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HIGHER EDUCATION

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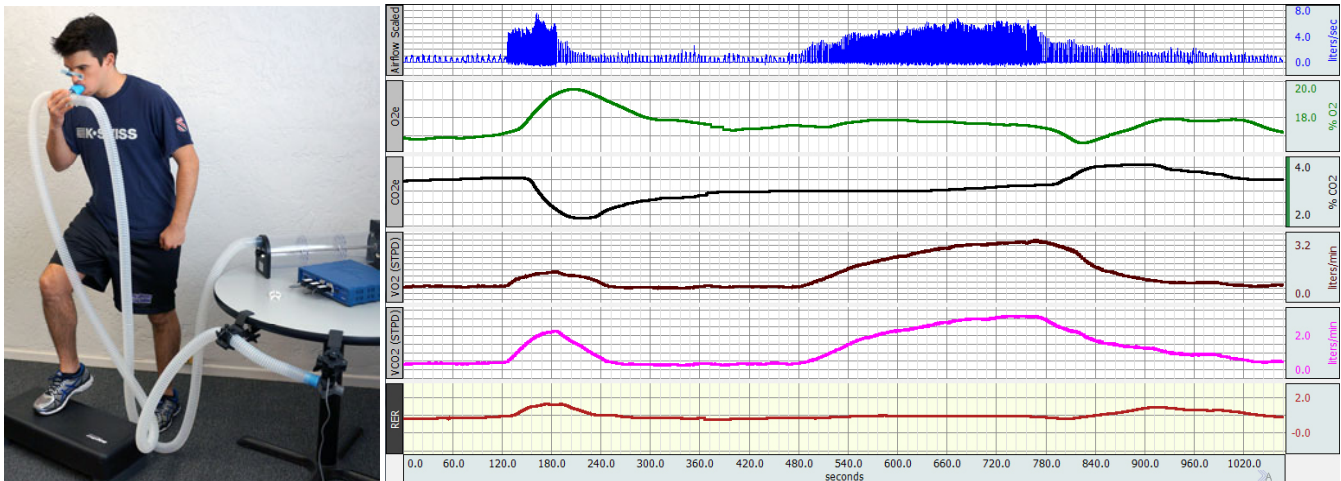
Updated 05.30.18

BSL *PRO* Lesson H19: $\dot{V}O_2$, $\dot{V}CO_2$, RER, O_2 deficit and EPOC Measurement

This BSL *PRO* lesson describes the hardware and software setup of the BSL System in order to measure $\dot{V}O_2$, $\dot{V}CO_2$ and RER using an open circuit indirect calorimetry technique.

Objectives:

1. Gain an understanding of $\dot{V}O_2$, $\dot{V}CO_2$, RER, O_2 deficit and EPOC and learn how they can be measured.
2. Learn how RER values indicate how much carbohydrate or fat is used for aerobic metabolism.
3. Record $\dot{V}O_2$, $\dot{V}CO_2$ and RER for a subject at rest, during and after hyperventilation, and during and after recovery from moderate submaximal exercise*****.
4. Examine the changes in $\dot{V}O_2$, $\dot{V}CO_2$ and RER over the recording period.
5. Measure O_2 deficit and EPOC from the recorded data.

   *The GASSYS2 module is intended for $\dot{V}O_2$ and RER studies ranging from resting to moderate submaximal exercise. For prolonged or $\dot{V}O_2$ max exercise studies where CO_2 levels may exceed 5%, BIOPAC recommends the Acq*Knowledge* Research system with O2100C and CO2100C gas sensing modules.

Equipment:

- Biopac Student Lab System:
  - MP36 or MP35 hardware
  - BSL 4.0.1 or greater software
- BSL *PRO* template file: "**h19_bsl4.gtl**"
- Airflow transducer (SS11LB or SS11LA))
- Gas Analysis System (GASSYS2-EA)
- 2 Liter Calibration Syringe (AFT26)
- Coupler, 35 mm OD, 35 mm OD (AFT11D)
- T-Valve, 35 mm OD (AFT21)
- Mouthpiece (AFT9)
- Disposable Nose Clip (AFT 3)
- 2x Tubing, 35 mm ID (AFT7-L. 3m)
- Coupler, 25 mm OD, 28.6 mm ID (AFT11H)

- Barometer and Thermometer gauge

OPTIONAL:
- Head support for T-Valve (AFT24)
- Clamp for SS11LB/LA (Chauvet CLP-10)
- Hose clamp (ADJ O-Clamp/1.5)
- 2x C-Clamp (Chauvet CLP-02)

Calibration Gas setup:
- Cal. Gas, 4% CO_2, 16% O_2 (GASCAL)
- Gas Regulator (GASREG)
- Gas cylinder cart
- PVC Tubing, 1/8" ID (M537)
- Luer Lock Male to 3/32" barb (CN192)

Background:

Cellular processes such as muscle contraction, nerve impulse conduction, and membrane transport require chemical energy. The universal currency for chemical energy in animals is adenosine triphosphate (ATP), a chemical compound containing large amounts of stored energy in some of its chemical bonds. Although chemical energy is present in the proteins, carbohydrates, and fats we eat as food, this energy cannot be directly used for powering cell processes. Some of the energy must first be transferred to ATP, which then provides the energy required for the performance of cell work. The basic building blocks of proteins, carbohydrates, and lipids are amino acids, monosaccharides, glycerol, and fatty acids, respectively. When these basic constituents are metabolized, oxygen (O_2) is consumed and energy is released. The production of ATP using oxygen is called **aerobic metabolism** and it utilizes a process known as **oxidative phosphorylation** that occurs in the mitochondria of cells.

Blood transports gases to and from the body's cells. The respiratory system supplies O_2 to the blood, and removes carbon dioxide (CO_2) from the blood with each respiratory cycle. Most of the gas exchange occurs at the level of the alveoli in the lungs, and the process is completely dependent on the maintenance of gas partial pressures favorable for adequate diffusion of O_2 and CO_2. Blood that has absorbed O_2 during inhalation (oxygenated blood) is transported by the cardiovascular system to systemic tissues throughout the body. Once in the tissues, the O_2 moves from the bloodstream into cells by diffusion, where it is used for ATP production. Although the majority of ATP is produced by aerobic metabolism, some ATP is produced via anerobic (without O_2) metabolism. Anaerobic metabolism can produce ATP much more quickly than aerobic metabolism, but can only sustain production for a short amount of time.
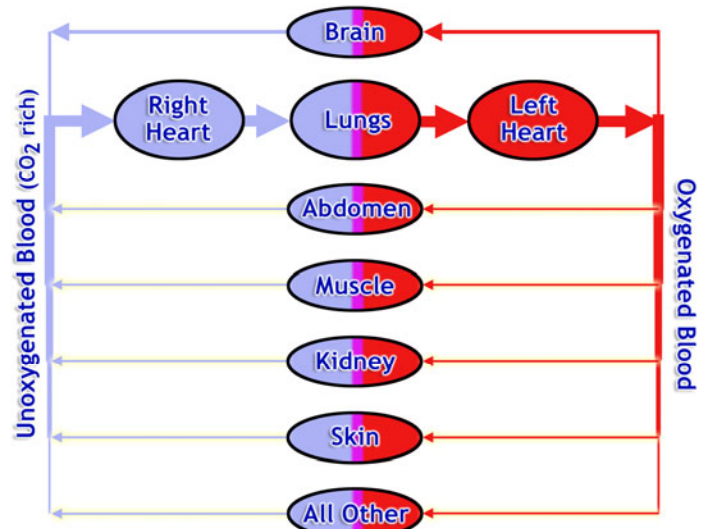

Figure 1

Oxygen consumption ($\dot{V}O_2$) is the amount of oxygen taken in and used by the body per minute. The "dot" above the V signifies the extraction of a **rate** measurement rather than a **volume** measurement. $\dot{V}O_2$ is dependent on the amount of air that can be moved in and out of the lungs during ventilation, the amount of O_2 extracted from air in the alveoli, the volume of blood that the heart can pump, and the tissues' capacity for extracting O_2 from the blood. $\dot{V}O_2$ can yield useful information and is one of the most common studied variables in exercise physiology.

