Exercise Physiology – Manual

Introduction

This program simulates some of the important physiological measurements which can be made to assess the cardio-respiratory performance of human subjects in the laboratory. The results presented are those from healthy individuals performing graded exercise on a bicycle ergometer and include:

- Heart rate
- Minute ventilation
- Oxygen consumption
- Blood lactate

Results from the first three of these parameters are presented on a simulated chart recorder. For the blood lactate experiment a simulated spectrophotometer is used.

The Investigations

You may investigate the effects of graded exercise on a number of physiological parameters.

- Heart rate
- Pulmonary (minute) ventilation
- Oxygen consumption
- Blood lactate concentration

The effect of exercise differs according to the subjects physical characteristics, sex, age and level of physical fitness which may be enhanced by training. It is therefore important to define a number of subject parameters before you begin the investigation.

Define a subject

You should enter details of sex (male or female), weight in kg, height in cm, age in years and whether you wish your subject to be trained (yes) or untrained (no).

NOTE: In this program training refers to endurance training. In order to illustrate differences in cardio-respiratory fitness between trained and untrained individuals the results for trained subjects are those from highly trained individuals.

Heart Rate

Heart rate results are presented on an animated screen display which simulates a chart recorder. Initially the pre-exercise heart rate is shown and the workload (0 watts) is indicated. The investigation is started, i.e. the subject is made to work. Immediately a work load of 20 watts is indicated on the screen and the clock is started. The increase

in heart rate associated with this load is displayed on the chart recorder. The subject works at this load for 1 minute when the work load is further increased by another 20 watts. This continues until the subject reaches exhaustion when the experiment is terminated.

Taking Measurements

It is advisable to measure the steady-state heart rate (where the trace levels off) for EACH work load, though this may not be possible particularly at higher work loads. You can then plot a graph of heart rate against work load for different subjects, determine the maximum heart rate and compare for example, male with female, trained with untrained individuals, etc.

Minute Ventilation (VE)

Results for minute ventilation (l/min) are displayed in the same way as for heart rate. You should measure the VE for each work load, determine the maximum VE and plot a graph of VE against work load for different subjects.

Oxygen Consumption (VO2)

Values for oxygen consumption (l/min) are again displayed on a simulated chart recorder. You should measure the VO2 at different work loads and the maximum VO2 for each subject studied. If the VO2 levels off at high work loads this is known as the VO2 max and is considered to be a measure of aerobic power.

Blood Lactate

Measurement of blood lactate levels during exercise can indicate when aerobic metabolism in muscle cells is supplemented by anaerobic mechanisms. You will need to:

a) Collect Samples

Repeat the heart rate experiment and briefly consider where and how many capillary blood samples you would collect from the subject for spectrophotometric analysis. Nine blood samples are collected automatically for you during the experiment. A control (pre-exercise sample) is taken automatically at the start of the experiment. Seven more are taken at regular intervals during the experiment and one immediately after the subject becomes exhausted.

b) Standard Curve

Lactate concentration can be measured using a spectrophotometer. The sample is mixed with certain reagents which react to give a coloured product which will absorb

light maximally at a particular wavelength (340 nm). The amount of light absorbed is proportional to the colour of the solution which in turn is proportional to the amount of lactate present. Thus the greater the concentration of lactate in the sample the greater will be the absorbance.

The simulated spectrophotometer allows you to measure the absorbance of the sample and measurements should be taken directly from the screen display.

In order to measure the lactate concentration of each blood sample you must first of all produce a standard or calibration curve. A range of lactate standard solutions of known concentration (0 to 16.0 mmol/l) are prepared and mixed with reagents to produce a coloured product. The intensity of the colour will depend on the concentration of the lactate standard. On the screen display each lactate standard is assigned a button. When you click the appropriate button the coloured band will move along the scale and give you the absorbance reading in arbitrary units. The spectrophotometer is calibrated such that the reagent blank or control (no lactate present) will give an absorbance of zero. You should note the absorbance reading for EACH lactate standard and then plot a graph of lactate concentration (mmol/l) on the x-axis against absorbance (arbitrary units) on the y-axis. The standard curve will then allow you to calculate the lactate concentration in each of the blood samples you obtained during the test.

c) Sample Analysis

This option allows you to measure the lactate concentration of each of the blood samples taken during the test. The screen display shows a simulated spectrophotometer and the 9 blood samples taken during the test, each being assigned a button. In the analysis each blood sample is treated identically to the lactate standards and the absorbance measured at 340nm. Clicking the appropriate button will allow you to measure the absorbance of that sample on the scale. You can then refer to your standard curve and determine the lactate concentration (mmol/l) equivalent to that absorbance reading. Repeat this for each sample and then plot work load (watts) against blood lactate concentration (mmol/l).

