How to Measure VO_2max

As you are becoming more aware, the concept and measure of VO_2max has a long history in exercise physiology. As such, VO_2max is a trademark measure and how to measure VO_2max is a fundamental skill set for the exercise physiologist. You must always remember that the three measurements for indirect calorimetry are ventilation, and the expired gas fractions for oxygen and carbon dioxide. This fact will be reinforced when you study the computations of expired gas analysis indirect calorimetry. However, for the measurement of VO_2max to be valid, you also need to develop the correct protocol for the test subject, and use a suitable exercise mode for the subject's training history.

The measurement of VO_2max requires knowing the physiology of exercise mode specificity, features of an **incremental protocol** conducive to inducing valid (truly maximal) VO_2max data, valid instrumentation, valid **post-acquisition** data processing and analyses to detect a VO_2max, and how any of the aforementioned criteria change for different subjects based on age, fitness status, diseases status, etc. I will explain the equipment components of valid VO_2max testing/measurement in this Topic, and devote an entire Topic to post-acquisition data processing and analyses. I will include pertinent issues about subject characteristics, when warranted, in each component explanation.

Exercise Mode Specificity

Basically, the more trained a subject is in a specific type of exercise, VO_2max testing should be done in that exercise mode. This is easier to do for cyclists and runners, thanks to the cycle ergometer and treadmill, respectively. However, for the swimmer, actual swim testing is more difficult as more elaborate equipment and protocol needs exist for in-pool testing, or for laboratories that have a swimming flume. Similarly, in-line skaters and cross country skiers require a wide belt and high performance (high maximal speed) treadmill (Figure 1), which are expensive and few exercise physiology laboratories have one.

Thus, for relevance to most individuals, let's keep discussion to cycle ergometry or treadmill running. It is easier for the technicians to perform incremental exercise on a cycle ergometer, and arguably this is a safer test than treadmill running as the subject is stationary while riding and there is far less risk for subjects falling off an ergometer than a treadmill. However, research is very clear in showing a 5-10% lower VO_2max for a given individual with cycle ergometry than treadmill running. Such a difference pertains to

Figure 1. Photograph of a wide belt high performance treadmill. This model can be controlled to have an incline or a decline.

untrained to moderately trained individuals only, as for highly trained cyclists, higher VO_2max data has been reported for the trained (cycling) exercise mode. The end result of all this is to always do treadmill running protocols for VO_2max measurement unless you are testing a highly endurance trained cyclist. The only caveat to this is if there are other purposes for the test. For example, if you also need to ascertain the Watts at the **ventilation threshold**, or Watts at VO_2max for the subject, then sticking to the trained exercise mode (cycling for the cyclist) is the way to go.

Protocol Development

The importance of the protocol in influencing the VO_2max measurement is the most overlooked issue in VO_2max measurement. Far too many exercise physiologists use protocols that are too long in duration, which as I will explain, causes lowering of VO_2max and therefore under-representation of the VO_2max measure. Yes, long duration protocols, as well as a protocol too short in duration, make the measurement of VO_2max invalid. This means there is an optimal range of protocol duration when testing VO_2max.

Historically, the field of exercise physiology has promoted a test duration approximating 12 min. This was based on research from the 1980's, where data was presented that showed highest VO_2max data for protocols close to 12 min. The problem with this data though was the researchers did not test enough people to meet the statistical criteria necessary to produce valid results. Similarly, an insufficient number of different protocol durations were studied. Since this time, several research studies have shown that higher VO_2max data results from protocols shorter in duration than 12 min, ranging between 8 to 10 min. These findings have been further refined to show that the fitness level of the subject is an important consideration in this protocol duration dependency of valid VO_2max measurement. The more trained the subject, the more important it is to have a shorter duration protocol, and test durations between 8 to 10 min (the closer to 8 min the better) should be adhered to (Figure 2). This appears to be true for both treadmill and cycle ergometer exercise modes (Figure 3). For such shorter protocols, subjects need to complete a preliminary **warm-up** first. We typically have subjects' warm-up for about 10 min, ending at an intensity that is close to their race

