

BSL PRO Lesson H29: Resting Metabolic Rate



This BSL PRO lesson describes the hardware and software setup of the BSL System in order to measure Oxygen Consumption ($\dot{V}O_2$) and Carbon Dioxide elimination ($\dot{V}CO_2$) using an open circuit indirect calorimetry technique.

Objectives:

1. Gain an understanding of how to indirectly measure energy metabolism.
2. Record $\dot{V}O_2$, $\dot{V}CO_2$, RER, RMR and REE.
3. Learn how RER values indicate how much carbohydrate or fat is used for metabolism.

Equipment:

- Biopac Student Lab System:
 - MP36 or MP36R data acquisition unit
 - Power supply (AC300A) + power cord
 - USB cable (CBLUSB)
 - Software: BSL 4.1.3 and higher or AcqKnowledge 5.0.3 and higher.
- BSL PRO file: "H29 Resting Metabolic Rate.gtl"
- Dialog files: "SubjectDetailsDialog.ui" and "AtmosphericConditionsDialog.ui"*
- Gas Analysis System (GASSYS3)
- GASSYS3 Power supply (AC400) + power cord
- Airflow transducer (SS11LB)
- Calibration Syringe: AFT27 (3 Liter) or equivalent 2, 3, 5, or 7-liter syringe
- Coupler, 35 mm (AFT11D)
- 2 x Tubing, 35 mm ID, 3 m (AFT7-L)

T-valve option 1:

- T-Valve, 35 mm OD (AFT21)
- Disposable filter with mouthpiece (AFT36)
- Disposable Nose Clip (AFT 3)

T-valve option 2:

- Facemask with integrated T-valve (AFT25)
- Syringe coupler, 35 mm to 25 mm (AFT11A)

OPTIONAL:

- Head support for AFT21 T-valve (AFT24)
- Tripod for securing SS11LB

If recording Heart Rate from ECG:

- Electrode lead set for ECG (SS2LB)
- 3 x Disposable ECG electrodes (EL503)
- Electrode Gel - GEL1 (50 g) or GEL100 (250 g)
- Skin Prep Gel (ELPREP) or alcohol

Note*: See [Appendix 4](#) for installation instructions.

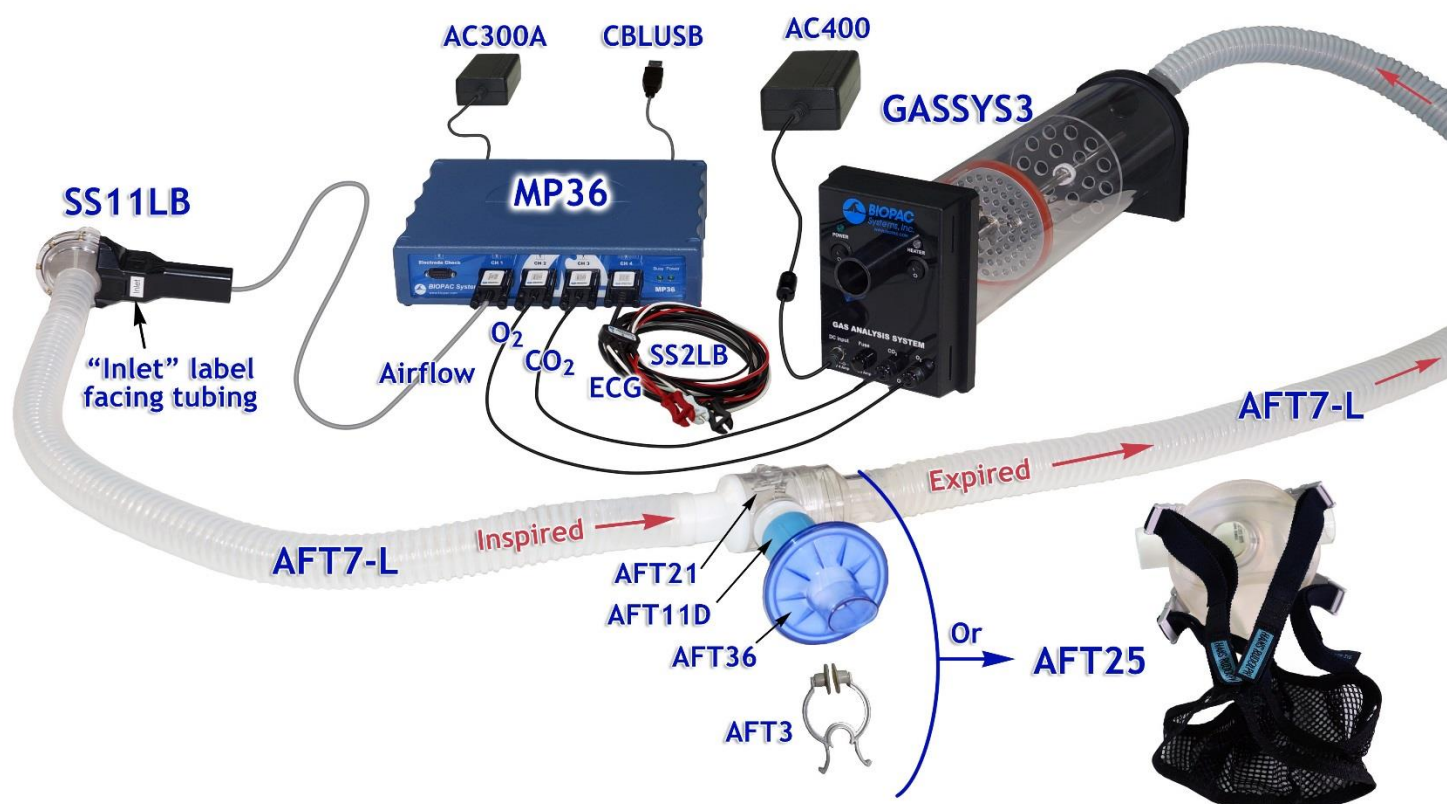


Fig. 1: Setup with airflow transducer on inspired side of T-valve



Fig. 2: Alternate setup with airflow transducer on expired side of T-valve

Setup Options

Airflow Placement: The airflow transducer (SS11LB) can be placed on either the inspired or expired side of the T-valve, but it is normally placed on the expired side (Fig. 2) for resting measurements. The main advantage is there is less air restriction during inhalations which helps the subject relax and breathe at their normal tidal volume. If the system will see heavy use, or if exercise lesson (H19) will be performed in succession, then the airflow transducer must be placed on the inspired side of the T-valve as shown in Fig. 1. This will prevent condensation from forming inside the airflow transducer which will affect its accuracy. If the airflow transducer is placed on the inspired side, it should be firmly secured as movement can cause signal artifact. One way to secure the transducer is to attach it to a tripod (Fig. 3).



Fig. 3

Mouthpiece or Facemask: For resting measurements, it is important for the subject to feel as relaxed as possible. The facemask is more comfortable, so it is often the preferred option. However, the facemask must be cleaned after each use, which can be impractical for some labs. The disposable mouthpiece/filter and nose clip provides a quick and easy way to switch between subjects.

Heart Rate from ECG: Recording heart rate is optional but can be useful as an indication of a subject's level of relaxation. If desired, the electrode lead set (SS2L) is plugged into Channel 4 and electrodes are placed on the Subject in order to record ECG. Heart rate is calculated based on the R–R intervals of the ECG signal.

Calibration Syringe: A calibration syringe is required for airflow transducer calibration. It is also used to flush the GASSYS3 with ambient air in order to obtain an ambient O₂ measurement prior to recording. The calibration syringe is connected to the T-valve with couplers, as shown below, to prevent any air leaks. Although the BIOPAC AFT27 3-liter calibration syringe is listed, any 2, 3, 5, or 7-liter syringe will work; however different couplers may be required.



Fig. 4

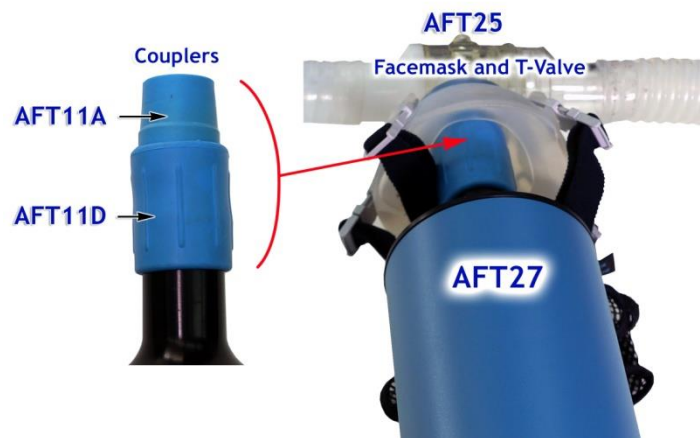
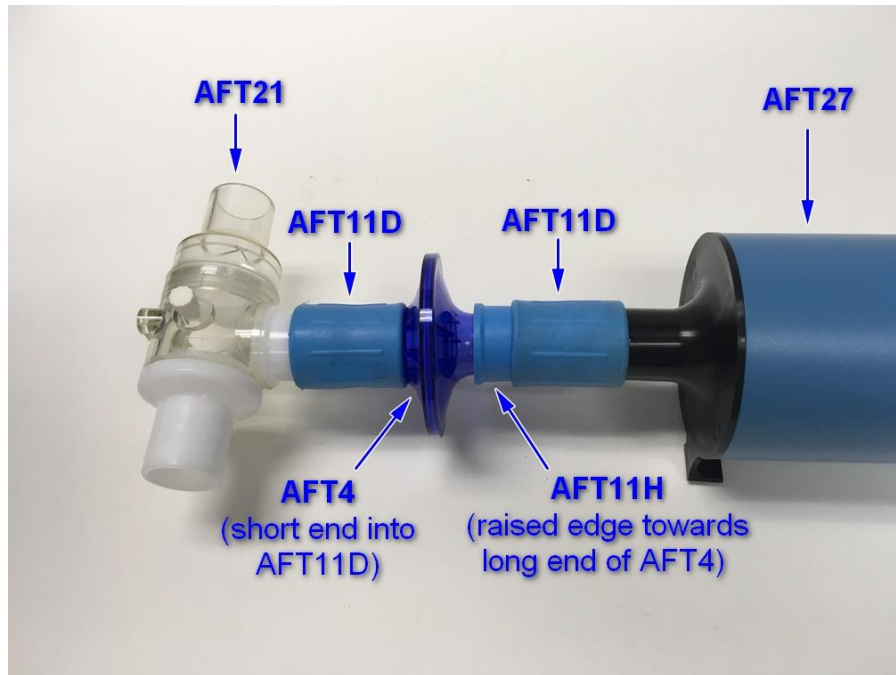


Fig. 5

Note: The calibration syringe is connected to the same T-valve port that the subject breathes through.

IMPORTANT

If the rest of the tubing and connections to the GASSYS3 have been sterilized, a filter should be incorporated when using the calibration syringe to maintain sterility. Additional equipment required: AFT4 bacterial filter, AFT11H coupler, and one additional AFT11D coupler.