Tutor's Notes

When you exercise the contraction of muscle requires energy which is supplied to muscle cells by the oxidation of substances such as muscle glycogen, lipids and glucose to yield ATP. Muscle only has sufficient stored ATP and phosphoryl creatine for a few seconds activity i.e. a short sprint and relies on aerobic metabolism to generate more ATP. The oxygen and nutrients need to be supplied to muscle cells at a rapid rate and this is a function of the cardio-respiratory "support systems". During exercise the oxygen demand of skeletal muscle increases some 20-30 times. This demand is met by physiological adjustments of both the cardiovascular and respiratory systems giving rise to increases in:

- blood flow to skeletal muscle
- pulmonary ventilation
- oxygen uptake by muscle cells

Cardiovascular Changes

The increased blood flow to skeletal muscle is brought about by:

- an increase in cardiac output
- changes in the systemic circulation

Cardiac Output (CO)

CO is the volume of blood ejected by each ventricle per minute and is a function of the heart rate (HR) and the stroke volume (SV) which is proportional to the force of contraction or contractility of the ventricles.

$$CO = HR \times SV$$

During exercise both heart rate and stroke volume are increased as a result of autonomic nervous system reflexes resulting in increased sympathetic activity, decreased parasympathetic activity and increased adrenaline release from the adrenal glands. There is also an increased flow of blood back to the heart (venous return).

The model used in this program calculates the maximum heart rate (beats/min) for each subject from: Mean = (220 - age) SD ± 10. Thus for different subjects of the same age the maximum heart rate will be different since it will be randomised from a normally distributed population with a mean of 220 - age (95% CI ± 19). This could be a useful exercise for a class of students, the data being used to teach elementary statistics.

Maximum heart rate is not significantly affected by gender, type of exercise or training. The difference between the predicted HR max and the observed maximum HR is known as the Heart Rate Reserve (HRR). This can be used as a measure of the stress of the cardiovascular system during exercise though it is also affected by factors

such as the normal population variability, motivation of the subject, use of drugs such as fl-blockers and heart disease.

Endurance training has the effect of decreasing the resting heart rate, increasing the resting stroke volume and increasing the systemic vascular conductance at rest.

Systemic Circulatory Changes

During exercise a high proportion of the CO goes to active muscle while simultaneously the blood flow to non-essential areas is reduced. This is due initially to the activation of autonomic reflexes. Sympathetic vasoconstrictor fibres arising in the medullary vasomotor centre cause a reduction in blood flow to the kidneys and other abdominal organs whilst sympathetic vasodilator fibres increase blood flow to skeletal and cardiac muscle. This increased blood flow is maintained by the action of vasodilator metabolites released by active muscle which cause dilatation of arterioles and capillary beds. These include lactic acid, CO2, K+, kinins, adenosine and a reduction in the pO2.

Respiratory changes

Pulmonary Ventilation (VE)

At rest the pulmonary ventilation is about 6 l/min; the tidal volume is 500-700 ml and the respiratory rate 12-15/min. During exercise this can increase up to 120 l/min and is called exercise hyperpnoea. The initial increase in pulmonary ventilation, the primary hyperpnoea, is due to autonomic nervous reflexes. The respiratory centres in the medulla are activated by an increased flow of sensory information from (a) the cerebral cortex, and (b) proprioceptors in contracting muscle. The respiratory centres then stimulate the respiratory muscles, the intercostal and diaphragm, to increase tidal volume and the frequency of breathing. The increased pulmonary ventilation is maintained by the increase in the concentration of CO2 (pCO2) in arterial blood as a result of muscle metabolism. Central and peripheral chemoceptors are sensitive to changes in the pCO2 and sensory stimuli from these receptors also stimulate the medullary respiratory centres giving rise to the secondary hyperpnoea. Respiratory centres are further stimulated by the decrease in p02 during prolonged exercise and the increase in body temperature.

VE depends on type of exercise and is influenced by factors such as the efficiency of ventilation, the degree of respiratory compensation for metabolic acidosis and the mechanical capabilities of the lungs, and respiratory muscles.

At moderate exercise levels, i.e. below the anaerobic threshold, the increase in VE is largely as a result of an increased tidal volume. Above the anaerobic threshold the frequency of breathing also increases (driven by the accompanying metabolic acidosis) and there is a further increase in tidal volume.

