Fitness Assessment - Aerobic Fitness Testing

Equipment Required

PC or Mac Computer and USB cable

IX-TA data acquisition unit and power supply

A-FH-1000 Flow head with flow head tubing

A-GAK-201 Reusable mask and non-rebreathing valve

6ft smooth bore tubing (35mm I.D.)

5 Liter Mixing Chamber

iWire-GA CO_2/O_2 Gas Analyzer with filter and Nafion gas sample tubing

A-CAL-150/A-CAL-200 Calibration kit

Treadmill with adjustable speed and gradient or Fitness Bike

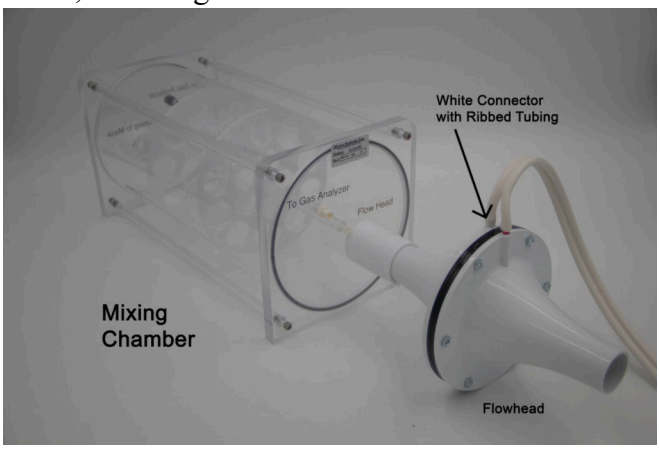
3 Liter Calibration syringe

Setup the IXTA and the iWireGA

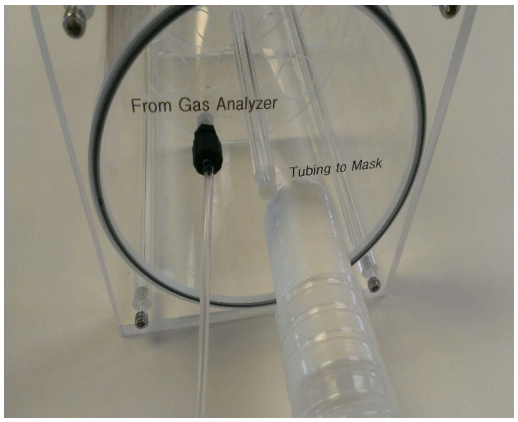
1. Place the IXTA on the bench, close to the computer.
2. Use the USB cable to connect the computer to the USB port on the rear panel of the IXTA.
3. **Connect the iWire-GA unit to the front of IXTA using the iWire-1 port.**
4. Plug the power supply for the iWire-GA into the electric outlet. Insert the plug on the end of the power supply cable into the labeled socket on the rear. Use the power switch to turn on the unit. Confirm that the power light is on.
5. Plug the power supply for the IXTA into the electrical outlet. Insert the plug on the end of the power supply cable into the labeled socket on the rear of the IXTA. Use the power switch to turn on the unit. Confirm that the power light is on.

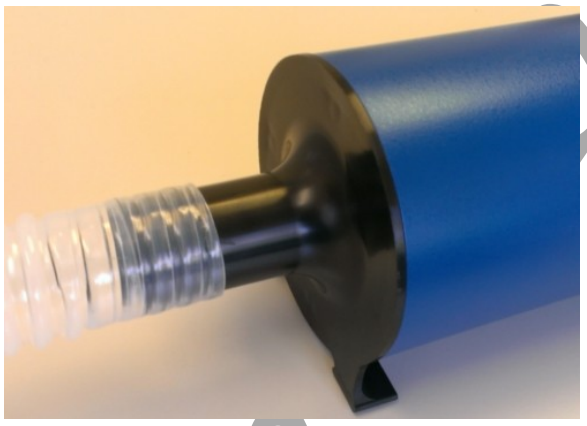
Setup the Metabolic Cart

1. Locate the A-FH-1000 flow head and tubing in the iWorx kit.
2. Plug the flow head tubing into channel A1 of the IX-TA, matching the colors.
3. Connect the other end of the flow head tubing to the flow head, making sure that the ribbed side of the tubing connects the red marked port on the flow head.
4. Locate the mixing chamber in the iWorx kit.
5. Connect end of the flow head to the flow head port of the mixing chamber, making sure that the white port faces the mixing chamber. Make sure the tubing is in an upright direction.
6. Locate the non-rebreathing valve, mask, and smooth bore tubing in the iWorx kit

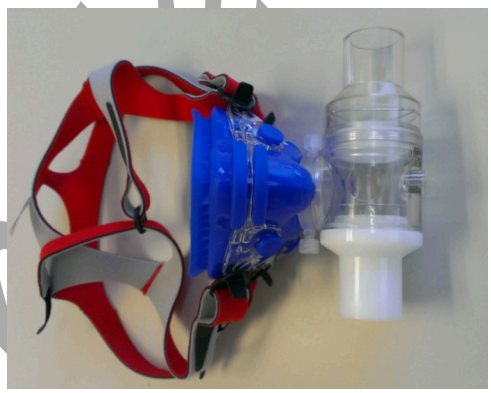


7. Attach one end of the smooth bore tubing to the "**Tubing to Mask**" inlet of the mixing chamber.
8. Attach the other end of the smooth bore tubing to the calibration syringe.

