

## Introduction:

Exercise tests are usually performed under constant or increasing work loads. Constant work loads are designed to reach a steady state of gas exchange, ventilation and heart rate, thereby investigating cardiopulmonary function under constant metabolic demand. Increasing work loads are used to reach exhaustion in the subject and determine a maximal level of oxygen consumption ( $VO_{2\max}$ ). Cycle ergometers and treadmills are often used for increasing work load protocols, while step and walking tests are used for constant work load tests.

There are two types of systems offered by ADInstruments: One is the PL3508B80 Exercise Physiology System for teaching laboratories to perform metabolic and exercise physiology studies. This system is discussed below under the PL3508B80 Exercise Physiology system. The second is a kit that can be used to adapt a PowerLab teaching system to perform the series of exercise experiments in LabTutor for teaching introductory courses on the physiological effects of exercise. These LabTutor experiments use the PTK20 Exercise Breathing Kit and MLA242 Exercise Breathing consumables. The contents of these kits are listed at the end of this document. This document does not discuss the LabTutor experiments.

## PL3508B80 Exercise Physiology System:

The PL3508B80 Exercise Physiology System contains hardware and software for recording expired  $CO_2$  and  $O_2$ , respiratory air flow, temperature and biopotentials such as ECG or EMG. The Metabolic Module automatically calculates and displays  $O_2$  consumption ( $VO_2$ ),  $CO_2$  production ( $VCO_2$ ) and respiratory exchange ratio (RER) in exercising and resting subjects.

### The PL3508B80 Exercise Physiology System contains:

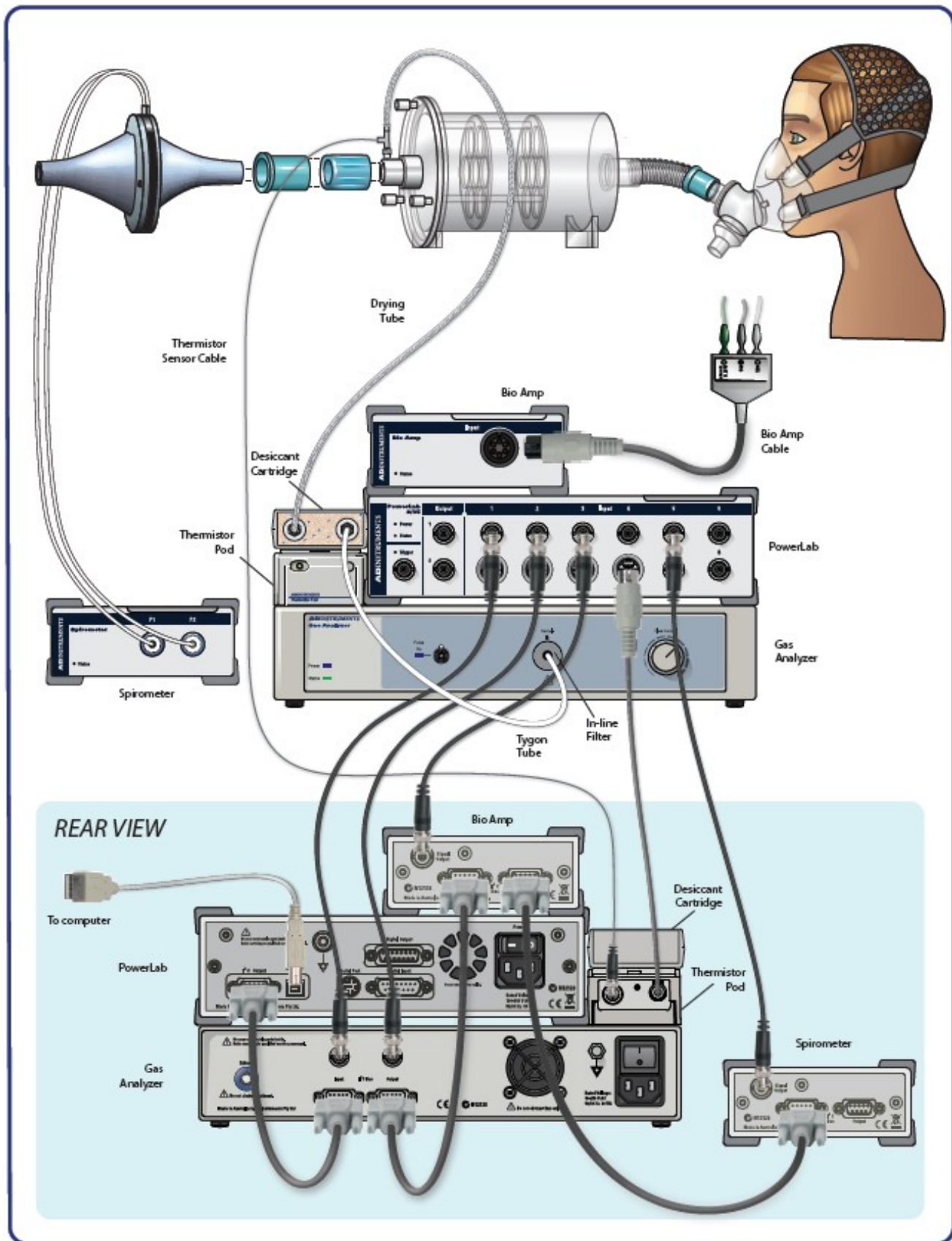
- PL3508 PowerLab 8/35
- MLS260/7 LabChart Pro software
- FE132 Bio Amp (Includes Shielded Subject Cable and Lead Wires)
- ML206 Gas Analyzer (includes 10 in-line filters)
- MLA246 Gas Mixing Chamber
- FE141 Spirometer
- ML309 Thermistor Pod
- MLA240 Exercise Physiology Accessory Kit (see below)

