EXERCISE PHYSIOLOGY

Aim

To explain the relationship between the body and health, fitness and exercise, with reference to physiological processes, including energy pathways during resting, work and recovery, and how energy is used in the human body to create work and power.

THE CARDIORESPIRATORY SYSTEM

This is a combination of two systems; the circulatory system (heart and blood vessels), and the respiratory system (lungs and air passages). Blood is a combination of specialised cells suspended in a liquid called plasma. The cells are primarily red blood cells (erythrocytes) as well as white blood cells (leukocytes) and platelets. The red blood cells contain iron-protein molecules called haemoglobin. Haemoglobin binds to carbon dioxide and oxygen, with a preference for the former. In this way, red blood cells collect oxygen from the lungs, and when they arrive at cells the cellular waste product carbon dioxide bumps the oxygen off the haemoglobin. The red blood cells then return carrying carbon dioxide to the lungs where they release it and take up fresh oxygen. The blood is therefore essential to life as it serves as a transport for the haemoglobin containing red blood cells necessary for cellular gas exchange.

The heart beats constantly, pumping the blood around the body. The heart contractions apply force to the blood and make it move and as it does the red blood cells move also, travelling to and from the heart, lungs and periphery with their cargo of oxygen and carbon dioxide. The rate at which blood is pumped through the body varies according to the needs of the body. When we exercise vigorously, the cells need more oxygen and produce more waste products due to increased energy production, so the heart pumps faster (your pulse rate increases) and with more force (stroke volume – the amount of blood pumped out of the heart with each beat - increases). For the average person, the heart pumps at approximately 60-80 beats per minute. This rate varies though according to the age of the individual, the health of the individual and other factors.

Rates of Heart Beats (pulse rates)

Although the heart beats continuously on its own, the nervous system does have some control over the rate of heartbeat and the strength of the contractions. During increased muscular activity such as running, the amount of blood returned to the heart increases. This causes the pressure in the heart and arteries to rise. In response, the rate of heartbeat increases by means of a reflex action. When the pressure in the heart and arteries drops sufficiently, the heartbeat will decrease, again by a reflex action.

There are two things which affect the amount of blood carried around the body:

- Pulse rate how often the heart beats each minute
- Stroke volume how much blood the heart pumps out with each contraction

With some people stroke volume is much larger than others, thus it might be quite feasible that they could have a much lower pulse rate and still be pumping as much blood around. The functional ability of the heart to pump blood is the key to successful muscular performance.

ENERGY AND WORK

Energy is derived from the metabolism of biomolecules in cells. The energy produced is used to power work, such as muscle contraction, and other cellular processes. Energy is produced in a variety of ways, with a preference for the aerobic (oxygen-requiring) system. However, the body cells are also able to produce energy in the absence of oxygen (anaerobic) for short periods of time. These reactions occur, for example, in muscle cells during strenuous exercise.

(NB: If you have not previously studied biochemistry you may find the following information more difficult, and hence require more reading. Do not become confused. If you are having difficulty - contact your tutor and ask for further reading or explanations. Be specific in your requests though. In the meantime you may continue reading other parts of this course).

ATP – The Body's Energy Reservoir

The cells of animals (and plants) require energy to perform many of their functions, from cell division, production of new molecules to movement and so on. The energy required is released through a chemical reaction where a nucleotide – Adenosine triphosphate (ATP) has one of its phosphate residues cleaved off, converting it into Adenosine diphosphate (ADP) and releasing energy in the process. In order for a cell to make use of its ATP stores, it must have many thousand times more ATP than ADP. As with all chemical systems, equilibrium (equal amounts of ATP and ADP) is favoured and if there is more ATP, then its conversion to ADP, with subsequent release of energy is favoured. If ATP and ADP are in equal amounts the cell could not derive energy from ATP.

Adenosine Triphosphate



Adenosine Diphosphate



Sources of ATP

ATP can be supplied to the body in several different ways:

• ATP-PC System (anaerobic)

Here the compound phosphocreatine is broken down to produce ATP. When phosphocreatine breaks down it produces phosphorus, creatine and energy. The energy produced is then able to be used by ADP to create ATP. Phosphocreatine is then able to be reconstituted with the addition of energy (which comes from foodstuffs -not from stored ATP/ADP reactions).