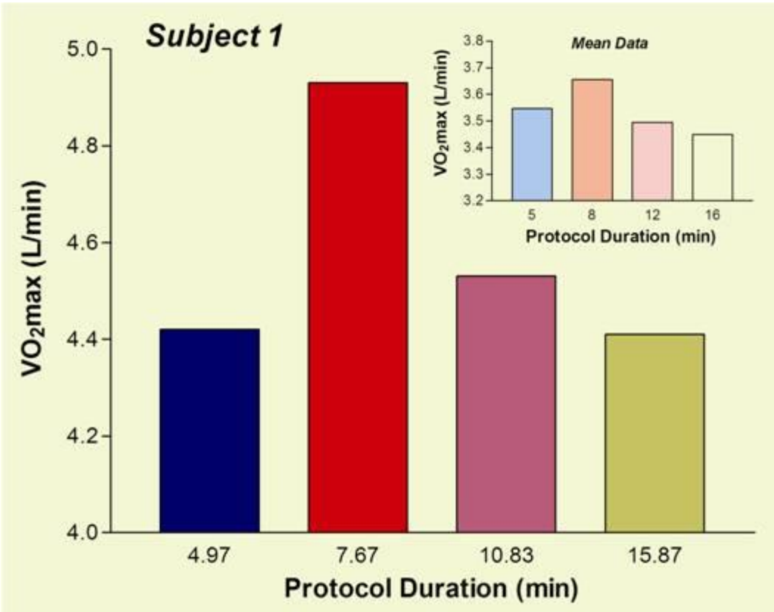

Figure 2. Comparisons between VO_2 responses to four different cycle ergometer VO_2max test protocol durations for a given subject from one of our recent studies. Mean data is provided in the graph insert.

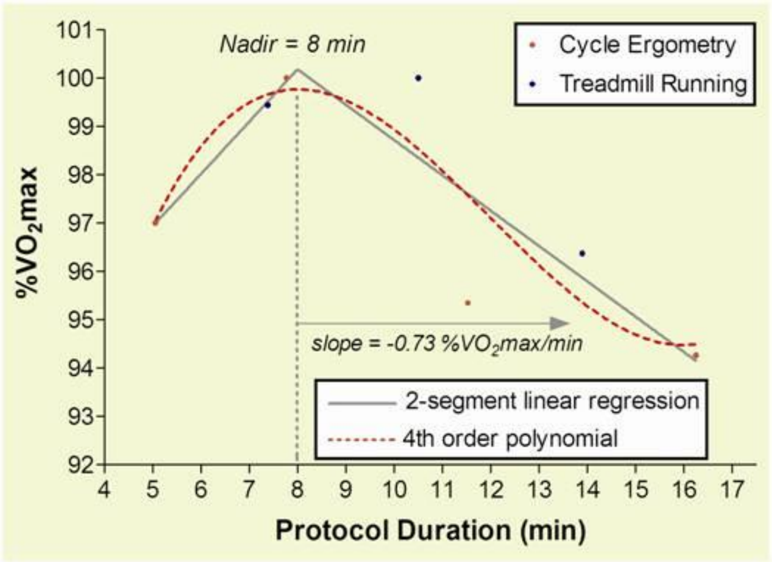

Figure 3. data from two studies (cycle ergometry and treadmill running) showing the consistent finding of the superiority of an 8 min test to measure VO_2max.

pace, maximal steady state, or a high quality training pace, whichever they relate to best. We then **calibrate** our equipment and start the test within 10 min after the warm up.

The need for a short protocol requires that exercise physiologists know how to develop subject-specific protocols, and I explain how to do this in the Topic on Incremental Protocol Development. Make sure you read and understand the Topic on Steady State VO_2 Estimation prior to learning about protocol development.