## **MLA240 Exercise Physiology Accessory Kit**

The MLA240 Exercise Physiology Accessory Kit is included with the PL3508B80 Exercise Physiology System but may also be purchased as a separate item. The Kit includes:

- MLT1000L Respiratory Flow Head (1000 L/min)
- MLA1029 Face Mask kit with one non-rebreathing valve, one SP0141 small and one medium adult mask
- MLA1081 Flow Head Adapter
- MLA1013 Tubing Adapter (35 mm I.D.)
- MLA1015 Breathing Tube (180 cm)
- MLA6024 Desiccant Cartridge
- MLA0343 Drying Tube
- Tubing 30 cm Length with Luers (x2)
- MLT415/M Thermistor Temperature Sensor

A summary sheet showing the connections for the Exercise Physiology System is included with each system and is shown below.



## Connection of Respiratory Apparatus

The Respiratory Flow Head attaches to the Spirometer and is used to record air flow. The ports of the flowhead should point upwards to minimise the chance of moisture condensing and blocking the tube connecting the flowhead to the spirometer. A non-rebreathing valve within the Face Mask separates inspired and expired breaths. Generally, a Face Mask is more comfortable for someone who is not accustomed to breathing with a mouthpiece and nose clip. Masks of two sizes are included for small and medium size adult faces. The non-rebreathing valve can be fitted to either mask. The breathing tube directs the expired breath to the Gas Mixing Chamber for sampling and allows the exercising subject to move freely.

## Connection of the Drying Tube and Desiccant Cartridge

Nafion tubing equilibrates the humidity levels between the surroundings and the air inside the tube. If the surrounding air is dry, like inside a Desiccant cartridge, then the air inside the tube is dried to the same moisture level of air in the Desiccant Cartridge. If the Nafion tubing is exposed to the room air then air inside the tube is dried to the ambient humidity levels. Using two lengths of tubing (one exposed to the room air and one exposed to air inside the desiccant cartridge) provides two sets of drying conditions, and helps keep the desiccant inside the cartridge remain drier for longer. Wet desiccant can be dried by heating it on a tray in a moderate oven.

When performing heavy exercise or in humid environments, the Desiccant Cartridge is recommended as it provides additional drying of the expired gases before gas analysis. The Desiccant Cartridge is connected to the Gas Mixing Chamber using Nafion Tubing. A silicon tube is then used to connect the Desiccant Cartridge to the sampling port of the Gas Analyzer. It is important that dry air is used in determining O<sub>2</sub> and CO<sub>2</sub> concentrations using the Gas Analyzer.

## Connection of Tubing and Adapters

The Flow Head Adapter and Tubing Adapters are used to connect the Respiratory Flow Head to the Gas Mixing Chamber.

## Temperature Sensor

The Thermistor Temperature Sensor incorporates a Nasal Temperature Probe in a fitting that allows the probe to connect to the Gas Mixing Chamber for measuring the temperature of the expired gas or to a tap to measure the temperature of mixed expired air before entering the gas analyser.