Increased Oxygen Uptake By Muscle Cells

The increased pulmonary ventilation ensures that the haemoglobin of red blood cells is saturated with oxygen even during strenuous exercise and the increased cardiac output and muscle blood flow ensures an effective supply of nutrient-rich, oxygenated blood to contracting muscle cells. Oxygen and nutrients are taken up, from capillary blood, by a process of simple diffusion which depends on a concentration gradient between the blood and muscle cell. During exercise the rate of diffusion is increased:

(a) the increased oxygen consumption of muscle cells lowers the pO2 of skeletal muscle and increases the diffusion gradient;

(b) the vasodilatation occurring in skeletal muscle increases the volume of, but reduces the rate of, blood flow through muscle tissue thus allowing more time for diffusion to occur. The distance over which diffusion has to take place is also shortened;

(c) the dissociation of oxygen from oxy-haemoglobin is increased as a result of the increased pCO2, H+ concentration, and temperature (Bohr effect);

(d) at very low muscle pO2 myoglobin releases oxygen.

Pulmonary ventilation is not a limiting factor in exercise. Haemoglobin is fully saturated even during the most severe exercise.

Oxygen consumption - VO2

The oxygen consumption, measured in l/min is calculated by multiplying the minute volume by the difference in the oxygen concentration in inspired and expired air. It is also necessary to measure the change in CO2 concentration to account for differences in the volume of inspired and expired air.

The maximum oxygen uptake (VO2 max) is the highest oxygen uptake that a healthy person can attain during exhaustive exercise of approximately 6 min duration. This is known as the aerobic capacity and is a measure of cardiorespiratory "fitness", and the ability to maintain strenuous exercise. The VO2 max is obtained by progressively increasing the work load until it exceeds the capacity for oxygen uptake and muscle depends on anaerobic power sources. The plateau of the plot of VO2 against work load is the VO2 max. Note that the maximal VO2 is only equivalent to VO2 max if, at the highest work loads, VO2 is seen to plateau.

VO2 max is a function of age, sex, weight, height and type of exercise. It decreases with age. Female values are approximately 77% of male values after adjustment for body size (muscle mass and not body mass is important). Endurance training significantly increases VO2 max.

Predicted VO2 Max

The VO2 max test described requires a subject to work at near-maximal intensity and reach a condition of very near complete exhaustion. For this reason it is difficult to perform, often produces low values and is not suitable for routine laboratory assessment of aerobic capacity. However, there is a direct relationship between heart rate and oxygen consumption. If a work load is chosen which can be sustained for 6 min, and results in a steady-state heart rate in the range 130-145/min for the 5th and 6th minute, the max V02 can be predicted by extrapolation, assuming that V02 max occurs at near-maximum heart rate for that subject (max HR is estimated as 220 - age in years). A table of normal values is available which relates work load to heart rate, for males and females of different ages and body weights, and allows the prediction of VO2 max.

Anaerobic Work

Both anaerobic and aerobic metabolism are involved in all intensities of muscular activity. Anaerobic power sources can be metabolised to release energy instantly without the delay required for oxygen uptake, and in exhaustive work they allow energy to be expended in excess of the capacity to metabolise aerobically. The most significant metabolite of anaerobic metabolism is lactate which accumulates in muscle and diffuses into the blood. Blood lactate needs to be buffered and this is achieved by the bicarbonate/carbonic acid system with the production of CO2 (an extra positive stimulus for respiration) and a lowering of the blood bicarbonate concentration. During recovery muscle lactate is reoxidised to pyruvate and metabolised via the TCA cycle, and blood lactate is converted to glycogen in the liver. Continued hyperventilation for up to an hour after exercise has ceased supplies the extra oxygen required for disposal of lactate. This is known as the oxygen debt and is incurred during exercise and paid back during recovery. Also during recovery blood bicarbonate concentration, ATP and phosphoryl creatine levels are restored.

The anaerobic threshold (AT) is the VO2 at which aerobic capacity is exceeded and aerobic energy sources are supplemented by anaerobic ones. In the program the AT is calculated as 56 ± 2% of VO2 max for untrained subjects and as 64 ± 2% of VO2 max for trained subjects.

Endurance training elevates the AT, increases the lactate tolerance allowing a greater accumulation of lactate to be tolerated, increases the activity of oxidative enzymes in muscle cells with no effect on glycolytic enzymes, increases the numbers of mitochondria in muscle cells and increases the use of fats and the conservation of muscle glycogen thereby postponing glycogen depletion and accumulation of lactate.

NOTE: We can compare the oxygen requirement and oxygen intake in three different running events: 200m sprint, 800m and 5,000m. In the sprint the oxygen requirement far exceeds the oxygen intake and 90% of energy expenditure is anaerobic; in the 800m oxygen consumption is higher and only 65% of energy production is from anaerobic sources; in the 5,000m the oxygen requirement is below the maximum oxygen intake and as a consequence a balance of oxygen supply and demand is reached which can be maintained for long periods.