Valid Instrumentation

Many exercise physiologists who have skills in electronics and software development, choose to develop their own custom systems of indirect calorimetry. I recommend this approach, as there is no easier way of guaranteeing valid measurements than knowing each component of your system, being responsible for calibration, and ensuring sensitive and rapid measurement of each of the three measurements of indirect calorimetry; ventilation, and expired gas fractions for O_2 and CO_2. Such control becomes more and more important for shorter and shorter intervals of measurement and metabolic calculations. This is not to say that there are no good commercial systems available. There are. However, you can arguably develop a superior system, with

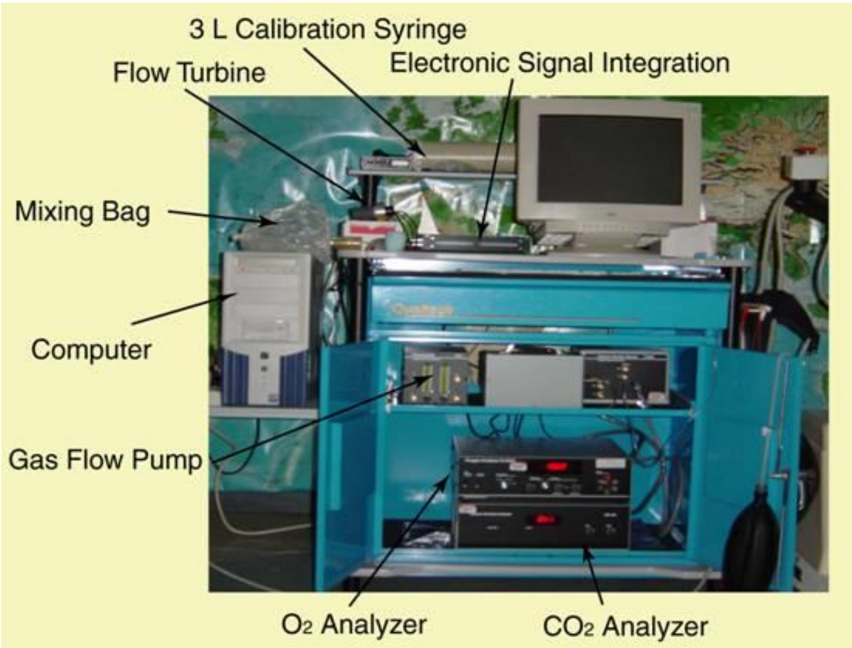

Figure 4. Photograph of a custom indirect calorimetry system developed at the University of New Mexico.

better gas analyzers and improved software, for less cost, if you build your own system. Take note for almost all aspects of your personal and professional life, just because a product is sold over the retail counter does not mean it is valid!

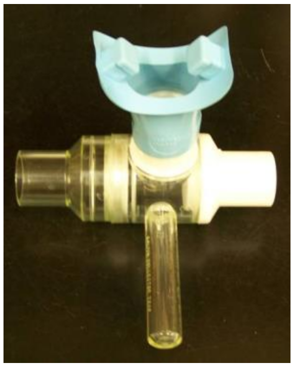

Figure 5. Photograph of a one-way T-valve apparatus.

Custom built systems, in being totally modular, are easier, cheaper and faster to repair when things go wrong, which they always, eventually do. I have built my own systems from state of the art components, and have patent protection for several of my hardware innovations and related software applications (Figure 4). Finally, but not of least importance, is that modular systems are superior for educating students about the science of expired gas analysis indirect calorimetry, and I like to think my students have gained enormously from exposure to custom systems in the laboratories I have developed in New Mexico and now Charles Sturt University, NSW, Australia.

Figure 6. Photograph of a subject fitted with a one-way T-valve apparatus and a nose clip.