## Gas Analyzer

The ML206 Gas Analyzer includes variable sampling flow rates from approximately 35 ml/min to 200 ml/min. An in-line filter must be attached to the sampling port of the Gas Analyzer to protect the transducers against moisture or damaging particulates. Ten hydrophobic filters (0.45 µm pore size) are supplied with the Gas Analyzer and additional filters may be ordered as MLA0110 In-line Filters (10 pack). These filters have male/female luer connections for direct connection to the Gas Analyzer sampling port and Drying Tube.

*NOTE: When using the Gas Analyzer for human subjects the flow rate should be set at maximal speed.*

## **Additional Items:**

### **PTK14 Exercise Physiology Kit**

For customers that already have a PowerLab, the PTK14 Exercise Physiology Kit is available. For metabolic studies, the PTK14 must be used with a PowerLab that has a minimum of four BNC channel inputs. For example, it cannot be used for metabolic studies with the PowerLab 15T, however it can be used with PowerLab 26T using MLAC22 adapters.

### **MLT3813H Heated Pneumotach and Heater Controller**

For laboratories that are regularly performing heavy exercise studies, the 800L/min heated pneumotach is recommended as an alternative to the MLT1000L Respiratory Flow Head. The heated pneumotach is designed to minimize excessive condensation on the gauze inside the flow head.

Condensation occurs within the MLT1000L Respiratory Flow Head after prolonged use or in excessively humid environments due to the large amounts of moisture in expired air. This condensation causes non-linearity within the flow heads and possible inaccurate recordings of respiratory volumes.

### **MLA5530 Calibration Syringe**

A 3-Liter Calibration Syringe is available for an easy and reliable method of calibrating respiratory equipment.

### **MLA6026 Desiccant Refill**

A 1 kg molecular sieve desiccant is available for refilling the MLA6024 Desiccant Cartridge.

### **MLA304 Disposable Filters and MLA0110 In-line Filters**

A pack of 50 disposable droplet filters (MLA304) are available to protect against cross-contamination between subjects without impeding air flow. The subject end has a 31 mm OD (outside diameter) while the tubing end has a 34 mm OD. It is the customer's responsibility to ensure that adequate filtering is provided to avoid crosscontamination between subjects. It is recommended that the MLA304 Disposable Filter is changed between subjects.

MLA0110 are supplied in packets of ten and must be used, in conjunction with the MLA0343 Drying Tube, to protect the ML206 Gas Analyzer transducers from moisture or damaging particulates.

*NOTE: The response times and related specifications of the Gas Analyzer were tested with the MLA0110 In-line Filter and MLA0343 Drying Tube attached. The inclusion of viral/bacterial filters and tubing may prolong the response time of the Gas Analyzer.*

### **Biopotential Accessories**

Various products are available for measuring biopotentials such as Disposable/Reusable ECG Electrodes, Abrasive Gel, Electrode Cream and Paste, EEG Flat Electrodes and more.

## Metabolic Module (Win & Mac):

The Metabolic Module is included with LabChart and the LabChart Pro modules and extends the capability of LabChart software. It is available in both Windows and Macintosh versions. The Module provides a comprehensive set of features for metabolic data acquisition and analysis from humans. The software is intended primarily for use with the Exercise Physiology System but can be used in combination with the following hardware: Any PowerLab data acquisition system with at least 4 input channels, any Spirometer and Flow Head, any Gas Analyzer, Gas Mixing Chamber and accessories.

The Exercise Physiology System measures and records inspired or expired air flow from a pneumotach (spirometer and flow head) and expired CO<sub>2</sub> and O<sub>2</sub> concentrations from a Gas Mixing Chamber. The Metabolic Module requires the following three channels of data (with the specified units labeled):

- CO<sub>2</sub> concentration with units of "%CO<sub>2</sub>"
- O<sub>2</sub> concentrations with units of "%O<sub>2</sub>"
- Air flow with units of "L/s"

*Note: Units for temperature, if recorded, must be specified as "°C", "°F" or "K".*

The simultaneous measurement of respiratory gas concentrations and air flow permits calculation of the following metabolic parameters:

- VO<sub>2</sub>, the rate of oxygen consumption [L/min]
- VCO<sub>2</sub>, the rate of carbon dioxide production [L/min]
- RER, the respiratory exchange ratio (VCO<sub>2</sub>/VO<sub>2</sub>)
- V<sub>E</sub>, the expired minute ventilation [L/min]

The Metabolic Settings dialog provides options for:

- Hardware Preferences: Choice of the PowerLab/8M or other "Custom" hardware ("Custom" would be selected if using the PL3508B80 Exercise Physiology System)
- Subject Details: Name, age, weight, height, gender, i.d. number, comments
- Calibration Preferences: Settings for data averaging and length of recording time
- Environment Settings: Expired/inspired air flow, atmospheric and air conditions