$\dot{V}O_2$ can be reported in absolute terms (liters/min) or it can be reported relative to body mass (milliliters of O_2 per kilogram of body weight per minute). Relative $\dot{V}O_2$ is more often used in practice because it is more specific to the actual Subject.

$$\dot{V}O_2 \textbf{(Relative)} = \frac{\dot{V}O2\textbf{(Absolute)}\frac{liters}{minute} \times 1000 \frac{milliliter}{liter}}{\textbf{(Body mass) kg}}$$

Expected Normal Values for resting $\dot{V}O_2$:

$\dot{V}O_2$ (Absolute):   0.2 – 0.5 liters/min (Men), 0.15 – 0.4 liters/min (Women)

$\dot{V}O_2$ (Relative):   3.5 ml/(kg * min) (Men and Women)

Under normal resting conditions, aerobic metabolism meets the body's energy demands by utilizing blood that is almost fully saturated with O_2 (97–99%). When going from rest to an exercise state, the ATP demands increase immediately and faster than aerobic metabolism processes can respond. Fortunately, during this period, fast acting anaerobic metabolism is able to provide the additional ATP. A few minutes into submaximal exercise, $\dot{V}O_2$ reaches a steady state and aerobic metabolism is again able to meet the ATP production requirements. The volume of O_2 that was unable to meet the ATP demands at the beginning of exercise is called **oxygen deficit**, as shown in Figure 2. At the end of exercise, O_2 uptake does not immediately revert to resting levels, but rather slowly returns over a period of a few minutes. This occurs for several reasons, including; (1) build up of lactate being converted to glucose, (2) heart rate, ventilation rate, and body temperature take time to return to normal levels, (3) ATP and phosphocreatine stores need to be replenished.

The volume of oxygen consumed after exercise that is above the resting level is called **excess post-oxygen consumption (EPOC)** and is shown in Figure 2.
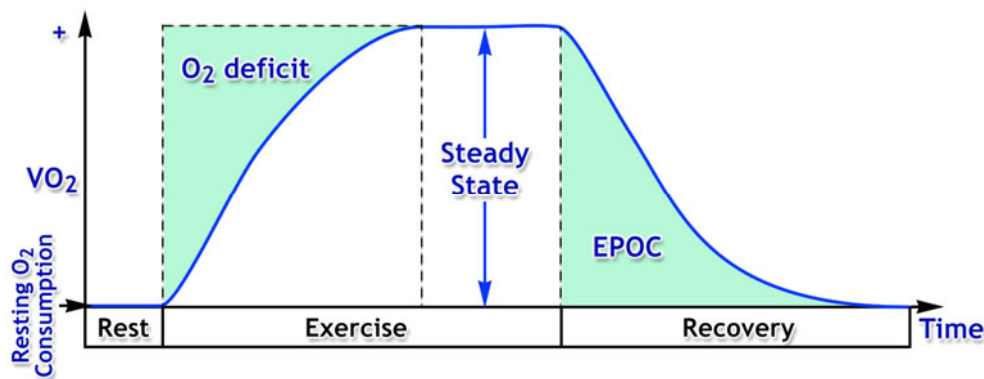
Figure 2

During the production of ATP, CO_2 is created as a by-product and must be removed, as it is toxic in large amounts. CO_2 is able to diffuse out of the cells and into the bloodstream, and is then carried by the blood back through the heart and into the lungs, where it is removed in the alveoli during exhalation. **Carbon dioxide production** ($\dot{V}CO_2$) is the amount of CO_2 produced by the body per minute. $\dot{V}CO_2$ can be reported in absolute terms (liters/min) or as relative to body mass (milliliters of CO_2 per kilogram of body weight per minute).

When hyperventilation occurs, alveolar ventilation increases beyond the requirement of aerobic metabolism. Although there are additional ATP requirements, there is not enough to justify the increase in ventilation. This results in more CO_2 being removed from the blood than the body can produce. The body will do its best to compensate metabolically, but if it cannot, a rise in blood pH, known as **respiratory alkalosis** will occur. Respiratory alkalosis can cause physical symptoms such as dizziness, weakness and fainting.

If $\dot{V}O_2$ and $\dot{V}CO_2$ are known, then it is possible to estimate the type of fuel being used for oxidation. The **respiratory exchange ratio** (**RER** or simply **R**) is the ratio of volume of CO_2 produced to volume of O_2 consumed per unit of time.

$$\textbf{RER} = \frac{\dot{V}CO2}{\dot{V}O2}$$

Due to the chemical composition differences between fats, carbohydrates and proteins, each differs in the amount of O_2 used and CO_2 produced during oxidative phosphorylation. The protein contribution to energy production has been found to be small for healthy subjects that are not starved or undergoing prolonged exercise. For this lesson, protein will be ignored and it will be assumed that only carbohydrates and fats contribute to energy production. The following are oxidation equations and corresponding RER values for a single molecule of carbohydrate and fat:

Carbohydrate: $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ $RER = \dfrac{\dot{V}CO2}{\dot{V}O2} = \dfrac{6\ CO2}{6\ O2} = 1$
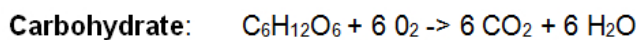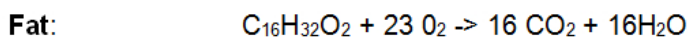
Fat: $C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16H_2O$ $RER = \dfrac{\dot{V}CO2}{\dot{V}O2} = \dfrac{16\ CO2}{23\ O2} = 0.7$

The human body is continually oxidizing a mixture of carbohydrates and fat rather than using any single food as the sole source of energy. This means typical RER values will vary between 0.70 and 1.0. The closer RER is to 0.70, the more fat is being oxidized, and the closer RER is to 1.00, the more carbohydrate is being oxidized. Because carbohydrate oxidation requires less O_2 than fat oxidation, carbohydrate produces more energy (ATP) per liter of O_2. RER is only useful for determining how much carbohydrate or fat is utilized during steady state conditions, because it is only during this time that RER is accurately reflective of O_2 uptake and CO_2 production in the tissues. Under non-steady state conditions, such as during recovery from severe exercise or during hyperventilation, RER can exceed 1.00 due to increased CO_2 being expired. After exercise, RER may fall below 0.5 or less as the oxygen debt incurred during severe exercise is paid off.

This lesson demonstrates how to obtain $\dot{V}O_2$, $\dot{V}CO_2$ and RER measurements using an open circuit indirect calorimetry technique. In the setup, a nose-clamped Subject is connected to a gas analyzer (GASSYS2) by way of plastic tubing, a non-rebreathing T-valve and a mouthpiece. The T-valve allows the subject to inspire from the atmosphere but diverts all of the expired air through the GASSYS2. The GASSYS2 consists of an air mixing chamber and O_2 and CO_2 sensors.

The sensors output voltages that are proportional to the $\%O_2$ and $\%CO_2$ in the chamber. The tubing and air mixing chamber act to average respiratory outflows. This averaging effect causes the CO_2 and O_2 concentrations to vary in accordance with the mean values resident over multiple expired breaths.

An airflow transducer (SS11LB/LA) is used to measure inspired air flow. It is placed on the inspired side rather than the expired side to minimize errors due to condensation (expired air is saturated with water vapor). Placing it on the inspired side also eliminates the need for a temperature transducer in order to make STPD corrections (explained below).

Because the airflow transducer is such a critical part of the system, it may be helpful to review Appendix 4 (at bottom of document) to get a better understanding of how it works and how to properly use it.

> *Note:* Changes occurring in expired air gas concentrations will not be immediately apparent in the recorded data. This is due to the time required for air to travel through the tubing, mix with the 5 liters of air in the mixing chamber and flow past the sensors. In addition, each sensor requires varying amounts of processing time. The delays are not important when taking steady state measurements, but will affect the accuracy of non-steady state $\dot{V}O_2$, $\dot{V}CO_2$ and RER measurements. To improve accuracy, assume a delay of 15 seconds for O_2e, CO_2e, $\dot{V}O_2$, $\dot{V}CO_2$ and RER with respect to the airflow signal and any manually entered event markers.

The system inputs three signals:

- **Airflow**: inspired air flow (Liters/sec)
- **O_2e**: % CO_2 in expired air
- **CO_2e**: % CO_2 in expired air

To determine $\dot{V}O_2$ and RER, the following calculations are performed in software:

- **Vi (ATP):** Volume of inspired air in liters/min at temperature and pressure (ambient conditions). This is calculated by integrating the airflow data using a 60 second (6000 samples@100 samples/second) box car average.

- **Vi (STPD):** Vi (ATP), is converted to Vi at standard temperature and pressure dry (STPD) to allow accurate comparisons between Subjects tested under different ambient air conditions. This conversion scales the volume to what it would be at a temperature of 0° C, pressure of 760 mmHg and with water vapor pressure removed. To make the conversion, the observed barometric reading (mmHg), and temperature of the ambient air (in lab) must be determined. A scaling (or normalization) factor is found using the table in Appendix 1 (at bottom of document) Fand manually entered into the software.