Valves and Mouthpieces

To collect expired air, subjects wear a nose clip to prevent nasal breathing and have a mouthpiece in their mouth connected to one-way valves to direct expired air to a mixing chamber (Figures 5 to 7). As you should now know, for expired gas sampling at the mouthpiece, there is no need for one way valve structures and the mixing chamber. The mouthpiece and valve apparatus consists of numerous parts (Figure 8), and students need to know how to put such components together. An alternative to a mouthpiece system is the face mask (Figures 9 and 10). However, the face mask is notorious for leaking and therefore preventing accurate measurement of ventilation and both expired gas fractions. Figure 11 shows a subject

Figure 8. Photograph of the individual components of a one-way T-valve apparatus.

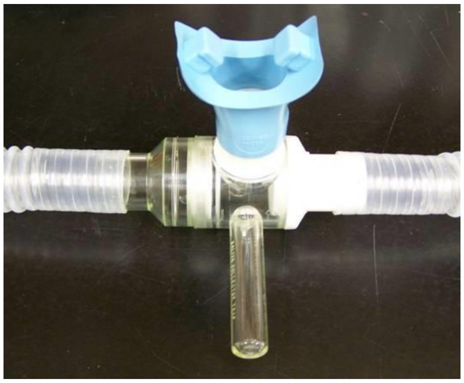

Figure 7. Photograph of the one-way T-valve apparatus connected to inspired and expired low resistance tubing.

Figure 9. Photograph of a face mask.

Figure 10. Photograph of a face mask fitted to a subject.

exercising using a custom developed compliant mixing chamber fitted to the expired side of the one-way valve apparatus. The turbine used in this example is bi-directional in that it measures both inspired and expired air flow.

Figure 11. A subject exercising using a custom developed compliant mixing chamber fitted to the expired side of a "Y" shaped one-way valve apparatus.

Ventilation Measurement

What ventilation equipment options are best for a valid system of indirect calorimetry? While all three measurements are crucial, the ventilation measure is by far the most influential in changing VO_2 and VCO_2 calculations if there are errors in the measurement. Thus, the method used for ventilation measurement is a priority. Historically, ventilation was measured by collecting expired air in large cylinders called **Tissot tanks** (Figure 12). Another early method was to collect air samples in large balloons, of which meteorological balloons were first used. These were later improved to minimize CO_2 loss through use of non-diffusive material, and such bags have been called **Douglas bags** after C.G. Douglas who was an early pioneer of this method (Figure 13). The main drawback of all these methods is the limit they present for the measurement of ventilation with high temporal resolution, as most expired air collections are from between 30 s to 1 min.

The next advancement in air flow or volume measurement was the **gas flow meter** (1960's), which was then (~1980's to 1990's) electronically integrated to a potentiometer to electronically record

Figure 12. A Tissot tank that is used to collect and measure expired ventilation. Vertical movement of the large bell is used with a known bell distance-to-volume calibration to compute gas volumes. The displaced bell floats in distilled water and based on a the barometric pressure and gas temperature measure for 100% humidity air, the volume of expired air can be converted to standard volume conditions (STPD).

circular displacement, and therefore the volume of each breath (Figure 14). However, use of the gas flow meter for breath-by-breath computations of VO_2 and VCO_2 could not be performed at this time due to limitations in the response time of electronic gas analyzers.

Figure 13. Use of the Douglas bag to collect expired air.

Figure 14. The air flow meter can be integrated via a potentiometer to computer acquisition for the recording of breath tidal volume during exercise.

With the continued development of the electronics age, an additional improvement to ventilation measurement was the development and application of the **pneumotach** to indirect calorimetry. This device allowed the measurement of flow based on the pressure difference across a heated metal screen. The integration of flow over time computes volume (Figure 15). However, to work well, the pneumotach needs to be heated above the sampled gas temperature to prevent condensation on the metal screen, and this temperature is required to be constant to prevent extraneous air flow signals and ventilation computation. This was and remains a major weakness of the pneumotach.

