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A STUDY OF BIOCHEMICAL AND HAEMATOLOGICAL VARIABLES WITH PERFORMANCE IN SHOWJUMPING HORSES

By

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Bachelor of Science (Human Biology)

EDITH COWAN UNIVERSITY

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DEFINITIONS

ADP : Adenosine Diphosphate

Aerobic Glycolysis : The complete breakdown of glucose into carbon dioxide and water, via pyruvic acid: a process that yields large amounts of ATP over a long period of time but requires the presence of mitochondria and oxygen. This is also called aerobic respiration.

Aerobic Threshold: The workload during an incremental exercise test where blood lactate increases to approximately 2 mmol/L.

Anaerobic Glycolysis : The cytoplasmic breakdown of glucose into lactic acid without the presence of oxygen. This energy system yields small amounts of energy supplied very quickly.

Anaerobic Threshold : The point during an incremental exercise test where the subject's ventilation and lactate levels show a non-linear increase with time.

ATP : Adenosine Triphosphate. A high energy compound used by the cells for energy.

CK : Creatine Kinase.

EDTA : Ethylenediaminetetraacetic Acid - an anti-coagulant.

FADH : Flavin Adenine Dinucleotide - a compound used for the transportation of protons in the aerobic energy process of oxidative phosphorylation.

GTP : Guanosine Triphosphate - a high energy compound similar to ATP.

Hb : Blood haemoglobin concentration, measured in grams per litre.

HCT : Haematocrit - the percentage of the volume of whole blood contributed to by cells: it is also called packed cell volume.

HR : Heart rate - measured in beats per minute.

HR_4 : The heart rate of a horse during an incremental exercise test where blood lactate is 4 mmol/L.

MCH :	Mean cell haemoglobin - this is a measure of the mean levels of haemoglobin within each red blood cell. This is measured in picograms.
MCHC :	Mean cell haemoglobin concentration - a measure of the mean concentration of haemoglobin within each red blood cell.
MCV :	Mean cell volume - a measure of the mean volume of each red blood cell.
mmol/L :	millimoles per litre
NADH :	Nicotinamide adenine dinucleotide. This is a proton carrying molecule used in the aerobic energy process of oxidative phosphorylation.
OBLA :	Onset of blood lactate accumulation.
RBC :	Red blood cells.
V_4 :	The velocity during an incremental exercise test corresponding to a blood lactate concentration of 4 mmol/L.
VO_2 max :	The rate of oxygen usage during maximal aerobic metabolism.
WBC :	White blood cells.

ABSTRACT

This honours project examined the biochemical and haematological changes induced by exercise in the showjumping horse to determine whether a relationship exists between these values and performance. To complete this study 7 geldings and 1 stallion who were considered competition fit by their trainers were subjected to 2 forms of exercise. The first phase of this study involved an incremental exercise test performed at the Byford City Council horse training facility. The horses were cantered/galloped around a 1300m all weather training track at velocities of 6, 8, 10, and 12 m/sec for a duration of 2 minutes for each workload. Blood samples were collected from the jugular vein at rest, 0, 3 and 5 minutes post exercise. A further sample was collected at 24 hours post for the determination of peak creatine kinase levels. Five of the 8 horses used in this study completed this test. In the second phase of this study the horses a) completed a competition standard showjumping course with no jumping efforts and then b) the horses completed the same course with the jumps placed in position. At the completion of each stage, blood was collected at 1, 3, and 5 minutes post. A further sample was also collected at 24 hours for analysis of peak CK levels. It was found that incremental exercise caused significant increases in heart rate, plasma lactate, RBC, HCT, Hb, and WBC values. Completion of the jump course produced increases in HR, plasma lactate, RBC, HCT, Hb, and MCH. There were no relationships observed between showjumping performance and the physiological changes induced by the jump test. With respect to the incremental exercise test, trends were observed between rates of lactate production and showjumping performance. A trend was also found between RBC values and showjumping performance, with the higher graded horses producing lower RBC values than the other horses. The plasma lactate values obtained from this study were lower

than those previously reported in the thoroughbred racehorse following comparable exercise. This suggests that the differences in training programs between these groups of horses cause a change in the rate of lactate production for individual horses. Following jumping, the lactate values obtained during the present study were significantly lower than values reported from European horses competing at similar levels. This may suggest some variation in skeletal muscle fibre types among different breeds of horses.

DECLARATION

" I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text."

Signature

Date: 10/1/97

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CHAPTER 1

INTRODUCTION

1.1- BACKGROUND

Showjumping is a sport which requires a large degree of acceleration and power type activities. To be successful in this sport, the performance horse must possess the ability to accelerate out of tight corners and power over jumps reaching heights of upto 1.6m. Although the successful showjumping horse is a highly skilled and trained animal, the basic ability to accelerate out of tight corners and power over large fences is, to a certain extent, determined at birth. The parameters of acceleration and power are governed by the type and functionality of each animal's skeletal muscle.

Mammalian skeletal muscle is divided into two major categories depending on its biochemical and contractile characteristics. Muscle fibres of the type I variety are slowly contracting and use predominantly aerobic energy pathways to obtain energy. Muscle fibres of the type II variety, are faster contracting and mainly use anaerobic glycolysis to obtain energy, with the production of lactate as a bi-product. Skeletal muscle fibre types exist in a ratio within each muscle group. The ratio of type I to type II fibres is determined at birth and, although each specific fibre type can be developed through training, the ratio of fibres will always remain the same. It is this ratio of fibre types that determines whether an individual is a "sprinter" or a "stayer". Individuals containing a large percentage of type II fibres will be best suited for sports consisting of short term, high intensity exercise, whereas individuals possessing a large percentage of type I fibres will be better suited for activities involving longer duration exercise (Farrell, Wilmore, Coyle, Billing and Costill, 1979; Ivy, Withers, Van Handel, Elger and Costill, 1980).

This relationship provides exercise physiologists with the basis for methods of identifying performance potential in young athletes. The percentages of skeletal muscle fibre types within a particular muscle can be determined by collecting a muscle sample (muscle biopsy) and directly counting the different fibres with the aid of special stains (Myosin ATPase stains). However, this method is invasive and not practical in the field situation.

