Introduction

In this laboratory, you will become familiar with measuring energy expenditure and substrate utilization during steady state exercise. The exercise involves measuring a participant’s heart rate (bpm), breathing rate (bpm), and the fraction CO₂ and O₂ expired. You will also assess changes in work rate intensity to the physiological measure of the participant.

Background

The human body constantly requires energy. Total Energy Expenditure (TEE) is the total amount of energy used to perform daily tasks. This includes mechanisms at a cellular level through to daily activities such as sleeping and eating, as well as exercise. TEE is comprised of three areas:

**Basal Metabolic Rate (BMR):** Sometimes referred to as resting metabolic rate (RMR). BMR is relatively stable but varies greatly between individuals depending on age, gender and free fat mass (FFM). An individual with a higher proportion of FFM will have a higher BMR. BMR decreases with age as a result of muscle atrophy and reduction in physical activity. The energy used in BMR corresponds to vital bodily functions (movements across membranes, chemical conversions, tissue turnover).

**Diet-Induced Thermogenesis (DIT):** Generally the consumption of food increases energy metabolism. This occurs from activation of the nervous system and the processes involved in digesting, absorbing and assimilating food. Some energy is lost as heat when consuming or digesting food, so not all of the energy in the food consumed is available for other activities.

**Activity:** is variable both between people and in the one person from day to day. An increase in activity will lead to an increase in energy used. Activity ranges from strolling on the flat, walking up the stairs to intensive exercise.

![Figure 1. The three areas that make up Total Energy Expenditure.](image)
Energy expenditure is usually measured in kJ or kCal, and these are often used interchangeably. The average person uses approximately 1,500 - 3,500 kcal (6,000 - 13,000 kJ) of energy per day. Compare this to a Tour de France cyclist with a daily TEE of approximately 6,000 kcal (24,000 kJ). To prevent weight gain, the energy consumed must equal the energy used.

Exercise is often regarded as either aerobic (with oxygen) or anaerobic (without oxygen). In aerobic exercise, the energetic cost can be estimated by indirect calorimetry by determining the rate of oxygen consumption (L min$^{-1}$). Two key factors affect the concentration of exhaled gases: the metabolic demand of the active tissues and the rate of ventilation of the lungs. Measuring both the composition and volume of exhaled gases, allows estimation of the metabolic rate or energetic cost of aerobically sustainable activities. During maximal exercise, ventilation can increase from a resting value of 5-6 L min$^{-1}$ to as much as 140-200 L min$^{-1}$.

During exercise, cardiac output (CO) increases from a resting value of approximately 5 L min$^{-1}$ to as much as 30 L min$^{-1}$. This is because the working muscle requires more oxygen-carrying blood to metabolize energy yielding substrates such as fat, protein and carbohydrate. During exercise, a greater proportion of arterial oxygen is extracted into the muscle per liter of blood compared to at rest.
Gas exchange in the lungs

Gas exchange between air and blood occurs in the alveolar air sacs of the lungs. The efficiency of gas exchange is dependent on ventilation; cyclical breathing movements that alternately inflate and deflate the alveolar air sacs. Inspiration draws air into the alveoli, where the oxygen is transferred to the blood and exchanged with carbon dioxide. Expiration removes the stale air, which contains increased carbon dioxide and reduced oxygen concentrations.

Estimation of substrate usage

As energy yielding molecules (carbohydrate and fats) are metabolized to generate ATP, oxygen consumption increases. Using spirometry and gas analysis techniques, the rate of oxygen (O\textsubscript{2}) consumption and carbon dioxide production (CO\textsubscript{2}) can be measured. The ratio between the VCO\textsubscript{2} and the VO\textsubscript{2} is called the respiratory exchange ratio (RER). Values less-than 1.0 indicate aerobic metabolism (CO\textsubscript{2} production equal to or less than O\textsubscript{2} consumption), whereas values greater-than 1.0 indicate that anaerobic metabolism is involved. Because the oxygen to carbon dioxide ratio is different for carbohydrate and lipid metabolism, the RER provides an indication of the predominant substrate being metabolized at a particular time during exercise. This is called indirect calorimetry and requires both gas analysis and measurement of the volumes of gases being breathed.

The Respiratory Quotient and the Respiratory Exchange Ratio

The ratio of carbon dioxide produced to oxygen consumed by the cells each minute, is called the Respiratory Quotient (RQ). When this ratio is calculated from the expired gases, it is referred to as the Respiratory Exchange Ratio (RER). Under steady state conditions, the respiratory exchange ratio provides a very good estimate of RQ. On a mixed diet, part of the total CO\textsubscript{2} produced and O\textsubscript{2} consumed by the cells comes from metabolism of carbohydrate (where the CO\textsubscript{2} produced and O\textsubscript{2} consumed are equal, so that RQ = 1) and part from metabolism of proteins (RQ = 0.82) and of fats (RQ = 0.71). Thus, on a mixed diet, RQ is always somewhat less than 1.0.

Note that RQ refers to metabolism at a cellular level, and indirect calorimetry (RER) excludes protein metabolism, which during acute exercise is assumed to stay relatively constant. In order to accurately measure protein oxidation, urinary and sweat urea analysis is required.
RER can be used to determine the substrate being used, as, during metabolism, fat requires more oxygen than carbohydrate (CHO), and CHO produces more carbon dioxide during its metabolism. If the RER value is greater than 1.0 indicates that aerobic metabolism is no longer taking place, as the rate of energy demand is exceeds the rate of oxygen being supplied. This is when anaerobic glycolysis occurs.