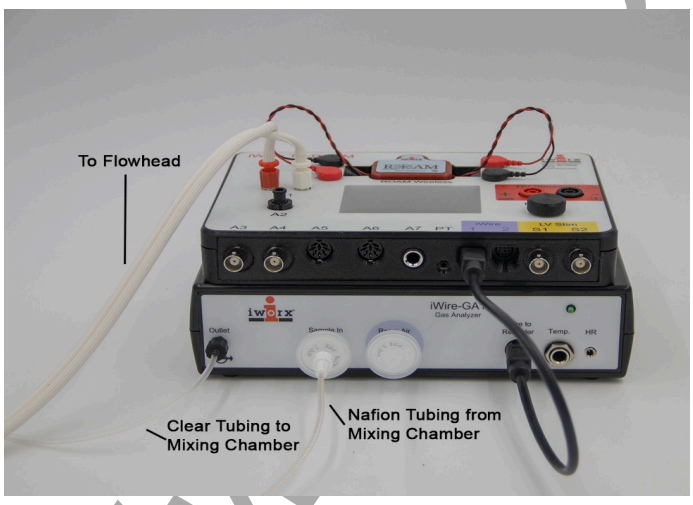




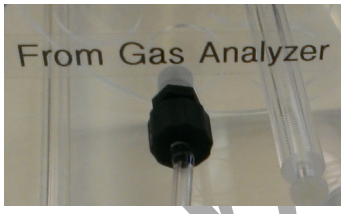
9. Attach the mask to the side port of the non-rebreathing valve, using the provided adaptor, if needed.
10. *Note: There are arrows on the valve that indicate the direction of air flow. The white port is the inlet and the clear port is the outlet. The Clean Bore tubing will be plugged into the clear port later when testing the subject.*



11. Place the filter on the Sample In port of the iWire-GA in the lower right front corner of the gas analyzer. Attach the braided end of the Nafion sampling tube to the filter.
12. Connect the other end of the Nafion sampling tubing to the white "**To Gas Analyzer**" port on the mixing chamber near the Flow Head

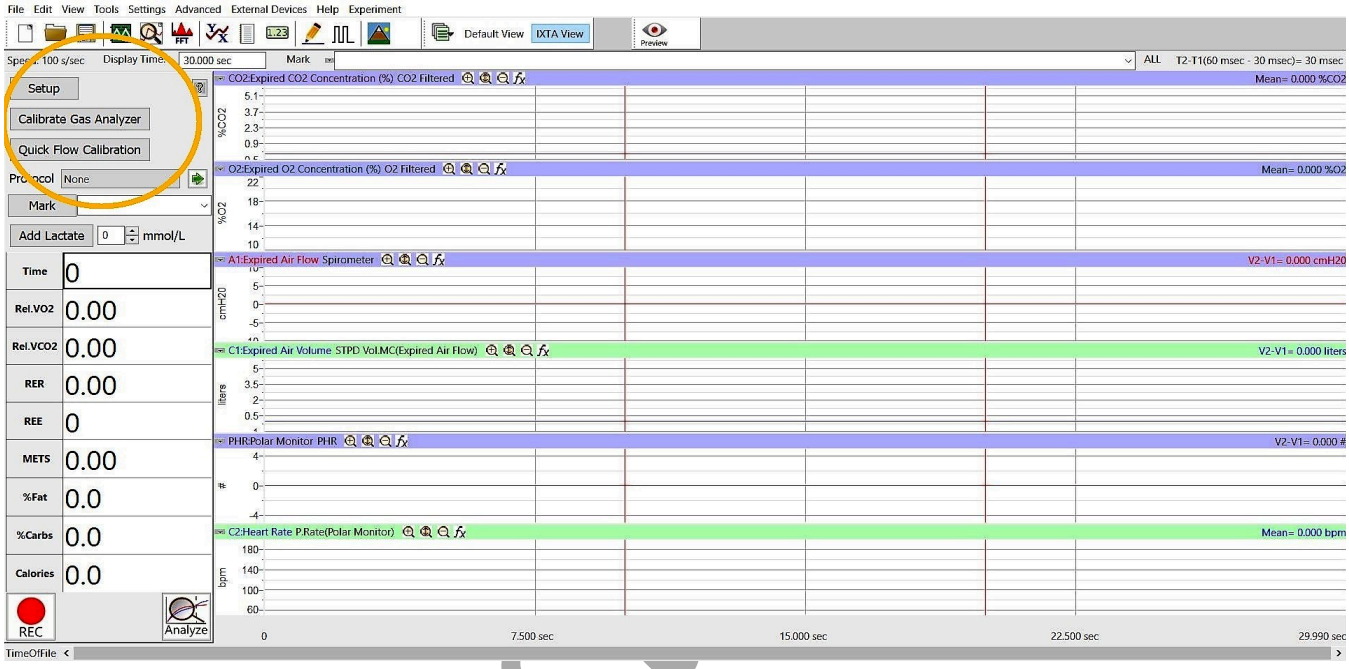


13. Attach a Clear tubing to the outlet gas analyzer.
14. Attach the other end of the clear gas analyzer tubing to the "**From Gas Analyzer**" connector on the mixing chamber next to the smooth bore tubing

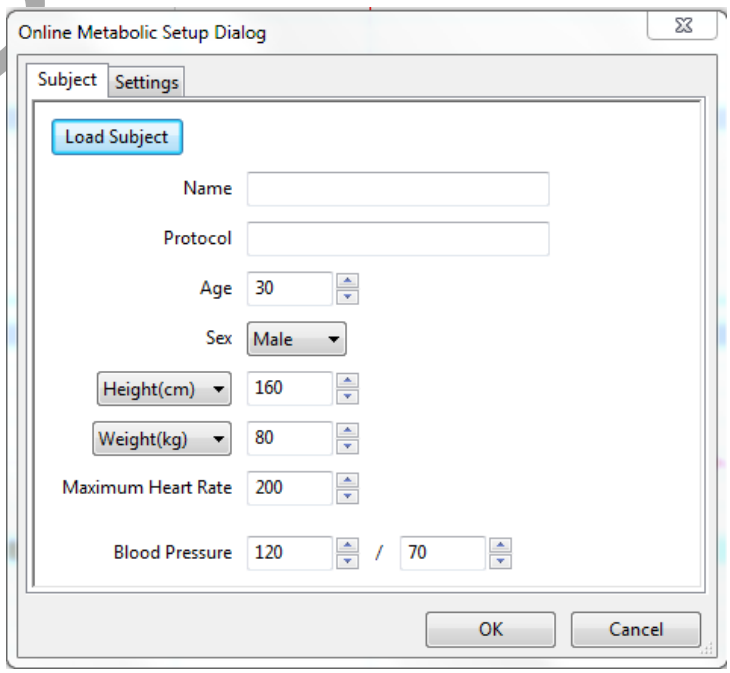


Software Setup:

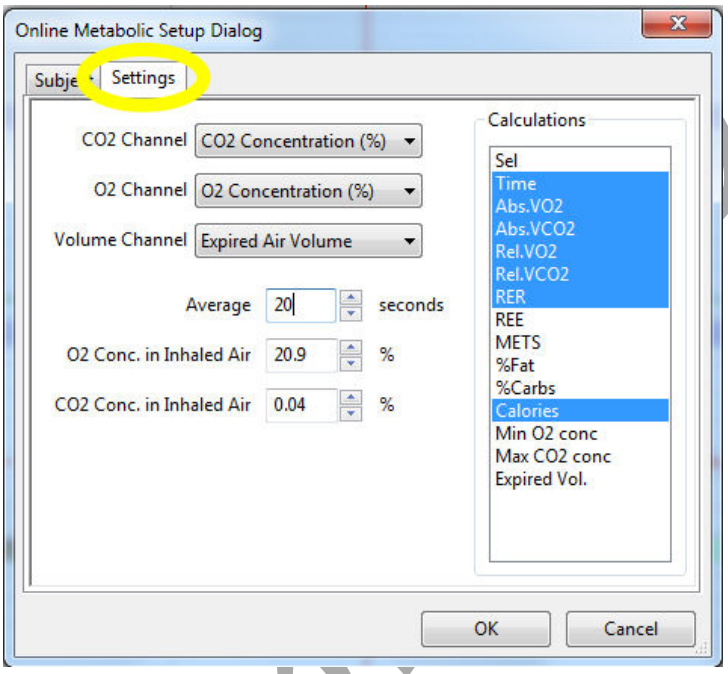
1. Load the settings for RMR-SubmaxVO2RER if you have not done so already.



2. Click the **Setup** button shown in the left side window. The Online Setup Dialog window will open
   - Enter your subject's information or Load a subject from a previously saved file.



- Click “Settings” to change any parameters you wish to view
- Click OK to save the changes.