Background:

The *First Law of Thermodynamics* states that heat is a form of energy, and that energy cannot be created or destroyed. It can, however, be converted to different types of energy and can be transferred from one location to another. Our bodies take in food containing chemical energy and convert it from one form to another in accordance to the First Law of Thermodynamics.

Body metabolism encompasses all the chemical reactions in all the cells of the body, and the **metabolic rate** is normally expressed in terms of the rate of the heat liberation during the chemical reactions. Essentially all the energy expended by the body is eventually converted into heat. The only significant exception to this occurs when the muscles are used to perform some form of work outside the body (lifting objects, etc.). But when external expenditure of energy is not taking place, it is safe to consider that all the energy released by the metabolic processes eventually becomes body heat¹. The normal unit of measure for expressing the quantity of energy released during metabolic processes is the calorie. Because the calorie is such a relatively small value, it is common to express energy expenditure in terms of the kilocalorie (kcal).

1 **calorie (cal)** = the amount of heat required to raise the temperature of one gram of water by one degree Celsius.

1 **kilocalorie (kcal)** = 1000 calories

Our current understanding of body metabolism stems from over 200 years of ingenious experiments performed by many scientists. The *process* of measuring heat production is called **calorimetry** and the *equipment* used for measurement is called a **calorimeter**. Although conceived in the eighteenth century, it was not until the early 20th century that accurate whole-body calorimeters were built². There are two general categories of calorimeters; Direct and Indirect.

A **Direct Calorimeter** measures the heat produced from a body. Imagine a resting subject in a sealed chamber as shown in Fig. 6, but without the front side cutaway. A layer of insulation prevents any heat from escaping the chamber. A continuous copper pipe encompasses the sides and top of the inner chamber. Water flows through the pipe, with cool water entering and warmer water exiting. The water is heated by the energy transfer between the warmer subject and the surrounding air, from the air to the copper pipes and then from the copper pipes to the water. The water flow rate is controlled such that the heat produced by the subject is carried away at the same rate that it is produced. By measuring the water temperature difference between the inlet and the outlet, along with the flow rate, the amount of energy (kcal) exchanged per minute can be determined.

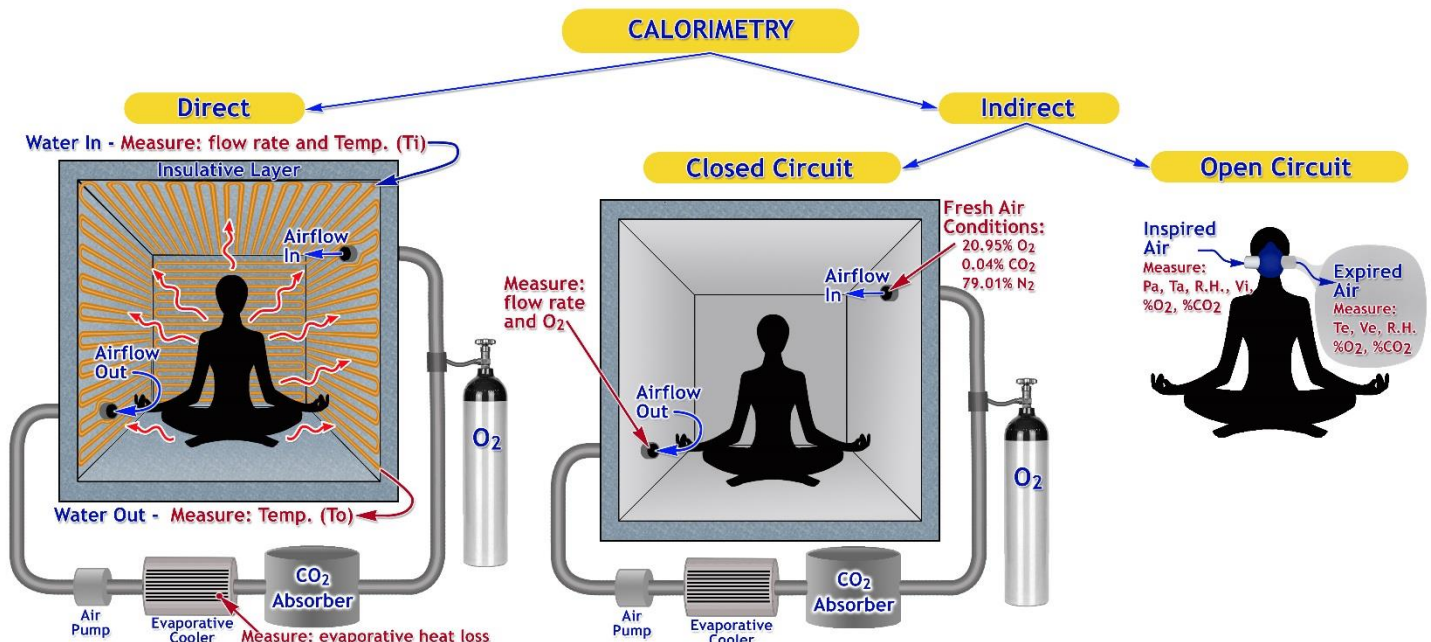


Fig. 6

To prevent the Subject from suffocating, the system must provide a constant supply of fresh/ambient air which ideally at **standard ambient conditions**; 20.95% oxygen (O_2), 0.04% carbon dioxide (CO_2) and 79.01% nitrogen (N_2). An air recirculation system can be used to maintain a constant supply of fresh air. Expired breath is near body temperature (about 35° C - 95° F), contains approximately 4% CO_2 , 16% O_2 , 79.1% N_2 and is saturated with water vapor (H_2O). The air recirculation system must cool the air, remove the expired CO_2 and H_2O and add back the O_2 prior to circulating through the inlet. The amount of water vapor in the outgoing air can be measured to determine the evaporative heat loss which is then added to the total energy exchanged. A regulation system can be used to force the air entering the chamber to be at the same temperature as the air inside the chamber so as not to add to or subtract from the energy measurement.

An **Indirect Calorimeter** estimates heat production based on oxygen consumption. This method came about from experiments in 1790 by the French Chemist Antoine Lavoisier who discovered that heat production can be predicted from oxygen consumption. This led to other experiments that showed the rate of oxygen consumption is directly proportional to metabolic rate. Many experiments have been performed using direct and indirect calorimeters that validate the correlation between oxygen consumption and heat produced by the body. Further experiments added CO_2 to the measurements, and it was found that the ratio of CO_2 eliminated by the body to O_2 consumed by the body differed depending on the types of food consumed. This is an important topic and will be discussed in more detail later. There are two categories of indirect calorimeters; closed circuit and open circuit.

In an **Indirect, Closed Circuit, Calorimeter**, the subject is in a sealed chamber that uses an air recirculation system as shown in fig. 6 (but without the front side cutaway). For this example, three assumptions are made; the air flow rate is constant, the air temperature in the chamber remains constant, and the incoming air is at standard ambient conditions. Measurements are made to determine the airflow rate (liters per minute) and the amount of O_2 (% or ppm) flowing through the outlet port. From these measurements, the volume of oxygen consumed by the Subject per minute can be determined.

For an **Indirect, Open Circuit, Calorimeter**, there is no air recirculation system or chamber. In the Fig. 6 illustration, the subject is wearing a facemask and non-rebreathing T-valve which allows for inspired and expired air to flow through different ports. The subject breathes in air at standard ambient conditions and the expired air is analyzed. The volume of air inspired (V_i) and air expired (V_e) are measured as are the concentrations of O_2 and CO_2 in the expired air. Because gas volume changes with temperature and pressure, the temperature of the inspired (ambient) air (T_a), temperature of expired air (T_e), and the barometric pressure (P_a) must be measured. In addition, because water vapor displaces the fractions of O_2 , CO_2 and N_2 in the air, the relative humidity (R.H.) of both inspired and expired are measured. These measurements are entered into well-established formulas which determine the volume of O_2 consumed and the volume of CO_2 eliminated (see Lesson Calculations section). A factor is then applied to convert volume of oxygen consumed to energy expended (kcal/min).

One common method for analyzing expired air is to first collect a sample of it over a specific time period such as one minute. The sampling container is called a **Douglas Bag** which is like a small weather balloon made of an impermeable material. Valves control whether expired air flows into the bag or into the ambient air. After the expired air is collected, the bag is then ported over to instruments that measure gas concentrations, temperature, relative humidity and air volume while the air in the bag is slowly depleted. The Douglas bag method has been around since the early 1900s and it was a necessity back when gas analyzers had very slow response times. The disadvantage of a Douglas bag system is that it can only take samples of air over short periods of time. With the advent of modern electronics, sensors can now measure gas concentrations, temperature and relative humidity much faster, some even in real time, which eliminates the need for air bag sampling.

Calorimeters have also been developed for determining the energy content of food. One type commonly used is the **Bomb Calorimeter** which determines the maximum amount of energy from food by measuring the amount of heat produced after complete combustion. Because the human body is not 100% efficient at extracting energy from food, the calorie (or kcal) value assigned to a food item is normally adjusted to reflect the typical amount of energy that can be extracted.

It was mentioned above that gas volumes vary with temperature and pressure. In order for measurements to be accurately compared to those taken at other locations, or at other times, the pressure and temperature variations must be removed. Gas volume is normally measured at **Atmospheric Temperature and Pressure (ATP)**. This volume is then converted to what is called **Standard Temperature and Pressure, Dry (STPD)** which is what the air volume would be if it were at 0° C (273° F), at a standard pressure of 1 atm (760 mmHg), and with all water vapor removed. The formula to convert from ATP to STPD is discussed in the Lesson Calculations section and shown in Table 2.

Physiology of Respiration

Blood transports gases to and from the body's cells. The respiratory system supplies O₂ to the blood and removes carbon dioxide (CO₂) 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 O₂ and CO₂. Blood that has absorbed O₂ during inhalation (oxygenated blood) is transported by the cardiovascular system to systemic tissues throughout the body. Once in the tissues, the O₂ moves from the bloodstream into cells by diffusion, where it is used for production of adenosine triphosphate (ATP). ATP is a compound containing high-energy bonds which the cell may later break to release energy and perform work, such as secretion, contraction, or membrane transport. Note that the terms breathing, and respiration do not have the same meaning. "Breathing" is the physical process and "Respiration" is the chemical process.

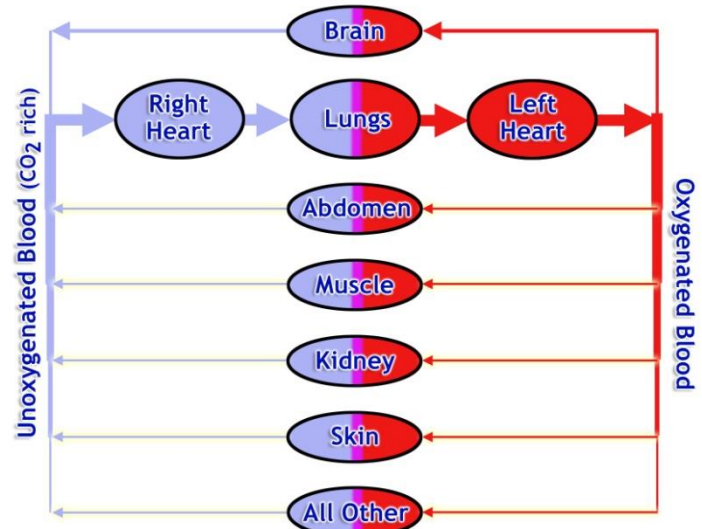


Fig. 7

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 O₂ extracted from air in the alveoli, the volume of blood that the heart can pump, and the tissues' capacity for extracting O₂ from the blood. $\dot{V}O_2$ can yield useful information and is universally studied 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 O₂ 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.