The major energy source of the type II muscle fibre is anaerobic glycolysis (Meyer and Terjung, 1979). This energy system is capable of providing the working muscle with large amounts of energy over a short period of time. (Astrand and Rodahl, 1986 pg 527 - 537). The major bi-product of this energy system is lactic acid. During exercise, lactate is produced within the muscle, it then diffuses out of the cell into the interstitial fluid and into the blood. Therefore the concentration of lactate in the blood is measured, providing valuable information about the energetics of the working muscles (Wasserman, Whip, Koyal and Beaver, 1973; Kindermann, Simon and Keul, 1979; Skinner and McLellan, 1980).

Numerous studies have been conducted involving exercise and plasma lactate concentrations (Asheim, Knudsen, Lindholm, Rulcker and Saltin, 1970; Krzywanek 1973; Wasserman, Whip, Koyal and Beaver, 1973, Milne, Muir, Skarda, 1975; Farrell, Wilmore, Coyle, Billing and Costill, 1979; Ivy, Withers, Van Handel, Elger and Costill, 1980; Jacobs, Schele and Sjodin, 1985) and it has been proven that sprint trained athletes (humans and horses) produce the largest concentrations of lactate. In addition a correlation has been shown to exist between plasma lactate and the percentage of type II

fibres within a muscle (Farrell, et al. 1979; Ivy et al. 1980; Ohkuwa, Kato, Katsumata, Nalao and Miyamura, 1984).

1.2- SIGNIFICANCE OF THE STUDY

Showjumping is an international sport which supports a multi-million dollar industry throughout the world. For many years, millions of dollars have been invested into the selection and development of young animals in an attempt to find the champion showjumping horse. Although it is widely recognised that family history is a major factor influencing the performance potential of young horses, there has been limited scientific research into this area. This project will address the paucity of literature in this field.

At present, the selection of young horses for showjumping is based on conformation, temperament and breeding. Although there is no doubt that these factors are vital for the selection of the animal, the ratio of Type I to Type II muscle fibres will also impinge on the showjumping potential of the animal. In addition to the methods currently employed to select young animals, a simple sub maximal exercise test to examine the lactate kinetics of the horse would undoubtably benefit individual trainers by providing them with specific information on performance potential. Using this information as a foundation, training programs could be developed and fitness could be more readily monitored. Further, trainers would circumvent the investment of a large amount of time in training only to discover that a particular animal might lack the appropriate performance ability, i.e. accelerating out of tight corners and subsequently powering over large jumps.

1.3- OBJECTIVES OF THE STUDY

The objectives of this study were to determine how well biochemical and haematological variables correlated with performance in the showjumping arena as a gauge of athletic potential in horses thus addressing a paucity of scientific data in this area. Further, it was hoped that this work would provide a foundation to develop a regime that can be used for screening potential animals for showjumping. This study also examined the effect of training procedures in relation to performance in the showjumping horse.

1.4- RESEARCH QUESTIONS

- Are lactic acid levels and the anaerobic threshold of showjumping horses affected by training?

- How do lactic acid levels and the anaerobic threshold compare with performance in showjumping horses?

- Do haematological and biochemical values vary between showjumping horses at different levels of competition?

- Is there a relationship between biochemical and haematological values with performance in showjumping horses?

1.5-HYPOTHESES

- Lactate levels and the anaerobic threshold correlate with level of competition.

- The anaerobic threshold and lactate production are affected by method of training in showjumping horses.

- Haematological and biochemical variables do vary between standardised horses at different levels of competition.

- There is a relationship between level of competition and haematological and biochemical values.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 INTRODUCTION

In order for skeletal muscles to contract, chemical energy in the form of adenosine triphosphate (ATP) must be converted into mechanical energy. During exercise skeletal muscles must contract more rapidly and with greater force than during sedentary conditions (Astrand and Rodahl, 1986 pg 311). As a result, the energy demands of the body are increased, and consequently, energy production must be increased to maintain continued muscle contraction (Guyton, 1991 pg 941-942). The duration and intensity of exercise is the major factor controlling the biochemical processes used by muscles to generate the required amounts of energy (Astrand and Rodahl, 1986 pg 535-537). In addition, the type and functionality of different skeletal muscle fibre will influence the major energy pathways used (Meyer and Terjung, 1979; Saltin and Gollnick, 1983).

During exercise of differing styles, the skeletal muscles must contract with varying rates and force in order to achieve the exercise goals. For example, activities involving large amounts of acceleration and power movements require high rates of muscle contraction with maximal muscular force being generated as quickly as possible (Astrand and Rodahl, 1986 pg 299-303, 535-537). It is the type and functionality of the working skeletal muscles adapting to specific exercise styles which will determine the success or otherwise of animals competing in sporting events.

Although this thesis is centred around the exercise physiology of the showjumping horse, there is a scarcity of literature in this area, with the majority of research being conducted on human athletes and the data extrapolated for equine use. As a result, this review of the literature will incorporate a range of studies concerning the human athlete, the thoroughbred racehorse as well as the showjumping horse.

2.2 ENERGY PRODUCTION

During exercise working muscles require energy for contraction. The methods of obtaining energy by muscle cells differ, depending on the intensity and duration of exercise (Astrand and Rodahl, 1986 pg 535-537). If exercise is of light intensity and long duration the energy demands of the working muscles are able to be met by energy production involving aerobic pathways (the breakdown of glucose, fatty acids or amino acids in the presence of oxygen) (Guyton, 1991 pg 941-942; Lehninger, Nelson and Cox, 1993 pg 400-405). However, if exercise is of high intensity and short duration, then the muscle's energy demands must be met predominantly by anaerobic energy production (Guyton, 1991 pg 941-942; Lehninger, Nelson and Cox, 1993 pg 400-405).