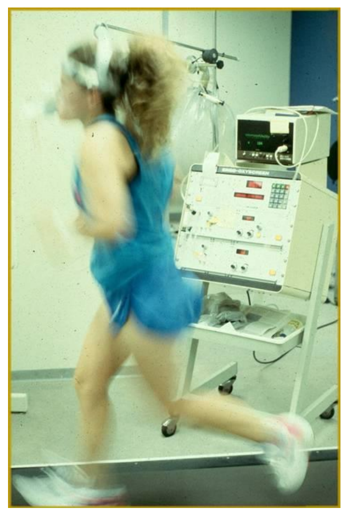

Figure 15. The expired air pneumotach, which in this example, is located just prior to the mixing bag to the left of the ECG unit.

The latest and most superior method of ventilation measurement today is the **flow turbine** (Figure 16). Such turbines are made to be either uni-directional, or bi-directional. However, as discussed for indirect calorimetry, uni-directional turbines are fine for indirect calorimetry based on the Haldane transformation for computing the

Figure 16. The modern air flow turbine which employs a low resistance impeller that rotates in proportion and direction to air flow.

opposing directional flow volume. The only potential concern with turbines is again moisture, which can interfere with the validity of the electronic signal from the rotating **impeller** (internal propeller).

Given the issue of moisture, I recommend that ventilation be measured on the inspired side when performing indirect calorimetry. This requires that room air temperature and relative humidity be known and unless measured constantly, be kept constant throughout the exercise test.

<u>Expired Gas Fraction Measurement</u>

As mentioned in the historical development sub-topic of the prior Topic, expired gas analysis originally required chemical assay of air samples to determine oxygen and carbon dioxide content. Such procedures required hours of meticulous work in a laboratory.

The electronics age changed everything regarding gas composition analysis. Since the 1960's, expired gas analysis has been done with electronic gas analyzers that indirectly measure the gas fraction in expired air (Figure 17). Many commercial systems for indirect calorimetry have their analyzers and ventilation components housed within a single module (Figure 18). As the method of measurement differs for oxygen and carbon dioxide, I will explain each gas analyzer separately.

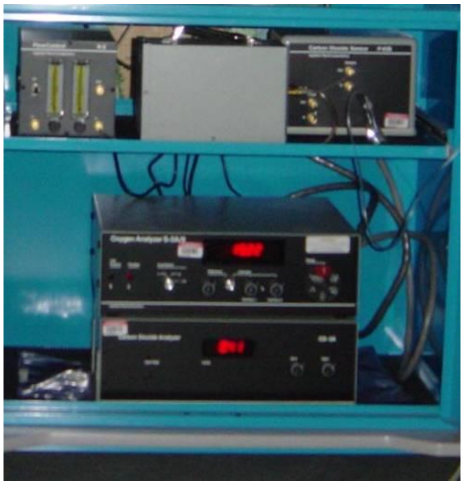

Figure 17. Electronic expired gas analyzers used in our custom-developed system.

<u>Electronic Oxygen Analyzers</u>

There are a variety of methods available for sensing and quantifying oxygen in a gas sample, consisting of electrochemical, paramagnetic, polarographic and zirconium oxide sensors. Of these four methods, only the paramagnetic and zirconium oxide sensors have wide application to expired gas sample measurement, and will be briefly explained below.

Figure 18. A commercial system for indirect calorimetry where each of the ventilation and electronic gas analyzer components are housed in a single module. This is convenient for space issues, but has limited educational application and renders the total system inoperable and expensive to maintain during times of problem solving and repair.

The **paramagnetic oxygen sensor** is based on the high magnetic property of oxygen. Thus, this analyzer has a sensor consisting of a nitrogen containing glass vessel (called a dumbbell) suspended on a platinum wire within a non-uniform magnetic field. Gas samples containing oxygen are pumped through this sensor chamber, and the higher the oxygen content, the greater the attraction of the oxygen gas to the stronger magnetic field. This directional movement causes rotation of the dumbbell. In some versions of this method, a light source measures this rotation, which is proportional to the oxygen content of the sampled gas. Another method of quantification is based on the electrical current required to restore the dumbbell to its normal position. This current is proportional to the oxygen content of the sampled gas.