The Metabolic Module provides the following eight windows for viewing results of the metabolic calculations, both online and offline:

- Metabolic Log Window
- V<sub>E</sub> (BTPS) vs. VO<sub>2</sub>
- V<sub>E</sub> (BTPS) vs. VCO<sub>2</sub> (WIN only)
- VCO<sub>2</sub> vs. VO<sub>2</sub>
- RER vs. time
- VO<sub>2</sub> vs. time
- VCO<sub>2</sub> vs. time
- V<sub>E</sub> (BTPS) vs. time

### Parameters Directly Recorded by the Exercise Physiology System

- Expired oxygen fraction- F<sub>EO2</sub>
- Expired carbon dioxide fraction- F<sub>ECO2</sub>
- Air flow (inspired or expired)- Air Flow

- Temperature of Expired Air- Temp

## **Additional Parameters Calculated Using the Metabolic Module**

- Body Temperature and Pressure Saturate- BTPS
- Standard Temperature and Pressure Dry- STPD
- Expired Minute Ventilation (BTPS)-  $V_E$
- Oxygen consumption (STPD)-  $VO_2$
- Carbon dioxide production (STPD)-  $VCO_2$
- Respiratory Exchange Ratio- RER
- Ventilatory equivalent for  $O_2$ -  $V_E / VO_2$
- Ventilatory equivalent for  $CO_2$ -  $V_E / VCO_2$

## **Derived Parameters (Manually Calculated by the User)**

- Tidal Volume-  $V_T$
- Maximal oxygen consumption-  $VO_{2\ max}$
- Ventilatory Anaerobic Threshold- VAT
- Excess post exercise oxygen consumption- EPOC
- Resting Energy Expenditure- REE

### **$F_{EO_2}$ Expired oxygen fraction**

Fractional amount of  $O_2$  in the expired air: Approximately 16-17 % at rest.

### **$F_{ECO_2}$ Expired carbon dioxide fraction**

Fractional amount of  $CO_2$  in the expired air: Approximately 4-6 % at rest.

### **Air Flow [L/s]**

The rate of air flow (commonly expressed in L/s) during the inspired or expired portion of the respiratory cycle. The integral of this signal provides the inspired or expired volume for each breath.

## **Expired or Inspired Air flow**

Generally, it is recommended that the researcher uses the expired air flow configuration as shown on page 3. Some researchers may choose to use inspired air flow to avoid condensation on the flow head during prolonged exercise.

For inspired air flow configurations the MLT1000L Respiratory Flow Head is connected to the inspiratory port of the Face Mask rather than the expiratory port of the Gas Mixing Chamber. If inspiratory air flow is measured then the signal in the spirometry flow channel must be inverted. This may be changed using the "Spirometer" dialog from the Channel pop-up menu and selecting the "invert" signal checkbox.

If inspired air flow is chosen, expired minute ventilation is assumed to be equivalent to inspired volumes. All of the "Environment Settings" of the Metabolic Module are still required for BTPS to STPD conversions (see below).

## **Temperature of the Expired Air**

This is used to convert expired volumes ( $V_E$ ; see below) from BTPS to STPD conditions. BTPS is the gas volume under conditions of body temperature and pressure, saturated with water vapour. STPD is the gas volume under conditions in which temperature and pressure have been standardized (0 °C, 760 mmHg) and the pressure of the water vapour has been removed. These correction factors are used to

account for the fact that a volume of inspired air increases when expired due to warming and humidification provided by the airways. As  $VO_2$  and  $VCO_2$  (see below) are reported at STPD,  $V_E$  needs to be converted from BTPS by removing the moisture and correcting the temperature and pressure to STPD conditions.

$$V_E(\text{STPD}) = \{(P_{\text{atm}} - P_{\text{vap}})/760\} \times \{273/(273 + T_{\text{chamber}})\} \times V_E(\text{BTPS})$$

where the term  $\{273/(273 + T_{\text{chamber}})\}$  corrects the expired breath temperature to standard temperature conditions, and the term  $\{(P_{\text{atm}} - P_{\text{vap}})/760\}$  corrects atmospheric pressure  $P_{\text{atm}}$ , with the water content removed  $P_{\text{vap}}$ , to standard pressure conditions.