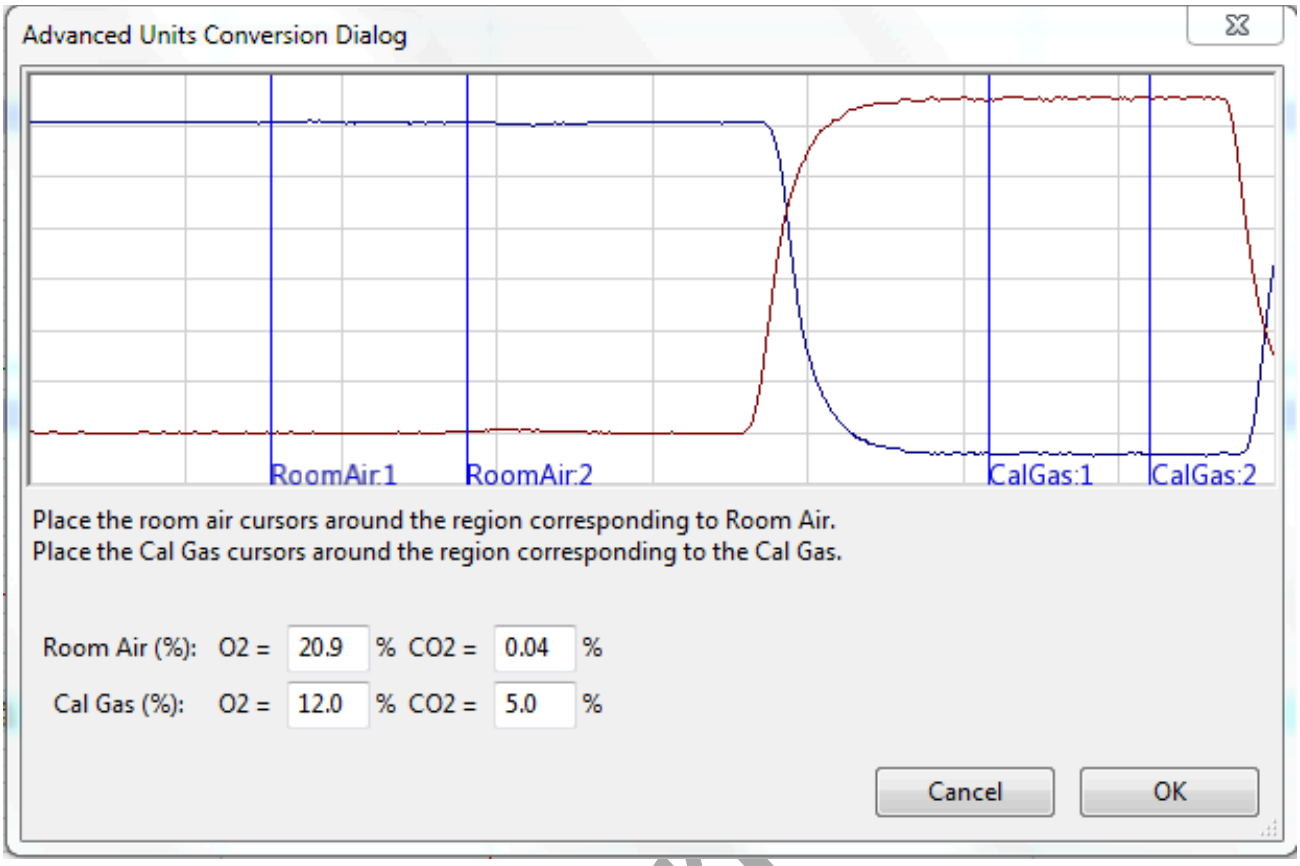


Calibrating the iWire-GA Gas Analyzer

Note: *Warm up the iWire-GA for at least 15 minutes prior to use. Make sure the calibration gas tank is located close to the Fitness Assessment equipment.*

This procedure will calibrate the O_2 and CO_2 channels.

1. Connect the gas sample tubing of the A-CAL-150 Calibration Kit to the output barbed connector of the gas regulator.
2. Click the **Calibrate Gas Analyzer** button. Click Perform Quick Software Gas Calibration.
3. Follow the directions as prompted. Room air will be sampled for 10 seconds. Calibration gas will be sampled for 20 seconds.
4. If necessary, move the cursors into correct position.
5. Click OK.



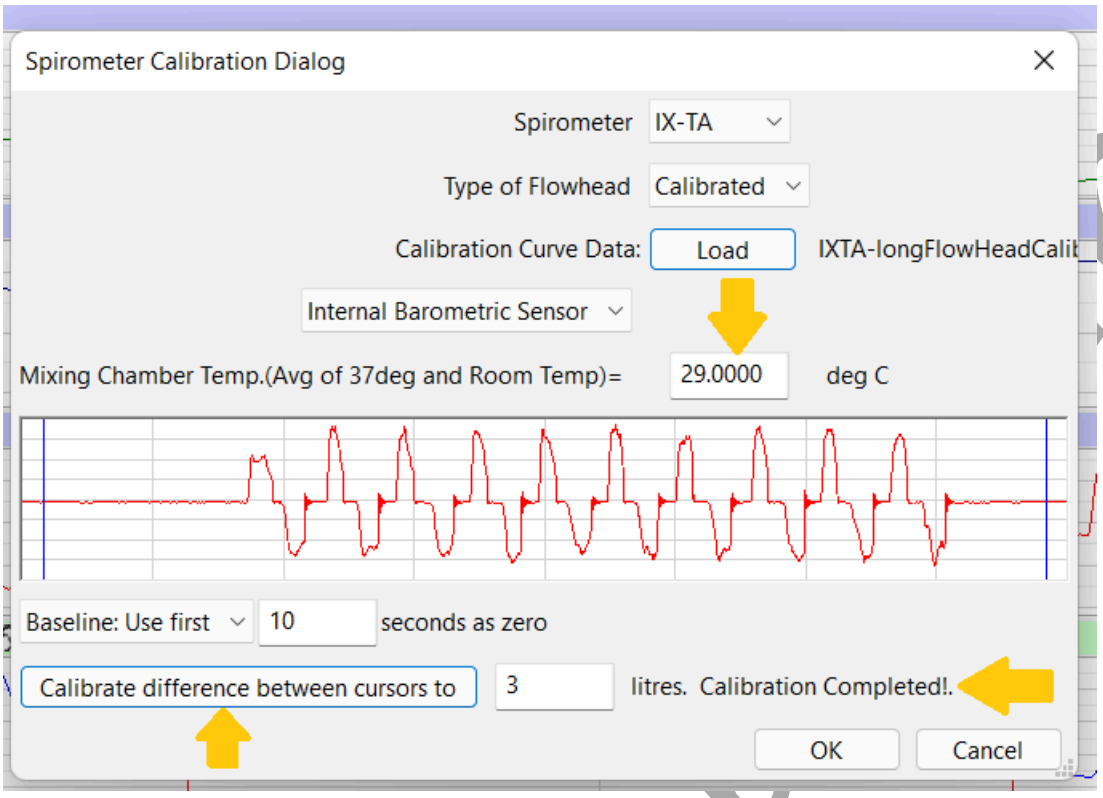
Quick Flow Calibration :

A pre-saved .iwxfcd (file IXTA-longFlowHeadCalibration) is already loaded for you.

If you prefer, click “Load”, to load the .iwxfcd file created when you performed the optional full flow head calibration.

Perform the Quick Flow Calibration by clicking the button and following the prompted directions as they pop up on the LabScribe Screen.

- After the calibration data has been collected, The Spirometer Calibration dialog window will pop-up.
- Position the two cursors on the flat line to either side of the recording shown.
- Change the temperature to 29 deg C.
- Click the 'Calibrate difference between cursors to' button.
- Click OK.



Prior to performing your test:

Determine the correct concentration of O2 in the mixing chamber:

- Click on the Preview button.
- Record room air by sampling air through the mixing chamber. Look at the value for O2 as you record for approximately 10 seconds. It should be reading between 20.7% and 21%.

NOTE – If the O2 channel is not reading between 20.7% and 21% - REPEAT the Quick Flow Calibration.

- Click on the Stop button.

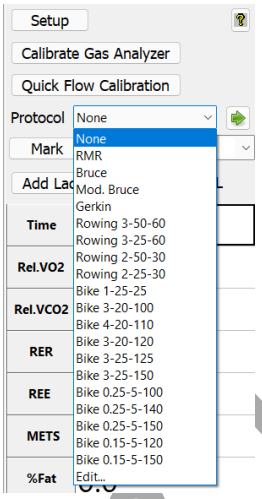
Now you are ready to perform your test. Some sample protocols are provided on the CD and on the website.