As there are no parts within this system that deteriorate, or are consumed in the process of gas detection, the operational life and reliability of these analyzers is exceptional. The only drawback is that other gases with magnetic properties can interfere with the oxygen signal, but for indirect calorimetry application, this is not a concern.

The **zirconium oxide oxygen sensor** is the typical method for rapidly responding analyzers. This method has two zirconium oxide probes, each externally plated with platinum. The two sensors are the “reference” and “source” sensors, respectively. Typically the reference gas sample is room air. Both sensors must be heated to at least 650 ^{o}C to operate effectively, which explains the warm-up time of about 30 min for these analyzers. At this high temperature, the zirconium layer becomes porous, allowing oxygen diffusion based on its concentration gradient. Oxygen diffusion into the zirconium generates a voltage, causing a voltage difference between the two sensors proportional to the oxygen content of the sampled air to the source sensor. The electrical generation principle of operation causes rapid response times, but some versions of these sensors deteriorate rather rapidly requiring sensor replacement and/or maintenance. Nevertheless, some zirconium oxide sensors are being developed with improved resiliency, extending the life of the analyzers.

Electronic Carbon Dioxide Analyzers

The main method of carbon dioxide measurement is via non-dispersed infrared (NDIR) technology. This principle is based on the **infrared light** absorption of carbon dioxide, with light absorption proportional to CO_2 concentration. Thus, the CO_2 gas analyzer is a special type of spectrometer, measuring infrared light absorption at a specific wavelength for a continuous sample of gas pumped through a light tube. All CO_2 analyzers used for indirect calorimetry use this method. Not surprisingly, electronic analyzers that use this technology are called **infrared CO_2 analyzers**.

Gas Mass Spectrometers

An alternative to the electronic gas analyzer is gas fraction measurement by mass spectrometry (Figure 19). The **gas mass spectrometer** generates a high vacuum within which a gas sample is drawn and exposed to an electron field which ionizes the gas particles. The ionized gases are then exposed to a magnetic field where the path of gas movement is dependent on the mass of the atoms, and such separation is quantified by an electronic detector, which also quantifies the relative amount of the different atomic masses.

Unfortunately, the gas mass spectrometer is an expensive option to an electronic gas analyzer, at almost twice the expense of an oxygen and carbon dioxide pair. While the response time of the gas mass spectrometer was a major plus 10 to 15 years ago, recent advances in electronic gas analyzer response times largely excludes the gas mass spectrometer from being a competitive alternative to electronic gas analyzers. When you add to this the labor intensive maintenance required to sustain a gas mass spectrometer, the typical exercise physiology laboratory within a university setting is better suited to the electronic gas analyzer.

Figure 19. An example of a gas mass spectrometer.

Purpose of the Test

As there are a variety of gas analyzer options that provide different response times, with faster response times costing more, how gas analyzes are intended to be used is a crucial issue. If there is no intended use for breath-by-breath analysis, then rapid response systems are not required. This is because a rapid response time (<500 ms) is inconsequential when using a system that averages gas signals over 15 to 60 s. Time average applications of indirect calorimetry can use cheaper, slower response gas analyzers.

Breath-by-breath expired gas analysis requires fast responding analyzers because most approaches to breath-by-breath analysis require that signals be acquired and complete within a breath cycle. For more intense exercise, this could be less than 1 s. I have personal preferences and arguments for why I view that such small time intervals invalidate most commercial approaches at breath-by-breath gas analysis. Basically, in my experience with several commercial systems, gas analyzers just do not have the resiliency to accurately profile expired gas signals over complete breathe cycles at high breathing rates week after week, month after month, and year after year. The results of this are repetitive and expensive repairs and maintenance of the analyzers, leading to a money pit analogy.