- **Ves (STPD):** Applies the **Haldane Transformation** to find the volume of expired air per minute using Vi (STPD), O_2e and CO_2e. The following assumptions are made:

  a) Air is made up of only N_2, O_2 and CO_2.

  b) The composition of ambient (inspired) air is constant at 79.03% N_2, 20.93 % O_2 and 0.04 % CO_2.

  c) Nitrogen is inert in terms of metabolism, so any changes in its concentration between inspired and expired air must be due to an imbalance between the number of oxygen molecules removed and carbon dioxide molecules produced during metabolism.

  Haldane Transformation formula:

  $$Ve(STPD) = Vi(STPD) \times \frac{\%N2i}{\%N2e} = Vi(STPD) \times \frac{79.03\%}{(100\% - \%O2e - \%CO2e)}$$

> *Note:* Additional trace elements are present in the atmosphere such as Ar, Ne, He, but their percentage by volume are very small and assumed negligible. The assumption that ambient air is made up of 0.04% CO_2 is accurate for well mixed outside air, but for air in a confined room, with multiple bodies expiring CO_2, it can be much higher (> 0.1%) and can vary over short periods of time. This variation does not greatly affect the $\dot{V}O_2$, and RER calculations because the amount of CO_2 in ambient air is small compared to the amount of O_2 and to N_2 and because the amount of CO_2 in expired air is much larger than that in ambient air. It is important to note, however, that ambient air calibration assumes 0.04% CO_2. Therefore, when returning the system to ambient conditions (flushing with ambient air) following expired air recordings, the %CO_2 reading may not return to 0.04% and can even read slightly negative.

- **$\dot{V}O_2$ (STPD):** Calculates the volume of O_2 consumed per minute by taking the volume of O_2 inspired per minute and subtracting the volume of O_2 expired per minute.

  $$\dot{V}O_2(STPD) = [Vis(STPD) \times \frac{20.93\%}{100\%}] - [Ves(STPD) \times \frac{O2e}{100\%}]$$

- **$\dot{V}CO_2$ (STPD):** Calculates the volume of CO_2 produced per minute by finding the volume of CO_2 expired per minute and subtracting the volume of CO_2 inspired per minute.

  $$\dot{V}CO_2(STPD) = [Ves(STPD) \times \frac{CO2e}{100\%}] - [Vis(STPD) \times \frac{0.04\%}{100\%}]$$

- **RER:** Calculates the ratio CO_2 produced per minute and O_2 consumed per minute.

  $$RER = \frac{\dot{V}CO2\ (STPD)}{\dot{V}O2\ (STPD)}$$

> *Note:* Additional calculations not included in this section are shown in Appendix 2 at bottom of document. Although the channel designations and formula arrangement used in the actual calculation channel expressions shown in Appendix 2 may differ from that shown above, they are still equivalent.

Setup:

General Connections

The MP36/35 should be connected to its power source but turned OFF. It is assumed that the MP36/35 is connected to the host computer (via USB cable) and that BSL *PRO* software has been installed and is known to work with the MP unit.

1. The GASSYS2 should be plugged into its power adapter and turned "ON."

2. Connect the equipment as shown in Figure 3. The Airflow transducer must be on the inspired side of the T-Valve and the "Inlet" label must facing the tubing. If using the optional Head Support (AFT24), refer to Figure 4. The connections to the MP36/35 must be as follows:

- CH 1: Airflow transducer   CH 2: "O_2" from GASSYS2   CH 3: "CO_2" from GASSYS2
     (SS11LB/LA)

Figure 3   *Figure 4*

Important: The majority of the calibration need only be performed once each semester, but in order for this to work, the hardware must be matched to the software. It is recommended to label each computer, MP device, Airflow Transducer (SS11LB/LA/L) and the GASSYS2 with a station number and make sure they are always matched.

Turn ON the hardware

3. After all cables are connected, the MP36/35 may be turned ON. Allow the system to warm up for at least fifteen minutes prior to calibration.

Secure the Airflow transducer and inlet tubing

4. The SS11LB/LA Airflow transducer should be held stationary and vibration must be minimized. Figure 5 shows one method for securing the SS11LB/LA and tubing (AFT7-L) to a table using the optional parts listed in the Equipment section.

Figure 5

Software Setup

5. Launch BSL 4, and from the Startup dialog choose the **Create/Record a new experiment** option. Choose **Open Graph template from disk** and then click **OK**. Navigate to the correct template file*****, select it and click **Open**.

    *The default BIOPAC template file is not normally used by students, as it does not include the last calibration settings. Check with your instructor to make sure the correct file is opened.

NOTE: In BSL 4.1.2 and higher, this Lesson Procedure PDF is also embedded in the graph template's Journal but is best viewed with the Journal set to "Floating display." See Appendix 5 for details.

Calibration Procedure:

Notes:

- The GASSYS2 and SS11LB/LA Airflow transducer must be warmed up at least 15 minutes prior to calibration.
- Prior to each lesson recording, it is important to zero the airflow baseline and apply the STPD Normalization factor. The remaining calibration steps are normally performed by a lab technician once each semester.

Zero airflow baseline

1. Navigate to the "CH1, Airflow" **Scaling dialog** (reference Appendix 3 at bottom of document).

2. With no air flowing through the SS11LB/LA, click **Cal 2**.

3. Add "3000" to the Cal 2 Input value and manually enter this as the new Input value for Cal 1. In the Figure 6 example, after clicking Cal 2, the Input reading is 172 microvolts (rounded). This value is added to 3000 microvolts and then entered as the new value (3172) for Cal 1 Input.

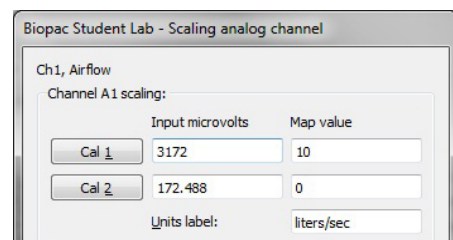4. Close both CH1 Setup dialogs by clicking **OK**.

Figure 6

Check polarity and verify calibration

5. Display Channel "1" (Airflow) by holding down the "Alt" key (Windows) or "option" key (Mac) and clicking the Channel Box.

6. Click **Start** and record for a few seconds with no air flowing through the system, then have the subject inhale into the mouthpiece and click **Stop**.

7. Verify that the airflow waveform is positive when air is inhaled into the mouthpiece. If it is not, then the tubing is connected to the wrong port of the airflow transducer (SS11LB/LA) and must be reversed before proceeding.

8. Using the "I" cursor, select an area of baseline (no flow) data as shown in Figure 7 and verify that the "Mean" measurement is close to zero (ideally less than 0.01 Liters/sec). If there is excessive offset, repeat steps 1 through 8.
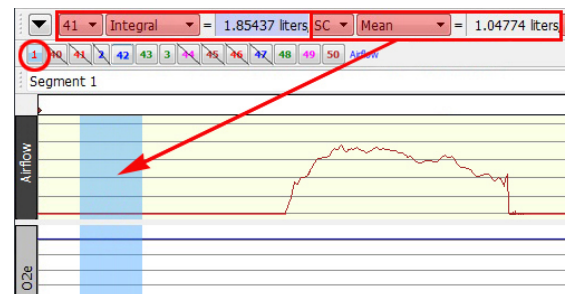
9. Hide Channel 1 by toggling the Channel Box.

Figure 7

Apply STPD Normalization factor

10. Obtain the temperature and observed barometric reading (OBR in mmHg) for conditions in the lab.

11. Find the Normalization factor using the table in Appendix 1 at bottom of document. Record this information in the Data Report section and/or the journal.

12. Navigate to the C7 **(Vis STPD)** Expression dialog (see Appendix 3 at bottom of document for details).

13. Replace the scaling factor in the expression dialog with the normalization factor found in the table. In the Figure 8 example, for a lab OBR of 728 mmHg and a temperature of 72° F, the normalization factor from the table is ."859."
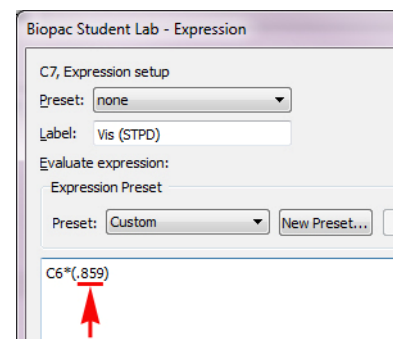
14. Click **OK** to close the Expression dialog.