2.2.1 ANAEROBIC ENERGY PATHWAYS

Anaerobic energy pathways consist of two major systems. These are the phosphagen system and anaerobic glycolysis (Guyton, 1991 pg 941-942). The phosphagen system involves the breakdown of the high energy phosphate bonds contained in the ATP stored within the skeletal muscle. Each of these phosphate bonds stores approximately 7300 calories of energy per mole of ATP. Within the ATP molecule three of these high

energy phosphate molecules are stored (Guyton, 1991 pg 941; Lehninger, Nelson and Cox, 1993 pg 374). Although this energy system is able to supply the muscle with immediate energy, the amount of intramuscular ATP in mammals is sufficient to sustain maximal muscle power for only about three seconds. In addition to the ATP stored within the muscle cell, another high energy phosphate compound, creatine phosphate, is also present and is used to generate energy. The high energy phosphate bond contained within the creatine phosphate molecule will release 10,300 calories per mole when broken down (Guyton, 1991 pg 942). This energy is used within the muscle cell to reconstitute the ATP molecule which is used for energy by the muscle cell. The energy transfer from creatine phosphate to ATP occurs within a fraction of a second. In effect, all the energy stored within the muscle is instantaneously available for muscular contraction (Astrand and Rodahl, 1986 pg 527-530; Guyton, 1991 pg 942). The combined energy reserves of phospho-creatine and ATP within the muscle are collectively known as the phosphagen energy system. This energy system produces ATP at a rate of 4 moles per minute which is enough energy to maintain maximal muscular power for approximately 8 to 10 seconds (Guyton, 1991 pg 942). As a result, except for a few seconds at a time, it is essential that new ATP be formed continuously through the process of anaerobic glycolysis (Astrand and Rodahl, 1986 pg 527-530; Guyton, 1991 pg 942).

The stored glycogen within the muscle can be converted into glucose, the glucose then being available as an energy source (Guyton, 1991 pg 942). The initial stage of the degradation of glucose to generate energy is termed anaerobic glycolysis. The process is termed anaerobic as it occurs entirely without the use of oxygen. During glycolysis each glucose molecule undergoes a series of enzyme catalysed reactions and is split into two

molecules of pyruvate (Lehninger, Nelson and Cox, 1993 pg 400-405). The breakdown of glucose into pyruvate causes the release of energy from the bonds within the glucose molecule. This energy is then used for the resynthesis of the ATP molecule which can be used to fuel muscular contraction. Under anaerobic conditions, most of the pyruvate produced by glycolysis is converted into lactic acid which diffuses out of the cell into the interstitial fluid and into the blood where it is either buffered by HCO_3^- ions or it is broken down by the liver and the heart (Stainsby and Brooks, 1990; Weltman, 1995 pg 17). The concentration of lactate in the blood is a commonly used indicator of the rate of anaerobic glycolysis. Anaerobic glycolysis can produce 4 molecules of ATP from 1 molecule of glucose at a rate of 2.5 moles of ATP per minute (Guyton, 1991 pg 942; Lehninger, Nelson and Cox, 1993 pg 400-405)

2.2.2 Aerobic Energy Pathways

Only a brief description of the aerobic pathways will be given as this project relates mainly to the processes involved in anaerobic respiration and lactate production.

In the presence of oxygen, glucose is oxidised and broken down to yield large amounts of continuous energy. Aerobic respiration can be divided into three major phases. Phase 1 is very similar to anaerobic respiration, however, in the presence of oxygen, pyruvate is broken down into two molecules of acetyl Co A. In the second phase of aerobic respiration acetyl Co A enters the citric acid cycle and, via a number of enzyme catalysed reactions, is converted into oxaloacetate. At the completion of the citric acid cycle, 3 molecules of NADH, 1 molecule of $FADH_2$ and 1 molecule of GTP (a high energy phosphate molecule) are produced. The molecules of NADH and FADH2 enter

the third phase of aerobic respiration, oxidative phosphorylation. In this process, NADH and $FADH_2$ are oxidised to yield energy which is used to resynthesise ATP which is then used for muscular contraction (Astrand and Rodahl, 1986 pg 530-533; Guyton, 1991 pg 942; Lehninger, Nelson and Cox, 1993 pg 446-447). Aerobic respiration can produce a total of 38 molecules of ATP from the complete oxidation of 1 molecule of glucose at a rate of 1 mole of ATP per minute (Guyton, 1991 pg 942; Lehninger, Nelson and Cox, 1993 pg 446-447).

At low exercise intensities, the energy demands of the working muscles are low, and the aerobic energy system is capable of totally supplying the muscle with ATP. However, as the exercise intensity increases, the metabolic demands of the muscles exceed the capacity of the aerobic system and therefore, anaerobic glycolysis must supplement the aerobic system (Stainsby, 1986). The major cause of the limitations of aerobic metabolism seem to be a failure to supply the working muscles with adequate amounts of oxygen. In this situation, glycolytic rate is increased and aerobic respiration is decreased. The resulting imbalance causes an increased formation of lactate (Astrand and Rodahl, 1986 pg 530-533; Stainsby, 1986; Guyton, 1991 pg 941-942 Weltman, 1995 pg 16-18).

2.3 SKELETAL MUSCLE ANATOMY AND BIOCHEMISTRY

In mammalian skeletal muscle there are two major fibre types(type I and type II). All skeletal muscles are composed of a mixture of these fibres and each fibre type has its own contractile and metabolic characteristics (Close, 1972 pg 130-134; Meyer and Terjung, 1979; Guyton, 1991 pg 945; Glenmark, 1994). Type II fibres have a short time to peak tension whereas type I fibres have a long time to peak tension (Close, 1972 pg 130-134; Astrand and Rodahl, 1986 pg 33-36). Therefore, those muscles that contract very rapidly are composed mainly of type II fibres with only small numbers of the type I variety. Conversely, the muscles that respond slowly but with prolonged contraction are composed mainly of fibres of the type I variety (Meyer and Terjung, 1979; Guyton, 1991 pg 945; Glenmark, 1994). For human skeletal muscles, there are studies indicating that the time to peak tension in a maximal isometric contraction is 80 - 100 ms for type I fibres and about 40 ms for type II fibres (Saltin and Gollnick, 1983).