• Lactic Acid System (anaerobic)

This system involves glucose (or glycogen) going through various chemical processes to produce ATP plus lactic acid. One glucose molecule is broken down into carbon dioxide and water, in the presence of oxygen, and in turn, produces two ATP molecules. The amount of ATP produced this way is small. This is a more complex procedure using only carbohydrates as its food fuel, and not requiring oxygen for the process. A problem with this process is that it can cause an accumulation of lactic acid in the body, which can affect blood Ph. Blood pH should be around 7.3, and never drop below 6.8. The lactic acid system is however self limiting, and should not normally develop such problems.

• Oxygen System (aerobic)

This process involves the formation of carbon dioxide, water and ATP, from fats, proteins and/or carbohydrates, in the presence of oxygen. This process can produce large amounts of ATP. One molecule of sugar can result in the production of 36 molecules of ATP. This is more complex than the ATP-PC system and the only limiting factor for this system is usually the supply of oxygen. The body will normally try to use this system, and only use other systems to produce ATP if oxygen is in short supply. The short supply of oxygen can occur when:

- Activity first starts
- Activity is placing higher demands on oxygen than what can be supplied by breathing.

The body uses anaerobic systems for energy supply only when aerobic systems cannot meet the demand.

Example:

If a person is running a marathon, breathing may not be supplying ample oxygen to produce ATP through this system, hence the lactic acid system may start to be used, resulting in a build up of lactic acid OR the ATP-PC system may be used resulting in a depletion of phosphocreatine in the muscles.

After completing exercise, there may be a lactic acid build up, and if so, the body needs to remove this excess. This lactic acid removal requires energy which is supplied aerobically; hence extra oxygen may be required. This extra oxygen requirement (after exercise) is called the "oxygen debt".

When the body needs extra energy, it always tries to use the most efficient system which is the aerobic system. The only limiting factor in this system is the supply of oxygen. If more energy is needed, the body will therefore tend to breathe faster and pump blood faster in order to increase the oxygen being supplied to the tissues. If activity gets too high, energy cannot come from aerobic sources because oxygen cannot be supplied fast enough, therefore anaerobic sources are drawn on. Anaerobic supply of energy can't last for much more than about 90 seconds though. It is dangerous to push the use of the anaerobic energy supply too hard, particularly if you are not trained. When exercise begins energy first comes from anaerobic sources but quickly moves to a combination of aerobic and anaerobic. After a while all energy virtually is coming from aerobic sources.

From the discussion above you should be able to see how important it is to develop good cardiorespiratory capacity in order to develop good physical fitness.

ENERGY REQUIREMENTS FOR DIFFERENT ACTIVITIES

Energy for activity is provided in the muscles in the form of a store of ATP. The small amount of ATP in muscle is only sufficient to support a single explosive muscle contraction, such as throwing a ball, or a golf swing. If sports performance demands repeated muscle contractions (such as running or cycling), the ATP required must be constantly replenished from other fuel sources in the muscle.

Short term energy supplies for sprints may be obtained by breaking down substances already in the muscle, without the need for additional oxygen (anaerobic reactions).

The ATP already in muscle, and another high energy substance, creatine phosphate together provide enough energy for 5-10 seconds of maximal effort. These energy supplies are being used during such things as 100 metre track events or short dashes of a soccer goal keeper. They are quickly rebuilt after an effort, to the extent that 50% of the energy source is available 30 seconds later, and all energy is restored within 2 minutes.

When a maximal effort is continued beyond the extent of the phosphate energy system, energy is provided from glycogen stored in the active muscles, using the lactic acid system. This energy supply is for example being used in 400 metre track races, and 100 metre swimming events. Continuous activities which lead to exhaustion in 45-50 seconds result in maximal values for lactic acid accumulation. Anaerobic energy release from glycogen produces lactic acid, resulting in a feeling of fatigue which will cause an athlete to slow down. Once lactic acid is produced, it requires 45 to 60 minutes to be removed, and for the athlete to recover.

As the duration of the performance is extended beyond one minute, oxygen is used to provide an aerobic supply of ATP for muscle contraction. The effective use of oxygen relies upon adequate sources of fat and glycogen fuel in the muscle. The longer an event (or period of activity), the more important becomes an adequate aerobic energy supply.