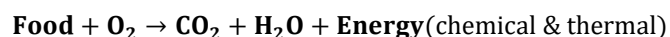
$$\dot{V}O_2 \text{ Relative (ml/kg/min)} = \frac{\dot{V}O_2 \text{ (Absolute)} \frac{\text{liters}}{\text{min}} \times 1000 \frac{\text{milliliters}}{\text{liter}}}{\text{Body mass (kg)}}$$

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

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

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

The human body's oxidation of organic food such as carbohydrate, fat, or protein, produces carbon dioxide and water, and releases chemical and thermal energy:



The chemical energy is used by the body's cells to generate ATP. The thermal energy (heat) is used to maintain a relatively stable, optimum internal body temperature.

Metabolic rate is the amount of energy released per unit of time. The amount of heat (measured in kilocalories) released during the oxidation of a food is directly proportional to the energy content of the food and directly proportional to the volume of oxygen required for complete oxidation. For example, the complete oxidation of one mole of glucose (in a calorimeter) requires 134.4 liters of O₂ (6 moles x 22.4 liters/mole = 134.4 liters of O₂) and yields 673 kcal of energy:



Thus, the complete oxidation of one mole of glucose yields 5.01 kcal per liter of O₂ consumed. This value is called the *caloric equivalent* of glucose. The caloric equivalent of other foods is 5.06 kcal/liter O₂ for starches, 4.70 kcal/liter O₂ for fats, and 4.6 kcal/liter O₂ for protein³.

The human body is continually oxidizing a mixture of carbohydrate, protein, and fat rather than using any single food as the sole source of energy. Based on an average utilization of all three foods, the average release of energy (in terms of oxygen consumed) is 4.825 kcal per liter of O₂ consumed. Metabolic rates under varying conditions may, therefore, be measured by determining oxygen consumption in liters per hour and multiplying by the caloric equivalent of **4.825 kcal** per liter of oxygen consumed.

Basal metabolic rate (BMR) refers to body metabolism as measured under a set of standard basal conditions designed to minimize the effects of as many influencing factors as possible. Basal conditions are as follows:

1. The Subject must ingest no food or stimulants for at least 12 hours prior to the test.
2. The test is performed in the morning.
3. The Subject must be in a supine position and must be mentally and physically relaxed.
4. The Subject's core body temperature must be normal.
5. Room temperature must be comfortable (65°F - 80°F).
6. The recording should last 45 minutes.

Meeting all these requirements can be difficult to achieve in a typically student lab. For this reason, it is more practical to perform a measurement of **Resting Metabolic Rate (RMR)** which only requires fasting for 7 hours and can be taken at any time of day. Note, however, that measurements of RMR taken in the afternoon will typically be 6% higher in comparison to that obtained in the morning. RMR can be calculated based on $\dot{V}\text{O}_2$.

There are different ways to express RMR which include factoring in body weight or body surface area and gender to normalize the measurement to specific body types. Table 1 shows the equations used in this lesson to calculate RMR.

Resting Metabolic Rate	
Units	Equation
[cal/day]	$\dot{V}\text{O}_2 \left(\frac{\text{l}}{\text{min}} \right) \times 4.825 \left(\frac{\text{kcal}}{\text{l}} \right) \times \left(\frac{1000 \text{ cal}}{\text{kcal}} \right) \times \left(\frac{1440 \text{ min}}{\text{day}} \right)$
cal/kg/day	$\frac{\left[\frac{\text{cal}}{\text{day}} \right]}{\text{Body Weight (kg)}}$
cal/m ² /day	$\frac{\left[\frac{\text{cal}}{\text{day}} \right]}{\text{Body Surface Area (m}^2\text{)}}$

Table 1

Note: There are many equations for calculating Body Surface Area (BSA) and this lesson provides several options in lesson preferences (see Table 3). The most commonly used is the De Bois equation which is based on the Subject's height and weight.

During the production of ATP, CO₂ is created as a by-product and must be removed, as it is toxic in large amounts. CO₂ can 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 elimination ($\dot{V}\text{CO}_2$)** is the amount of CO₂ eliminated by the body per minute. $\dot{V}\text{CO}_2$ can be reported in absolute terms (liters/min) or as relative to body mass (milliliters of CO₂ per kilogram of body weight per minute).

Although the oxygen consumption ($\dot{V}O_2$) is the main determinant of **Energy Expenditure (EE)** and thus metabolic rate, CO_2 plays a small role because it makes up part of the volume of expired air. If a Subject is not relaxed or is hyperventilating, the measured $\dot{V}O_2$ is affected. A more accurate way of calculating energy expenditure is to use the WEIR formula⁴ which factors in $\dot{V}CO_2$:

$$\text{Energy Expenditure (cal/min)} = (3.9 \frac{\text{cal}}{\text{l}}) \times \dot{V}O_2 (\frac{\text{l}}{\text{min}}) + (1.1 \frac{\text{cal}}{\text{l}}) \times \dot{V}CO_2 (\frac{\text{l}}{\text{min}})$$

From this, we can calculate **Resting Energy Expenditure (REE)**:

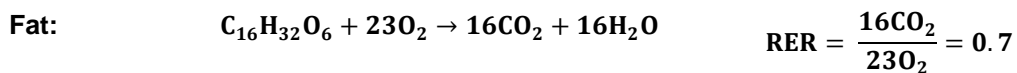
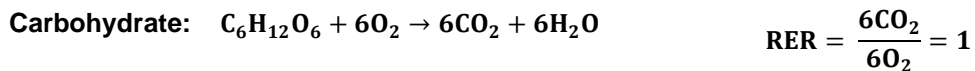
$$\text{Resting Energy Expenditure (cal/day)} = \text{EE} (\frac{\text{cal}}{\text{min}}) \times 1440 (\frac{\text{min}}{\text{day}})$$

In this lesson, RMR and REE have the same meaning, even though they are calculated differently.

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.

$$\text{RER} = \frac{\dot{V}CO_2}{\dot{V}O_2}$$

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:



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 $\dot{V}O_2$, $\dot{V}CO_2$, RMR, REE and RER using an open circuit indirect calorimetry technique.

The GASSYS3 consists of a sealed 5-liter air mixing chamber, a heater plate, a blower and seven sensors. Four of the sensors are located inside the chamber, near the exhaust port, and they measure %O_{2e}, %CO_{2e}, temperature (T_c) and relative humidity (RH_c) of expired air. The notation “e” references expired and “c” references the chamber.

Another three sensors are located inside the “O₂” connector housing (sampling port on underside) and they measure ambient air temperature (T_a), barometric pressure (P_{ba}) and relative humidity (RH_a).

The heater plate and blower are used to prevent condensation from forming in and around the sensors. The blower also helps mix the air and gives the CO₂ sensor a faster response time. A more detailed description of the GASSYS3 internals is provided in [Appendix 5](#).

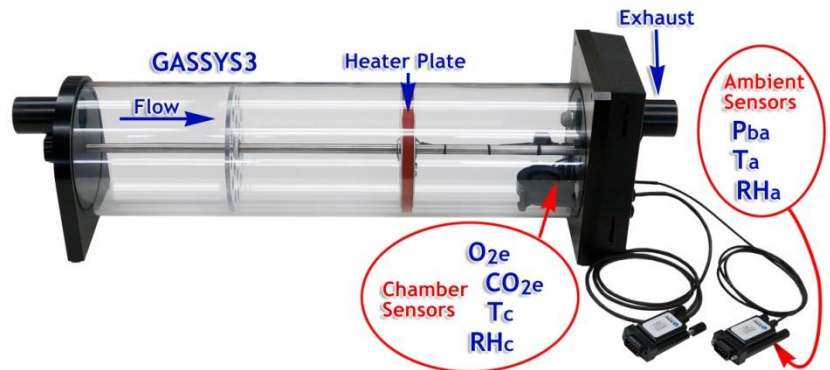


Fig. 8

In the hardware setup, a Subject is connected to the GASSYS3 by way of plastic tubing and a non-rebreathing T-valve (Fig.1 and Fig. 2). The T-valve allows the subject to inspire from the atmosphere but diverts all the expired air through the GASSYS3. The tubing and air mixing chamber act to average respiratory outflows. This averaging effect causes the CO₂ and O₂ concentrations to vary in accordance with the mean values resident over multiple expired breaths. An airflow transducer (SS11LB) is used to determine the air volume (l/min). A more detailed description of the SS11LB is provided in [Appendix 6](#).

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 has a specific response time. The delays are not important when taking steady state measurements but will affect the accuracy of non-steady state VO₂. To improve accuracy, assume a delay of 30 seconds for O_{2e}, CO_{2e}, VO₂ with respect to the airflow signal and any manually entered event markers.

Lesson Calculations

This lesson uses an embedded script to control lesson flow and to perform calculations that are not available in a standard lesson template. The following describes the lesson flow and specifies the calculations that are made. Refer to Table 2 and Table 3 for the formulas that are used.

1. Upon launch, request a folder/file name where data will be saved and then request the Subject's gender, age, height (H) and weight (W). After dialogs are closed, the software will calculate estimations and record to the journal:
 - Body Surface Area (**BSA**) based on formula chosen in Lesson Preferences.
 - Body Mass Index (**BMI**).
 - Resting Energy Expenditure (**REE**).
2. Readings of the ambient sensors (**Pba**, **Ta** and **RHa**) are made and displayed in a pop-up dialog. The assumed ambient/inspired % CO₂ value (**CO_{2i}**) is also displayed. The user can change these values if needed. Upon closure of the dialog, the values will be displayed in the journal.
3. A series of dialogs will be displayed guiding the user through Airflow calibration. The first step adjusts the baseline (zero flow) value and the second step examines the output after a single full stroke of the calibration syringe. It then determines the gain correction factor which will be displayed in the journal upon completion.
4. If the Heart Rate from ECG is enabled, this step allows for a check of the signals. It is important to work out any problems with the electrodes before the recording begins. This data is not saved.
5. The GASSYS3 chamber is flooded with ambient air by cycling the calibration syringe for 60 seconds. Upon completion, the ambient/inspired % O₂ value (**O_{2i}**) is measured along with the relative humidity.