The differences between contractile properties of type I and type II fibres can be attributed to variations in individual cell biochemistry and energetics. Type I fibres are characterised by low actomyosin ATPase activity, low glycolytic and high respiratory activities (Close, 1972 pg 130-134; Meyer and Terjung, 1979; Glenmark, 1994). That is, these fibres contract slowly and use predominantly aerobic pathways to obtain energy. Type II fibres can be sub-divided into type IIa and type IIb fibres. Type IIa fibres are characterised by high ATPase activity, high glycolytic activity and a low respiratory capacity. Type IIb fibres are characterised by high ATPase activity, high glycolytic activity and a high respiratory capacity (Close, 1972; Meyer and Terjung, 1979; Astrand and Rodahl, 1986 pg 33-36; Glenmark, 1994). Therefore, type IIa fibres are the fastest

contracting and use predominantly anaerobic glycolysis for energy production. Type IIb fibres are fast contracting and use both anaerobic and aerobic energy pathways (Close, 1972 pg 130-134; Meyer and Terjung, 1979; Astrand and Rodahl, 1986 pg 33-36).

As a result of differing energy and contractile characteristics, the rate of lactate production within a muscle fibre is dependent upon its fibre type. Type I fibres produce the lowest amounts of lactate due to low glycolytic capacities and type IIa fibres produce the greatest amount of lactate due to high anaerobic capacities (Farrell, Wilmore, Coyle, Billing and Costill, 1979; Ivy, Withers, Van Handel, Elger and Costill, 1980; Jacobs and Kaiser, 1982; Ohkuwa, Kato, Katsumata, Nakao and Miyamura, 1984; Gollnick, Bayly, and Hodgson, 1986; Jacobs, 1986). The maximal rate of lactate production in human muscle is approximately 0.5 μmol/gram wet weight/second for muscle composed primarily of type II fibres, whereas it is about half that for muscle composed of type I fibres (Gollnick, Bayly and Hodgson, 1986).

Despite the strong correlation between muscle and blood lactate concentrations observed during exercise, it is incorrect to assume that blood lactate concentrations are directly reflective of muscle lactate production (Weltman, 1995 pg 16). The total blood lactate concentration measured during or following exercise is the result of the balance between adding lactate to the blood (as a result of anaerobic glycolysis within the muscle) and the removal of lactate from the blood (Stainsby and Brooks, 1990; Weltman, 1995 pg 16). It has been reported (Stainsby and Brooks, 1990) that the major sites of lactate clearance from the blood are the liver, the heart and skeletal muscles. Lactate is broken down by the process of gluconeogenesis and converted into glycogen and is re-used for energy. As a result, it must be noted that when measuring blood

lactate, the time taken for the lactate to diffuse from the muscle into the blood (peak value) before it is broken down by the liver, heart and skeletal muscle should be calculated and used in experiments to ensure true lactate concentrations are reported.

2.3.1 Muscle Metabolic Characteristics and Exercise

In 1936, Bang first pointed out that during incremental, non-steady state exercise there is a phase where the metabolic demands of the working muscles cannot be satisfied by the aerobic energy systems. As a result, the energy demands of the body are met by anaerobic respiration which is noted as an increase in blood lactate levels. Subsequently, an anaerobic threshold concept was introduced by Wasserman, Whip, Koyal and Beaver (1973) in order to define the point when metabolic acidosis, and its associated changes in gas exchange in the lungs, occur during incremental exercise. It was also noted by Wasserman et al. (1973) that during incremental exercise a point is reached at which the subject's ventilation begins to show a non-linear increase with time. This has been termed the ventilatory threshold by Walsh and Banister (1988). In concordance with the ventilatory threshold, a non-linear increase in blood lactate concentration occurs which is termed the lactate threshold (Ryan, Joiner and Ryan, 1976; Kindermann, Simon and Keul, 1979; Skinner and McLellan, 1980; Aunola et al. 1988; Walsh and Banister, 1988; Weltman et al. 1990). Together, the ventilatory and lactate thresholds form what is termed the "anaerobic threshold" (AT).

During the transition from low to maximal work intensities during incremental exercise, three phases and two thresholds for blood lactate levels have been reported (Kindermann, Simon and Keul, 1979; Skinner and McLellan, 1980) as shown in figure

2.1. During phase 1, the exercise intensity is low and the aerobic energy system is dominant. Minute ventilation and heart rate increase slightly while blood lactate levels remain close to resting; 1.0 to 1.5 mmol/L (Kindermann, Simon and Keul, 1979). The second phase begins when the first threshold, (the aerobic threshold), has been reached. The aerobic threshold occurs when a workload corresponding to approximately 2 mmol/L of lactate is reached. In theory, the exercise should be able to be sustained at this intensity for 3 to 4 hours (Kindermann, Simon and Keul, 1979; Skinner and McLellan, 1980). As the intensity of exercise continues to increase, the second phase is reached. This phase incorporates an aerobic to anaerobic transition. Blood lactate levels approach approximately 4 mmol/L with heart rate and oxygen consumption also increasing. At the completion of the second phase, the anaerobic threshold is reached. The third phase of incremental exercise consists mainly of anaerobic respiration. During this phase, blood lactate levels rise in an exponential manner with heart rate and oxygen consumption approaching maximum (Kindermann, Simon and Keul, 1979; Skinner and McLellan, 1980).

