with a wrench making sure there are no leaks in the system.
3. Disconnect the nafion tubing from the small animal chamber.
4. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a sample of room air.
- Place the gas sampling tubing away from the users to prevent the sampling of exhaled air. Allow room air to be pumped through the gas analyzer for 10 seconds before recording the outputs of the sensors.

- Type **Room Air** in the Mark box to the right of the Mark button.

- Click on the Record button. The recording should scroll across the screen.

- While recording, press the Mark button to mark the recording with information about the room air gas sample.

- Record the outputs of the two gas sensors for about ten seconds. The recording which should be like the first segment of data in **Figure AMe-1-S3**.

- Continue to record while moving to the next series of steps.

5. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a second sample of a gas mixture containing known concentrations of oxygen and carbon dioxide.

   - Open the valve on the regulator all the way.

   - While the gas sample is flowing from the regulator, connect the gas sample tubing of the A-CAL-200 Calibration Kit (**Figure Ame-1-S2**) to the Luer-Lock connector on the output of the regulator.

   - Connect the outlet from the A-CAL-200 Calibration Kit to the inlet filter port on the gas analyzer. The gas analyzer will pull the air in from the calibration kit.

   - Type **Gas Sample** in the Mark box.

   - While continuing to record with the sample gas flowing into the gas analyzer, press the Mark button to mark the recording with information about the second gas sample.

6. Once the recordings of the gas concentrations reach a steady level, record for another ten seconds. This may take a minute or so to fully level out.

7. Click the Stop button.

8. Turn off the regulator and reconnect the nafion tubing to the small animal chamber.

9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

**Convert the Units on Gas Concentration Channels**

1. Use the Display Time icons to adjust the Display Time of the Main window to show the complete calibration data on the Main window at the same time. The required data can also be selected by:

   - Placing the cursors on either side of data required.

   - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the entire segment of data to the width of the Main window.
2. Click the 2-Cursor icon (Figure AMe-1-S4) so that two blue cursors appear on the Main window. Place one cursor on the section of data recorded when gas analyzer was collecting a sample of room air and the second cursor on the section of data recorded when the second sample was collected.

Figure AMe-1-S3: The voltage outputs of the two sensors in the gas analyzer, carbon dioxide on the top and oxygen on the bottom. Other recording windows have been minimized to show detail.

4. Convert the voltages at the positions of the cursors to concentrations using the Advanced Units Conversion dialogue window (Figure AMe-1-S5).

5. To convert the voltages on the Expired CO2 Concentration (%) channel, click on the arrow to the left of the channel title to open the channel menu. Select Units from the channel menu, and select Advanced from the Units submenu.

Animal Metabolism – RER-iWireGA – SetupIXTA
6. On the Units Conversion window:
   - Make sure Apply units to the next recorded block and Apply units to all blocks are selected in the menu under the displayed graph on the left side of the window by putting a check mark in the boxes next to each statement.
   - Click on and move the cursors so that they are in position such that:
     - the first 2 cursors are on the area where room air values were recorded. Leave a space between the cursors so that you have an average value being calculated while room air was moving into the iWire-GA gas analyzer.
     - the second 2 cursors are on the area where the gas sample values were recorded. Leave a space between the cursors so that you have an average value being calculated while the gas sample was moving into the iWire-GA gas analyzer.
   - Notice that the voltages from the positions between the cursors are automatically entered into the value equations. Enter the two concentrations of carbon dioxide measured from the two samples in the corresponding boxes on the right side of the conversion equations.
     - Using room air - the concentration of CO2 = 0.04
     - The second gas concentration will be the one from the gas cylinder. Generally a 5% CO2 concentration is recommended.
   - Enter the name of the units, %, in box below the concentrations.
   - Click the OK button in the lower right corner of the window.

Figure AMe-1-S5: The Advanced Units Conversion dialogue window with the voltages between the cursors set to equal the concentrations used in calibration.
7. Repeat Steps 4 and 5 on the Expired O2 Concentration (%) channel.
   - Room air = 20.9
   - Second gas concentration will be the one from the gas cylinder. Generally a 12% O₂ concentration is recommended.

8. Click on the Save button.
Experiment AMe-1: Small Animal Respiratory Exchange Ratio (RER)

Exercise 1: Changes in CO$_2$ and O$_2$ in a closed chamber, and RER in an endothermic animal.

Aim: To determine mean RER of an endotherm at rest.