6. The following calculation are made at the end of calibration:
 - a. Saturation vapor pressure of ambient air (**PH2OSATa**) based on (Ta) using the Buck Equation.
 - b. Vapor pressure of ambient air (**PH2Oa**) based on RH_a and PH2OSAT_a.
 - c. Percentage of water vapor in ambient air (**%H2Oa**) based on PH2O and P_{ba}.
 - d. Percentage of Nitrogen in ambient air (**N2a**) based on O_{2a}, CO_{2a} and H_{2Oa}.
 - e. If the airflow transducer is placed on the inspired side of the T-Valve, calculate the ambient air volume correction factor (N) to convert from volume at Actual Temperature and Pressure (ATP) to volume at Standard Temperature and Pressure Dry (STPD) based on Ta, P_{ba} and PH2O_a. This allows accurate comparisons between Subjects tested under different ambient air conditions. The 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.
7. Once the recording begins, all analog and calculation channel data are recorded at a sample rate of 125 samples/sec, except for the optional ECG channel which is recorded at 500 samples/sec. The following measurements and calculation are then made every 10 seconds:
 - a. Chamber temperature (**Tc**), Relative Humidity (**RHc**) are measured.
 - b. Saturation vapor pressure of chamber air (**PH2OSATc**) based on (Tc) using the Buck Equation.
 - c. Vapor pressure of chamber air (**PH2Oc**) based on RH_c and PH2OSAT_c.
 - d. Percentage of water vapor in chamber air (**%H2Oc**) based on PH2Oc and P_{ba}.
 - e. Percentage of Nitrogen in expired air (**N2e**) based on O_{2e}, CO_{2e} and H_{2Oe}.
 - f. If the airflow transducer is placed on the expired side of the T-Valve, calculate the chamber air volume correction factor (N) to convert from volume ATP to volume at STPD based on T_c, P_{ba} and PH2O_c.
 - g. Calculate \dot{V}_{iSTPD} based on \dot{V}_{eSTPD} or vice versa depending on whether the airflow transducer is on the inspired or expired side of the T-Valve. This calculation is made using the Haldane equation which assumes that 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.
8. For the first 60 seconds, the data is invalid because the airflow integrator is initializing. Beginning at 90 seconds and continuing every 30 seconds thereafter, channel measurements (chosen in Lesson Preferences) will be placed in the journal.
9. At the end of the recording, the Resting data summary will be placed in the journal. This includes calculations of RMR (cal/day) and REE (cal/day).

Notes:

- The assumption is made that Air is made up of only N₂, O₂ and CO₂. Additional trace elements are present in the atmosphere such as Ar, Ne, He, but because, like nitrogen, they are inert in terms of metabolism, they are added to the nitrogen content.
- The default value for ambient CO₂ is 0.04%. This is normally true for outside air, but for air in a confined room, with multiple bodies expiring CO₂, 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 CO₂ in ambient air is small compared to the amount of O₂ and to N₂ and because the amount of CO₂ in expired air is much larger than that in ambient air.

Description	Notation	Units	Formula (see Key for variable references)	Reference
Saturation vapor pressure at temp. (°C) via Buck Equation	PH2OSAT	mmHg	$\left(\frac{6.1121}{1.3332239}\right) \times e^{\left(\frac{18.678 - \left(\frac{T}{234.5}\right)}{\left(\frac{T}{T+257.14}\right)}\right)}$	5
Vapor pressure	PH2O	mmHg	$\frac{\text{PH2OSAT} \times \text{RH}(\%)}{100(\%)}$	6
Percentage of water vapor in air	H2O	%	$\left(\frac{\text{PH2O}}{\text{Pb}}\right) \times 100 \%$	
Percentage of Nitrogen in air	N2	%	$100\% - (\text{O2}(\%) + \text{CO2}(\%) + \text{H2O}(\%))$	
Air volume correction factor to convert from ATP to STPD conditions.	Vf		$\left(\frac{273}{(273 + T)}\right) \times \left(\frac{\text{Pb} - \text{PH2O}}{760}\right)$	7
Volume expired from Volume inspired (Airflow transducer on inspired side of T-valve)	\dot{V}_{ESTPD}	l/min	$\dot{V}_{\text{ISTPD}} \times \frac{\text{N2i}(\%)}{\text{N2e}(\%)}$	8 Haldane Transformation
Volume inspired from Volume Expired (Airflow transducer on expired side of T-valve)	\dot{V}_{ISTPD}	l/min	$\dot{V}_{\text{ESTPD}} \times \frac{\text{N2e}(\%)}{\text{N2i}(\%)}$	
Volume of O ₂ consumption (at STPD)	$\dot{V}\text{O2}_{\text{STPD}}$	l/min	$\left(\dot{V}_{\text{ISTPD}} \times \frac{\text{O2i}(\%)}{100(\%)}\right) - \left(\dot{V}_{\text{ESTPD}} \times \frac{\text{O2e}(\%)}{100(\%)}\right)$	7
Volume of CO ₂ elimination (at STPD)	$\dot{V}\text{CO2}_{\text{STPD}}$	l/min	$\left(\dot{V}_{\text{ESTPD}} \times \frac{\text{CO2i}(\%)}{100(\%)}\right) - \left(\dot{V}_{\text{ISTPD}} \times \frac{\text{CO2e}(\%)}{100(\%)}\right)$	
Respiratory Exchange Ratio	RER		$\frac{\dot{V}\text{CO2}_{\text{STPD}}}{\dot{V}\text{O2}_{\text{STPD}}}$	9
Energy Expenditure via Weir formula	EE (Weir)	cal/min	$(3.9 \times \dot{V}\text{O2}_{\text{STPD}}) + (1.1 \times \dot{V}\text{CO2}_{\text{STPD}})$	10
Volume of O ₂ consumption (at STPD) relative to body weight	$\dot{V}\text{O2 Rel}$	ml/kg/ min	$\frac{\dot{V}\text{O2}_{\text{STPD}} \times 1000}{W}$	
Body Mass Index	BMI	Kg/m ²	$\frac{W}{H^2}$	11
Est. Resting Energy Expenditure (Female)	REE	cal/day	$(10 \times W) + (6.25 \times H \times 100) - (5 \times \text{Age}) - 161$	12 Mifflin- St Jeor
Est. Resting Energy Expenditure (Male)			$(10 \times W) + (6.25 \times H \times 100) - (5 \times \text{Age}) + 5$	

Table 2 Formulas used in lesson

Key		
Variable	Definition	Units
CO2i, CO2e	CO ₂ inspired or expired	%
H	Subject's Height	m
N2i, N2e	Nitrogen inspired or expired	%
O2i, O2e	Oxygen inspired or expired	%
Pb	Barometric Pressure	mmHg
RH	Relative Humidity	%
T	Temperature	°C
W	Subject's Weight	kg

Description	Units	Formula (see Key for variable references)	Key	Ref.
Mosteller	m ²	$\sqrt{\frac{W \times H}{3600}}$	W = Weight (kg) H = Height (cm)	13
Du Bois, Du Bois		$0.007184 \times W^{0.425} \times H^{0.725}$		14
Haycock		$0.024265 \times W^{0.5378} \times H^{0.3964}$		15
Gehan and George		$0.0235 \times W^{0.51456} \times H^{0.42246}$		16
Boyd		$0.0003207 \times W^{(0.6157 - (0.0188 \times \log_{10} W))} \times H^{0.3}$		17
Fujimoto		$0.008883 \times W^{0.444} \times H^{0.663}$		18
Takahira		$0.007241 \times W^{0.425} \times H^{0.725}$		19
Schlich		$0.000975482 \times W^{0.46} \times H^{1.08}$ (Women) $0.000579479 \times W^{0.38} \times H^{1.24}$ (Men)		

Table 3 Body Surface Area Formulas

References:

- Guyton, Arthur C. (1991). Textbook of Medical Physiology, 8th ed. W. B. Saunders Company: Pg 792.
- Kenny Glen P., Notley Sean R., Gagnon Daniel, Direct calorimetry: a brief historical review of its use in the study of human metabolism and thermoregulation, Eur J Appl Physiol 2017; 117:1765-1785.
- Lusk G. Food values, Science. 1917;45:345-350
- WEIR, J.B. De V. (1949). J Physiol (109, I-9).
- Buck, A. (1981). J Appl Meteorol 20, 1527-1532.
- Consolazio C, Johnson R, Pecora L. (1963). Physiological Measurements of Metabolic Functions in Man. New York NY: McGraw Hill.
- Brown D, Cole TJ, Dauncey MJ, Marrs RW, and Murgatroyd PR (1984). Analysis of gaseous exchange in open-circuit indirect calorimetry. Med Biol Eng Comput 22: 333-338, 1984.
- Branson RD, Johannigman JA (2004) The measurement of energy expenditure. Nutr Clin Pract 19: 622-636.
- Schmidt-Nielsen, Knut (1997). Animal Physiology. Cambridge, UK: Cambridge University Press. p. 171.
- Weir, J. B. De, (1949). New Methods for Calculating Metabolic Rate with Special Reference to Protein Metabolism. J. Physiol 109, I-9
- Blackburn H, Jacobs D (June 2014). Commentary: Origins and evolution of body mass index (BMI): continuing saga". International Journal of Epidemiology. **43** (3): 665–9.
- Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr. 1990; 51:241–247.
- Mosteller, RD (1987). Simplified calculation of body-surface area. N Engl J Med. 317 (17): 1098.
- DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Medicine. 1916; 17:863-71.
- Haycock, GB; Schwartz, GJ; Wisotsky, DH (1978). "Geometric method for measuring body surface area: A height-weight formula validated in infants, children and adults". J Pediatr. 93: 62–66.
- Gehan EA, George SL, Cancer Chemother Rep 1970, 54:225-235
- Boyd, Edith (1935). The Growth of the Surface Area of the Human Body. University of Minnesota. The Institute of Child Welfare, Monograph Series, No. x. London: Oxford University Press.
- Fujimoto S, Watanabe T, Sakamoto A, Yukawa K, Morimoto K. Studies on the physical surface area of Japanese. 18. Calculation formulae in three stages over all ages. Nippon Eiseigaku Zasshi 1968;5:443–50.
- Schlich, E; Schumm, M; Schlich, M (2010). "3-D-Body-Scan als anthropometrisches Verfahren zur Bestimmung der spezifischen Körperoberfläche". Ernährungs Umschau. **57**: 178–183

Setup:

General Connections

The MP36 must be connected to the AC300A power supply but initially turned OFF. It is assumed that the MP36 is connected to the host computer (via USB cable) and that BSL PRO software has been installed and is known to work with the MP36 unit.

1. The GASSYS3 must be plugged into the AC400 (12 Volt, 5 Amp) power supply and the POWER switch should be turned ON. The HEATER switch should be turned OFF.
2. Connect the hardware as shown in Fig. 1 and Fig. 2.

Notes:

- Make sure the correct power supply is plugged into the GASSYS3 as it requires higher amperage than the MP36.
- Because the GASSYS3 requires at least 45 minutes of warm-up, it should be powered ON well in advance of running the lesson.
- Regardless of whether the airflow transducer is placed in the inspired or expired side of the T-valve, the side with the "Inlet" label must be facing towards the T-valve as shown in Fig. 1 and Fig. 2.

IMPORTANT

The GASSYS3 and the connected tubing are not sterilized and therefore must only process expired air (Fig. 6). Make sure that the output port of the T-valve is connected to the GASSYS3 tubing. If necessary, test the T-valve prior to connecting the tubing to make sure the correct port is used. If using BIOPAC's AFT21 or AFT25, the inspired side of the T-valve is the white side as shown in Fig. 7.