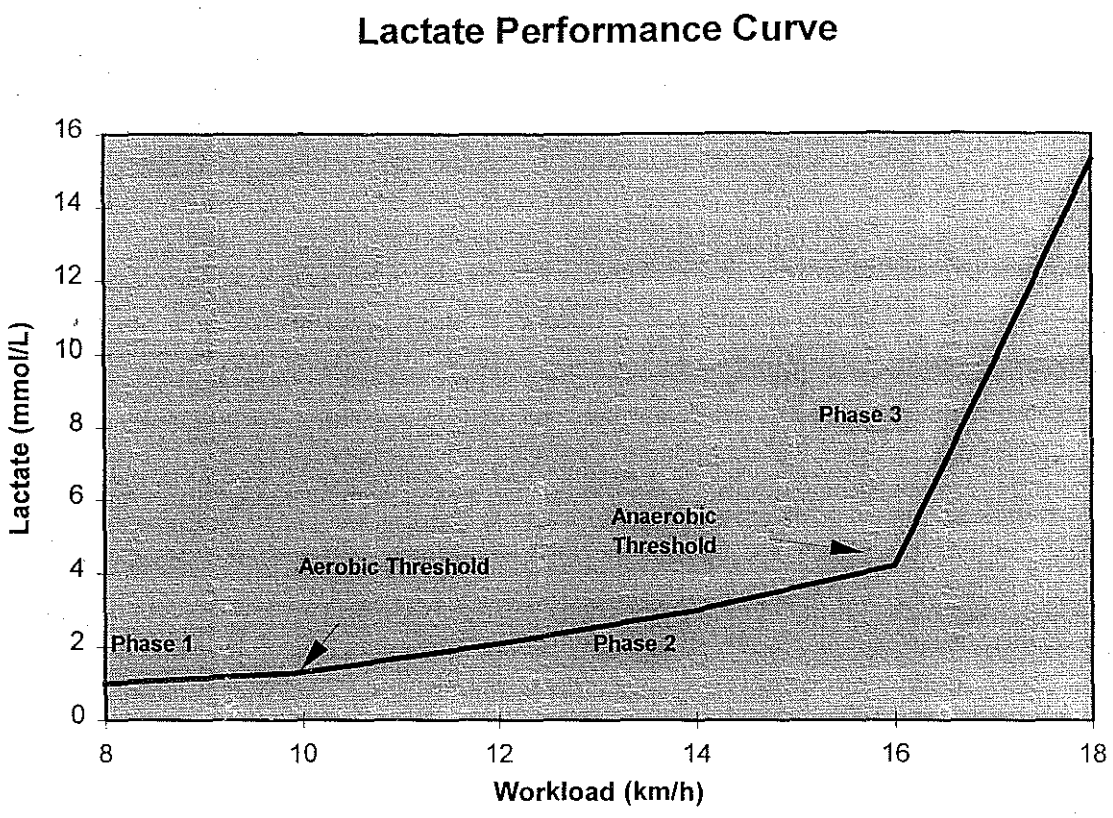

Figure 2.1 - A typical lactate performance curve showing the 3 phases and 2 thresholds observed during the transition from low to high exercise intensities (Adapted from Kindermann, Simon and Keul, 1979, Skinner and McLellan, 1980; Jacobs, 1986)

The mammalian anaerobic threshold has been determined by many authors using various methods, all centred around the measurement of glycolytic respiration. In 1973, Wasserman, Whip, Koyal, and Beaver found that during an incremental exercise test, the volume of oxygen consumed by the working muscles remains linear when plotted against workload. A "threshold" is reached when oxygen consumption increases in an exponential manner. These authors have defined this deflection as the anaerobic threshold (A.T.). Other authors have used the concentration of blood lactate as a measure of the anaerobic threshold. In 1976, Ryan, Joiner and Ryan determined the anaerobic threshold by the work rate or time just before the initial departure from the linearity of the lactate linear regression residuals. Davis, Bassett, Hughes and Gass

(1983) defined the anaerobic threshold as a sudden sustained rise in lactate concentrations over resting levels.

During exercise it has been shown that the concentration of lactate in the muscle and blood is lower at the same work intensity in endurance trained persons when compared to untrained individuals (Gollnick, Bayly and Hodgson, 1986; Aunola et al. 1988; Mognoni, Sirtori, Lerenzelli, Cerretelli, 1990). When lactate performance curves are plotted for endurance trained and non-trained persons, the resulting curve of a trained individual is below and to the right of the non-trained person (Gollnick, Bayly and Hodgson, 1986; Aunola et al. 1988; Weltman et al. 1990) as shown in figure 2.2. Conversely, if lactate performance curves are plotted for sprint trained individuals, the resulting curve will be above and to the left of the non-trained subject (Jacobs, 1986; Cadefau et al. 1990) as shown in Figure 2.2.