Figure 2. Energy Systems

Figure 3. Energy Metabolism Pathways.
Measurement of gas volumes

Historically gas analysis was commonly done by breathing into a bell spirometer; where the level of a floating bell tank gave a measure of changes in lung volume. Flow ($F$) was calculated from the rate of change of the volume ($V$).

Today, airflow can be measured directly with a pneumotachometer (from Greek roots meaning "breath speed measuring device"). The ADInstruments pneumotachometer is shown below.

![Pneumotachometer Diagram](image)

*Figure 4. The ADInstruments pneumotachometer.*

The flow head contains a fine mesh. Air breathed through the mesh gives rise to a small pressure difference proportional to flow rate. Two small plastic tubes transmit this pressure difference to the Spirometer Pod, where a transducer converts the pressure signal into a changing voltage that is recorded by the PowerLab and displayed in LabTutor. The volume, $V$, is then calculated as the integral of flow:

$$V = \int F \, dt$$

*Figure 5. Equation for the determination of volume from flow integration.*

Respiration consists of repeating cycles of inspiration followed by expiration. During the respiratory cycle, a specific volume of air is drawn into and then expired from the lungs; this volume is the Tidal Volume (VT). In normal ventilation, the breathing frequency ($f$) is approximately 15 respiratory cycles per minute. The product of $f$ and VT is the Expired Minute Volume (VE), the amount of air exhaled in one minute of breathing. Tidal Volume and Expired Minute Volume change according to the level of activity.

Energy expenditure and production

The rate of mechanical energy expenditure, or power, is readily determined by use of a known workload (watts). A bicycle ergometer is ideal for this. The rate of metabolic energy consumption can be estimated from measurement of the rate of oxygen consumption or carbon dioxide production. On a mixed diet, the complete combustion of food stuffs yields 20 kJ of energy per L of oxygen consumed. This is the so-called
'energetic equivalent of oxygen'. By measuring the rate of oxygen consumption in L min⁻¹, the rate of metabolic energy consumption can be calculated for any rate of mechanical energy expenditure.

Note: to use oxygen consumption as a measure of total metabolic rate, you must be exercising in an aerobic steady state condition.

**Figure 6. Typical heart rate trend showing an individual has reached steady state.**

Steady state exercise refers to the "level of exercise at which the physiological responses remain relatively stable for an extended period of time" (McArdle, Katch and Katch, 1994). There are four stages that can be seen when establishing steady state exercise and can be measured while observing heart rate, ventilation, oxygen consumption and cardiac output. Figure 6 illustrates the heart rate changes when establishing steady state exercise.

1. A rapid increase in the first minute.
2. A more gradual rise during the second minute.
3. Between three and six minutes, a steady state is reached which is then maintained provided the exercise rate remains constant.
4. If exercise persists for several hours, a gradual drift up reflecting effects of variables such as the rising core body temperature.

Factors which affect steady state exercise are:
- O₂ delivery to working muscles
- O₂ utilization in cells during aerobic metabolism
- The ability to off-load heat.

At higher exercise intensities, some of the ATP used may be generated via anaerobic glycolysis rather than aerobic respiration, in which case oxygen consumption underestimates the true metabolic rate.
Calculating the rate of O₂ consumption and CO₂ production

O₂ consumption

The volume of O₂ consumed each minute is the difference between the volume inspired and expired per minute.

\[ V_{O_2} = V_{I_{O_2}} - V_{E_{O_2}} \]

If we know the fraction of the volume occupied by O₂ under STPD conditions, then

\[ V_{O_2} = V_{I} \times F_{I_{O_2}} - V_{E} \times F_{E_{O_2}} \]

And, assuming an RQ of 1,

\[ V_{O_2} = V_{I} \times (F_{I_{O_2}} - F_{E_{O_2}}) \]

CO₂ production

The volume of CO₂ produced each minute is, again, the difference between the volume inspired and expired per minute. But inspired air has virtually no CO₂ so the equation is simpler.

\[ V_{CO_2} = V_{E} \times F_{E_{CO_2}} \]

And, assuming an RQ of 1,

\[ V_{CO_2} = V_{I} \times F_{E_{CO_2}} \]

What you will do in the laboratory

This lab is designed to be performed with a single volunteer, who performs 15 minutes continuous exercise on a bicycle ergometer, divided into three 5 minute blocks that increase in intensity (watts). However, if time permits, the whole Lab can be run through a second time with another volunteer.

During the lab, measurements will be made at rest and then during 3, 5 minute blocks of exercise at increasing levels of intensity:

1. **Variables recorded**: respiratory rate and air flow, from which respiratory minute volume can be calculated; ECG, from which heart rate can be derived; CO₂ and O₂ in inspired and expired air; and the work rate set on the bicycle ergometer.

2. **Results calculated from variables recorded**: minute volume (STPD); tidal volume; rates of O₂ consumption and CO₂ production; and the Respiratory Exchange Ratio (RER); and energy expended during the different exercise rates.