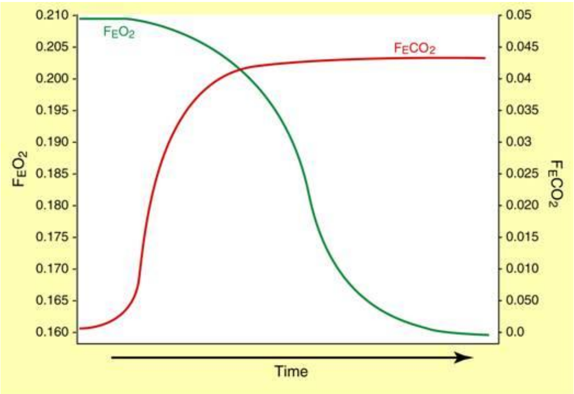

Figure 20. The time dependence for the change in expired oxygen (FEO_2) and carbon dioxide ($FECO_2$).

Figure 20 reveals the changing expired gas fractions for oxygen and carbon dioxide over time for a typical sampled breath. For higher exercise intensities, integrating the expired gas fraction curves is prone to error because there are less signals (fewer data points) compared to lower intensity exercise, meaning that any one data point has greater influence over the integrated signal. Any limitation in response time influences the end of the signal acquisition, which is the end tidal air, preventing inclusion of signal and resulting in lowering the integrated signal.

Some commercial systems get around this dilemma by applying averaged expired gas signals from mixing chamber hardware, aligned to each breathe. The need to do this each breathe still requires rapid response analyzers, but as there is no profiling of gas fractions across a complete expired breath, and only the ability to detect average changes in the gas fractions of the mixing device is needed. This is how I developed my own system with preference to sampling the expired air at the end of an exhalation.

Glossary Words

incremental protocol is a sequence of changing exercise intensities that progressively induce a linear increase in metabolic demand over time.

post-acquisition refers to after the data has been collected.

ventilation threshold is the point in time and intensity when the exercise intensity has caused a more rapid increase in ventilation compared to VO_2, indicating the onset of altered features of ventilation control such as metabolic acidosis, altered motor unit recruitment, increasing neural effort in motor unit recruitment, and more complex movement patterns and control.

warm-up refers to the exercise performed prior to a more focal exercise or performance activity. This is a misleading term, as not all warm-up needs to induce a change in body or muscle temperature, and the benefits of the warm-up may have nothing to do with temperature at all!

calibrate refers to the procedures where an method of measurement is compared to known standard conditions or samples, and constants derived from this mathematical comparison are used to increase the accuracy of the measurements.

Tissot tanks have an aluminum bell cylinder within a larger open aluminum water tank. Air is directed to and/or from the upper cylinder. Air moved into the bell causes it to rise as it floats in the water of the lower tank. Air that is removed from the bell cylinder causes the bell to lower.

Douglas bags are specialized bags for expired air collection during exercise.

gas flowmeter is a mechanical device that measures the volume of air as it passes through an internal bellows-type device. Inflation of the bellows device is calibrated to volume and this is displayed by the rotation of a dial.

pneumotach is an electronic device for measuring air flow as it passes across a heated metal screen. Air flow is proportional to the pressure difference between the upstream and downstream sides of the screen.

flow turbine is an electronic device that measures air flow based on the rotation of an internal impellar. The faster the flow, the large the number of rotations per unit time.

Impellar is a light weight, low friction propeller-type device within a flow turbine.

paramagnetic oxygen analyzer is a common method of measurement of the oxygen content of air samples based on the magnetic properties of gaseous oxygen.

zirconium oxide oxygen sensor is a common method of measurement of the oxygen content of air based on the capacity of a heated zirconium plated sensor to oxygen.

infrared light is light emitted at wavelengths between …. to …… nm.

infrared CO_2 analyzers are electronic devices that are used to quantify the content of carbon dioxide in air samples.

gas mass spectrometer is a sophisticated electronic device that generates a high vacuum from which to separate and measure the content of gases of different mass.