If disposable bacteriological filters (AFT36) are not being used, then the T-valve and facemask (if applicable) must be properly disinfected (high level) or sterilized prior to each subject's use. Always follow the manufacturer's recommended disinfecting procedures. If using the AFT21 T-valve:

<http://www.rudolphkc.com/img/uploads/pdf/691200%201117%20D.pdf>

If using the AFT25 Facemask with integrated T-valve:

<http://www.rudolphkc.com/img/uploads/pdf/691195%200915%20D.pdf>



Fig. 9



Fig. 10

3. The MP36 channel input connections must be as follows:

CH 1: Airflow transducer (SS11LB)	CH 2: "O2" from GASSYS3
CH 3: "CO2" from GASSYS3	CH 4: (optional) Electrode Lead Set (SS2L) for ECG
4. Connect the calibration syringe to the T-valve as shown in Fig. 4 and Fig. 5. Make sure there is a tight seal between the two.

ECG Setup (optional)

- If ECG and Heart Rate will be recorded, attach the electrodes and leads on the Subject as shown in Fig.11.

Notes:

- Clean electrode sites with ELPREP Skin Prep Gel or alcohol before abrading.
- Always apply a drop of gel (GEL1) to the sponge portion of electrodes before attaching.
- Remove any jewelry on or near the electrode sites.
- Place one electrode on the medial surface of each leg, just above the ankle. Place the third electrode on the right anterior forearm at the wrist (same side of arm as the palm of hand).
- For optimal electrode contact, place electrodes on skin at least 5 minutes before start of Calibration.



Fig. 11

Turn ON the hardware

- After all cables and electrodes are connected, the MP36 may be turned **ON**.
- The subject should be in a relaxed, supine position. If this is not practical, a comfortable seated position can be used.

Note: The T-valve or facemask is not yet placed on the subject.



Fig. 12

Software Setup

Note to Instructor: Prior to running the lesson for the first time, set up lesson preferences (see [Appendix 1](#)).

- Launch the software. From the Startup dialog, choose **Create/Record a new experiment** and **Open Graph template from disk** and then click **OK**. Navigate to the file “H29 Resting Metabolic Rate.gtl”, select it and click **Open**.

Calibration Procedure:

Subject Details

- Enter a folder/file name for the Subject in the first dialog and click **OK**.
 - After clicking OK, a folder with this name will be automatically created and a file name will be assigned (i.e. “Ryan-H29.acq”). If the subject’s folder name already exists, it can still be used, and older files within it will not be overwritten. If the file name already exists, a new name will be created with a Revision number (i.e. “RyanR2-H29”).
- Enter the Subject’s profile and click **OK**.
 - The data will be used to estimate BSA, BMI and REE.
 - Different height and weight units can be chosen from the pull-down menus, and these will be retained.

Note: If instead of the dialog, a prompt “Unable to find required custom dialogs!” appears, you must quit the program and follow the instructions in [Appendix 4](#).

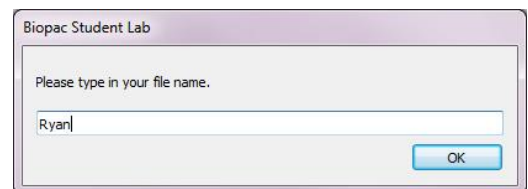


Fig. 13

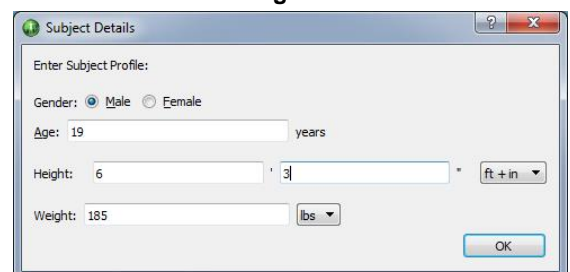


Fig. 14

Check Setup Configuration and Set Preferences

- Check the “Hardware Setup” summary in the journal (Fig. 15). If any settings are incorrect, change them by clicking on the Preferences button and then choose and change the specific option(s). See [Appendix 1](#) for more details.

Notes:

- Do not proceed until the hardware setup information in the journal is correct.
 - Any changes to Preferences will be retained.
- To continue, click **Start**.

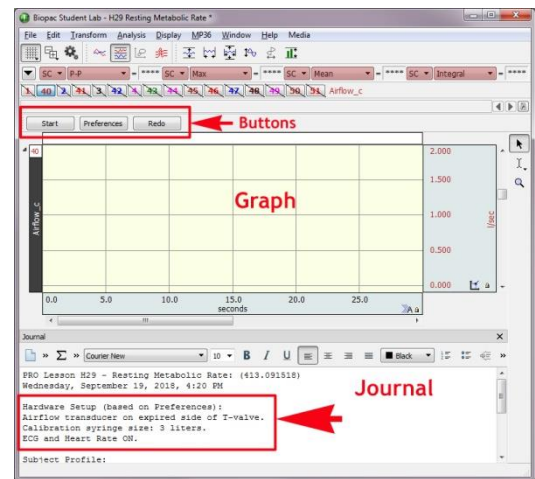


Fig. 15

Ambient Air Check

- The dialog displays the current ambient air conditions (Fig. 16). You may manually change any value.

Notes:

- If this dialog does not appear, see the note in step 2.
 - All measurements except for the CO₂ value are read from sensors that are built into the O₂ connector housing. Any values may be manually changed if, for example, the lab uses on a different ambient air monitor. In addition, the units can be changed via the pull-down menus and they will be retained the next time the lesson is run.
 - The ambient CO₂ value is assumed. This is because the CO₂ sensor in the GASSYS3 is calibrated to be accurate in the 3 – 8% range for measurement of expired human breath and the sensor cannot maintain high accuracy down to the low (almost zero) ambient air CO₂ levels.
- Click **OK** to continue.

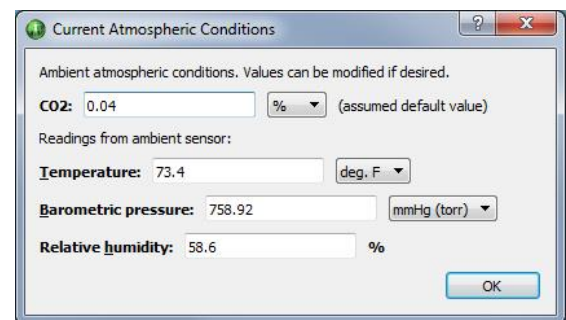


Fig. 16

Airflow Calibration

- Click **Airflow Check** to bring up the “Zero Baseline” dialog.
- Make sure no air is flowing through the system and click **Zero Baseline**.
- After two short recordings are performed, a dialog will appear for the next step. Before proceeding, examine the data:

The “Mean” measurement for data in the selected area is displayed. The Mean value should be very close to zero (+/- 0.01 l/sec) as shown in Fig. 17.

- If there is excessive baseline offset, click **Redo**.
- If baseline offset is correct, follow the directions in the dialog, and when ready, click **OK**.

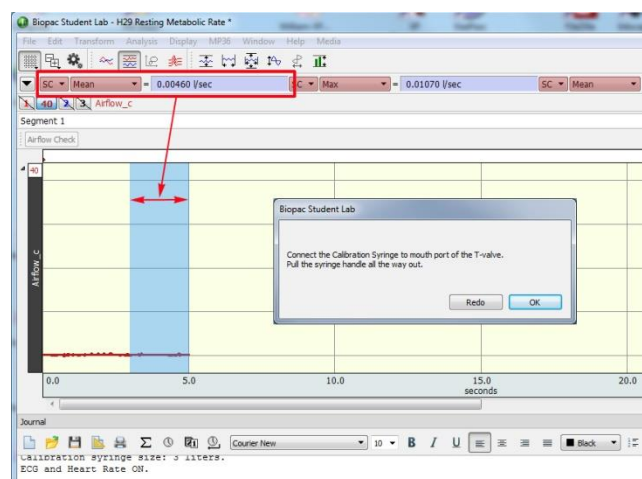


Fig. 17

Calibrate airflow amplitude

10. Read the next dialog carefully as it explains what needs to be done after clicking OK.

Note: The directions to push or pull the calibration syringe handle will depend on the location of the airflow transducer.

11. Click **OK**.
12. Wait until a few seconds after the recording begins and then either push the handle all the way in or pull the handle all the way out as per instructions in the previous dialog. After completion, click the lesson **Stop** button.
13. The data should look similar to that in Fig. 18; one pulse of air going upwards from baseline. If the data is not correct, recheck the setup, making sure the "Inlet" label on the SS11LB is oriented correctly (Fig. 1 and Fig. 2), and click Redo.
14. Read the instructions in the dialog and click **OK**.
15. Using the "I" beam cursor, select all of the airflow pulse data, while excluding as much baseline data as is reasonable, and then click **Adjust Airflow**.

Notes:

- The Integral measurement will display the volume calculated from the selected airflow data. This should be very close to the value of the calibration syringe.
- If the volume is incorrect, the wrong calibration syringe size in Preference may be selected. A dialog will appear allowing you to check and/or change this preference setting. If there was some other problem, click **Redo**.

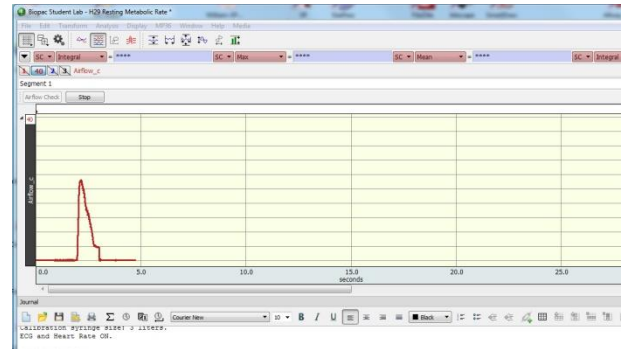


Fig. 18

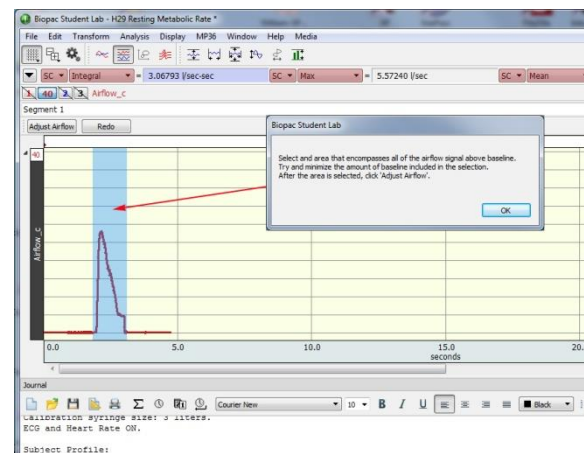


Fig. 19

Get Ambient O₂

The GASSYS3 chamber will be flushed with ambient air for 60 seconds. Upon completion, an ambient O₂ measurement will be read by the software.

16. Click **Get Ambient O₂**.
17. After reviewing the dialog instructions, click **OK**.
18. Cycle the calibration syringe in and out continuously until the recording stops.

