

Multi Chamber Small Animal Respiratory Exchange Ratio (RER)

Equipment Required

PC or Mac Computer

iWorx IX-TA-220, USB cable, and power supply

iWire-GA1 or GA-200 Gas analyzer

Upto 4 Small Animal Chambers

Gas sample tubing

A-CAL-200 Calibration Kit

Gas Pump (aquarium pump can work)

Barometer and thermometer

Mouse or Rat or Snake, Frog or Medium sized Lizard

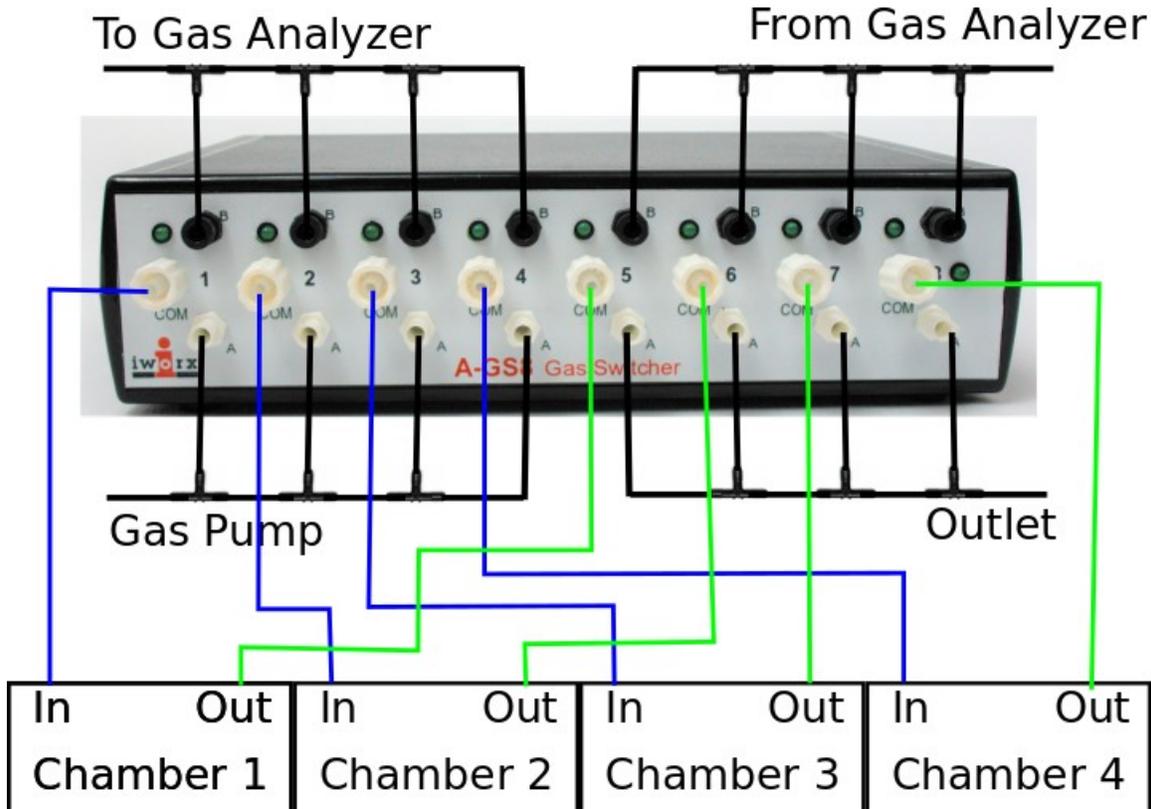
Balance or scale

Setup the IX-TA-220 and Gas Analyzer

1. Connect the Gas Analyzer to the IX-TA-220.
 - For iWire-GAx : plug the iWire-GA into the iWire port of the IX-TA-220
 - For the GA-200: Turn on the GA-200 and connect the oxygen outputs of the GA-200 to channel 3 of the IX-TA-220, and the CO₂ output to Channel 4 of the IX-TA-220
2. Connect the IX-TA-220 to the wall outlet and, using the USB cable, to the computer.
3. Turn on the Gas Analyzer and the IX-TA-220. The unit must warm up for 15 minutes prior to use.
4. Click on LabScribe
5. Load the IX-TA-220 Research settings group, if it has not been done already.
 - Click on Load Group → Choose the ResearchSettingsFiles\IX-TA-220\ResearchTA.iwxgrp
6. Click Settings → MultiChamber-SmallAnimal-ClosedSystem
7. Once the settings file has been loaded, Setup (opens automatically)

Closed System Setup

To measure the gas concentrations in multiple closed systems



Setup the Metabolic Cart