Performing the Test:

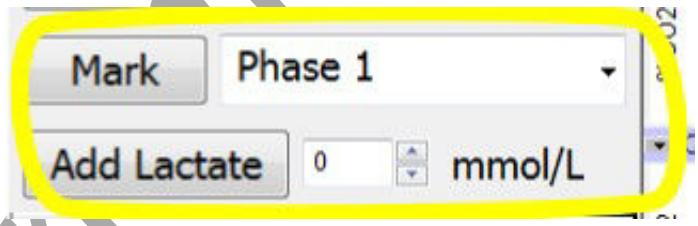
1. Attach the head gear to the mask.
2. Instruct the subject to try on the assembly. Adjust the straps so that the mask fits the subject comfortably. Make sure there are no leaks around the mask.
3. Connect the smooth-bore tubing to the clear outlet of the non-rebreathing valve. There are arrows on the valve that indicate the direction of air flow.
4. Make sure the flaps on the non-rebreathing valve are facing the right way.

5. Remove the smooth-bore tubing from the mixing chamber to record baseline.
6. Click the **Record** Button to start recording data 

7. Or choose a protocol from the protocol list and click the green arrow to start recording.



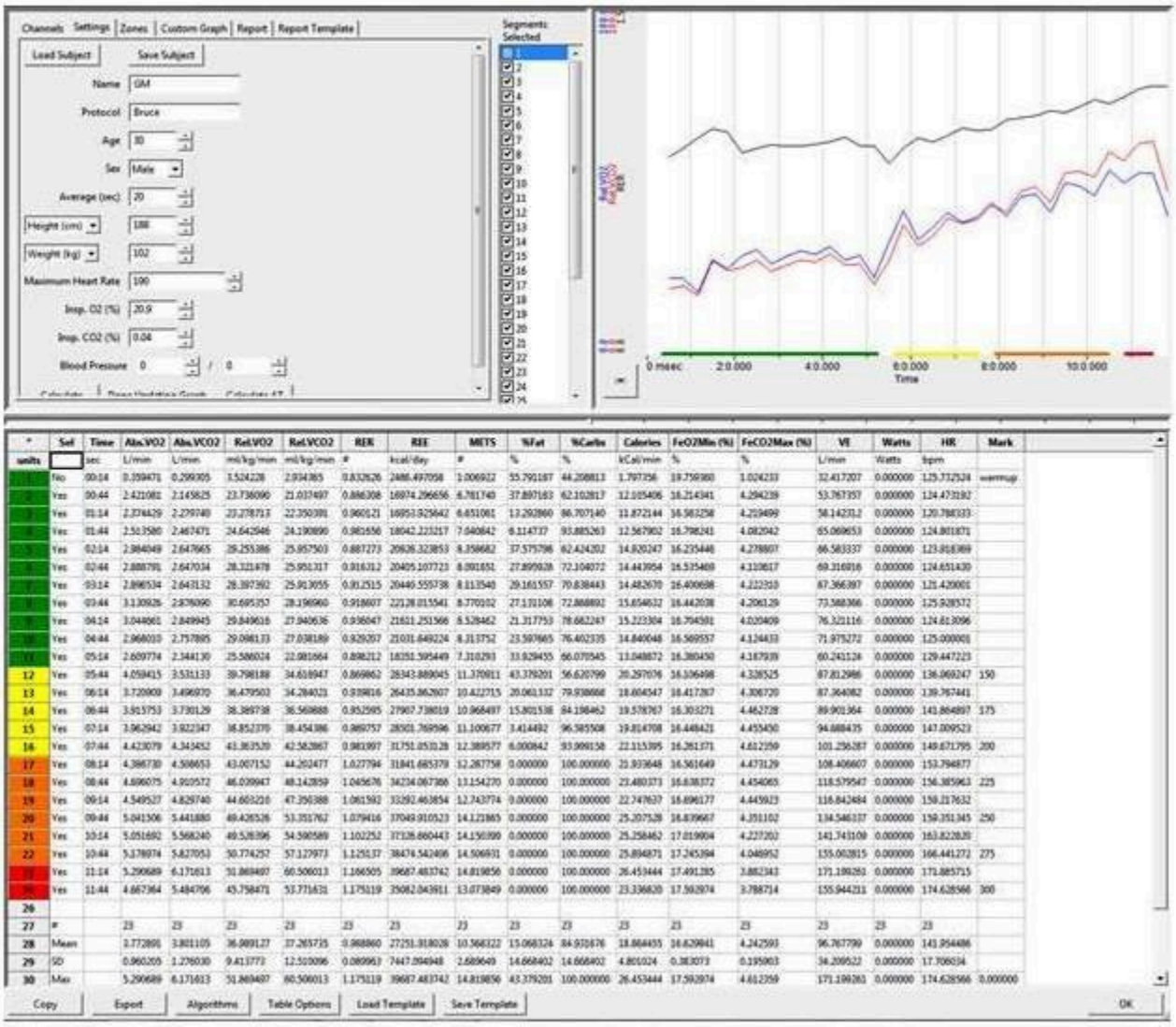
8. Wait at least 10 seconds for the system to zero, then reconnect the smooth-bore tubing to the mixing chamber.
9. The test has now started.
10. Mark the data for each stage of the exercise protocol you have chosen to use. You can enter text in the text area next to the **Mark** button and click on the **Mark** button, or choose one of the preset marks from the drop down list.



11. If lactate values are measured during the test, you may enter them in the record using the **Add Lactate** button.
12. Click **Stop** and then Click Save As to save your data 

Analyze your data

1. Click **Analyze** to pull up the automated metabolic calculations and chart generator.
2. Make sure channels are set correctly.
3. Click Settings: Your subject information should be automatically populated into the settings.
4. Click Calculate to generate the table as seen below.
5. To generate a Report, click on the Report Tab. Reports can be customized as well.
6. Refer to the metabolic module manual in the LabScribe manual for more information.



