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

NOTE: The iWire-GA must be plugged into the IXTA prior to turning both machines on.

Setup the IXTA and iWire-GA

1. Connect the iWire-GA to the iWire1 port on the front of the IX-TA, and plug it into the wall using the power supply.
2. Plug the IX-TA into the wall and, using the USB cable, to the computer.
3. Turn on the iWire-GA and then the IX-TA.

Setup the Metabolic Cart

1. Locate the small animal chamber.
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.
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.

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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 leur lock, and connect the other end to the inlet port of the small animal chamber.

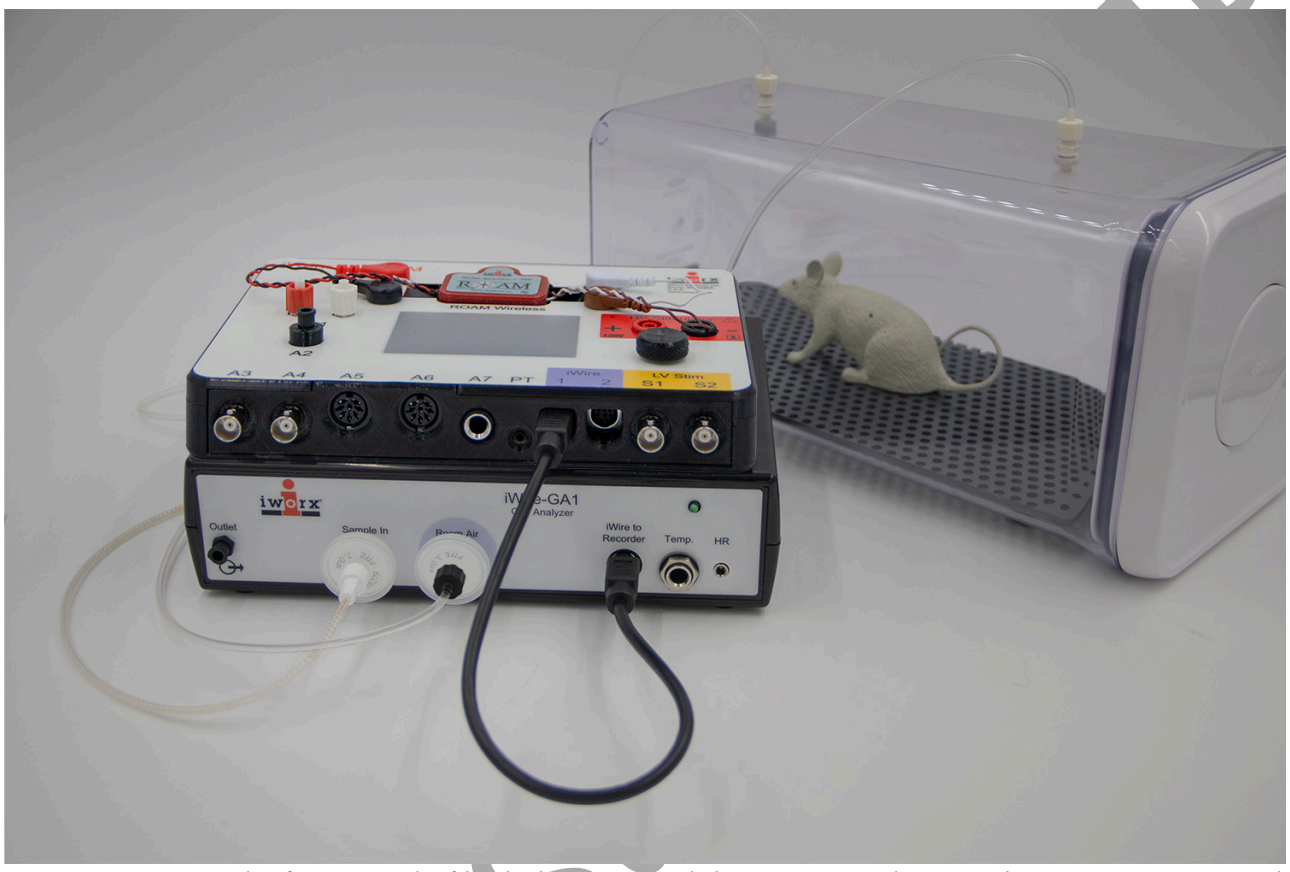

Figure AMe-1-S1: The front panel of both the IXTA and the iWire-GA showing the connections to and from the small animal chamber with the mouse inside.