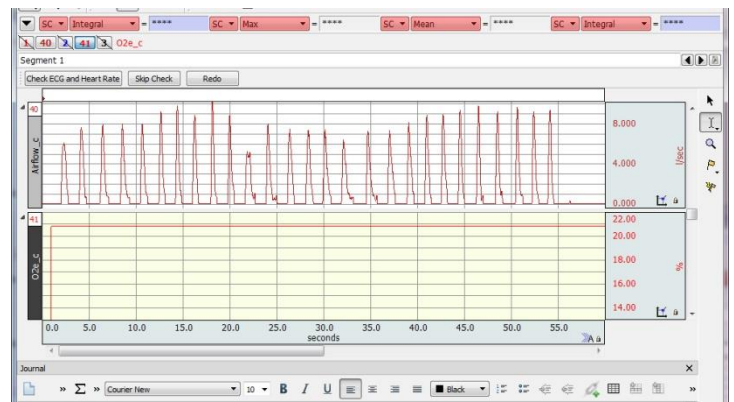


Fig. 20

Check ECG and Heart Rate. *If not used, skip to Recording Procedure.*

If “ECG and Heart Rate” are turned On in Preferences, then this step allows for a check of ECG signal quality and whether Heart Rate is accurately and consistently calculated from the ECG. It is assumed that the ECG electrodes and cables are attached to the subject and that they are in a relaxed supine or seated position. If this preference is turned Off, the lesson buttons will not appear.

19. Click **Check ECG and Heart Rate** or, if you feel confident, click Skip Check.
 - The data should look similar to Fig. 21. The ECG signal should have little EMG artifact and little baseline drift. It takes a few cardiac cycles before the heart rate data becomes accurate. After a few seconds, have the subject breath in and out deeply and check whether the Heart Rate is still tracking.
20. Click **Stop** to complete the check.

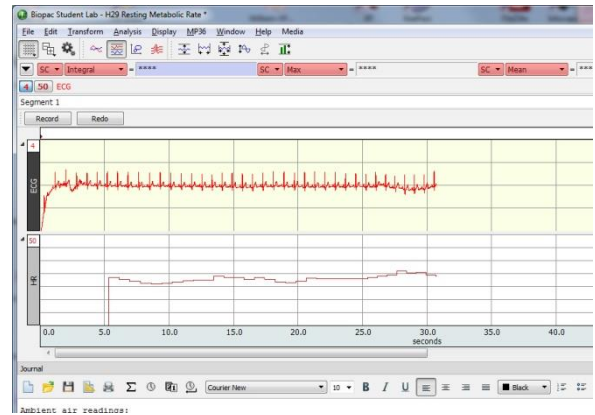


Fig. 21

Recording Procedure:

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

Subject's resting state:

The subject should ideally be in a supine position (Fig. 19). If this is not possible, then they should be in a seated position with arms supported and legs raised as shown in Fig. 22.

Note: For "Resting" conditions to be met, the subject must:

- Ingest no food or stimulants such as caffeine or nicotine for at least 7 hours prior to the test.
- Have not performed strenuous exercise for 24 hours prior to the test.
- Be mentally and physically relaxed with eyes closed and should not be listening to music.
- Have a normal core body temperature.
- Be in quiet room at a temperature between 65°F and 80°F.
- Have been in the resting position for at least 10 minutes prior to the test.

If recording Heart Rate from ECG (Setup step 6), make sure the cable and electrode leads are positioned such that the leads are not pulling on the electrodes.



Fig. 22



Fig. 23

IMPORTANT

- The sensors in the GASSYS3 must not be exposed to air with relative humidity greater than 95% as they are sensitive to condensation. It is normal for condensation to form in the chamber up to the point of the heater plate which, when off, is a cooler surface than the air.
- If condensation begins to form after the heater plate and near the sensors, you must turn the HEATER switch ON. The heater will raise the air temperature which will lower the relative humidity, without changing the percentages of O₂, CO₂, N₂ and H₂O in the air

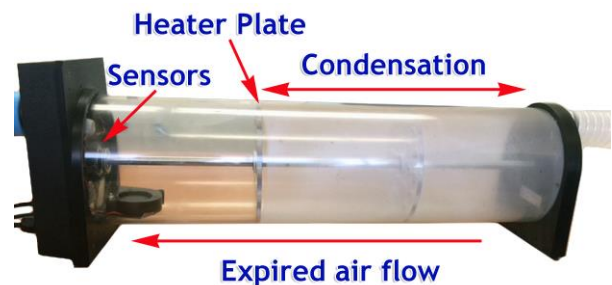


Fig. 24

1. Subject puts on facemask or inserts the mouthpiece/filter + T-valve. If using a mouthpiece, place the nose clip over nose.
2. Subject breathes normally for at least 10 minutes to acclimate breathing through the T-valve and to allow expired air to saturate the tubing and GASSYS3.
3. Click **Record**.

Notes:

- Data is not valid until 60 seconds after the start of a recording due to the 60 second integrator that derives inspired and expired volume from the airflow signal.
- Measurements will begin to be logged in the journal 90 seconds after the start of a recording. Journal measurements will then be updated every 30 seconds.

4. Continue to record for at least 10 minutes and more if necessary, until the VO₂ and RER data has reached a somewhat steady state for approximately 2 minutes.

5. Click **Stop**.

Notes: After the recording stops, the software will summarize measurements taken from the last 10 seconds of data in the journal. It will also calculate the Resting Energy Expenditure (REE) and the Resting Metabolic Rate (RMR) in units of “cal/day” “cal/kg/day” and “cal/m²/day”. You may need to scroll down to see these measurements.

6. If more recordings are desired using the same Subject, repeat steps 3 through 5 and new graph and journal data will be appended onto the previous data. If finished recording data on this Subject:

- a) Click **Done** to automatically save the data and bring up the dialog shown in Fig. 25.
- b) Have subject remove his/her facemask or mouthpiece and nose clip.
- c) Thoroughly flush the GASSYS3 with ambient air.

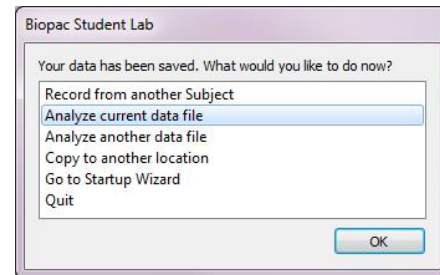


Fig. 25

Note: If recordings using other Subjects will be made in quick succession, choose “Record from another Subject”. In this mode, the initial airflow calibration steps can be skipped. It is always necessary, however, to thoroughly flush the GASSYS3 with ambient air after each Subject use.

Data Analysis:

All data channels have been saved and are accessible, even though they may be initially hidden. Data Analysis requires familiarization with the display and measurement tools (see [Appendix 3](#) for basic details).

Note: Data is not valid until one minute after the start of a recording as the airflow to volume integrator is averaging over 1 minute.

Sample data is shown in Fig. 26. The resting metabolic data is summarized in the journal.

The example in Fig. 27 shows the measurement of tidal volume for one respiratory cycle. The following procedure was used:

- All channels except for CH 40 (Airflow_c) were temporarily hidden.
- The CH 40 toolbar button was pressed to make it the Selected Channel (SC) for measurement (channel label highlighted).
- The zoom tool was used to expand the data to clearly see individual cycles.
- The area around one airflow pulse was selected with the "I" cursor and the Integral measurement displays the calculated volume.

Note: The Integral units are liters/sec-sec which is the equivalent of liters - (liters/sec) x sec = liters.

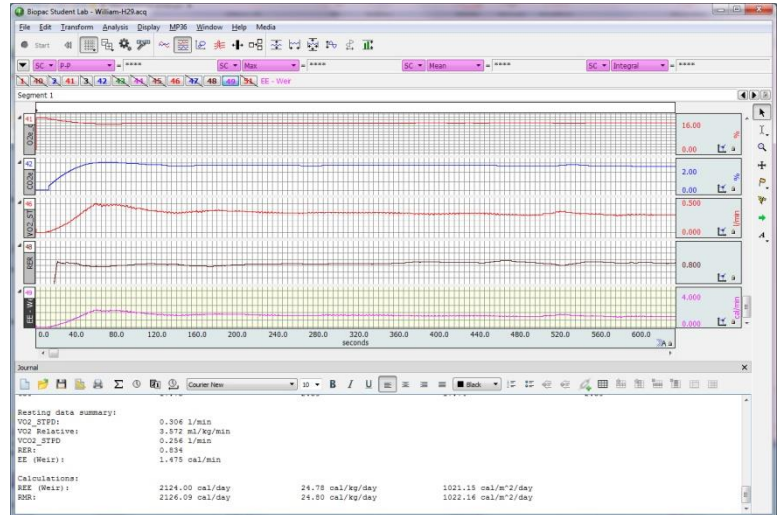


Fig. 26

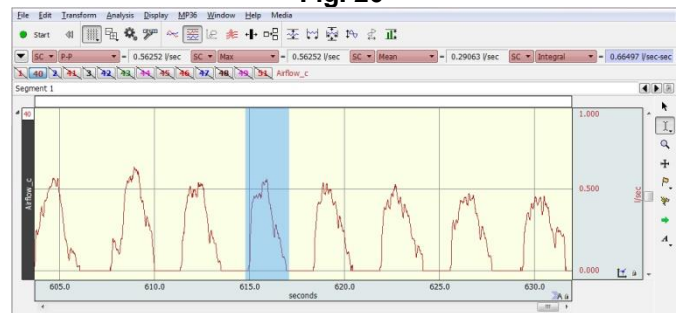


Fig. 27

Appendix 1: Lesson Preferences

Prior to a lab running the lesson for the first time, the Instructor must adjust Preferences to match their setup. All changes are retained for future lesson runs.

When the lesson is opened and after the initial subject entry dialogs, the **Preferences** button becomes available.

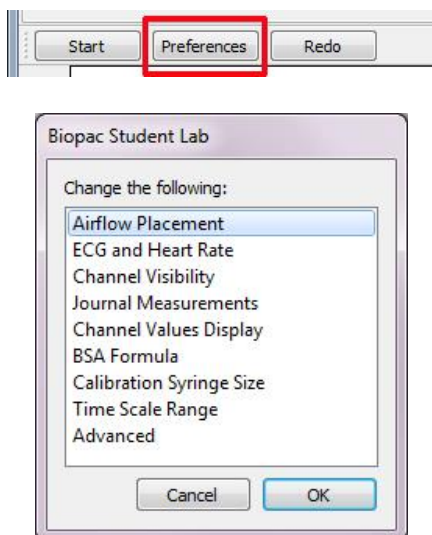


Fig. 28

Airflow Placement is important as it determines whether inspired or expired air will be measured.

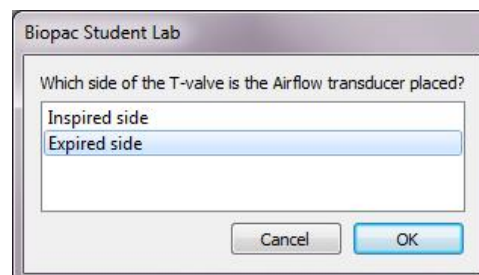


Fig. 29

ECG and Heart Rate enables or disables the feature.