Figure 8

Note: The remaining calibration steps are normally performed by a lab technician once prior to the start of each semester. Students should proceed to the **Recording Procedure** section.

Connect calibration syringe

15. Connect the Calibration Syringe to the T-valve (in place of the mouthpiece) as shown in Figure 9.

16. Cycle the syringe for several cycles (2 to 3 minutes) at a moderate rate to completely flush GASSYS2 with ambient air.

Figure 9

Calibrate airflow amplitude

17. Click **Start** to begin a recording.

18. Cycle the Syringe all the way in and out at a moderate rate and then click **Stop**.

19. Select an area of data that encompasses the airflow pulse as shown in Figure 10. Note the value of the "Integral" measurement. If this value does not closely match the volume of the calibration syringe, then a scaling factor must be used.

    Scaling factor = Syringe volume / Integral value.

    For example, if a 2 Liter syringe is used and the integral measures 1.85 Liters, then a scaling factor of 2.0/1.85 = 1.08 must be applied.

Figure 10

20. *Apply the Scaling factor*. Navigate to the Calculation channel **C3** (Airflow Scaled) Expression dialog.

21. Enter the calculated scaling factor into the C2 expression. For the Figure 11 example, the previous value was replaced with "1.08."

22. Click **OK** to close the Expression dialog.

23. Check the airflow calibration by recording another syringe cycle and verify that the Integral measurement more closely matches the Syringe volume.
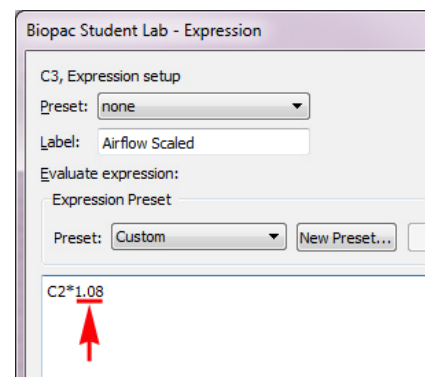

Figure 11

Gas calibration part 1 – using ambient air

Note: If the lesson objective only examines <u>relative</u> changes in a Subject's VO_2 and RER, then only ambient air calibration is necessary. If, however <u>absolute</u> measurements are required, it will be necessary to perform the additional "Gas calibration part 2" using a calibration gas.

O_2 Sensor

1. Flush the GASSYS2 with ambient air by cycling the calibration syringe at a moderate rate for 1 minute.

2. Wait 2 minutes.

3. Navigate to the **CH2** (O_2e) **Scaling** dialog (see Appendix 3 at bottom of document).

4. Record the <u>initial</u> Input values of Cal 1 and Cal 2.

5. Click **Cal 1** to obtain the <u>actual</u> Input voltage value.
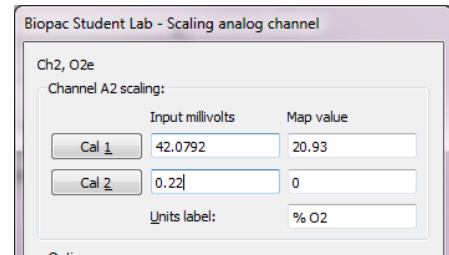
Figure 12

Note: If you will be performing Gas Calibration part 2 (with calibration gas), skip the next step.

6. Manually calculate a new Cal 2 Input value as follows:

   Cal 2 New = Cal 2 initial + (Cal 1 actual – Cal 1 initial)

   Enter this new value for Cal 2 Input.

   In the Figure 12 example, the Cal 1 initial Input value was 41.86 millivolts and the Cal 2 initial value was 0 millivolts. After clicking Cal 1, the Input changed to 42.08 millivolts (rounded). Cal 2 New was calculated as: 0 + (42.08 - 41.86) = 0.22 and this was then manually entered as the new Cal 2 Input value.

CO_2 Sensor

7. Navigate to the **CH3** (CO_2e) **Scaling** dialog.

8. Record the <u>initial</u> Input values of Cal 1 and Cal 2.

9. Click **Cal 2** to obtain the <u>actual</u> Input voltage value.

Note: If you will be performing Gas Calibration part 2 (with calibration gas), proceed to Step 12.

Figure 13

10. Manually calculate a new Cal 1 Input value as follows:

    Cal 1 New = Cal 1 initial + (Cal 2 actual – Cal 2 initial)
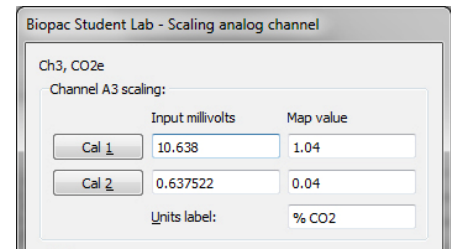
    Enter this new value for Cal 1 Input.

In the Figure 13 example, the Cal 1 initial Input value was 10 millivolts and the Cal 2 initial value was 0 millivolts. After clicking Cal 2, the Input changed to 0.638 millivolts (rounded). Cal 1 New was calculated as: 10 + (0.638 - 0) = 10.638 and this was then manually entered as the new Cal 1 Input value.

11. Skip the Gas Calibration part 2 steps and save the calibration settings.

Gas Calibration part 2 – Using calibration gas

12. Connect the calibration gas to the "Calibration Port" on the GASSYS2 and turn on the flow. It can take several minutes for the gas to saturate the chamber and sensors.

13. Click **Start** to begin a recording and monitor the CO_2e and O_2e channels. Wait until both have completely stabilized and then Click **Stop**.
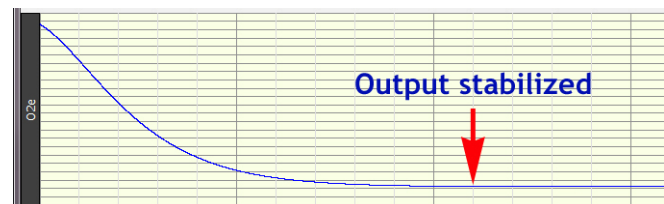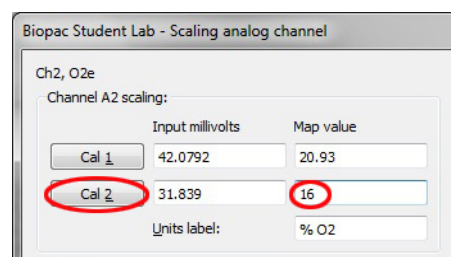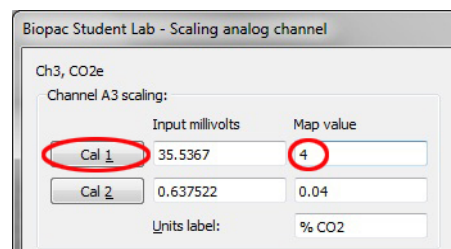
Figure 14

O$_2$ Sensor

14. Navigate to the **CH2** (O$_2$e) **Scaling** dialog.

15. Enter the calibration gas O$_2$ % value into the Cal 2 Map value.

16. Click **Cal 2** to update the Input voltage value.

17. Click **OK** to close the Scaling dialog.

Figure 15

CO2 Sensor

18. Navigate to the **CH3** (CO$_2$e) Scaling dialog.

19. Enter the calibration gas CO$_2$ % value into the Cal 1 Map value.

20. Click **Cal 1** to update the Input voltage value.

21. Click **OK** to close the Scaling dialog.

Figure 16

22. Erase all calibration data by holding down the "Ctrl" key (Windows) or "option" key (Mac) and clicking the Rewind toolbar button.

Save the calibration settings

23. Choose **Save As** from the File menu.

24. Navigate to the location where the lesson files (templates) are stored.

25. Make sure "**Graph Template (*.gtl)**" is selected as the "Save as type."

26. Enter a file name. Include the station number in the name if the files are not stored on the local computer.

27. Click **Save**.
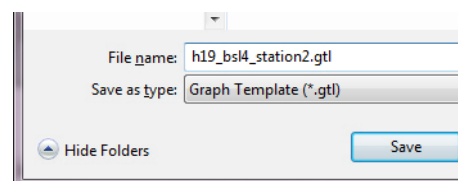
Figure 17

Recording Procedure:

Note: The Subject must be healthy and have no history of respiratory or cardiovascular problems.

Resting and Hyperventilation

The Subject should be in a seated and relaxed position.

1. Subject places the nose clip over nose, inserts the mouthpiece, and begins breathing through the T-Valve. Subject continues to breathe normally for at least 5 minutes to allow expired air to saturate the tubing and 5 Liter mixing chamber (GASSYS2).