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%.

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This procedure will calibrate both the O_2 and CO_2 channels.

Connect the gas sample tubing of the A-CAL-200 Calibration Kit to the Luer-Lock connector on the output of the regulator.

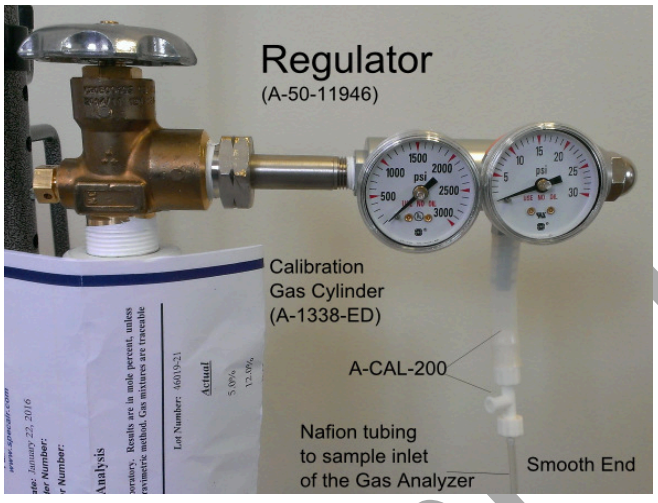

Figure AMe-1-S2: Calibration Kit (A-CAL-200).

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

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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 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 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.
3.

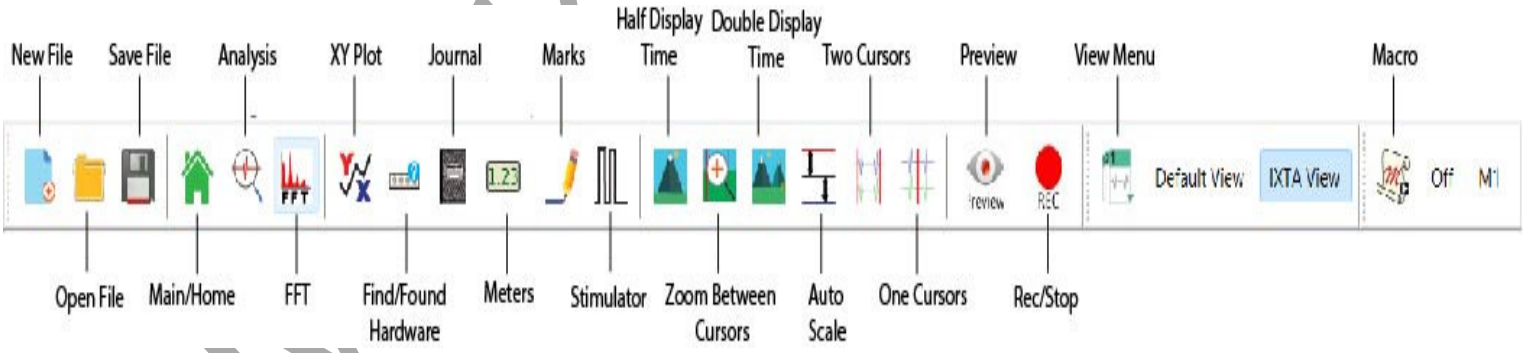

Figure AMe-1-S3: The LabScribe toolbar.