Approximate Time: 15-20 minutes or longer depending on the animal

**Procedure**

1. Place the animal in the chamber and close the lid securely.
2. Click on the Record button. Type <Mouse/Rat> in the Mark box to the right of the Mark button. Press the Mark button to mark the recording.
3. Click the AutoScale All button.
4. On the Expired CO$_2$ Concentration (%) channel, notice that the CO$_2$ concentration shows a continuous steady rise in level throughout the recording.

**Note:** During the first few minutes or so of the recording, the small animal chamber is filling with expired air from the organism inside. Since this is a closed system, the concentration of CO$_2$ continues to rise as the animal exhales into the chamber.

- The time that it takes the chamber to be filled with expired air and a steady increase in the level of carbon dioxide will depend on the volume and respiration rate of the organism and the volume of the small animal chamber. It will take longer to fill the chamber if the organism’s respiration rate and tidal volume are low, or the animal is very small.

**Warning:** The CO$_2$ concentration will continue to rise throughout the experiment and levels can become toxic to the animal in the chamber. Remove the animal immediately if the CO$_2$ levels reach 3.5%.

5. On the Expired O$_2$ Concentration (%) channel, notice that the O$_2$ concentration will show a steady decrease as the animal is using the O$_2$ in the chamber. As pointed out in the previous step, the size of the chamber, the tidal volume, and respiration rate of the organism, determine the time it takes for the concentration of oxygen to decrease significantly.

6. On the RER channel, an equation programmed in the software determines RER based on the relative levels of CO$_2$ (which is increasing) and O$_2$ (which is decreasing).

7. Continue to record until the concentration of carbon dioxide in the chamber reaches 3%.
Note: Remember to remove the animal from the chamber immediately if the carbon dioxide concentration reaches 3.5%.

8. Once the appropriate data is recorded, click Stop to halt the recording. Your recording should be similar to the data displayed in Figure AMe-1-L1.

9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

10. Remove the animal from the chamber and place it back into its container.

11. Clean and dry the small animal chamber if necessary.

RER Channel Set Up

1. Display the complete data recording in the Main window. Use the Display Time icons to adjust the Display Time of the Main window to show the complete recording on the Main window.

2. Select and display a section of data that shows the change in CO₂ concentration at 1% (Figure AMe-1-L1).

3. This can be done by:
   - Clicking on and dragging the cursors to either side of the data, and looking at the V2-V1 value in the upper right corner of the Expired CO₂ channel.
   - When V2-V1 value is equal to 1%, look at the T2-T1 value in the upper right to determine the time it took for the CO₂ concentration to change by 1%.

4. Click on RER Expired CO₂ Concentration (%) on the RER channel. Choose Setup Function from the drop down list. This will open the RER Calculation Dialog window (Figure AMe-1- L2).

5. Setup the RER calculations:
   - Choose the appropriate RER Type by clicking on the down arrow and choosing Closed Small Animal Chamber.
   - Check the O₂ and CO₂ channel information so that they are being calculated from the correct channels.
   - Change the Time(s) to average to 30 seconds
   - Change the Delta Time(min) to be the T2-T1 value noted in Step 2. This is the time it took for the CO₂ concentration in the small animal chamber to change by 1%.
   - Click OK.

6. The recording on the main window will now have a histogram on the RER channel.
Figure AMe-1-L1: The recording of the expired CO2 over a period of 28 minutes showing a change in concentration by 1%.

Figure AMe-1-L2: RER Calculation Dialog window.

**Data Analysis**

1. Scroll to the beginning of the recording where the histogram is not a straight line. You should be able to see a step-like histogram.
2. Click and drag the cursors to either side of the step-like histogram.

3. Click the Zoom between Cursors button on the toolbar to expand this section of data to fill the window.

4. Click AutoScale on all channels. Your data should look like the data seen in Figure AMe-1-L3.

5. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window (Figure AMe-1-L4).

6. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The functions, Mean, T2-T1, and V2-V1 should appear in this table. Values for these parameters on each channel are seen in the table across the top margin of each channel.

7. Once the cursors are placed in the correct positions for determining the CO$_2$ and O$_2$ concentrations, the values for these parameters can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal.

8. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of these parameters in the Journal. To use them:
   - Place the cursors at the locations used to measure the concentrations and RER.
   - Transfer the names of the mathematical functions used to determine these data to the Journal using the Add Title to Journal function in the Expired CO2 Channel pull-down menu.

\textit{Figure AMe-1-L3: The RER channel showing the histogram for the ratio of CO2 to O2 in the endothermic organism.}
9. Transfer the values for the data to the Journal using the Add All Data to Journal function in the Expired CO2 Channel pull-down menu.