2. Click **Start** to begin recording.

    • *While recording, monitor the RER data (bottom channel).*

3. After 5 minutes of recording with the Subject is at rest, examine the trend of the RER data. Continue the at-rest recording, if necessary, until the RER data has reached a somewhat steady state for approximately 2 minutes.

    • *Do not Stop the recording.*

Figure 18

4. Subject begins hyperventilating (breathing as quickly and deeply as possible) for 1 minute and then returns to normal breathing.

    Note: Subject should stop hyperventilating if feeling sick or excessively dizzy.

5. Record for 5 additional minutes to capture the recovery period and then click **Stop**.

6. Subject should remove the mouthpiece and nose clip and return to a relaxed state before starting the exercise recording.

Troubleshooting

If the values $\dot{V}O_2$, $\dot{V}CO_2$, or RER values seem incorrect, check that the airflow baseline is zero and that the airflow amplitude is scaled properly (calibration steps 5 – 9 and 17 – 19). Recalibrate if necessary.

Moderate exercise

This recording is performed before, during and after moderate submaximal (50 – 75% maximal) exercise. The chosen exercise must allow the Subject to remain in close proximity to the GASSYS2. Both the cardio step and stationary bicycle are exercises that work well.

Note: To more easily determine oxygen deficit, the Subject should make a quick transition from rest to a moderate submaximal exercise state and avoid a slow ramp-up.

Figure 19

Figure 20

1. Subject gets into position to start exercising. Place the nose clip over the nose, insert the mouthpiece, and begin breathing through the T-Valve.

2. Subject breathes normally (at rest) for 5 minutes.

3. Click **Start** to begin recording.

4. Record for 3 minutes while Subject is in resting state, breathing through the T-Valve.

5. Subject now makes a quick transition from rest to a moderate submaximal exercise level.

   - *Press the "ESC" key to place an event marker and type in the label "Start of exercise."*
   - *While recording, monitor the RER and $\dot{V}CO_2$ data.*

7. After 5 minutes, examine the trend of the RER data. Continue the exercise recording, if necessary, until the RER data has reached a somewhat steady state for at least 2 minutes.

   - *If at any point in the recording, $\dot{V}CO_2$ reaches 5% (limit of CO_2 sensor), continue to the next step.*
   - *<u>Do not</u> Stop the recording.*

6. Subject stops exercising but remains breathing through T-Valve.

   - *Press the "Esc" key to place an event marker and type in the label "End of exercise."*

7. Record for 5 additional minutes to capture the recovery period and then click **Stop**. Subject should remove the mouthpiece and nose clip.

Save the Data

1. Select **Autoscale Horizontal** from the Display menu followed by Display > **Autoscale Waveforms** so that all of the data can be seen.

2. Save the data by selecting: **File** menu > **Save As**… Enter a file name and then click **Save**.

Data Analysis:

1. Fill out Subject Profile section of Data Report.

2. Take measurements of VO₂ (STPD), VCO₂ (STPD) and RER at various locations in the recording and transfer them to Table 1 of the Data Report.

   Notes:

   - Data Analysis requires familiarization with the measurement tools (see Appendix 3 for basic details).
   - $\dot{V}O_2$, $\dot{V}CO_2$ and RER data are not valid until at least 1 minute after the start of a recording as the airflow to volume integrator is averaging over 1 minute.
   - Measurements of $\dot{V}O2$, $\dot{V}CO_2$ and RER taken during steady state conditions use a selection interval of 60 seconds while those taken under non-steady state conditions use a 20 second selection interval. For the non-steady state measurements, a 15 second delay is added for the start of the selection interval (see note in Background section).

Measurement examples:

Figure 21 shows an example of "at rest" measurements.

   a. The graph is set to display all of the recorded data.

   b. A 60 second data interval is selected (utilizing the **Delta T** measurement) near the end of the "At Rest" recording (prior to hyperventilation).

   c. The "Mean" measurements for the following are transferred into Table 1:

- $\dot{V}O_2$ (STPD) <Absolute> – CH 48
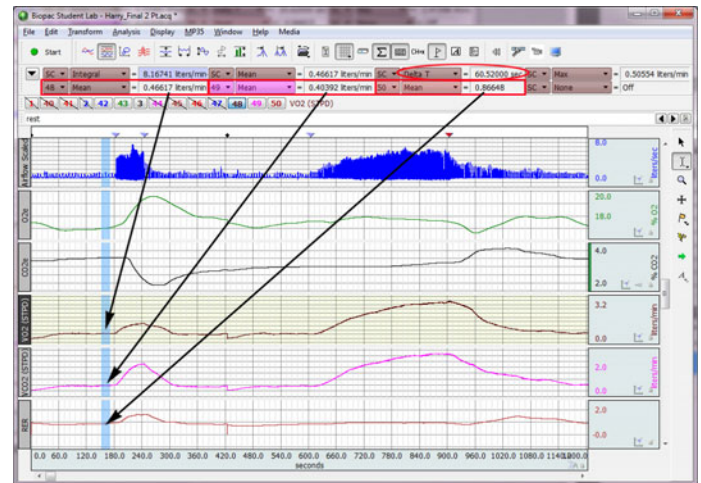- $\dot{V}CO_2$ (STPD) <Absolute> - CH 49
- RER - CH 50

Figure 21

Figure 22 shows measurements taken 2 minutes after start of exercise. The start of exercise can be determined by the event marker (if present) or by the sudden change in amplitude of the airflow signal.

   a. The "I" cursor is placed at the start of exercise event marker and a data interval of 2 minutes + 15 seconds (135 seconds) is selected.

   b. A new data selection interval of approximately 20 seconds is made beginning where the above selection ended.

   c. The "Mean" measurements are transferred into Table 1.
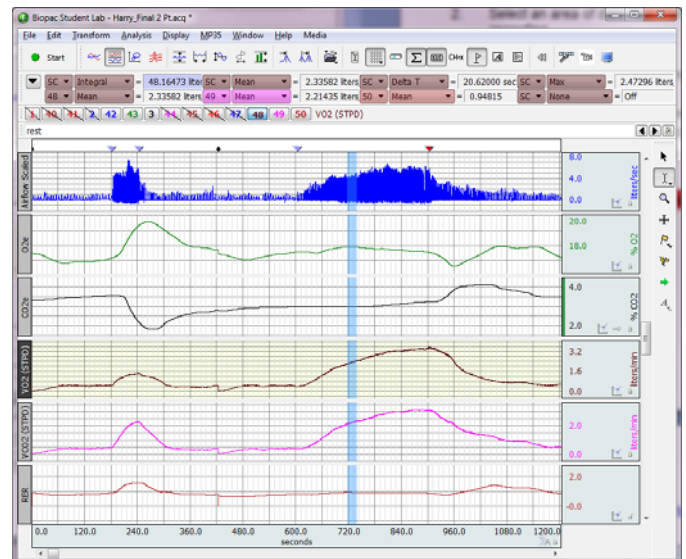
Figure 22

3. Calculate the relative values for $\dot{V}O_2$ and $\dot{V}CO_2$ in order to complete Table 1 of the Data Report.

$$\dot{V}(\text{Relative}) = \frac{\dot{V}(\text{Absolute})\frac{\text{liters}}{\text{minute}} \times 1000 \frac{\text{milliliter}}{\text{liter}}}{(\text{Body mass}) \text{ kg}} \qquad \text{Body mass (Kg)} = \frac{\text{Body mass (Lbs)}}{2.2}$$

4. Determine the amount of oxygen deficit at the start of exercise and the EPOC during recovery from exercise and enter the values into the Data Report.

Notes:

- The event markers will precede the changes in $\dot{V}O_2$ data for reasons noted in the Background section.

- For data that are all positive, the Integral measurement will compute the area under the curve with respect to zero baseline and not the resting $\dot{V}O_2$ level. Because resting $\dot{V}O_2$ values are not zero, the area below the resting VO2 level must be subtracted from the integral measurement in order calculate EPOC. In addition, because the time scale is in seconds and $\dot{V}O_2$ is in units of liters/minute, the Integral measurement must be divided by 60 seconds/minute to obtain the proper units of (Liters/minute)*minutes = liters.

- To make it easier to obtain these measurements:
    - o Zoom in on only the data pertaining to the beginning of exercise and the recovery period.
    - o Hide all channels with the exception of VO$_2$ (STPD).
    - o Setup the top row of measurements to: [SC] Integral, [SC] Min, [SC] Max, and [SC] Delta T.