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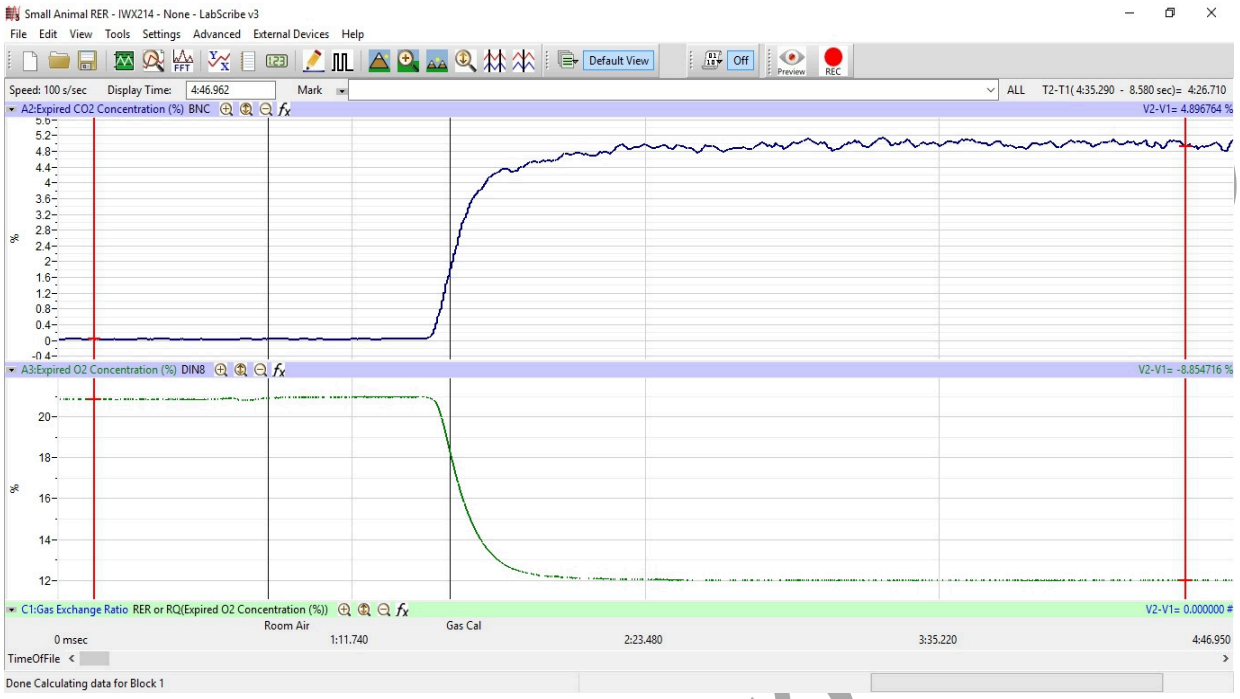

Figure AMe-1-S4: The voltage outputs of the two sensors in the gas analyzer, carbon dioxide on the top and oxygen on the bottom. Other recording windows have been minimized to show detail.

4. Convert the voltages at the positions of the cursors to concentrations using the Advanced Units Conversion dialogue window.
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.