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CARBOHYDRATE OXIDATION

Glucose is the major biomolecule used to generate energy in the body. It enters the body in foodstuffs and as they are broken down, the glucose is absorbed into the blood stream, and then taken up by cells for storage or use.

Glucose in cells undergoes *cellular respiration* in the mitochondria (intracellular organelles, introduced in the first lesson). This involves glucose being oxidised to form carbon dioxide, water and ATP (the energy source). This is an oxygen dependant reaction, where the cell takes in fresh oxygen, and at the end of the process delivers waste carbon dioxide back the circulation for expulsion via the lungs.

The energy produced by cellular respiration is stored in the body in the form of ATP. When the cell is ready to use the energy, ATP is converted to ADP and this reaction provides the energy to drive work, such as muscle contraction. Oxidation of glucose is divided into two stages: glycolysis and the citric acid cycle.

Glycolysis

Glycolysis is anaerobic (doesn't require oxygen) and is the process by which one molecule of glucose is converted into 2 molecules of pyruvate, with the energy released from the reaction captured in the form of 2 ATP molecules.

Glucose + 2 ADP + 2 P_i + 2 NAD⁺ \rightarrow 2 Pyruvate + 2 ATP + 2 NADH + 2H₂O

(Pi is an ionic phosphate group)

This reaction takes place in the cells cytoplasm after glucose molecules are transported into the cell.

Citric Acid Cycle

Known by several names including the Krebs Cycle, although this can also refer to other metabolic pathways, so the Citric Acid Cycle is the preferred name. There is an intermediate step between glycolysis and the citric acid cycle, where pyruvate is converted into Acetyl-CoA. (CoA is short for coenzyme A, and acetyl CoA is coenzyme A with an acetyl group attached)

Pyruvate + CoA + NAD⁺ \rightarrow Acetyl CoA + CO₂ + NADH

The acetyl CoA then combines with a 4 carbon molecule, oxaloacetate forming citric acid. The citric acid cycle then proceeds in the mitochondria as follows:

Citrate	
↓	
cis-Aconitate	
\downarrow	
Isocitrate	
↓ ──►	NADH
Oxalosuccinate	
\downarrow	
α-ketoglutarate	
\downarrow ———	NADH
Succinyl-CoA	
↓►	GTP
Succinate	
↓►	FADH
Fumarate	
\downarrow	
Malate	
↓	NADH
Oxaloacetate	

Oxaloacetate then combines with another molecule of acetyl-CoA and the cycle repeats.

So where is the ATP? NADH molecules from the Citric Acid Cycle are each converted into three molecules of ATP; GTP is readily converted to ATP. Each glucose molecule is converted by glycolysis and subsequent pyruvate decarboxylation into two molecules of acetyl CoA. So, the cycle runs through twice for each glucose molecule.

So the yield of ATP for each glucose molecule is:

Glycolysis	4 ATP + 4 NADH	= 16 ATP
Pyruvate Decarboxylation	2 NADH	= 6 ATP
Citric Acid Cycle	6 NADH + 2 GTP	= 20 ATP
		= 42 ATP

However, it takes 4 ATP molecules to phosphorylate and activate glucose so it can enter the citric acid cycle, so the final yield is 38 ATP.

Some key points about the citric acid cycle:

- Glycolysis produces pyruvate from phosphorylated glucose.
- This pyruvate enters the cycle, giving off carbon dioxide which is transferred to the lungs and breathed out.
- Electrons are removed from hydrogen to produce H⁺ ions, and electrons. These electrons enter the electron transport chain for further chemical reactions.
- Eventually water is formed from hydrogen ions and electrons removed in the cycle.
- Energy is released as electrons are transported in the Electron Transport Chain and this energy is used in the formation of ATP from ADP and free phosphate ions.

SUMMARY OF THE AEROBIC ENERGY SYSTEM:

 $C_6H_{12}O_6 + 6O_2 ----> 6CO_2 + 6H_2O + ATP$

COMPARING ENERGY PATHWAYS FROM DIFFERENT FOODS Carbohydrates

- Carbohydrates break down to glucose (simple sugar)
- Glycolysis of simple sugars produces pyruvate
- Pyruvate produces Acetyl CoA which enters the Citric Acid Cycle and results in the production of energy in the form of ATP.