Examples:

Determining O$_2$ deficit:

a. An area of data is selected that begins just before $\dot{V}O_2$ rises above resting level and ends just after $\dot{V}O_2$ reaches a steady state exercise level (Figure 23).

b. The Delta T measurement is converted from seconds to minutes: 260 seconds = 4.33 minutes

c. The area of a rectangle of width Delta T (in minutes) and of height equal to the steady state exercise level (Max measurement) is computed: (3.37 Liters/min)*4.33 min = 14.59 Liters

d. The area under the curve is found by dividing the integral measurement by 60 seconds/minute:
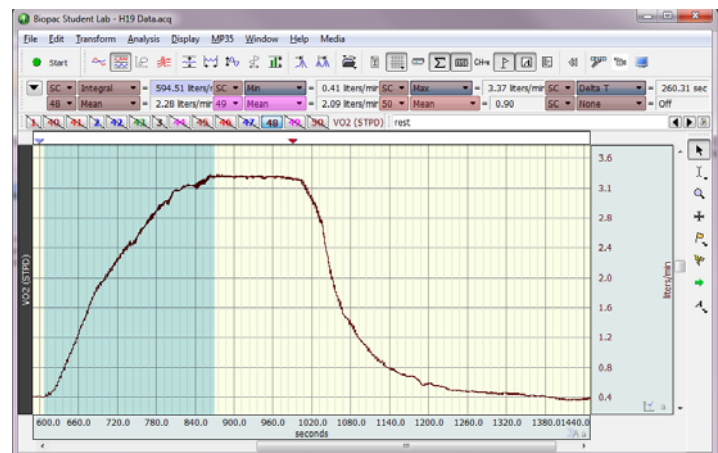
Figure 23

$$594.51(\text{liters*sec/min}) /60 \text{ sec./min} = 9.91 \text{ liters}$$

e. The area approximating O$_2$ deficit (see Figure 2) is the difference in areas: O$_2$ deficit = 14.59 liters – 9.91 liters = 4.68 liters.

Determining EPOC:

a. An area of data is selected that begins just before $\dot{V}O_2$ falls from its steady state exercise level and ends just after $\dot{V}O_2$ returns to resting level (Figure 24).

b. The Delta T measurement is converted from seconds to minutes: 387 seconds = 6.45 minutes

c. The area of a rectangle of width Delta T (in minutes) and of height equal to the resting state level (Min measurement) is computed: (0.42 Liters/min)*6.45 min = 2.7 Liters

d. The area under the curve is found by dividing the integral measurement by 60 seconds/minute:
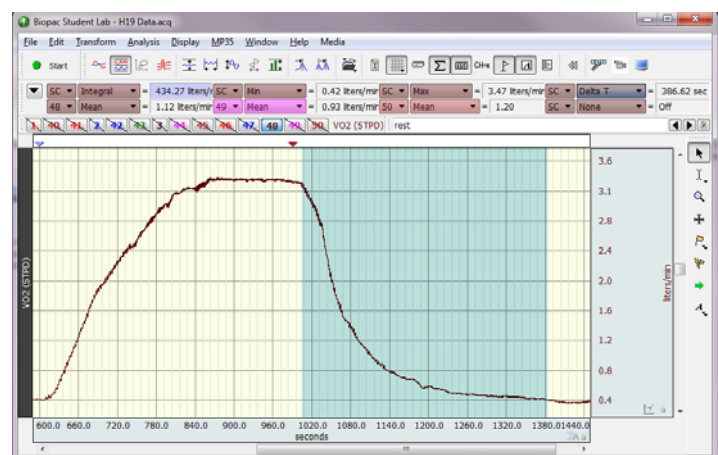

Figure 24

$$434.27(\text{liters*sec/min}) /60 \text{ sec./min} = 7.23 \text{ liters}$$

e. The area approximating EPOC (see Figure 2) is the difference in areas: EPOC = 7.23 liters – 2.7 liters = 4.53 liters.

Data Report:

Name: _____

Lab Section: _____   Date: _____

Ambient Conditions: Temperature : _____ °F _____ °C   Barometric pressure: _____ mmHg

STPD normalization factor (from Appendix 1): _____

Subject Profile: Name: _____   Height:_____

Age: _____   Weight: _____ Kg _____ Lbs

Gender: Male / Female   Time of day: _____

Data selection	VO$_2$ (STPD) <Absolute> $\dfrac{liters}{second}$ [48 ▼] [Mean ▼]	VO$_2$ (STPD) <Relative> $\dfrac{milliliters}{kg * minute}$ [Calculate]	VCO$_2$ (STPD) <Absolute> $\dfrac{liters}{second}$ [49 ▼] [Mean ▼]	VCO$_2$ (STPD) <Relative> $\dfrac{milliliters}{kg * minute}$ [Calculate]	RER [50 ▼] [Mean ▼]
At rest (prior to hyperventilation)					
End of hyperventilation					
1 min. after hyperventilation					
2 min. after hyperventilation					
Prior to exercise					
1 minute into exercise					
2 minutes into exercise					
3 minutes into exercise					
During steady-state exercise					
1 minute after exercise					
2 minutes after exercise					
3 minutes after exercise					
4 minutes after exercise					

Table 1

O$_2$ Deficit: _____ liters

EPOC: _____ liters

1. Does the resting RER value indicate more carbohydrate or fat being utilized?

2. Are there differences in RER between resting and steady state exercise? Why might there be a difference?

3. Do the subject's fitness level and/or diet correlate to the resting RER values?

4. Are the subject's resting $\dot{V}O_2$ values in the expected range?

5. During hyperventilation did $\dot{V}CO_2$ increase more than $\dot{V}O_2$? Was this expected?

6. Examine the O_2e and CO_2e changes that occur during and after hyperventilation and during and after exercise. How do they compare?   Why might they be different?

7. Examine the changes in $\dot{V}O_2$, $\dot{V}CO_2$ during and after exercise. How do these changes affect the RER values? Explain why these changes occur.

8. Was EPOC greater than O_2 deficit? Why might EPOC be greater than O_2 deficit during some exercise tests?

Appendix 1: Normalization Factors

The following table lists the factors required to reduce volume of moist gas to volume occupied by dry gas at 0° C, 760 mmHg. **OBR** is observed barometric reading (mmHg), uncorrected for temperature.

Important! All factors are **0.x**, with x being the value from the table. Be sure to include the **leading decimal** when completing the calculation.

º C	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
º F	**59**	**61**	**63**	**64**	**66**	**68**	**69**	**72**	**73**	**75**	**77**	**79**	**81**	**82**	**84**	**86**	**88**	**90**
OBR																		
700	855	851	847	842	838	834	829	825	821	816	812	807	802	797	793	788	783	778
702	857	853	849	845	840	836	832	827	823	818	814	809	805	800	795	790	785	780
704	860	856	852	847	843	839	834	830	825	821	816	812	807	802	797	792	787	783
706	862	858	854	840	845	841	837	832	828	823	819	814	810	804	800	795	790	785
708	865	861	856	852	848	843	839	834	830	825	821	816	812	807	802	797	792	787
710	867	863	859	855	850	846	842	837	833	828	824	819	814	809	804	799	795	790
712	870	866	861	857	853	848	844	839	836	830	826	821	817	812	807	802	797	793
714	872	868	864	859	855	851	846	842	837	833	828	824	819	814	809	804	799	794
716	875	871	866	862	858	853	849	844	840	835	831	826	822	816	812	807	802	797
718	877	873	869	864	860	856	851	847	842	838	833	828	824	819	814	809	804	799
720	880	876	871	867	863	858	854	849	845	840	836	831	826	821	816	812	807	802
722	882	878	874	869	865	861	856	852	847	843	838	833	829	824	819	814	809	804
724	885	880	866	872	867	863	858	854	849	845	840	835	831	826	821	816	811	806
726	887	883	879	874	870	866	861	856	852	847	843	838	833	829	824	818	813	808
728	890	886	881	877	872	868	863	859	854	850	845	840	836	831	826	821	816	811
730	892	888	884	879	875	871	866	861	857	852	847	843	838	833	828	823	818	813
732	895	890	886	882	877	873	868	864	859	854	850	845	840	836	831	825	820	815
734	897	893	889	884	880	875	871	866	862	857	852	847	843	838	833	828	823	818
736	900	895	891	887	882	878	873	869	864	859	855	850	845	840	835	830	825	820
738	902	898	894	889	885	880	876	871	866	862	857	852	848	843	838	833	828	822
740	905	900	896	892	887	883	878	874	869	864	860	855	850	845	840	835	830	825
742	907	903	898	894	890	885	881	876	871	867	862	857	852	847	842	837	832	827
744	910	906	901	897	892	888	883	878	874	869	864	859	855	850	845	840	834	829
746	912	908	903	899	895	890	886	881	876	872	867	862	857	852	847	842	837	832
748	915	910	906	901	897	892	888	883	879	874	869	864	860	854	850	845	839	834
750	917	913	908	904	900	895	890	886	881	876	872	867	862	857	852	847	842	837
752	920	915	911	906	902	897	893	888	883	879	874	869	864	859	854	849	844	839
754	922	918	913	909	904	900	895	891	886	881	876	872	867	862	857	852	846	841
756	925	920	916	911	907	902	898	893	888	883	879	874	869	864	859	854	849	844
758	927	923	918	914	909	905	900	896	891	886	881	876	872	866	861	856	851	846
760	930	925	921	916	912	907	902	898	893	888	883	879	874	869	864	859	854	848