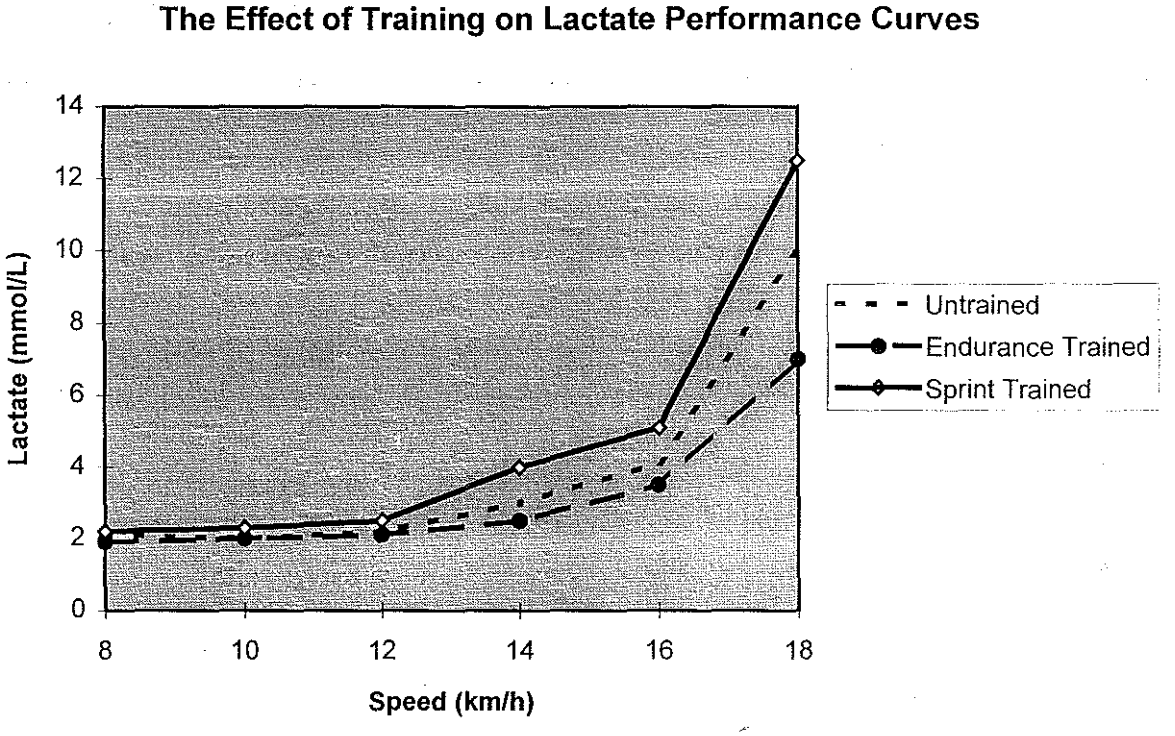

Figure 2.2 - The effect of different training methods on lactate production. (Adapted from Hermansen and Stensvold, 1972; Kindermann et al., 1979; Jacobs, 1986)

Lactate performance curves and the anaerobic threshold have been shown by several authors to be a valuable tool in the prediction of endurance performance (Wasserman, Whip, Koyal and Beaver, 1973; Ivy, Withers, Van Handel, Elger and Costill, 1980; Jacobs, Schele and Sjodin, 1985; Mognoni, Sirtori, Lorenzelli and Cerretelli, 1990; Weltman et al. 1990). It has been suggested that the characteristics of the working musculature such as the percentage of type I fibres, the capillary density and the respiratory capacity of the working muscle correlate highly with an onset of blood lactate accumulation (OBLA) (Jacobs, 1986).

A study conducted by Farrell, Wilmore, Coyle, Billing and Costill (1979), investigated the relationship between blood lactate accumulation, VO_2 max and distance running performance. In this investigation muscle fibre composition (expressed as percent of type I fibres), VO_2 max, and anaerobic threshold were determined for eighteen male distance runners. The results of this study were significantly correlated ($r > 0.91$) to performance at all race distances examined (3.2, 9.7, 15 and 19.3 km). The authors concluded that the runner who had the highest percentage of type I fibres would produce the least lactate, have the highest VO_2 max, and would therefore have the fastest race time. Similar findings have also been reported by Hagberg and Coyle, (1983), Fay, Londeree, LaFontaine and Volek, (1989), and Coyle et al. (1991).

Ivy, Withers, Van Handel, Elger and Costill, (1980) examined the relationship of fibre type with lactate production and performance. In this study, lactate thresholds were calculated for thirteen male volunteers using an incremental exercise test (on a cycle ergometer), which was performed until exhaustion. Muscle biopsies were taken from the subjects' vastus lateralis muscles, and VO_2 max was also calculated. The results of this

study indicated that the percentage of type I fibres was found to be significantly related to the lactate threshold, (r=0.74) terms. When the fibre area was considered, significant positive correlations were found between the percent relative area of type I fibres and the lactate threshold. In addition, positive correlations were noted between the percentage of type I fibres and VO_2 max and percent relative area of type I fibres and VO_2 max. The authors concluded that the percentage of type I fibres was related to both absolute and relative lactate thresholds. This, the authors suggested, would indicate that the ratio of type I to type II fibres which is genetically determined may exert an influence over the lactate threshold and possibly control the range in which the lactate threshold can shift. This could partly explain the high submaximal capacities of elite marathon runners who possess both a high relative lactate threshold and a high percentage of type I fibres. Further studies (Komi, Ito, Sjodin, Wallenstein and Larlsson, 1981; Sjodin and Jacobs, 1981; Aunola et al. 1990) have supported the findings of this study.

In 1984, Ohkuwa, Kato, Katsumata, Nakao and Miyamura examined blood lactate kinetics after 400m and 3000m runs in sprint and long distance runners. The purposes of this study were to test whether peak blood lactate levels could be used as an index of anaerobic work capacity and to examine the differences in running performance between 400 and 3000 metre runners. During sprinting, the mean running velocities of the sprinters were significantly higher than those of the long distance runners and untrained subjects. Further, peak blood lactate values were significantly higher in the sprinters than in the long distance runners and untrained subjects. On analysis of muscle biopsies it was found that the leg muscles of the sprinters contained a higher percentage of type II fibres than the long distance runners and untrained subjects. In the 3000m run,

the long distance runners had a higher mean running velocity than the sprinters and the untrained subjects. The authors found that there was no significant difference in the peak lactate levels of all three groups. The results of this study suggest that athletes whose muscles contain a high percentage of type II fibres will have faster running velocities than athletes whose muscles contain a high percentage of type I fibres. In addition, athletes whose muscles contain high percentages of type II fibres will produce greater volumes of lactate than athletes containing high percentages of type I fibres following short duration, high intensity exercise.

Further support for the relationship between fibre type and fibre type recruitment with blood lactate concentrations has been shown by Ball-Burnett, Green and Houston. (1991). These authors examined energy metabolism in human type I and type II fibres during cycle exercise. During this study it was found that type II fibres produce more lactate during exercise than the type I fibres. The authors therefore conclude that the increases in lactate of the type II fibre are the result of an increased level of anaerobic metabolism.

2.4 LACTATE RESEARCH IN THE HORSE

Research into the exercise physiology of the performance horse is a major contributor to the training and selection of these animals. Research has been conducted into the physiology of the thoroughbred racehorse (Harris, Marlin and Snow, 1987; Valette, Barrey and Wolter; 1991; Harkens, Beadle and Kamerling, 1993), the trotter (Asheim, Knudsen, Lindholm, Rulcker and Saltin, 1970; Gottlieb, Essen-Gustavsson and Lindholm, 1987 pg 384-390), the endurance horse (Rose, Purdue and Hensley, 1977; Rose et al. 1980), but there has been very little studies involving the showjumping horse. This research has been conducted to develop more effective training procedures, to monitor the progress and adaptations to training and as a means of identifying performance potential in young horses.