If using the Desiccant Catridge and drying the expired breath completely, all of the water content is removed and  $P_{\text{vap}}$  is the saturated vapour pressure at the temperature of the air flow measurement  $T_{\text{chamber}}$ . If using Nafion tubing and only drying the expired breath to room humidity, then  $P_{\text{vap}}$  is the relative humidity (in mmHg) of the room air. Generally the inspired humidity of the room air is ignored.

## $V_E$ Expired Minute Ventilation [L/min]

Minute ventilation is the total volume that is either inspired ( $V_I$ ) or expired ( $V_E$ ) over a one minute period. It is expressed in liters per minute. Expired minute ventilation is more commonly reported than inspired minute ventilation.

At rest  $V_E \sim 5$ -10 L/min but it increases to up to 150 L/min in trained athletes and up to 50 L/min for a healthy adult. The increase in ventilation removes  $CO_2$  from working muscles.

At low to moderate levels of exercise,  $V_E$  increases linearly with the workload (and oxygen consumption [ $VO_2$ ]). At high levels of exercise,  $> 60\%$   $VO_{2\text{max}}$ , aerobic (ventilatory) mechanisms fail to meet the demands required for energy production and non-aerobic means are used to generate energy (i.e. anaerobic glycolysis produces lactate).

As lactate concentration increases in the muscles, buffering of lactic acid by serum  $-HCO_3$  occurs, subsequently increasing the amount of  $CO_2$  in the blood.  $VCO_2$  then increases as the  $CO_2$  is removed through the cardiorespiratory system.

## $VO_2$ Oxygen Consumption [L/min]

$VO_2$  is the volume of  $O_2$  consumed by a subject in L/min or mL/min at STPD. Resting  $VO_2$  is around 0.25 L/min and increases to about 3.5 L/min for exercising subjects and may increase to well over 4 L/min for trained subjects.  $VO_2$  is calculated from the fractional concentrations of expired  $O_2$  ( $F_{EO_2}$ ) and  $CO_2$  ( $F_{ECO_2}$ )

$$VO_2 = \text{Inspired } O_2 - \text{Expired } O_2$$

$$VO_2 = \{[(1-F_{EO_2}-F_{ECO_2})/(1-F_{IO_2})] \times F_{IO_2} - F_{EO_2}\} \times V_E(\text{STPD})$$

where  $F_{IO_2}$  is the inspired oxygen fraction, and the term  $(1-F_{EO_2}-F_{ECO_2})/(1-F_{IO_2})$  corrects for small differences between the inspired and expired volumes.

## $VO_{2\text{max}}$ Maximal Oxygen Consumption [L/min]

$VO_{2\text{max}}$  denotes oxygen consumption at the highest level of work. It is characterized by a plateau of  $VO_2$  despite increasing work loads, and the onset of fatigue in a subject.  $VO_{2\text{max}}$  is useful when comparing the exercise capacity between subjects for sports that do not involve moving against gravity, such as swimming or rowing where a larger  $VO_{2\text{max}}$  means more output. When comparing  $VO_{2\text{max}}$  for exercises that involve shifting weight, such as running, then a  $VO_{2\text{max}}$  normalized by the weight ( $VO_{2\text{max}}/\text{kg}$ ) is

used to compare  $VO_{2\max}$  between subjects.

## **VCO<sub>2</sub> Carbon Dioxide Production [L/min]**

VCO<sub>2</sub> is the volume of CO<sub>2</sub> produced by a subject in L/min or mL/min at STPD. For a normal person it is around 0.2 L/min at rest and can increase to over 4 L/min at maximal exercise. VCO<sub>2</sub> is directly proportional to the alveolar carbon dioxide concentration.

$$VCO_2 = \text{Expired } CO_2 - \text{Inspired } CO_2$$
$$VCO_2 = F_{ECO_2} \times V_E(\text{STPD}) - F_{ICO_2} \times V_I(\text{STPD})$$

$F_{ICO_2}$  is usually the concentration of room carbon dioxide (generally taken to be 0.0003). Under heavy exercising conditions in a lightly air conditioned room, this value may vary. Some people use a value greater than a fraction of 0.0003, for example 0.0005 for the CO<sub>2</sub> ambient concentration.

As the concentration of inspired CO<sub>2</sub> is very small, the second term for VCO<sub>2</sub> is often ignored and VCO<sub>2</sub> ~  $F_{ECO_2} \times V_E(\text{STPD})$  is often used to approximate VCO<sub>2</sub>.