Experimental Method and Protocol

The Bicycle Ergometer

The bicycle ergometer is the work form used in all experiments and is suitable for measuring physiological changes during graded exercise in the laboratory. Several large muscle groups are used and, at submaximal work loads, it demands about the same energy output for subjects of different ages, weight, fitness and sporting background.

It consists of a single wheel turned by means of pedals and braked mechanically by a belt running around the rim. One complete turn of the pedals moves a point on the rim through 6 metres. Subjects are asked to make 50 full pedal turns per minute (timed by a metronome), equivalent to 300 m/min. A range of work loads (measured in watts) is achieved by applying different resistances to the wheel by stretching the belt. The rate of pedalling is kept constant at all resistance settings.

A standard protocol of work is used for all experiments. Subjects warm up by pedalling for several minutes with no work load which allows time to adjust to the rate of pedalling described above. The experiment is commenced when the first load (20 watts) is applied. The subject works at this load for 1 minute after which a further 20 watt load is added. The rate of pedalling and the duration of work at each load are kept constant throughout. The work load is increased by increments of 20 watts until the subject is exhausted.

Methods

Heart Rate

Heart rate is monitored by positioning electrodes on the chest wall which will record the electrical signals associated with contraction of cardiac muscle. These signals are then used to produce a continuous record of heart rate (beats/min).

Minute Ventilation

Minute ventilation (VE) is a product of the tidal volume and the frequency of breathing. Subjects breathe in room air through a mouthpiece and exhaled air is collected in a Douglas bag. A new Douglas bag is connected each minute as the work load is increased. The volume of air breathed out per minute is measured using a gas-meter and the oxygen and carbon dioxide concentration of a sample of this air is also measured using O2/CO2 analysers. In the experiments illustrated in this program equipment was used which gave a breath-by-breath analysis of exhaled air. By convention VE is reported in l/min at body temperature saturated with water vapour at

ambient pressure (BTPS) and measured at ambient temperature (ATPS). Tables are available to convert BTPS volumes to ATPS.

Oxygen Consumption

Oxygen consumption (VO2) is calculated by multiplying VE (corrected to STPD) by the difference in oxygen concentration in inspired and expired air. STPD is standard temperature (0∞ C), pressure (760 mm Hg), Dry.

Blood Lactate

Capillary blood samples (200 µl) are taken before and at intervals during graded exercise. Samples are collected into a small volume of a solution which will precipitate blood proteins (e.g. perchloric acid, metaphosphoric acid) and then centrifuged. The clear supernatant can then be stored at 4∞ C for several days.

Principle: Lactate in the presence of NAD+ and lactate dehydrogenase (LD) is oxidised to pyruvate forming NADH, the absorbance of which can be measured spectrophotometrically at a wavelength of 340nm. NADH production is then a measure of lactate concentration.

$$\text{lactate} + \text{NAD}^+ \quad \text{pyruvate} + \text{NADH} + \text{H}^+$$

A pH of 9.0 - 9.6 (Tris buffer), removal of pyruvate with hydrazine and an excess of NAD+ will drive the reaction to the right.

Blood Lactate Assay

A number of methods are available for the assay of blood lactate. One possible method is given here.

Reagents

1. Lactate dehydrogenase (LD) - use crystalline beef heart LD (Sigma; 10 mg protein/ml).

2. Metaphosphoric acid MPA (5g/100 ml).

3. Tris-hydrazine buffer pH 9.6 (Tris 79 mmol/l; hydrazine 400 mmol/l).

4. NAD+ solution 27 mmol/l.

5. Lactate Standards prepared from lithium lactate stock solution (2-16 mmol/l).

Procedure

Standard Lactate Calibration Curve

To a cuvette containing 2.0 ml of Tris-hydrazine buffer and 0.1 ml of MPA add 0.1 ml of standard lactate solution. Mix well and add 30 µl of LD and 0.2 ml NAD+. Mix again, leave for 15 minutes at room temperature and measure absorbance at 340 nm using a spectrophotometer. A reagent blank (control) containing 0.1 ml MPA should be used alongside lactate standards and test samples. Plot absorbance against lactate concentration.

Blood Samples

Substitute 0.1 ml of supernatant from each sample for the lactate standard in previous procedure.

Normal resting levels for blood lactate are approximately 0.9 mmol/l rising to 12-14 mmol/l during strenuous exercise.

Bibliography

Wasserman K. et al (1987) Principles of Exercise testing and interpretation. Lea & Febiger.

Tietz N. W. (Ed) (1976) Fundamentals of clinical chemistry. W. B. Saunders Company.

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