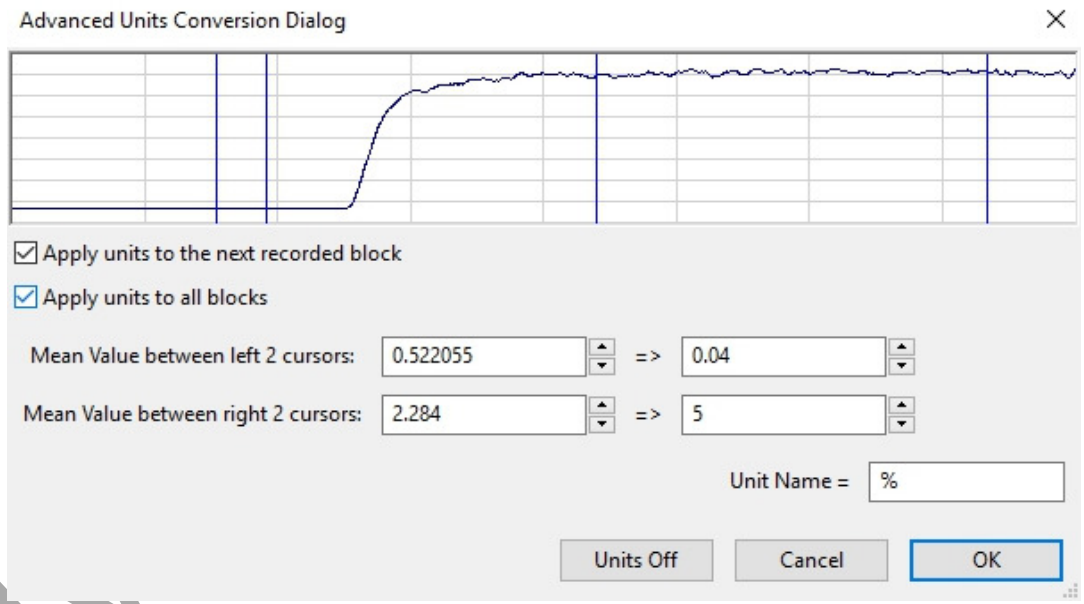

Figure AMe-1-S5: The Advanced Units Conversion dialogue window with the voltages between the cursors set to equal the concentrations used in calibration.

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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% CO_2 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.

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% O_2 concentration is recommended.
8. Click on the Save button.

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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 CO2 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 O2 Concentration (%) channel, notice that the O_2 concentration will show a steady decrease as the animal is using the O2 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%.

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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 shown below.
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 CO_2 concentration at 1%.
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 CO_2 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.
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.

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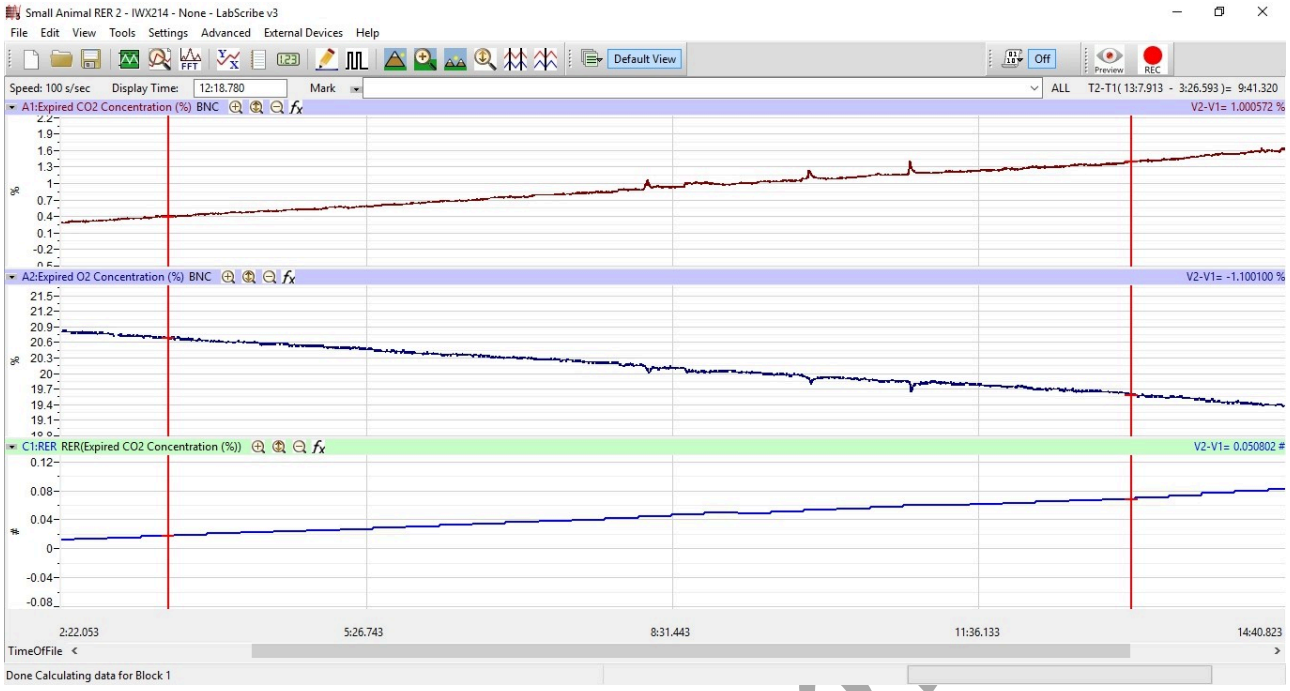

Figure AMe-1-L1: The recording of the expired CO2 over a period of 28 minutes showing a change in concentration by 1%.