1. Locate the 2 inlet filters, room air sampling tube, and a 6ft long Nafion sampling tubing.
2. Position the gas analyzer on the desktop, so that the analyzer can be connected to the small animal chamber.
3. On the Gas Analyzer, place a filter on the "Sample In" port. Attach the braided end of the Nafion sampling tube to the filter on the "Sample In" port.
4. Place the other end of the Nafion sampling tube to the port marked "To Gas Analyzer".
5. Connect the Outlet port to the tubing with the black leuc lock, and connect the other end to the port marked as "From Gas Analyzer"
6. Connect the system, as shown above.
7. Connect the Digital Output of the IX-TA-220 to the Digital Input of the A-GS-8 Gas switcher

The A port is connected to the common port when the digital input is zero. Thus the gas pump will be connected to the chambers and the air will exit to the outlet,

When a measurement is to be made on a chamber1, Turn on the Digital input 1 and input 5, This will connect the chamber to the Gas analyzer.

To measure chamber 2, turn off all digital inputs except, digital input 2 and 6.

To measure chamber 3, turn off all digital inputs except, digital input 3 and 7.

To measure chamber 4, turn off all digital inputs except, digital input 4 and 8.

Calibrating the 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.

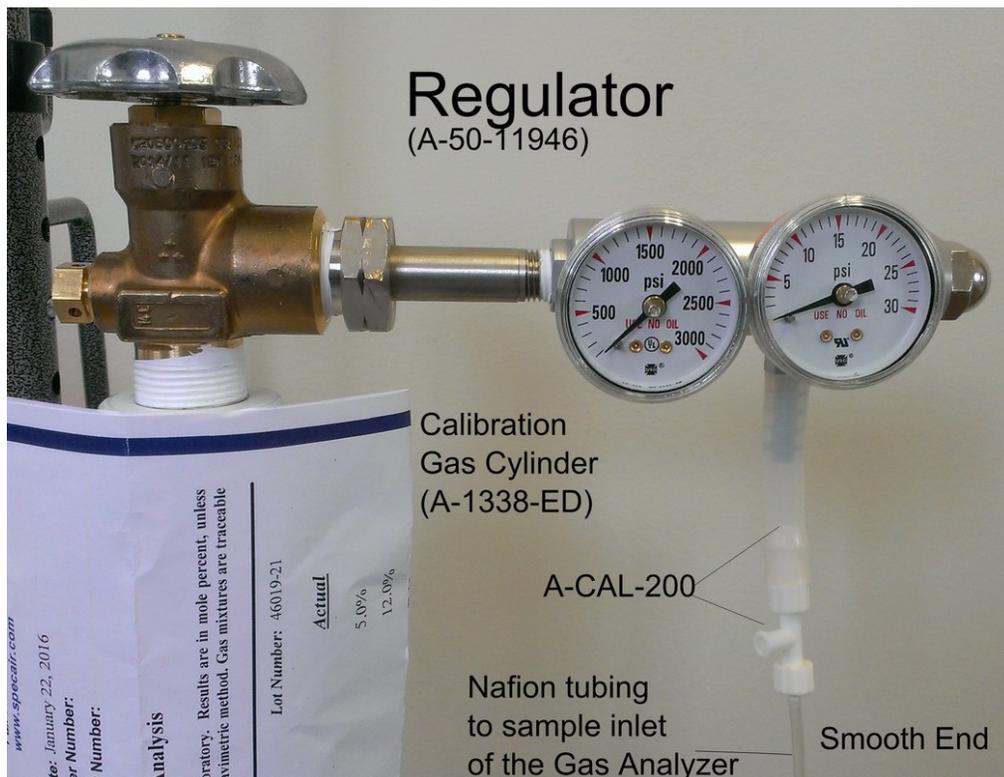
The recommended gas mixtures include:

- 12% O₂ and 5% CO₂ with the balance of the mixture being N₂; or,
- 16% O₂ and 4% CO₂ with the balance of the mixture being N₂.

Room air can provide both the high concentration of O₂ at 20.90%, and the low concentration of CO₂, 0.04%.

This procedure will calibrate both the O₂ and CO₂ channels.

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

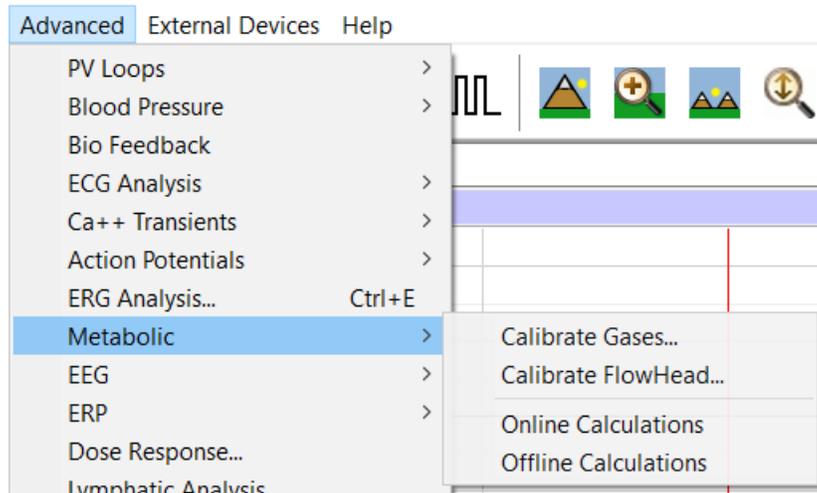


Turn on the gas analyzer for at least 15 minutes before performing a calibration.

Prepare the equipment that will deliver any gas samples, other than room air, to the IX-Peak:

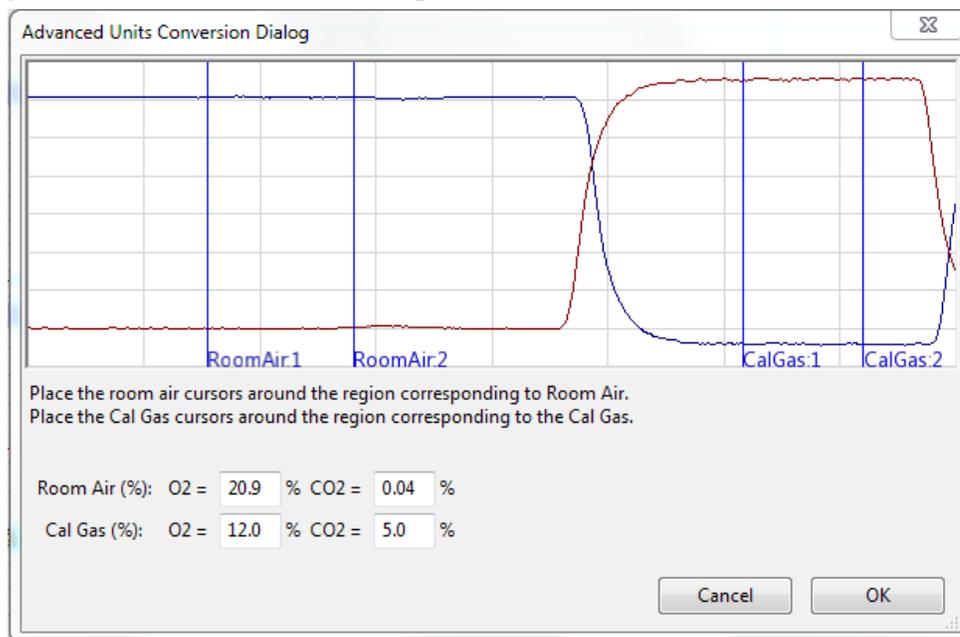
- Clamp and secure any gas cylinders that will be used to provide gas samples near the gas analyzer.
- Attach the regulator to the gas cylinder with a wrench making sure there are no leaks in the system.
- Disconnect the nafion tubing from the small animal chamber and connect it to the A-CAL-200