Fats (Triglycerides)

- · Hydrolysis of triglycerides produces glycerol and free fatty acids
- · Beta oxidation of glycerol produces acetyl CoA
- Acetyl CoA enters the Citric Acid Cycle and results in the production of energy in the form of ATP

Proteins

- · Proteins break down into amino acids
- Deamination may occasionally occur to produce keto acid which in turn can move into the Citric Acid Cycle, however this is not the typical pathway for proteins, as they are only a last resort energy source.

THE UREA CYCLE

As the name suggests, this pathway produces urea. The purpose of the cycle is to remove ammonia (NH_3) the body in the form of urea, which is in turn excreted in the urine. Ammonia is a by product of the catabolism (break down) of proteins (which are made up of individual amino acid groups) as well as some other processes. It can act as both a base and a weak acid. Build up of ammonia in the body can result in severe liver and brain damage.

The urea cycle progresses as follows:



Things to Note About the Urea Cycle

- Each molecule of urea is synthesised from TWO amino groups, the original group in step 1 as well as an amino group from arginine.
- The ornithine produced in step 5 is then used as a reactant in Step 2, forming a continuous cycle.
- Steps one and two occur in the mitochondria, steps three, four and five occur in the cytosol
- Each step requires enzyme catalysis, defects in any of the enzymes can cause defects in this pathway
- The conversion of ammonia to urea requires two molecules of the co-enzyme NAD+. Ammonium may be supplied to the urea cycle by the breakdown of the amino acid glutamate and the enzyme for this reaction requires NAD+ which is converted to NADH. The fumarate that is produced in step 4 is converted to oxaloacetate in a two step process that also requires NAD+.
- This is an energy dependant and energy producing pathway. It requires 3 ATP and it produces 5 ATP from the two molecules of NADH it produces.
- This pathway is linked to the citric acid cycle. The oxaloacetate produced is an intermediate of the citric acid cycle, this is another way protein can be used for energy it is broken down to give ammonium, which proceeds through the urea cycle, producing oxaloacetate which then feeds the energy producing citric acid cycle.

CREATINE

Creatine is a natural mineral obtained when meat and fish is consumed, but also by our bodies combining three amino acids (arginine, glycine and methionine). It is believed an average sedentary person uses about 2 grams creatine per day, however only about 1 gram is replaced as a result of diet. This means the body must therefore synthesis the additional 1 gram.

In terms of the three main energy systems (being ATP/PC; Anaerobic glycolysis; and Aerobic) all three are interrelated, ie. the body will not automatically cut off anaerobic glycolysis system and switch on the aerobic system after three minutes.

Creatine is essential to the ATP/PC system. During muscular activity (ie: ATP --> ADP + P + Energy), the body must re-establish ADP back to ATP in order to continue activity. To do this ADP must combine with phosphocreatine (PC) (ie: ADP + PC ---> ATP + C)

This continues as long as creatine is present. Should creatine be depleted, ATP/PC system will stop producing energy. Therefore ensuring a stock of creatine in the body ensures the continuance of energy, and also delays the onset of fatigue. The idea of creatine supplements in Australia is relatively new, but early indications show that supplements will optimise the availability of creatine prior to intense exercise. It will allow extended periods of training without early onset of fatigue. In simple terms this can be translated as 'the longer you can train at your max, the greater potential for hypertrophy to your muscles'.

Early signs indicate it would be best suited to sports like weightlifting, tennis, boxing, and volleyball - all sports that involve explosive actions. Body weight increases have been recorded with creatine use. This is believed to be the result of creatines ability to increase intracellular water content. It is believed this characteristic is important for muscle hypertrophy and therefore creatine may assist strength and other individuals who are looking to 'bulk up'.

It is also important to note other research carried out has not shown any benefits. Further research in the future will help clarify the significance of this type of supplement for energy.

ENERGY

Energy is the capacity to do work. Work is the transferring of a force over a distance (ie. Work = force X distance). Power is a measure of work carried out per unit of time.

Efficiency = <u>Work Output</u> X 100 Energy Input

The Nature of Energy

- Energy can not be created or destroyed, it simply changes form.
- All energy in a person's body is derived from food.
- Chemical energy is a most important form of energy for man.