## **RER Respiratory Exchange Ratio**

RER is the ratio of VCO<sub>2</sub> to VO<sub>2</sub> normally expressed as a fraction. At rest RER is around 0.7-0.8 and increases slowly with an exercising subject. Often when first breathing into a circuit, RER is elevated, as initially the subject hyperventilates. It remains elevated until the subject begins to settle or exercise. It becomes greater than unity (>1) when the anaerobic metabolism produces CO<sub>2</sub> from the buffering of lactate, and VCO<sub>2</sub> becomes greater than VO<sub>2</sub>.

## **V<sub>E</sub>/VO<sub>2</sub> Ventilatory equivalent for O<sub>2</sub>**

The ventilatory equivalent for oxygen is calculated when dividing ventilation (L/min, BTPS) by VO<sub>2</sub> (L/min, STPD). It reflects the ventilatory requirement for a specific level of oxygen uptake and the efficiency of the ventilatory pump to cope with work at various loads. At rest, V<sub>E</sub>/VO<sub>2</sub> is usually around 20-30 L/L. A decrease in V<sub>E</sub>/VO<sub>2</sub> is usually seen at the onset from rest to sub-maximal exercise as increased cardiac output and ventilation combine to enable increased oxygen uptake. However, at around 60-70% of VO<sub>2max</sub>, V<sub>E</sub> is more closely related to VCO<sub>2</sub>. Consequently, V<sub>E</sub>/VO<sub>2</sub> will increase as V<sub>E</sub> increases in response to the need to buffer lactate and reduce CO<sub>2</sub>.

## **V<sub>T</sub> Tidal Volume (expressed in mL or L)**

Tidal volume is the volume of a single expired or inspired breath. At low exercise loads V<sub>T</sub> increases until it approaches ~60% of vital capacity (VC), following which ventilation is further increased by increasing respiratory rate. V<sub>T</sub> is not displayed by the Metabolic Module but may be estimated by dividing the V<sub>E</sub> by respiratory rate for each block shown in the Metabolic Log Window. Tidal volume can be shown in LabChart by setting up a channel to calculate an integral using the Flow channel as the source, or using one of the options for Spirometry Volume from the Spirometry Extension.

## **VAT Ventilatory Anaerobic Threshold**

Ventilatory anaerobic threshold (VAT) can be determined non-invasively from the analysis of V<sub>E</sub>, VCO<sub>2</sub> and VO<sub>2</sub>. It is a reproducible point during exercise when ventilation increases above the linear increase in work and after which fatigue shortly follows. VAT occurs when the energy demands of the exercising muscles are no longer met by aerobic means. At low to moderate exercise V<sub>E</sub> increases with VO<sub>2</sub> (work load) while aerobic mechanisms are used. The VAT is usually associated with the true anaerobic

threshold at which muscle cell hypoxia induces lactate production. The primary product of anaerobic metabolism, lactate, increases the amount of CO<sub>2</sub> produced by exercising muscles due to the buffering of lactate to maintain constant pH. VAT usually occurs around 60-70% of VO<sub>2 max</sub>.

The inflexion point from a plot of VCO<sub>2</sub> against VO<sub>2</sub> can be used to determine the VAT. The inflexion point is usually determined by fitting two lines through high and low work loads to determine the onset of lactate production. This is often called the V slope determination method of ventilatory anaerobic threshold (VAT). The ventilatory equivalents for O<sub>2</sub> and CO<sub>2</sub> can also be used to identify VAT. Anaerobic metabolism is accompanied by a steady increase in V<sub>E</sub>/VO<sub>2</sub> while V<sub>E</sub>/VCO<sub>2</sub> remains constant or falls slightly. The VAT method uses plots of V<sub>E</sub>/VO<sub>2</sub> and V<sub>E</sub> against time to determine VAT.

## **EPOC Excess Post Exercise Oxygen Consumption**

Sustained exercise levels greater than VO<sub>2 max</sub> can be maintained for short periods of time and the difference between the work equivalent VO<sub>2</sub>, and is the O<sub>2</sub> debt which must be repaid at the end of exercise. The amount of O<sub>2</sub> used to repay the debt is called the EPOC, which is greater than the deficit.

## **Resting Energy Expenditure (REE)**

REE is the minimum amount of calories required each day. There are many different formulas to estimate REE and they are mainly used by clinical dietitians to determine the dietary requirements for bed-ridden patients. An example is the Abbreviated Weir Equation:

$$\text{REE} = 1.44 \times (3 \times \text{VO}_2 + 1.1 \times \text{VCO}_2), \text{ where } \text{VO}_2 \text{ and } \text{VCO}_2 \text{ are recorded at rest.}$$