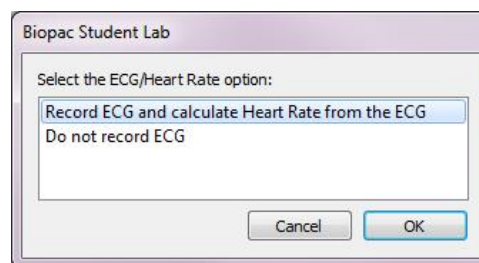


Fig. 30

Channel Visibility sets which channels are initially displayed during the recording. Channel visibility can be manually changed after the start of the first recording, using the channel buttons (see [Appendix 3](#)).

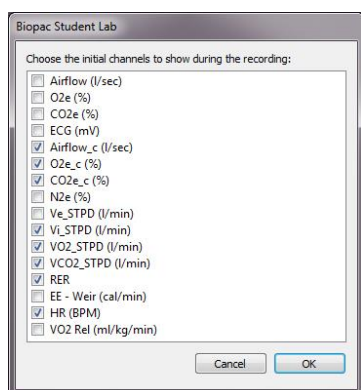


Fig. 31

Journal Measurements selects which measurements will be logged in the journal during the recording. Journal measurements begin 90 seconds after the start of a recording and then are updated every 30 seconds thereafter.

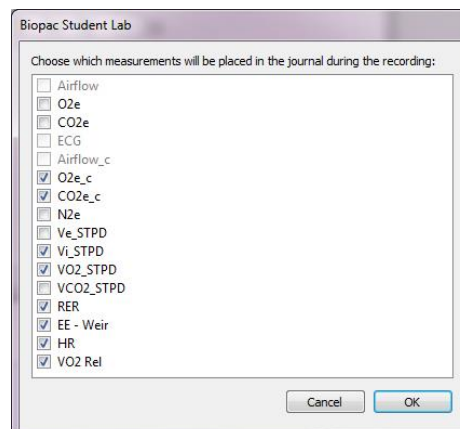


Fig. 32

Note: Journal measurements for Airflow and ECG are disabled because the standard measurements options for these data types do not provide meaningful information.

Channel Values Display is an additional window that displays data in a bar graph format. If the “Show” option is chosen, the next dialog will allow specific channel selection.

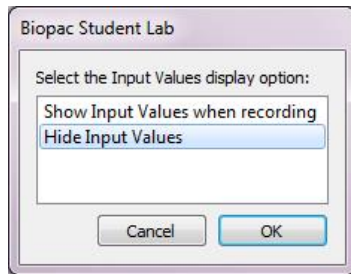


Fig. 33

BSA formula allows selection of the formula used to estimate Body Surface Area based on the subject's gender, weight and height. BSA is used to calculate RMR.

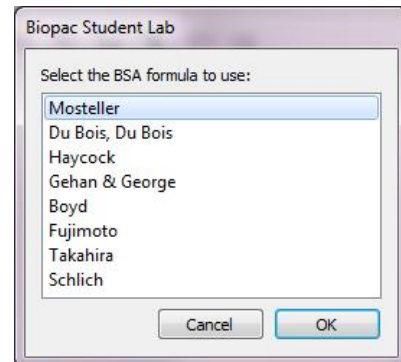


Fig. 34

Calibration Syringe Size is important as it is used during airflow calibration. The BIOPAC AFT27 is a 3 liter syringe.

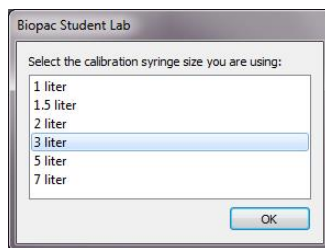


Fig. 35

Time Scale Range sets the initial time range on the horizontal axis when in the recording mode. The time range can still be changed either manually or when zooming or auto scaling.

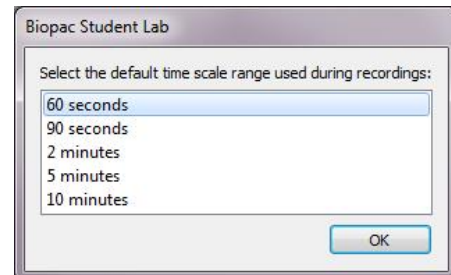


Fig. 36

The lesson template contains both a *PRO* graph template and an embedded script (program) that controls the lesson. The **Advanced** preference allows for certain changes to be made to the lesson's *PRO* graph template only. Changes such as waveform grids, waveform color, initial vertical scales, and initial measurements can be made.

Notes:

- Before opening the lesson template and making changes, save a backup copy of the original template. Certain changes, such as altering calculation channel expressions, may cause conflicts between the graph template and the embedded script.
- Do not record data while in this mode.
- Changes made to journal text will be overwritten by the lesson script.

Appendix 2: Channel Processing Summary:

Channel	Label	Description/Processing
CH 1	Airflow	Airflow from SS11LB
		Gain: x50, High Pass filter: Off (DC), Scaling: 28.5 mV maps to 10 l/sec (ATP), 0 mV maps to 0 l/sec (ATP) 3 IIR Filters: (Low Pass, 66.5 Hz, Q=.5), (Low Pass, 38.5 Hz, Q=1), (Band Stop -Line Freq, Q=1)
CH 2	O2	O₂ Expired from GASSYS3
		Gain: x5, High Pass filter: Off (DC), IIR Filters: None
CH 3	CO2	CO₂ Expired from GASSYS3
		Gain: x5, High Pass filter: Off (DC), IIR Filters: None
CH 4	ECG	ECG using SS2L Electrode Lead Set (Optional)
		Gain: x1000, High Pass filter: 0.5 Hz 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_c	Airflow corrected (l/sec): Multiplies CH 1 (Airflow) data by a gain correction factor that is determined during airflow calibration. If the airflow transducer is located on expired side of T-valve, the correction factor will be negative to invert the signal and display positive polarity data. C1.1 zeros any data that is less than 0.02 Liters/sec. which reduces noise artifact caused by mechanical vibration and also prevents the signal from going negative. The signal can go negative briefly prior to valves inside the T-valve fully closing which can be exacerbated when valves are worn out.
		C1.0: $Ch1 \times J$ J = airflow scaling and polarity factor C1.1: IF (LESS(R, C1.0), C1.0, 0) = if Airflow_c < R, then Airflow_c = 0 R = Threshold value: -1 l/s prior to calibration (for zeroing baseline) and 0.02 l/s after calibration.
C2 [41]	O2e_c	O₂ expired and corrected (%): Filter and delay adjusts response time to more closely match that of the slower responding CO ₂ sensor. Also filters out high frequency artifact that can occur due to rapid pressure changes during exhalations.
		C2.0: SC-CH 2 (O ₂), Low Pass filter, 0.04 Hz, Q = 0.5 C2.1: Delay 1 second
C3 [42]	CO2e_c	CO₂ expired and corrected (%): Because the CO ₂ sensor samples air every 2 seconds, a low pass filter is used to remove the abrupt changes in output signal. The filter also removes signal artifact caused by rapid fluctuations in sensor current during air sampling. C3.1 forces any signal below 0.5% to the ambient CO ₂ value manually entered in software (T). C3.2 performs a correction based on the difference between current barometric pressure and the barometric pressure measured during factory calibration per CO ₂ sensor specifications.
		C3.0: SC-CH 3 (CO ₂), Low Pass filter, 0.15 Hz, Q = 0.5 C3.1: IF (LESS(0.5, C3.0), C3.0, T) T = % CO ₂ in ambient air (CO _{2a}) C3.2: $C3.1 * O$ O = correction factor based on barometric pressure per sensor specs.
C4 [43]	N2e	N₂ expired (%): Calculates % Nitrogen by subtracting %O ₂ , %CO ₂ and %H ₂ O (K) from 100%.
		$100 - (C2 + C3 + K) = 100\% - (O2e_c + CO2e_c + K)$ K = % H ₂ O in expired air
C5 [44]	Vi_STPD Or Ve_STPD*	Volume of air inspired or expired* at STPD (l/min): Calculates Volume by integrating airflow and averaging over 60 seconds. Factors in barometric Pressure, temperature and relative humidity to output data corrected to <u>S</u> tandard <u>T</u> emperature and <u>P</u> ressure <u>D</u> ry (STPD) conditions.
		C5.0: SC-C1 (Airflow_c), Integrate, Average over 7500 samples (@ 125 s/s) = 60 seconds C5.1: $C5.0 * N$ N = correction factor to STPD calculated based either on inspired or expired air*

C6 [45]	Ve_STPD Or Vi_STPD*	Volume of air expired or inspired* at STPD (l/min): Applies the Haldane Transformation to calculate volume of <u>expired</u> air per minute or volume of <u>inspired</u> air per minute.
		If Ve_STPD: $(L/C4)*C5 = (\frac{L}{N_{2e}}) \times Vi_STPD$, If Vi_STPD: $(C4/L)*C5 = (\frac{N_{2e}}{L}) \times Ve_STPD$ L = % N ₂ in ambient air (N _{2a}) = 100% - (%O _{2a} + %CO _{2a} + %H ₂ O _a)
C7 [46]	VO2_STPD	Volume of O₂ consumed in liters per minute (l/m)
		If C5 is Vi_STPD: $(1/100)*((C5*M)-(C6*C2)) = (\frac{1}{100}) \times ((Vi_STPD \times M) - (Ve_STPD \times O_{2e_c}))$ If C5 is Ve_STPD: $(1/100)*((C6*M)-(C5*C2)) = \text{same as above}$ M = % O ₂ in ambient air (O _{2a}) measured during calibration (after ambient air syringe flush)
C8 [47]	VCO2_STPD	Volume of CO₂ eliminated in liters per minute (l/min)
		If C5 is Vi_STPD: $(1/100)*((C6*C3)-(C5*T)) = (\frac{1}{100}) \times ((Ve_STPD \times CO_{2e_c}) - (Vi_STPD \times T))$ If C5 is Ve_STPD: $(1/100)*((C5*C3)-(C6*T)) = \text{same as above}$ T = % CO ₂ in ambient air (CO _{2a} assumed)
C9 [48]	RER	Respiratory Exchange Ratio: Calculated from VCO2_STPD / VO2_STPD. Values limited to between 0 and 2, and values cannot change more than 0.1 in one second. This prevents unreasonable values that can occur prior to reaching steady state conditions.
		C9.0: IF(LESS ((C8/C7), 2.0), (IF(LESS(0.0, (C8/C7)), (C8/C7), 0.0)) , 2.0) C9.1: Slew Rate Limiter, 1 second time window, maximum change 0.1
C10 [49]	EE (Weir)	Energy Expenditure via Weir formula (cal/min):
		$((3.9*C7)+(1.1*C8)) = ((3.9 \times VO2_STPD) + (1.1 \times VCO2_STPD))$
C11 [50]	HR	Heart Rate (BPM): Determined from ECG R to R intervals. First filter ECG data to optimize R-wave detection (C11.0 and C11.1). Then calculate RMS to make sure R-wave has positive polarity (C11.2). Then calculate Heart Rate using Rate parameters as shown in C11.3.
		C11.0: SC-CH 4 (ECG), IIR Filter: Band Pass Low + High, 5 – 25 Hz, Q = 0.707 C11.1: IIR Filter: Band Pass, 17 Hz, Q = 5 C11.2: Integrate RMS, Average over samples, 40 samples, Root mean square, C11.3: Rate, Positive peaks, Remove baseline (window 100 ms), Auto threshold detect, Noise rejection 5% of peak, Cycle Interval Window: 40 to 120 BPM.
C12 [51]	VO2 Rel	Volume of O₂ consumed per minute relative to body weight (ml/kg/min):
		$(C7*1000)/P = \frac{(VO2_STPD \times 1000)}{P}$ where VO2_STPD is in l/min. P = Subject's weight in kg.