Units of Measurement

Work and power are measured as: -Kilopond metre (ie. Kpm) or -Watts (NB: 1 kilopond = 1 kilogram)

Power is measured as work per unit of time (eg. kilopond metre per minute)

Energy is expressed as:

- Kilocalories (kcal) or calories (1 kcal = 1000 calories)
- Kilojoules (kJ) or Joules (1 kJ = 1000 Joules)
- Litres of oxygen per minute (1L O₂/min = 5 k/cal approximately)

To convert between joules and calories:

- 1 Joule = 0.24 Calories
- 1 kJ = 240 calories
- 1 Calorie = 4.19 Joules
- 1 kcal = 4190 Joules

PRODUCTION AND STORAGE OF ENERGY

Adenosine triphosphate is a particularly important chemical for storing energy in the human body. When one mole of ATP breaks down into ADP and free phosphorus; 7.5 k/cal of energy is produced. The ATP PC store of energy is only good for a maximum of 10 seconds. ATP and PC cannot be transported, it must be produced and used in the same location. Muscle cells are the main storage sites for these chemicals, but all cells are able to store them.

Sugars (glucose or glycogen) are absorbed into the blood (ie. blood sugars), and transported to various parts of the body (particularly muscles and the liver). In most cases (except nerve tissues), the enzyme "insulin" is needed to facilitate the transfer of these sugars through the cell walls (eg. inside muscle cells).

Other chemicals (ie. Phosphatises and hexokinases) are also needed to facilitate transfer of

sugars through the cell wall. Glucose is not stored, but can change to glycogen, which is stored (NB: Glycogen is essentially a string of glucose molecules linked together by single phosphorus atoms).

Enzymes

Enzymes are essential for most biological reactions. Without them some reactions would go very slow, some would stop all together and others would proceed in the wrong direction. Enzymes themselves are large globular proteins that facilitate a reaction, without being themselves altered in the process. This makes them biological catalysts. Enzymes have an optimum pH range, and are less efficient if the pH drops or increases. Because of this pH problem, the enzyme controlled reactions that produce energy are self limiting. They can proceed for about 40 seconds, and then production of lactic acid causes the pH to drop to a level where the functioning of the enzymes slows. They are also sensitive to temperature.

RESPIRATION

Respiratory Quotient

This is a measure of the relationship between carbon dioxide produced and oxygen consumed.

- If you are metabolising fats the respiratory quotient = 0.7
- If you are metabolising glucose, the respiratory quotient is 1.0
- If you are metabolising protein, the respiratory quotient is 0.8

This means; if you are metabolising fat, you need more oxygen than if metabolising glucose.

Resting Quotient

When resting it is normal for the body to be metabolising a combination of both fat and glucose. If the body is metabolising 66% fat and 33% glucose, the respiratory quotient would be 0.8

Aerobic Capacity

This is determined by measuring a person's ability to extract and utilise oxygen. This measurement is called " O_2 uptake" (ie. VO_2 max). People can utilise fat deposits when energy consumption is less than 50% of VO_2 max. Energy must come from anaerobic sources when intensity is high. Any running in excess of approximately 1 mile tends to be dominated by aerobic metabolism. The energy required for activity is determined by not just duration of exercise, but also the intensity.

What Happens During Exercise

As exercise starts:

- Heart rate and respiration rate increase
- VO₂ increases
- Lactate initially increases
- RQ drops at first, then increases

As intensity of exercise increases, the use of fat decreases; and at 50% VO₂ max, fat use can reach zero.

RECOVERY FROM EXERCISE

Metabolic pathways involved in the transition from exercise to rest are different to those involved from rest to exercise.

The purpose of this recovery phase is to return the body to its pre exercise condition. To do this the following needs to be achieved:

- Replenish energy stores depleted by exercise.
- Remove any build up of lactic acid (ie. lactates).

As exercise slows:

- Energy demand decreases
- Oxygen consumption continues at a higher than normal level for a period ("Oxygen debt" is a term used to describe any excess of oxygen consumed during recovery which is over that normally consumed at rest)
- For the first 2 or 3 minutes following exercise there can be a high rate of oxygen consumption. After this oxygen consumption declines slowly to near pre exercise levels.

Oxygen Debt

Oxygen debt is not simply oxygen required to replace oxygen stores which were used during exercise. In fact very little oxygen from the muscles needs replacing. It is the sources of energy (ie. ATP, PC and glycogen) which need to be replenished, and oxygen is needed to fuel this replenishment.