762	932	928	923	919	914	910	905	900	896	891	886	881	876	871	866	861	856	851
764	936	930	926	921	916	912	907	903	898	893	888	884	879	874	869	864	858	853
766	937	933	928	924	919	915	910	905	900	896	891	886	881	876	871	866	861	855
768	940	935	931	926	922	917	912	908	903	898	893	888	883	878	873	868	863	858
770	942	938	933	928	924	919	915	910	905	901	896	891	886	881	876	871	865	860
772	945	940	936	931	926	922	917	912	908	903	898	893	888	883	878	873	868	862
774	947	943	938	933	929	924	920	915	910	905	901	896	891	886	880	875	870	865
776	950	945	941	936	931	927	922	917	912	908	903	898	893	888	883	878	872	867
778	952	948	943	938	934	929	924	920	915	910	905	900	895	890	885	880	875	869
780	955	950	945	941	936	932	927	922	917	912	908	903	898	892	887	882	877	872
º F	59	61	63	64	66	68	69	72	73	75	77	79	81	82	84	86	88	90
º C	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

From Peters and Van Slyke, *Quantitative Clinical Chemistry*, vol. 11. (Methods) Baltimore: Williams and Wilkins, 1932, reprinted 1956.

Appendix 2: Channel Processing Summary:

| Channel	Label	Description/Processing
Ch1	Airflow	Airflow from SS11LB/LA
		3 IIR Filters: Low Pass, 66.5 Hz, Q=.5, Low Pass, 38.5 Hz, Q=1, Band Stop, Line Freq, Q=1
Ch2	O_2e	O_2 Expired from GASSYS2
		3 IIR Filters: Low Pass, 66.5 Hz, Q=.5, Low Pass, 38.5 Hz, Q=1, Band Stop, Line Freq, Q=1
Ch3	CO_2e	CO_2 Expired from GASSYS2
		3 IIR Filters: Low Pass, 66.5 Hz, Q=.5, Low Pass, 38.5 Hz, Q=1, Band Stop, Line Freq, Q=1
C1 [40]	Airflow Threshold	Applies zero to any airflow signal that is less than 0.02 Liters/sec. This reduces noise artifact caused by mechanical vibration and also prevents the signal from going negative which can occur briefly when valves inside the T-valve slam shut.
		IF (LESS(0.02, ABS(Ch1)), Ch1, 0)
C2 [41]	Airflow corrected	Polynomial correction that extends the linear range of the SS11LB/LA from 5 Liters/sec. to 10 Liters/sec.
		(2.0*C1)/((0.247951736452952*C1)+(-0.0928767685027268*C1^2)+(0.0224986269987362*C1^3)+(-0.00222581575367371*C1^4)+(7.53176506967997E-005*C1^5)+1.71749281362047)
C3 [42]	Airflow Scaled	Applies a scale factor to the airflow signal for amplitude calibration (using syringe) – see Figure 11.
		C2*1.0
C4 [43]	O_2e	Acts as a delay on O_2 data to more closely align the response time of the O2 sensor with the slower responding CO_2 sensor.
		Low Pass Filter on Ch2 data (O_2e), 0.01Hz, Q = .5
C5 [44]	N_2e	Calculates percentage of Nitrogen in expired air by subtracting out percentages of O_2 and CO_2.
		100-(Ch2+Ch3)
C6 [45]	Vi (ATP)	Integrates the corrected airflow data (C3) to produce volume of air Inspired per minute. This volume is At Temperature and Pressure which is to say it is not yet corrected for Standard Temperature and Pressure Dry (STPD).
		INTEGRATE <C3 Data!> 6000 samples, Average over samples, no parameters - do not rectify
C7 [46]	Vis (STPD)	Applies STPD Normalization Factor to volume of inspired air per minute
		C6*(.874)
C8 [47]	Ves (STPD)	Applies the Haldane Transformation to find volume of <u>expired</u> air per minute (Ves) from volume of <u>inspired</u> air per minute (Vis)
		(C7*79.03)/C5
C9 [48]	VO_2 (STPD)	Calculates volume of O_2 consumed per minute (difference between Vis and Ves).
		(1/100)*((C7*20.93)-(C8*Ch2))
C10 [49]	VCO2(STPD)	Calculates volume of CO_2 produced per minute
		(1/100)*((C8*Ch3)-(C7*0.04))
C11 [50]	RER	RER = Volume of CO_2 produced per minute divided by volume of O_2 consumed per minute
		C10/C9

Note: "Channel" displays both the calculation channel reference (i.e., "C1") and channel graph reference (i.e., "40").

Appendix 3:

This section offers a quick overview of software essentials. For a full explanation of features, see the BSL *PRO* Tutorial or BSL *PRO* Manual.

Show/Hide channels:

Channel visibility can be toggled on and off by holding down the "Alt" key (Windows) or "option" key (Mac) and clicking on the Channel Box.
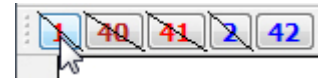
Navigating to channel Scaling dialog:

Select **Set Up Channels** or **Set Up Data Acquisition** (depending on software version) from the MP3x menu to bring up the Input channels setup dialog and then select the **Analog** tab. Click on the desired channel (i.e., CH 1, Airflow) and then click **Setup.** From the setup dialog, click **Scaling**.
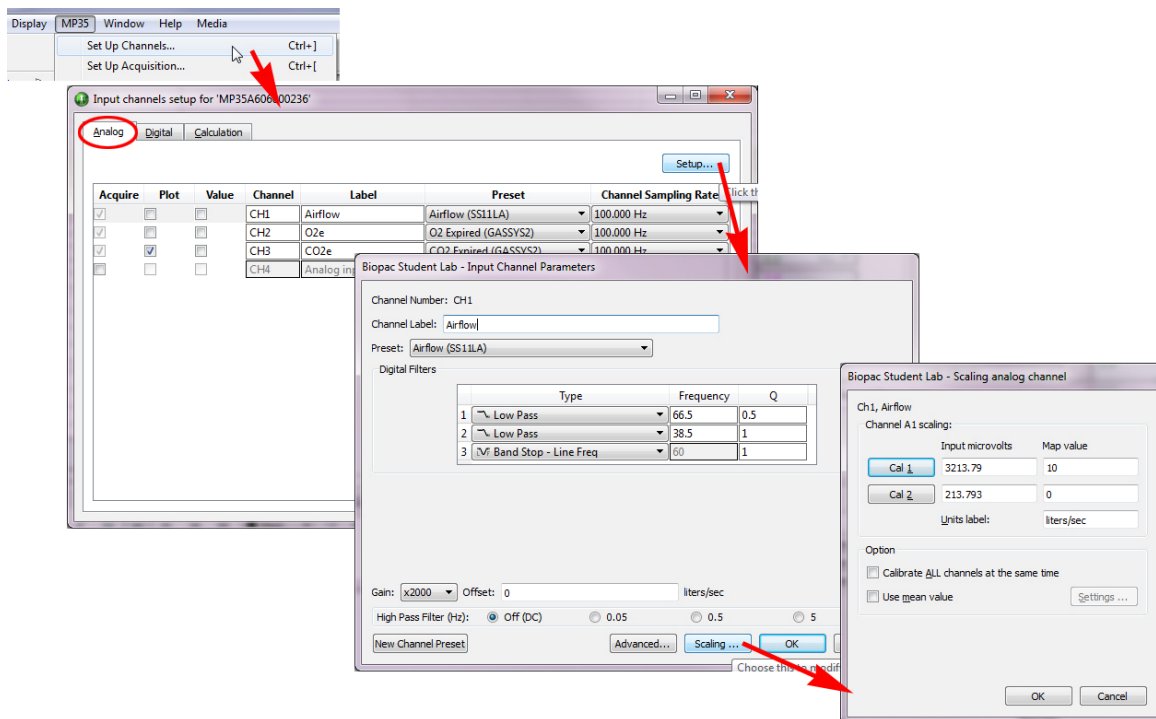
Figure 25

Navigating to calculation channel Expression dialog:

Select **Set Up Channels** or **Set Up Data Acquisition** (depending on software version) from the MP3x menu to bring up the Input channels setup dialog and then select the **Calculation** tab. Click on the desired calculation channel (i.e., C3 (Airflow Scaled)) and then click **Setup.**

Autoscaling data:

Graph data can be autoscaled vertically and horizontally for enhanced viewing.