10. Repeat this procedure for the Expired O2 Concentration (%) channel.

11. The values for the following parameters are determined when the cursors are positioned as directed:
   - Mean concentration of CO$_2$ in expired air, which is the value for Mean on the Expired CO2 Concentration channel.
   - Mean concentration of O$_2$ in expired air, which is the value for Mean on the Expired O2 Concentration channel.

12. Mean RER, which is the value for Mean on the RER channel. This is calculated by dividing the expired CO$_2$ value by the expired O$_2$ value to get the RER ratio as setup previously.

13. Record the T2-T1 value that is used to measure the mean values of CO$_2$, O$_2$ and RER.

14. Record the values in the Journal using one of the techniques described in Steps 6 or 7.

15. Record the values for the Mean CO$_2$ and O$_2$ concentrations in expired air in Table AMe-1-L1. Also record the RER and T2-T1 values.

**Exercise 2: Changes in CO$_2$ and O$_2$ in a closed chamber, and RER in an ectothermic animal.**

Aim: To determine the changes in CO$_2$ and O$_2$ volumes of an ectotherm and make a comparison with the values obtained from an endotherm.

Approximate Time: 15-20 minutes, depending on the animal.

**Procedure**

1. Use the same procedures used in Exercise 1 to record the CO$_2$, O$_2$ and RER values from an ectotherm.

2. Place the ectotherm (lizard, frog or snake) into the clean, dry small animal chamber and close the lid securely.

3. Mark the recording with comments that indicate the organism in the chamber.

4. Click Record to begin the recording.

5. Record until a concentration of 3% carbon dioxide is reached.

   **Note:** Remove the animal from the chamber immediately in the CO$_2$ concentration reaches 3.5%.

6. Click Stop to halt the recording and Save your data file.

7. Remove the animal from the chamber and place it back in its container.
RER Setup and Data Analysis

1. Use the same procedures used in Exercise 1 to set up the RER channel showing a 1% change in CO₂ value.

2. Make any necessary changes on the RER channel by opening the RER Calculation dialog window (Figure AMe-1-L2).

3. Follow the procedures in Exercise 1 for data analysis of the beginning section of the recording for the ectotherm showing the step-like histogram.

4. Determine the oxygen (O₂), carbon dioxide (CO₂), and respiratory exchange ratio (RER) values. Note the T2-T1 value for the section of data with the step-like histogram.

5. Record the values for the Mean CO₂, O₂ concentrations, RER, and the T2-T1 value on Table AMe-1-L1.

Questions

1. During which experimental period was the endotherm’s expired CO₂ the highest? In which period was it the lowest?

2. During which period was the endotherm’s expired O₂ the highest? In which period was it the lowest?

3. During which period did the endotherm have the highest RER? In which period was the RER the lowest?

4. How do the values obtained from the endothermic animal compare to those from the ectothermic animal?

5. How does the RER values from these organisms relate to their overall metabolic rate?

6. Does ambient temperature have anything to do with their overall metabolism?

7. What would happen to the RER values for the ectotherm if you lowered the ambient temperature? The ectotherm?

8. For what reason should the animal not have eaten within an hour of performing these experiments? Evaluate the diet of these animals. How does diet correlate to the RER values?
Figure AMe-1-L4: The expired oxygen and carbon dioxide concentration and RER of a small animal as displayed in the Analysis window. The cursors are in position to measure the mean values of the selected interval.

Optional Exercise

RER is a temperature dependent value. As an optional exercise, the animals can be cooled and RER can be calculated as they return to normal body temperature or to room temperature.

Table AMe-1-L13: Table for recording Mean CO2, O2 and RER values for the endothermic and ectothermic organisms. T2-T1 values are also recorded.

<table>
<thead>
<tr>
<th>Environmental Conditions</th>
<th>Organism</th>
<th>Mean Concentration (%) in the Chamber</th>
<th>Mean RER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>T2-T1 (min/sec) for RER calculation</td>
<td>CO₂</td>
<td>O₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endotherm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ectotherm</td>
<td></td>
</tr>
</tbody>
</table>
Weight Normalization Calculation

Normalized Expired O₂

\[
\text{O₂ norm} = \frac{\text{O₂}}{((\text{weight}(g) / \text{mass unit}))^{\text{effective mass}}}
\]

Example: O₂ = 0.83 ml/min, weight = 25 grams, mass unit = KG, effective mass factor (slope) = 0.75

\[
\text{O₂ norm} = \frac{0.83}{(25/1000)^{0.75}} = 13.2 \text{ ml/KG/min}
\]

Using an effective mass factor of 1 eliminates any effective mass correction. This can be repeated for the CO₂ values. When RER is calculated using normalized values, the weight of the organism is taken into account.