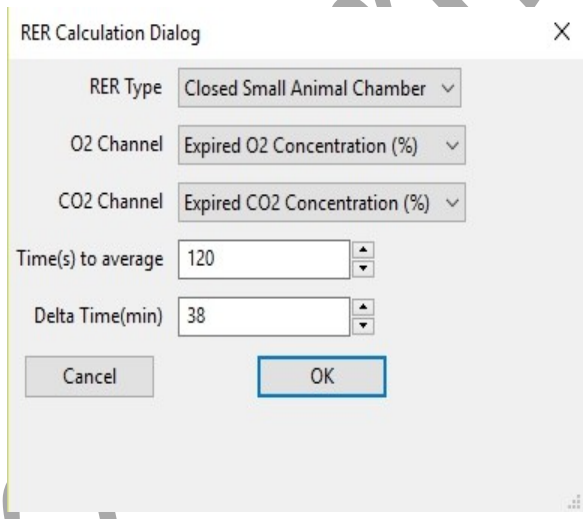

Figure AMe-1-L2: RER Calculation Dialog window.

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.

Work in Progress

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 the image below.

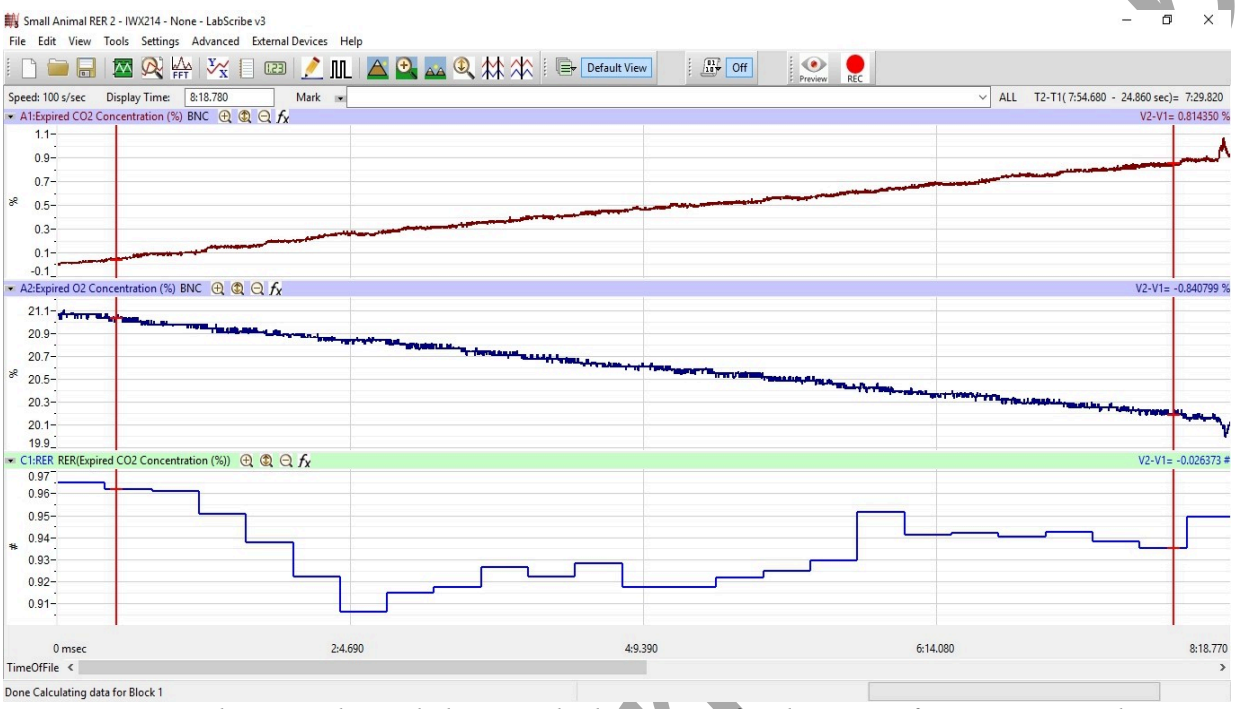

Figure AMe-1-L3: The RER channel showing the histogram for the ratio of CO2 to O2 in the endothermic organism.

5. Click on the Analysis window icon.
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.
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.

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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 1. 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.