Choose Advanced→Metabolic→Calibrate Gases.



Follow the directions as prompted. Room air will be sampled for 10 seconds. Calibration gas will be sampled for 15 seconds.

If necessary, move the cursors into correct position



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

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

Merasuring Changes in CO₂ and O₂, and calculating RER in a Multiple closed chambers

Aim: To determine mean RER of multiple animals at rest.

Procedure

1. Place the animals in the chambers and close the lid securely.
2. Click on the **RER-4Chamber** button on the LabScribe Toolbar.
3. The Recording will start and the Program will start sampling from each chamber for 2 minutes.
4. Click the AutoScale All button.
5. On the Expired CO₂ Concentration (%) channel, notice that the CO₂ concentration shows a continuous steady rise in level throughout the recording from a Chamber
6. Marks will be placed in the record when data is recorded from a chamber.

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 CO₂ continues to rise as the animal exhales into the chamber.*

6. 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 CO₂ concentration will continue to rise while data is recorded from a chamber and levels can become toxic to the animal in the chamber. Remove the animal immediately if the CO₂ levels reach 3.5%.*

7. On the Expired O₂ Concentration (%) channel, notice that the O₂ 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.
8. On the RER channel, an equation programmed in the software determines RER based on the relative levels of CO₂ (which is increasing) and O₂ (which is decreasing).
9. The recording will stop after each chamber is sampled 10 times.
10. The Macro controlling this experiment can be modified to suit the experimental setup as required.

Note: Remember to remove the animal from the chamber immediately if the carbon dioxide concentration reaches 3.5%.

11. Your recording should be similar to the data displayed in [Figure AMe-6](#).
12. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
13. Remove the animals from the chambers and place them back into their containers.
14. Clean and dry the small animal chambers 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-7](#)).
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-8](#)).
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%.
6. Click OK.
7. The recording on the main window will now have a histogram on the RER channel.