2.4.1 THE IMPORTANCE OF LACTATE RESEARCH

Equine exercise physiology in the past has closely followed research into the physiology of human athletes. The early works of Asheim, Knudsen, Lindholm, Rulcker and Saltin, (1970), Milne, Muir and Skarda (1975), Anderson (1975) and Snow and Mackenzie (1977) illustrate the relationship between exercise and lactic acid production in the horse. In 1983, Wilson, Isler and Thornton made a significant contribution to the equine literature by establishing that lactate performance curves could be developed on the training track during an incremental exercise test. Following this publication, the use of incremental exercise tests became widely accepted as a useful tool in the research of the equine athlete (Harris and Snow, 1988; Wilson, Isler and Thornton, 1983 pg 487-496; Marlin, Harris and Snow, 1991 pg 188-195; Valette, Barrey and Wolter, 1991 pg 337-

342; Gauvreau, Staempfli, McCutcheon, Young and McDonell, 1995). In addition to the use of incremental exercise tests, other equine researchers chose to employ a one step exercise protocol where the horse was worked for a single bout of exercise or several bouts of exercise at a fixed intensity. Samples were then taken at designated times following the completion of these bouts of exercise (Bayly, Grant and Persson, 1987 pg 426-437; Snow, Harris and Gash, 1985; Harris, Marlin and Snow, 1991 pg 173-178; Harkins, Beadle and Kamerling, 1993; Evans, Harris and Snow, 1993). These tests are designated as "standardised exercise tests" (SETs).

As in humans (as previously discussed), there is evidence that short term high intensity exercise in the horse results in the intramuscular production of lactate (Anderson, 1975; Asheim, Knudsen, Lindholm, Rulcker and Saltin, 1970; Brooks and Gaesser, 1980; Bayly, Grant, and Persson, 1987; Lindholm and Saltin, 1974; Milne, Skarda, Gabel, Smith and Ault et al., 1976; Snow, Harris and Gash, 1985; Harris, Marlin and Snow, 1991 pg 173-178). The rate of lactate accumulation in skeletal muscle and its release into the blood is exponentially related to the rise in oxygen consumption which accompanies an increase in exercise intensity (Bayly, Grant and Persson, 1987). It is based on this relationship that blood or plasma lactate concentration may provide valuable information about changes occurring in the blood and the working muscles during exercise. As a result, it is proposed that the measurement of plasma lactate during and after exercise can be used as an indicator of performance and/or fitness in horses.

2.4.2 Research Involving Standardised Exercise Tests (SETs)

In 1987, Bayly, Grant and Persson undertook studies involving the thoroughbred racehorse. In one study, venous blood samples were collected from 58 thoroughbreds over a 3 month period. Plasma lactate was measured in 183 blood samples taken from these horses following work intensities of 600, 800 and 1000 metres. In addition, blood samples were taken following racing (1000m to 1700m) and a comprehensive history of the animals was recorded. This study showed that mean plasma lactate increased with distance. The authors also concluded that the fastest running animal had the lowest plasma lactates at workloads of 800 meters. However, at a distance of 1000m there was a trend towards higher lactate values in the faster horses. This suggests that the fastest horses produce the most lactate over racing distances of 1000 to 3200m. Despite the relationships reported by the authors of this study between lactate levels and performance, blood sampling times following exercise were not consistent. Blood samples were collected between 5 and 10 minutes post exercise. Lactic acid is quickly buffered by HCO_3^- in the blood and broken down by the heart and liver (Stainsby and Brooks, 1990; Weltman, 1995, pg 17). Therefore lactate concentrations following exercise decrease with time. If comparisons between different groups of horses are made, blood sampling times must be consistent or the results obtained will not be accurate. In addition, the above study was conducted on the track over a period of 3 months. The authors of this study made no comment regarding their methods of standardising the tests.

Another study, conducted by Harkins, Beadle and Kamerling (1993) involved galloping twenty five healthy, race fit thoroughbred racehorses over three distances (1200, 1600 and 2000m). The objectives of this study were to determine how well the measured

variables (lactic acid, maximal oxygen volume uptake, heart rate, and hydrogen carbonate levels) correlated with racetrack performance as a gauge of athletic potential in horses. It was concluded that there is a positive correlation between running speed and the slope of a scatter plot of all the above variables. This suggests that heart rate increases more rapidly for faster horses than for slower horses during exercise of similar intensities. The authors also concluded that faster horses can attain a heart rate of 200 beats/minute at a lower running speed than do slower horses. However, faster horses are able to perform sub-maximal work at lower steady state heart rates than can slower horses. Therefore, an attribute of a successful racehorse is the ability to attain the heart rates mentioned above which may be a useful indicator in the selection of young horses for competition. When heart rate is used as a measure of performance potential, it is important to note that the heart rate of the horse can be influenced by stress or excitement. Therefore, when studies are conducted on the heart rate of the horse, care must be taken to reduce any elevations in heart rate values as a result of stress. The authors of the above study do not mention how this was achieved. Therefore, the heart rate results observed may be due to excitement or stress rather than the physiological changes induced by exercise as reported (Thornton, 1985).

2.4.3 PLASMA LACTATE AND EXERCISE

The correlation of plasma lactate after exercise with performance in thoroughbred racehorses was investigated by Evans, Harris and Snow (1993). These authors tested 26 race fit thoroughbred racehorses. In the first stage of this investigation, the horses were galloped once at maximal pace over 800 metres. Blood samples were taken 2 and 5 minutes after the gallop and analysed for lactate concentrations. The results were then correlated with timeform ratings. A timeform rating is a score out of 100 with the fastest horse having the highest score, with these ratings being updated after every race meeting. The results of this stage of the study, unlike those of other investigators, showed no correlation between blood lactate and performance. The second stage of this study examined the effects of blood lactate levels during a standardised exercise test on an inclined treadmill. Fourteen horses were used in this study which involved exercising at four different velocities ranging from a walk to a canter. Blood samples were collected at 2 and 5 minutes after exercise and analysed for blood lactate levels. The results of this test showed a negative correlation ($r = -0.69$) between blood lactate levels and timeform ratings. In concluding their results, the authors of this study suggested that longer workloads (> 3 mins) and blood samples taken regularly to measure peak levels might improve the correlation of lactate with performance. In addition, the authors of this study compared lactate values obtained from treadmill exercise with racetrack performance, however, no comment was given on the transferability of data obtained from the treadmill with the field situation.