*	Sel	Time	Abs.VO2	Abs.VCO2	Rel.VO2	Rel.VCO2	RER	REE	METS	%Fat	%Carbs	Calories	FeO2Min (%)	FeCO2Max (%)	VE	Watts	HR	Mark
units		sec	L/min	L/min	ml/kg/min	ml/kg/min	#	kcal/day	#	%	%	kCal/min	%	%	L/min	Watts	bpm	
1	No	00:14	0.359471	0.299305	3.524228	2.934365	0.832626	2486.497058	1.006922	55.790187	44.209813	1.797356	19.759360	1.024233	32.417207	0.000000	125.732524	warmup
2	Yes	00:44	2.421081	2.145825	23.736090	21.037497	0.886308	16974.296656	6.781749	37.897183	62.102817	12.105406	16.214341	4.294239	53.767357	0.000000	124.473192	
3	Yes	01:14	2.374429	2.279740	23.278713	22.350391	0.960121	16953.929642	6.651081	13.292860	86.707140	11.872144	16.981258	4.219499	58.142312	0.000000	120.788333	
4	Yes	01:44	2.513580	2.467471	24.642946	24.190890	0.981656	18042.223217	7.040842	6.114737	93.885263	12.567902	16.798241	4.082942	65.069653	0.000000	124.801871	
5	Yes	02:14	2.384049	2.647665	29.255386	25.957503	0.887273	20828.323853	8.358682	37.575798	62.424202	14.920247	16.225446	4.278807	66.583337	0.000000	123.818389	
6	Yes	02:44	2.888791	2.647034	28.321478	25.951317	0.916312	20405.107723	8.001851	27.899928	72.100072	14.443954	16.535469	4.210817	69.316916	0.000000	124.651430	
7	Yes	03:14	2.898534	2.643132	28.397392	25.913055	0.912515	20449.555738	8.013540	29.161557	70.838443	14.482670	16.400698	4.222310	67.366397	0.000000	125.429001	
8	Yes	03:44	3.130926	2.876090	30.695357	28.196960	0.918607	22128.015541	8.770102	27.131108	72.868892	15.654632	16.442038	4.206128	73.588366	0.000000	125.928572	
9	Yes	04:14	3.044861	2.849945	29.849618	27.940636	0.936047	21611.251366	8.528462	21.317753	78.682247	15.223304	16.794581	4.020409	76.321116	0.000000	124.813096	
10	Yes	04:44	2.968010	2.757895	29.098133	27.038189	0.929297	21031.649224	8.313752	23.597665	76.402335	14.840048	16.569557	4.124433	71.975272	0.000000	125.000001	
11	Yes	05:14	2.659774	2.344130	25.586024	22.981664	0.898212	18351.595449	7.310293	33.829455	66.070545	13.048872	16.380450	4.187939	60.243124	0.000000	129.447223	
12	Yes	05:44	4.058415	3.535133	39.798188	34.618947	0.869862	28343.889045	11.370911	43.379201	56.620799	20.297976	16.106498	4.328525	87.812986	0.000000	138.069247	150
13	Yes	06:14	3.720809	3.496970	36.479503	34.284021	0.939816	26435.862607	10.422715	20.061332	79.938668	18.604547	16.417287	4.306720	87.364082	0.000000	139.767441	
14	Yes	06:44	3.915753	3.730129	38.389738	36.563688	0.952595	27907.738019	10.968497	15.801538	84.198462	19.578767	16.303271	4.462728	89.901364	0.000000	141.864897	175
15	Yes	07:14	3.962942	3.922347	38.852379	38.454386	0.989757	28501.768596	11.100677	3.414492	96.585508	19.814708	16.448421	4.455450	94.688435	0.000000	147.009523	
16	Yes	07:44	4.423079	4.343452	43.383520	42.582867	0.981897	31751.051028	12.389577	6.000842	93.999158	22.115395	16.261371	4.612359	101.256287	0.000000	149.671795	200
17	Yes	08:14	4.386730	4.506653	43.007152	44.202477	1.027794	31841.685379	12.287758	0.000000	100.000000	21.933648	16.361649	4.473129	108.406607	0.000000	153.794877	
18	Yes	08:44	4.696075	4.910572	46.039947	48.142859	1.045676	34234.067388	13.154270	0.000000	100.000000	23.480373	16.638372	4.454065	118.579547	0.000000	156.385963	225
19	Yes	09:14	4.549527	4.829740	44.603220	47.350388	1.061592	33292.463854	12.743774	0.000000	100.000000	22.747627	16.896177	4.445923	116.842484	0.000000	159.217632	
20	Yes	09:44	5.041506	5.441880	49.428526	53.351762	1.079416	37049.910523	14.123865	0.000000	100.000000	25.207528	16.839667	4.351102	134.546107	0.000000	159.351345	250
21	Yes	10:14	5.051692	5.568240	49.528396	54.590589	1.102252	37328.660443	14.150399	0.000000	100.000000	25.258462	17.019904	4.227202	141.743109	0.000000	163.822820	
22	Yes	10:44	5.178074	5.827053	50.774257	57.127973	1.125137	38474.542406	14.506931	0.000000	100.000000	25.894871	17.245394	4.046952	155.002815	0.000000	166.441272	275
23	Yes	11:14	5.290689	6.171613	51.869497	60.506013	1.166505	39687.483742	14.819856	0.000000	100.000000	26.453444	17.491285	3.882343	171.189281	0.000000	171.885715	
24	Yes	11:44	4.667364	5.484706	45.758471	53.771631	1.175119	35082.043911	13.073849	0.000000	100.000000	23.336820	17.592974	3.788714	155.944231	0.000000	174.628566	300
26																		
27	#		23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	
28	Mean		3.772891	3.801105	36.989127	37.265735	0.988860	27251.318028	10.366322	15.068324	84.931676	18.864455	16.629841	4.242593	96.767799	0.000000	141.954486	
29	SD		0.960205	1.276030	9.413773	12.510096	0.080963	7447.094948	2.689649	14.668402	14.668402	4.801024	0.383073	0.195903	34.209522	0.000000	17.706034	
30	Max		5.290689	6.171613	51.869497	60.506013	1.175119	39687.483742	14.819856	43.379201	100.000000	26.453444	17.592974	4.612359	171.189281	0.000000	174.628566	0.000000