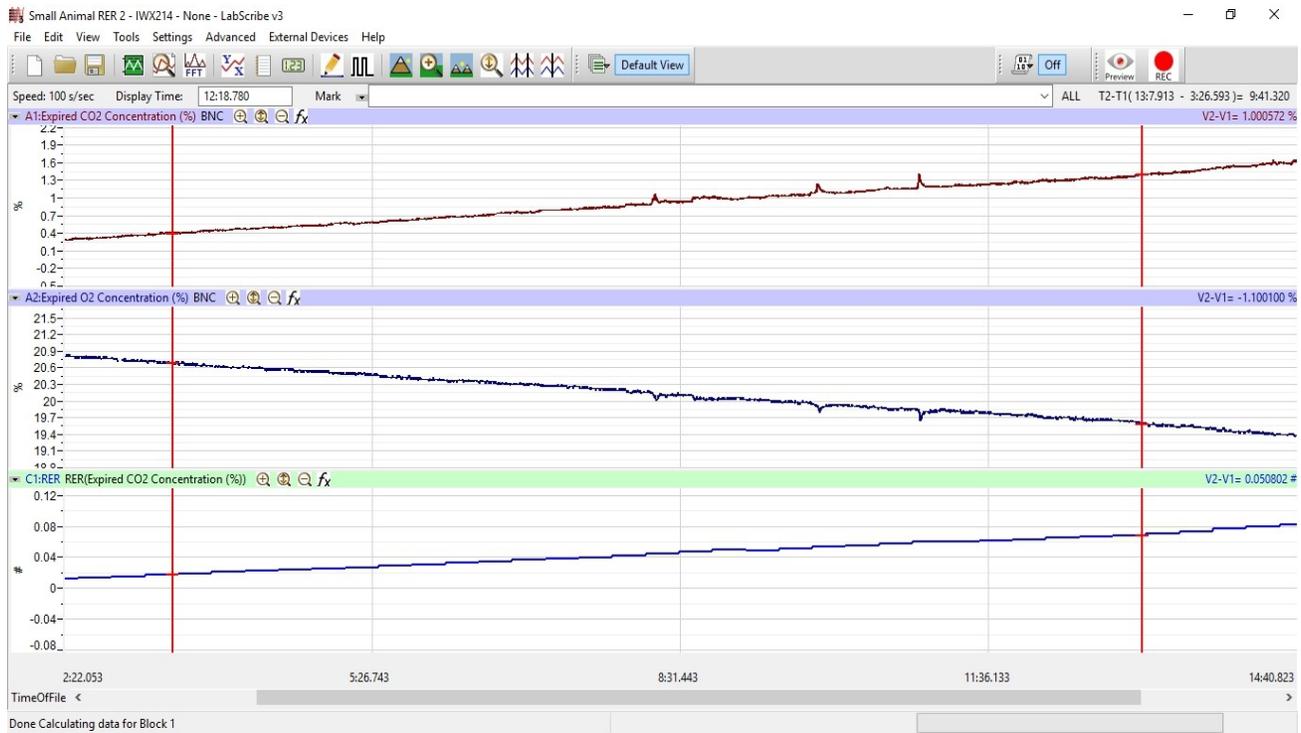


Figure AME-6: The recording of the expired CO₂ over a period of 28 minutes showing a change in concentration by 1%.

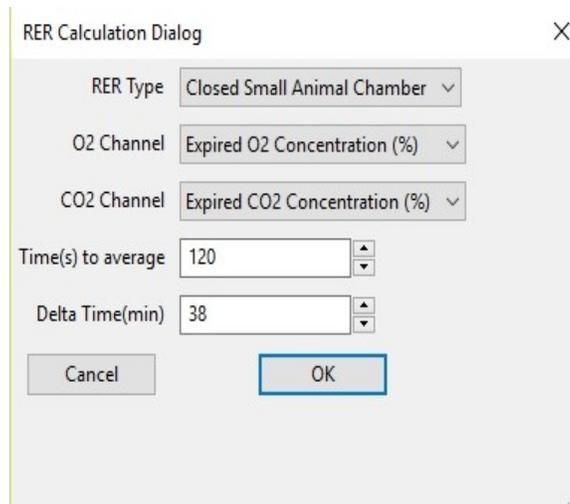


Figure AME-7: RER Calculation Dialog window.

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.

- Click the Zoom between Cursors button on the toolbar to expand this section of data to fill the window.
- Click AutoScale on all channels. Your data should look like the data seen in [Figure AMe-7](#).