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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 CO_2 value.
2. Make any necessary changes on the RER channel by opening the RER Calculation dialog window.
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 (O_2), carbon dioxide (CO_2), 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 CO_2, O_2 concentrations, RER, and the T2-T1 value in Table 1.

Questions

1. During which experimental period was the endotherm's expired CO_2 the highest? In which period was it the lowest?
2. During which period was the endotherm's expired O_2 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?

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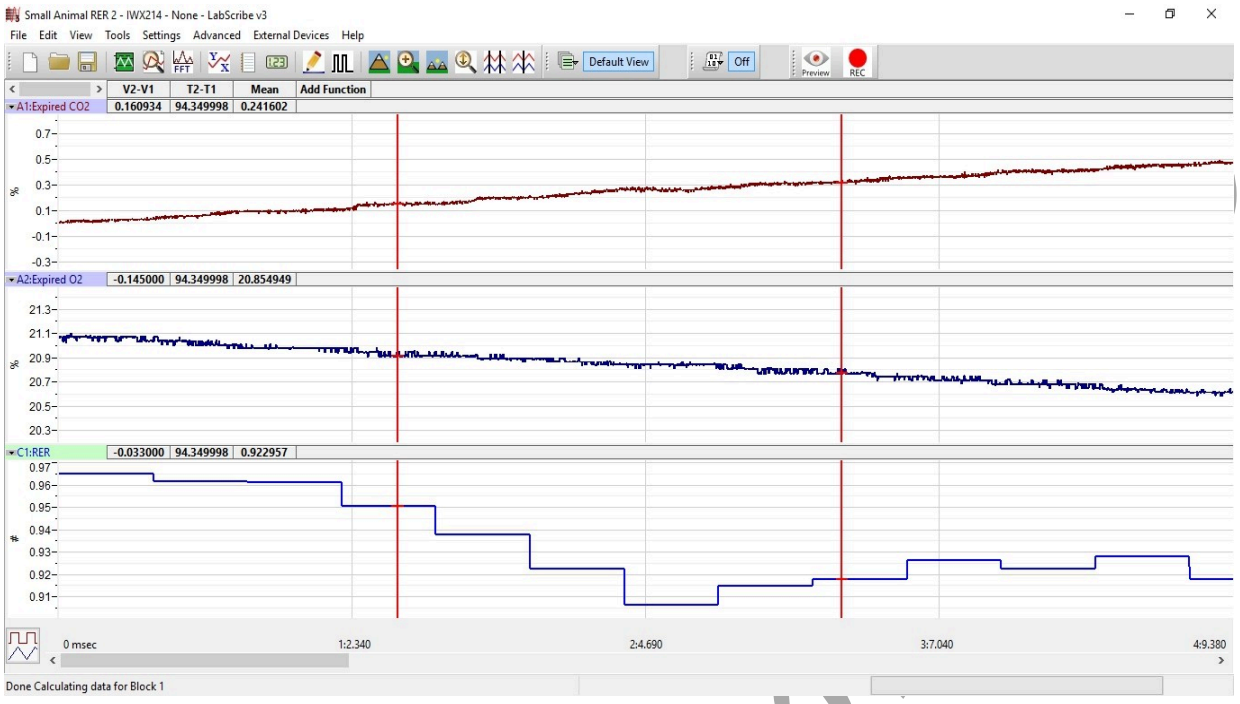

Figure AMe-1-L4: The expired oxygen and carbon dioxide concentration and RER of a small animal as displayed in the Analysis window. The cursors are in position to measure the mean values of the selected interval.

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.

Environmental Conditions		Organism	Mean Concentration (%) in the Chamber		Mean RER
Temperature (°C)	T2-T1 (min/sec) for RER calculation		CO_2	O_2	
		Endotherm			
		Ectotherm			

Work Sample

Weight Normalization Calculation

Normalized Expired O_2

$O2 / [(weight(g) / mass\ unit)]^{*effective\ mass}$

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

$O2_{norm} = 0.83 / (25/1000)^{0.75} = 13.2$ ml/KG/min

Using an effective mass factor of 1 eliminates any effective mass correction.
This can be repeated for the CO_2 values. When RER is calculated using normalized values, the weight of the organism is taken into account.

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