Metabolic parameters and plots of VO_2, VCO_2, and RER vs. Time, displayed in the Offline Metabolic Calculations window used to analyze data collected during an aerobic fitness test.

Notice that the VO_2 and VCO_2 values increase quickly as the subject performs more strenuous segments of the test.

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%.

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.

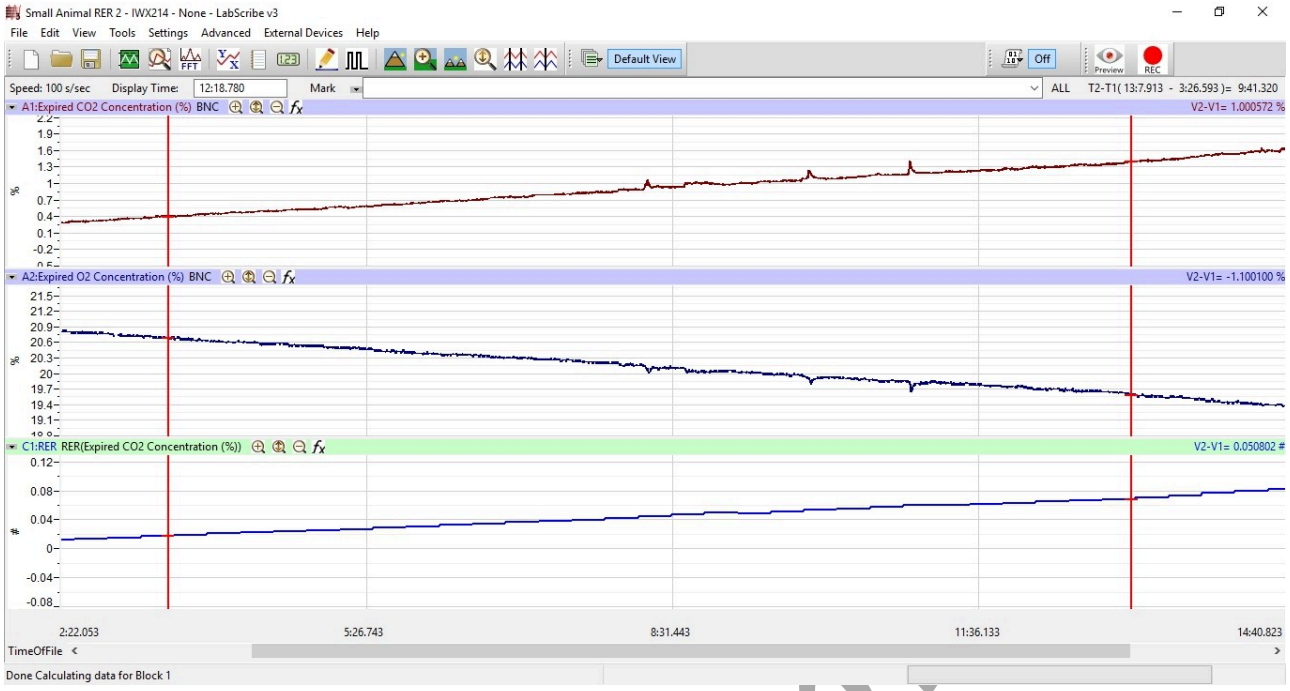


Figure AMe-1-L1: The recording of the expired CO2 over a period of 28 minutes showing a change in concentration by 1%.

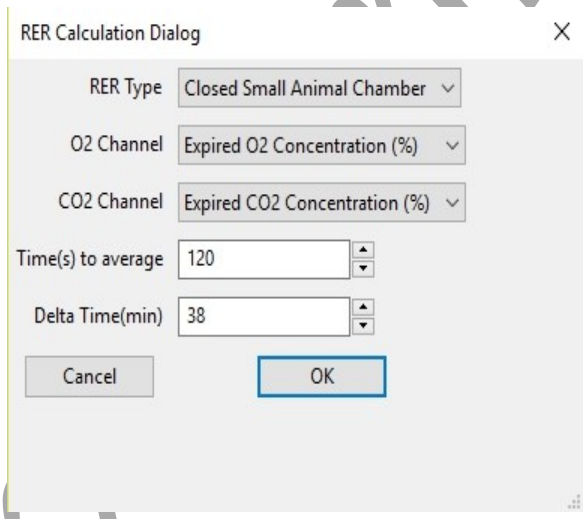


Figure AMe-1-L2: RER Calculation Dialog window.

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 the image below.