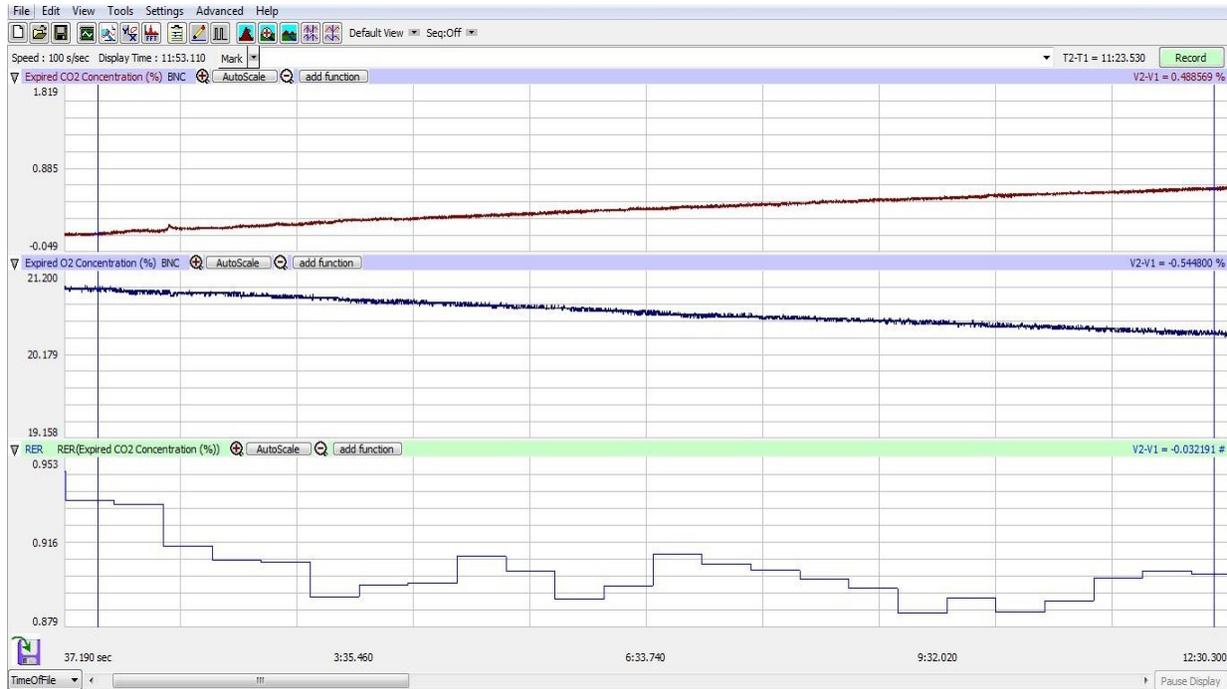


Figure AMe-8: The RER channel showing the histogram for the ratio of CO₂ to O₂ in the endothermic organism.

- 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-9](#)).
- 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.
- Once the cursors are placed in the correct positions for determining the CO₂ and O₂ 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.
- 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.
- 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.
- Repeat this procedure for the Expired O₂ Concentration (%) channel.

13. The values for the following parameters are determined when the cursors are positioned as directed:
14. Mean concentration of CO₂ in expired air, which is the value for Mean on the Expired CO₂ Concentration channel.
15. Mean concentration of O₂ in expired air, which is the value for Mean on the Expired O₂ Concentration channel.
16. Mean RER, which is the value for Mean on the RER channel. This is calculated by dividing the expired CO₂ value by the expired O₂ value to get the RER ratio as setup previously.
17. Record the T2-T1 value that is used to measure the mean values of CO₂, O₂ and RER.
18. Record the values in the Journal using one of the techniques described in Steps 6 or 7.
19. Record the values for the Mean CO₂ and O₂ concentrations in expired air in [Table AMe-1](#). Also record the RER and T2-T1 values.

Weight Normalization Calculation

Normalized Expired O₂

$$O_2 / [(weight(g) / mass unit)]^{effective\ mass}$$

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

$$O_{2_{norm}} = 0.83 / (25/1000)^{0.75} = 13.2\ ml/KG/min$$

Using an effective mass factor of 1 eliminates any effective mass correction.

This can be repeated for the CO₂ values. When RER is calculated using normalized values, the weight of the organism is taken into account.