Notes:

- * If Airflow transducer is on Inspired side of T-valve, C5 will be Vi_STPD, and C6 will be Ve_STPD. If Airflow transducer is on expired side of T-valve, the channels will be reversed.
- The "Channel" column displays both the calculation channel reference (i.e. "C1") and channel graph reference (i.e. "[40]").
- The Processing field shows the calculation channel expression syntax and the equivalent formula based on channel labels.
- "SC" designates the Source Channel, which is the data channel that is used for processing.
- If a calculation channel uses a MetaChannel, then multiple, successive, processing is established. For example, C3 uses Metachannels; C3.0 processes data from CH 3 (CO₂), C3.1 processes data from C3.0 and C3.2 processes data from C3.1. The data output from C3 is the C3.2 data.
- Single bolded letters shown in the Processing descriptions are variables that are calculated and updated via the lesson script.

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.




Autoscaling data:

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

- To reset all vertical scales to the default template settings, select Display > Show Default Scales.
- Show All Data  toolbar button or Display > Show All Data adjusts vertical and horizontal scales to make all data visible.
- 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:

The measurement boxes are above the marker region in the data window. Each measurement has three sections: channel number*, measurement type, and value. The first two sections are pull-down menus that are activated when you click them.

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.

Note*: When “SC” (Selected Channel) is assigned as the channel number, the measurement will apply to the active channel (channel button and channel label highlighted).

Appendix 4:

Special file setup instructions:

1. The lesson template “**H29-Resting Metabolic Rate.gtl**” can be placed at any convenient location.
2. This lesson requires two external dialogs; “**AtmosphericConditionsDialog.ui**” and “**SubjectDetailsDialog.ui**”. These files must be placed in one of the locations listed below. The first location listed under the Operating System (OS) is the preferred location, but if users do not have permission to save to this location, the second, shared, location can be used.

Windows OS:

1st choice, place the two files within the Application folder at the following location:

If running AcqKnowledge 5.0.3 or higher: <Main Drive>:\Program Files\BIOPAC Systems, Inc\AcqKnowledge 5.0

If running Biopac Student Lab 4.1.3 or higher:

<Main Drive>:\Program Files (x86)\BIOPAC Systems, Inc\Biopac Student Lab 4.1\PROLessonsSupport\all-lang

2nd choice, place the two files in the following shared location:

<Main drive>:\Users\Public\Documents\BSL PRO Lessons¹

Mac OS:

1st choice, place the two files within the Application folder at the following location:

If running AcqKnowledge 5.0.3 or higher:

1. **Navigate to** Macintosh HD/Applications/AcqKnowledge 5.0.
2. Right-click the application icon (Acqknowledge.app) and choose “**Show Package Contents**”.
3. Copy the two files into the “Contents” folder.

If running Biopac Student Lab 4.1.3 or higher:

1. **Navigate to** Macintosh HD/Applications/BSL 4.1.3.
2. Right-click the application icon “BIOPAC BSL 4.1.app” and choose “**Show Package Contents**”.
3. Navigate to: /Contents/Resources/PROLessonsSupport/all-lang
4. Copy the two files into the “all-lang” folder.

2nd choice, place the two files in the following shared location: Macintosh HD/Users/Shared/BSL PRO Lessons¹

Note¹: If the folder “**BSL PRO Lessons**” does not exist, you must manually create it. Make sure to use the exact name (case sensitive).

Note²: On some older Mac Operating Systems, the template may not open properly by dragging the template icon over the application icon. If this is the case, the application should be opened first and then, in the launcher dialog, choose “Create/Record a new experiment” and then “Open a graph template from disk...”. Navigate to the template file and choose it.

Appendix 5:

GASSYS3 Internals

Figure 37 shows the heater plate and sensors that are located inside the GASSYS3 assembly.

O₂ sensor: Zirconia-Solid Electrolyte that measures between 1 to 25%. The sensor contains a heater, a zirconium oxide disk, and a pair of electrodes (Anode and Cathode) on either side of the disk. The heater creates a regulated temperature of 450°C and at this temperature; oxygen gas is converted to ions at the cathode, and the zirconium oxide disk becomes penetrable by oxygen ions. A constant voltage is applied across the electrodes which cause oxygen ions to flow from the cathode to the anode. The amount of current is proportional to the amount of oxygen ions flowing through the zirconium oxide disk which is in turn, is proportional to the amount of oxygen in the air.

The amount of current flow is converted into a proportional voltage value for output to the MP36.

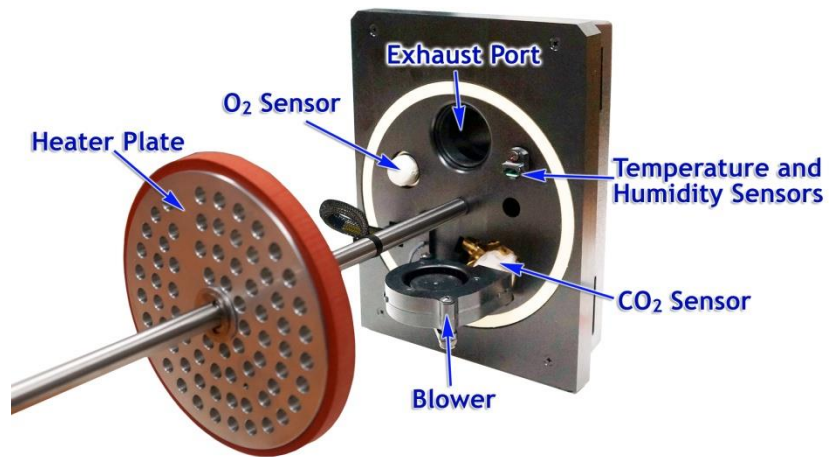


Fig. 37

CO₂ sensor: Utilizes non-dispersive infrared waveguide technology to measure CO₂ in the range of 0 – 10%. It is “non-dispersive” because the infrared energy is allowed to pass through the air sampling chamber without deformation. An infrared (IR) LED directs light through a tube filled with air toward an IR light detector, which measures the amount of IR light that hits it. As the light passes through the tube, any gas molecules that are the same size as the wavelength of the IR light absorb the IR only, while letting other wavelengths of light pass through. Next, the remaining light hits an optical filter that absorbs every wavelength of light except the exact wavelength absorbed by CO₂. Finally, an IR detector reads the amount of light that was not absorbed by the CO₂ molecules or the optical filter. The difference between the amount of light radiated by the IR lamp and the amount of IR light received by the detector is proportional to the number of CO₂ molecules in the air inside the tube. The sensor outputs an analog voltage which can be read by the MP36.

Blower: Circulates air in and around the CO₂ sensor which reduces the chance of condensation forming inside the sensor and improves the response time. The blower also helps circulate and mix the air around the other sensors.

Temperature and Humidity Sensors: Temperature and humidity sensors are integrated into a single package. Processing inside takes the measured temperature and absolute humidity and calculates relative humidity (RH). Readings from the sensors are output serially to the MP36.

Heater Plate: The sensors inside the GASSYS3 cannot tolerate condensation. The heater is used to reduce the chance of condensation by raising the air temperature which increases the capacity for air to hold water vapor and decreases the relative humidity (RH). Note that the heater does not dry the air, and that the percentage of water vapor in the air does not change. After the warm air exits the GASSYS3, if it comes in contact with cooler surfaces (i.e. SS11LB Airflow screen), the RH will increase and condensation can form. The larger the RH value, the greater the chance is for condensation to form when air contacts a cooler surface. When performing resting measurements, in most cases, by the time the Subject's expired air reaches the sensors, the RH is not high enough to cause condensation. In this case, the heater is not needed and the SS11LB can be placed on the exhaust port. It is important, however, to monitor the GASSYS3 chamber, and if condensation begins to form near the sensors, the heater should be turned ON. For exercise measurements, the heater must always be ON and the SS11LB airflow transducer should always be placed on the inspired side of the T-valve. It is also helpful to flush the GASSYS3 with ambient air after each recording to remove condensation that may have formed prior to the heater plate.

Appendix 6:

SS11LB Airflow transducer operation

The BIOPAC SS11LB airflow transducer works by funneling air through a sealed head which is divided in half by a fine mesh screen (Fig. 38). 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 between the two sides of the screen.

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

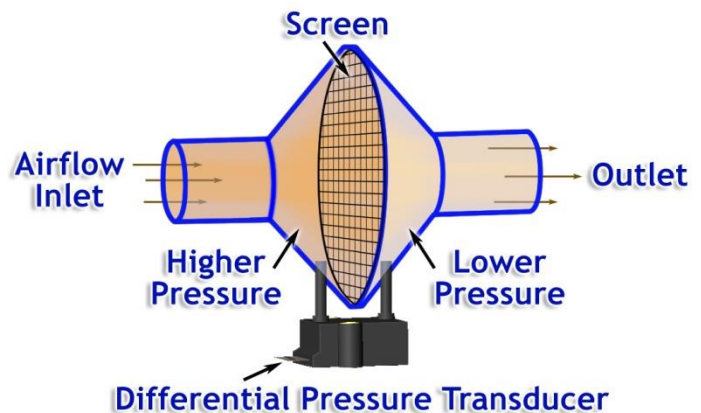


Fig. 38

- Baseline offset will vary slightly during the transducer warm-up period. For this reason, it is important to perform calibration at least 10 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 and the orientation used during calibration should be maintained during the 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 works best for this lesson when it is placed on the inspired side of the T-valve to minimize the chance of condensation forming on the screen which will alter the airflow accuracy. Condensation occurs when warm humid air (i.e. expired breath) flows against a cooler surface.
- Normally the SS11LB does not require calibration before each use. However, because the metabolic system uses a T-valve to control the direction of air flow, the valves change the air dynamics enough to make it necessary to calibrate the airflow transducer prior to each set of recordings. This calibration adjusts the zero-flow baseline offset and adjusts the gain correction factor.

Note* The relationship between the airflow and differential pressure is not perfectly linear. Non-linearity's arise due to air turbulence at higher flow rates. The SS11LB maintains linearity over its rated flow range because the airflow response has been modeled and polynomial correction equations have been established to linearize the response. Because the response is slightly different depending on the direction the air is flowing, two polynomial expressions are required. Memory inside the SS11LB circuitry saves two sets of polynomial coefficients that are determined during factory calibration. The software will automatically read in the coefficients and apply the correction equations. Because different correction equations are applied depending on the direction of airflow, it is important to always orient the side of the SS11LB marked with the "Inlet" label towards the T-valve. This will maintain the intended signal polarity of inspired air flow being positive and expired air flow being negative.