Alactacid oxygen debt component

This refers to the rapid breathing phase in perhaps the first 2 or 3 minutes following exercise. During this period the oxygen debt is used to replenish muscular stores of ATP and PC. The alactacid debt may range from 2 to 2.5 litres.

Lantacid oxygen debt component

This is the slower phase of recovery where oxygen consumption gradually decreases. Lactic acid is removed in this phase. This phase is about 30 times slower than the alactacid phase, because it takes longer to metabolise lactic acid and restore phosphagens. Light exercise will however speed up this phase.

Replenishing Muscular Glycogen

This was once thought to occur during the lantacid oxygen debt period, but that is largely untrue. Muscle glycogen can take at least 2 days to resynthesise, and is affected largely by diet. Muscle glycogen may increase to more than double normal levels by firstly depleting stores through strenuous exercise and a low carbohydrate diet; then following that exercise with rest and a high carbohydrate diet.

Lactic Acid

About 10% of lactic acid removed during recovery is converted to glucose and 75% is oxidised through the oxygen system to produce CO_2 and O_2 . The remainder is unaccounted for. A gradual cooling down following exercise (rather than abrupt stop) will eliminate lactate. Excessive lactate can cause pain in the muscle and affect blood pH.



SELF ASSESSMENT

Perform the self assessment test titled 'Test 2.1', Test 2.2 and 'Test 2.3'. If you answer incorrectly, review the notes and try the test again.

SET TASK

1. Do either A or B. You will need someone to assist you, first to monitor you, then to

undertake the exercise themselves. If they are below average health ensure they have a medical certificate before proceeding. Do not have someone perform the test if they have cardiac or respiratory problems.

Rockport Walking Test

You will need access to a treadmill with a distance reader, and if possible a heart rate monitor and speed reader (km/h).

Walk as fast as you can, without breaking into a run or jog, but not so fast you have to pause before you complete the full 1 mile/1.61km distance. Make a note of how long it takes you to complete the distance and also take your heart rate as soon as you finish.

During the test you also need to monitor changes every 2-3 minutes (in breaths per minute) during the exercise. Develop a graph which shows a relationship between energy cost (or use - oxygen is required to obtain energy in the body) and rate of exercise or work. This information will be used in your assignment.

Calculate the VO2 max using the following formula:

VO2 max = 132.853 - (0.1692 x weight of person in kilograms) - (0.3877 x age of person in years) + (6.315 x gender of person*) - (3.2649 x time taken in minutes) - (0.1565 x heart rate at the end of the test)

* for females, gender is given the value 0, for males, the value is 1

Now, have your friend undertake the same activity, monitor them as described above and remember to record the age and weight of subject.

Cooper Test

You will need access to a treadmill with a distance reader

For 12 minutes run as fast as you are able on the treadmill. At the end of the test, you can calculate your VO2 max using this formula:

VO2 max = (the distance you covered in metres in the 12 minutes – 505) divided by 45.

During the test you also need to monitor changes every 2-3 minutes (in breaths per minute) during the exercise. Develop a graph which shows a relationship between energy cost (or use - oxygen is required to obtain energy in the body) and rate of exercise or work. This information will be used in your assignment.

Now, have your friend undertake the same activity and monitor them and calculate their VO2 max.

Use an ergo bike that reads workload based on rpm

First do a 3 minute warm up such as walking.

Set the machine at a constant rev (use a low rev range such as 45rpm).

Ride the bike at 45rpm for at least 10 minutes monitor changes every couple of minutes (breaths per minute) and any other changes you notice

Get off the bike and sit down (or stop pedalling).

Take one minute heart rate readings after the first minute, second minute and third minute after sitting down. (ie at one minute take a heart reading over one minute; at two minutes take another one minute reading, and do the same at the third minute). Alternatively, take a 15 second reading then multiple that figure by 4 to get the beat/minute figure.

Add those three beat per minutes together.

Divide this figure by 2.

This figure is known as the Recovery Index. It is used as a Heart Rate Recovery Benchmark. It is less technical than VO2, but is considered more accurate at estimating recovery.

- 2. Resource file. Add to your resource file on the following topics:
- Professional competency resources in your area (how will you maintain your competency when you have finished this course?)
- Sources of industry legal advice in your area



ASSIGNMENT

Download and do the assignment called 'Lesson 2 Assignment'.