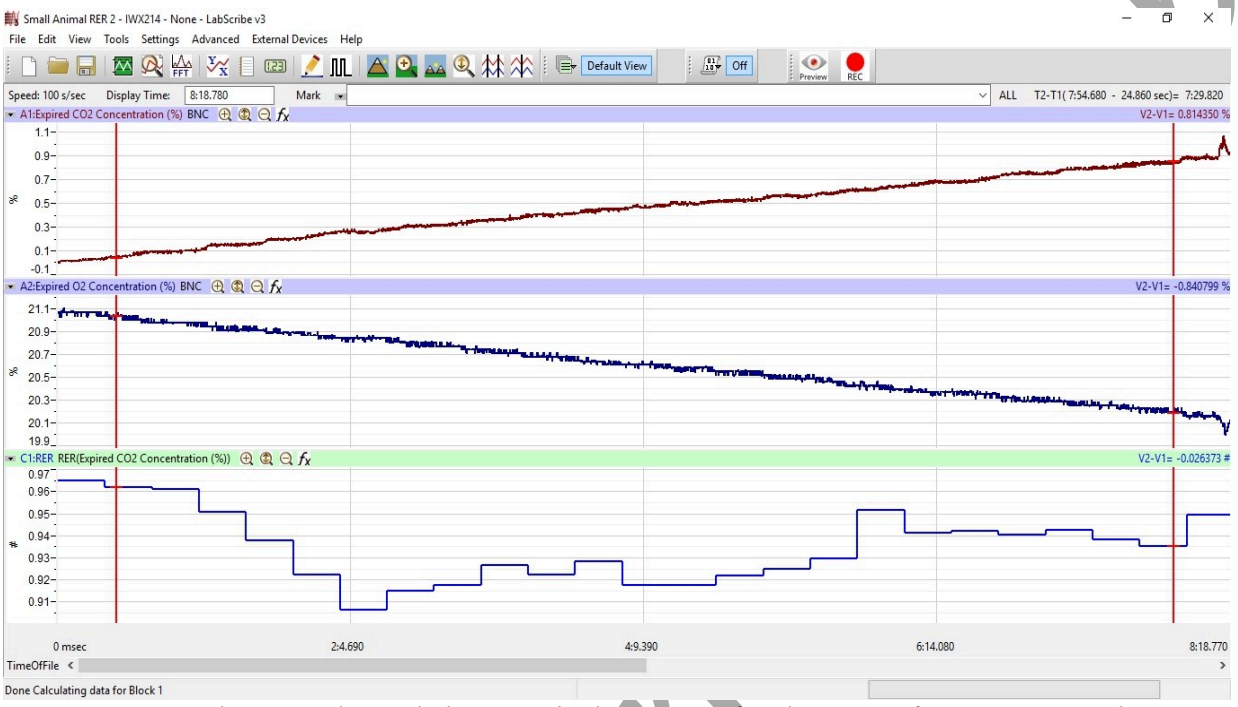


Figure AMe-1-L3: The RER channel showing the histogram for the ratio of CO2 to O2 in the endothermic organism.

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.

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.

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?

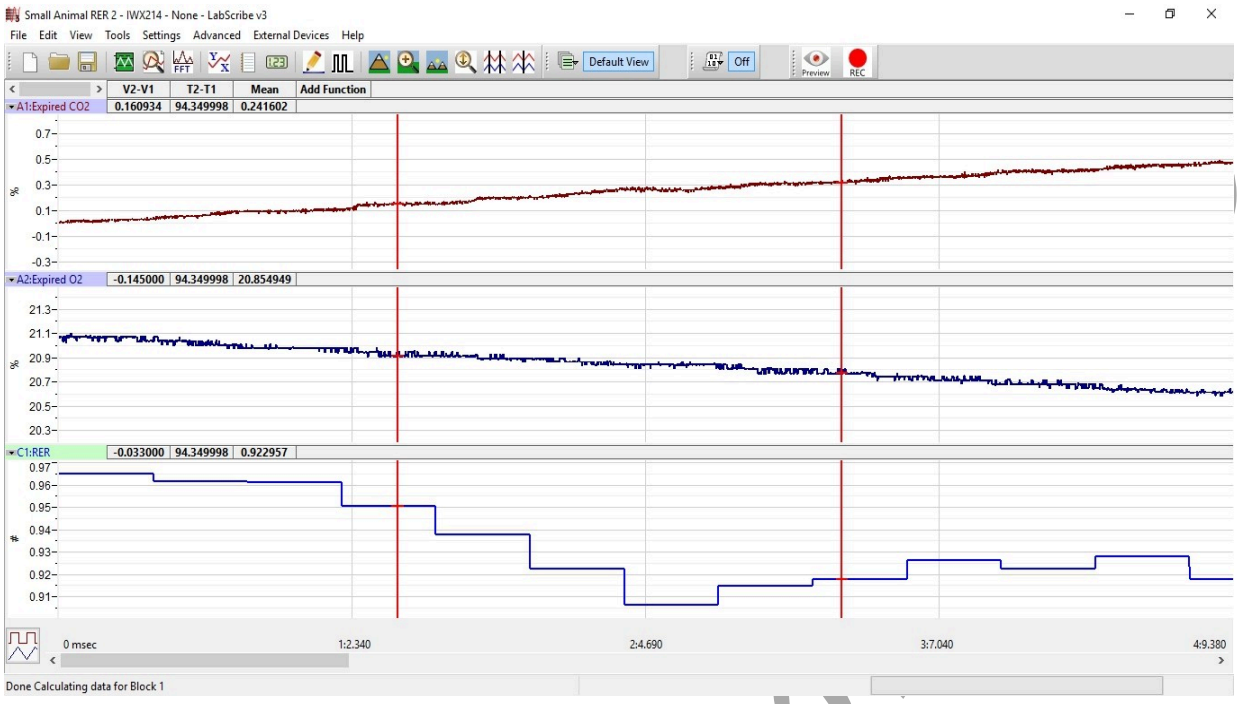


Figure AMe-1-L4: The expired oxygen and carbon dioxide concentration and RER of a small animal as displayed in the Analysis window. The cursors are in position to measure the mean values of the selected interval.

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			

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.