- Autoscale vertically by using the ⊞ toolbar button or Display > Autoscale Waveforms to optimize the vertical display and allow closer examination of the waveform.

- Autoscale horizontally by using the ⊞ toolbar button or Display > Autoscale Horizontal to display the entire horizontal time scale in a single graph window.

Zooming in and out of data:

Use the Zoom tool to magnify portions of the waveform for a closer look.

- Zoom in by selecting the 🔍 toolbar icon and click/drag over the area of interest.
- To zoom back, use Ctrl – (minus) or "Display > Zoom Back."

Measurements used in this lesson:

Graph data measurements are taken by using the I-beam tool to select an area of interest. The following basic measurements are used in this experiment:

Delta-T	Shows the difference in time between the last and first sample of the selected area.
Max (Maximum)	Shows the maximum amplitude value in the selected area.
Mean	Shows the mean amplitude value of data samples within the selected area.
Min (Minimum)	Shows the minimum amplitude value in the selected area.
P-P (Peak-to-Peak)	Shows the difference between the maximum amplitude value and the minimum amplitude value in the selected area.
Value	Shows the data value at the cursor position, or if an area is selected, the data value at the end of the selection.

Appendix 4:

Airflow transducer operation

The BIOPAC SS11LB/LA airflow transducer works by funneling air through a sealed head which is divided in half by a fine mesh screen (Figure 26). The screen creates a slight resistance to air flow resulting in a higher pressure on one side than the other. This pressure differential is closely proportional* to the air flow rate (liters/second). A differential pressure sensor outputs a voltage that is proportional to the difference in pressure across its two inlet ports.

In software, air volume is determined by integrating air flow. This integration technique is simple and accurate as long as the air flow signal is precise. Small errors in the air flow signal, such as a non-zero baseline offset, can cause larger errors in the calculated volume. For this reason, it is important to note the following:
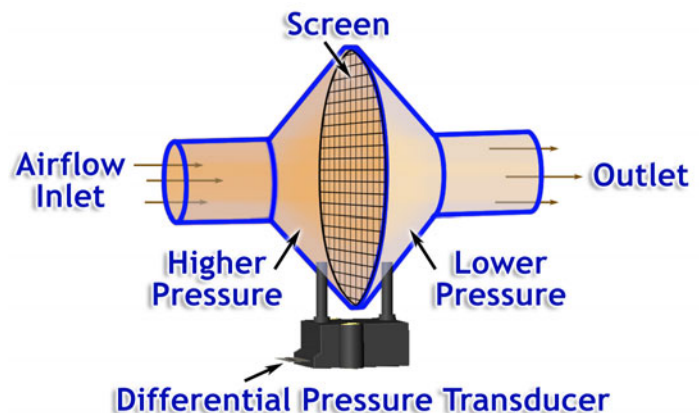

Figure 26

- Baseline offset will vary slightly during the transducer warm-up period. For this reason it is important to perform calibration at least 15 minutes after the transducer has been turned ON.

- The differential pressure sensor is a micro-electromechanical system (MEMS) that is subject to the effects of gravity. This means orientation changes can cause slight shifts in baseline offset. To prevent this, the airflow transducer must be held stationary during Subject recordings.

- Vibration can cause noise artifact in the airflow signal. It is recommended that the airflow transducer and its tubing be held in a fixed, secure position.

- The SS11LB/LA works best for this lesson when it is placed on the inspired side of the T-valve. This is because condensation can clog the screen and change relationship between air flow and differential pressure. Condensation occurs because expired air exits the body at 37°C and is saturated with water vapor. As the air travels through the exhaust tubing and into the GASSYS2, the air is cooled and condensation forms.

- To obtain the best accuracy, the airflow transducer should be calibrated using a calibration syringe at least once a semester. This calibration is described in the "Calibrate airflow amplitude" section above. A scaling factor is determined and applied to the airflow signal. Because air volume is calculated by integrating the air flow, the scaling factor is directly applied to the volume.

*The relationship between the air flow and differential pressure is not perfectly proportional. Non-linearities can arise due to air turbulence hitting the screen. The SS11LB/LA maintains good linearity over its rated flow range of 0 to 5 liters/second, but becomes increasing non-linear for flow rates from 5 to 10 liter/second. Flow rates in the 5 to 10 liter/second range can be produced by some subjects during exercise. This problem is resolved by modeling the airflow response and creating a polynomial expression to correct (linearize) the response. For this lesson, calculation channel expression C2 performs this airflow correction (see Appendix 2).

Appendix 5:

Enabling the "Floating window" Journal Display

BSL 4.1.2 and higher: In addition to the Help menu, lesson-specific *PRO* Lesson PDFs are also available in the lesson's Journal and viewable by clicking the Journal's "lesson procedure" tab. To enhance viewing of lesson PDFs from within the Journal, BIOPAC recommends changing the Journal display preference from the default "Docked at bottom of graph window" setting to "**Floating window**." This option allows for easy resizing and repositioning of the onscreen lesson Journal while allowing full access to the graph. "Floating window" also provides a higher resolution PDF display and positions any Output Control panels directly below the graph for easier viewing.

To change the Journal display to "Floating window":

1. In BSL *PRO*, choose "Display > Preferences" (or click the Preferences toolbar button. ⚙).

2. Highlight the "Journal" option in the Preferences window.

3. Under "Display Style," select "Floating window" and click OK.
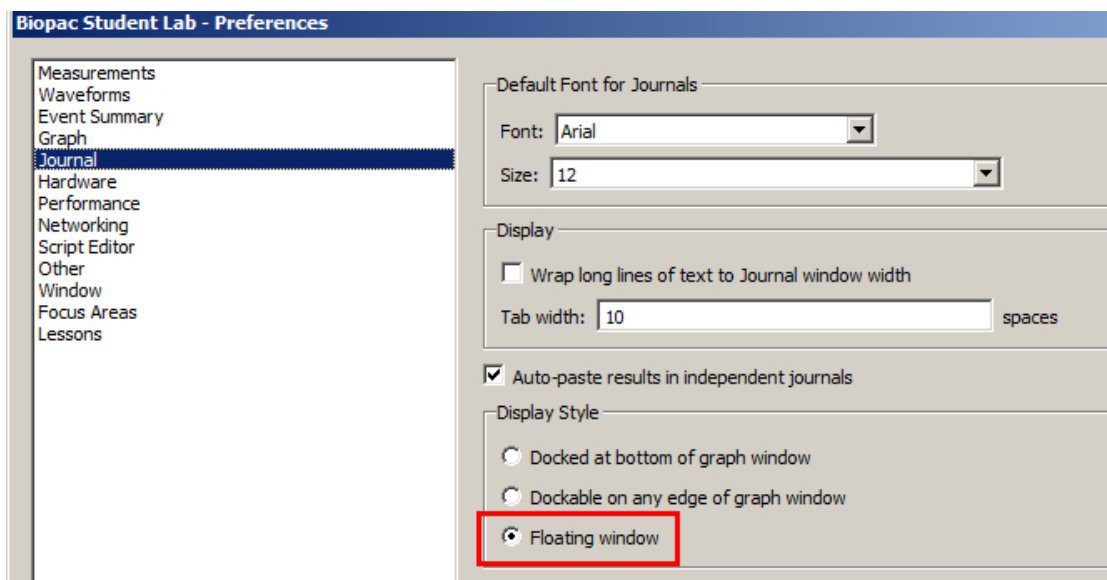
4. The Journal will now appear in a separate window from the BSL graph. (It may appear behind the graph display. Drag the graph sideways and click the Journal window to bring it to the front.

5. Click the "lesson procedure" tab to view the PDF. Reposition and resize the Journal window as necessary. Toggle between Journal text (notes you've entered) and the lesson procedure PDF by clicking the "Journal" and "lesson procedure" tabs.