Saibene et al. (1985) also studied the effects of exercise on plasma lactate levels in the thoroughbred racehorse. These authors and Evans, Harris and Snow, (1993) have

demonstrated that faster horses attain higher lactate levels than do their slower counterparts over short distances (1000-2000m). In addition, lactate levels in the faster horses rise more quickly than in the slower horses. Based on these findings it appears that for a thoroughbred racehorse, rapid lactate production to high levels is advantageous for successful competition over distances between 1000-2000m (Harkins, Beadle, and Kamerling, 1993).

2.4.4 Plasma and Muscle Lactate Changes With Exercise

A study conducted by Snow, Harris and Gash (1985) investigated the response of equine skeletal muscle to intermittent maximal exercise. In this study, 4 thoroughbred horses performed 4 maximal gallops over a distance of 620m with 5 minutes rest between gallops. Muscle biopsies and blood samples were taken before, during and after the exercise. On examination of blood lactate levels and the individual performances, the two horses that had the fastest times in the first gallop produced the highest amounts of lactate. However, the times of these animals for the following three gallops decreased more than any of the other horses. In addition, the final lactate values for these two horses were significantly higher than for the other animals (160-180 mmol/kg of dry muscle compared to 90-110 mmol/kg of dry muscle). Conversely, the horse that had the slowest running time in the first gallop (53.1 seconds, or 7.4 seconds slower than the fastest horse) had the fastest running time in the final gallop (50.9 seconds, or 10.7 seconds faster than the 2 horses mentioned earlier). Blood and muscle lactate values for this horse were significantly lower than the three other horses for galloping efforts. The overall changes in lactate levels reported in this study suggest that faster horses have a greater rate of lactate production within the working muscles than do slower horses

during exercise of short duration and high intensity. This study was repeated by Harris, Marlin and Snow (1991) a similar result were reported. When conducting studies involving the collection of muscle biopsy samples before an exercise bout, if the results obtained are to be correlated with performance, attention must be given to the state of the animal during the experiment. If the horse has had a biopsy taken, it will undoubtedly be in pain during the test. It is therefore doubtful whether that animal is performing at a similar intensity to that during a race or competition. Therefore, the results obtained from the study may not be reflective of that animal's true performance potential.

2.4.5 THE EFFECTS OF EXERCISE ON MUSCLE ENERGY RESERVES

In addition to muscle and plasma lactate, Snow, Harris and Gash (1985) investigated the changes in muscle adenosine triphosphate (ATP), inosinate monophosphate (IMP) levels after four gallops. The two fastest horses (J and L) showed the greatest depletions in muscle ATP following gallops 1-4 (22 mmol/kg dry muscle to 10-12 mmol/kg d.m.). Conversely, one horse (S) showed only a minor decrease in muscle ATP levels from resting to gallop 4 values (24 mmol/kg d.m. to 21 mmol/kg d.m.) In explanation of the variations seen in gallop times, lactate levels and intramuscular ATP, the authors of this study suggested differences in skeletal muscle fibre types as the cause of the discrepancy. The likelihood of this is supported by reports that the breakdown of ATP occurs readily in type II fibres but not in type I fibres during exercise of short duration and high intensity (Meyer and Terjung, 1979; Guyton, 1991). In addition, the rate of lactate production in the type II fibre is approximately double that of the type I fibre (Meyer and Terjung, 1979). If the authors of this study had assayed the muscle biopsy

samples for percentages of fibre types using the myosin ATPase method, a relationship may have been observed between percentages of fibre type and the above variables as has been reported in the human literature (Farrell, Wilmore, Coyle, Billing, and Costill, 1979; Ivy, Withers, Van Handel, Elger and Costill, 1980; Ohkuwa, Kato, Katsumata, Nakao and Miyamura, 1984).

2.4.6 Research Using Incremental Exercise Tests

The production of lactic acid in horses during single bouts of exercise or several bouts of exercise at a fixed intensity has been well documented (Bayly, Grant and Persson, 1987 pg 426-437; Snow, Harris and Gash, 1985; Harris, Marlin and snow, 1991 pg 173-178; Harkins, Beadle and Kamerling, 1993; Evans, Harris and Snow, 1993).

2.4.7 The Relationship Between Lactate and Potassium

A study conducted by Harris and Snow (1988) investigated the alterations in plasma potassium and lactate at different intensities of exercise in five well-trained thoroughbred geldings. Following a warm-up period consisting of a walk and trot, horses were cantered or galloped at speeds ranging from 8 to 14 m/sec on a treadmill at a 5° incline. The duration of gallops was 2 minutes with the exception of the 14 m/sec exercise which was maintained for 1.5 minutes. Blood samples were taken at 2 minute intervals during the warm up period and the last 8 minutes of recovery. During the exercise period and the first 2 minutes of recovery, blood samples were taken every 30 seconds and then at 1 minute intervals between 2 and 4 minutes of recovery. The authors of this study report a small but significant increase in plasma potassium levels

and no change in plasma lactate levels during the warm up period. However, the commencement of cantering or galloping was followed by an abrupt increase in both potassium and lactate levels. Blood lactate levels for all horses tested increased from resting values of approximately 0.8 - 1.0 mmol/L to 16.5 - 29.6 mmol/L at a workload of 12 m/sec. Plasma potassium levels increased in a similar fashion ranging from approximately 3.5 mmol/L at resting to 7.1 - 10.0 mmol/L at a workload of 12 m/sec. The authors reported a high correlation between peak potassium and peak lactate levels.

Harris and Snow (1988) concluded that the high correlation seen between peak potassium and peak lactate levels indicated that potassium alterations were related to an increased rate of anaerobic metabolism. In explanation of their results, the authors suggest that the accumulation of lactate and hydrogen ions increases the osmolarity of the muscle cell, and therefore this may retard the re-uptake of the potassium released during muscle contraction. This failure to re-uptake all the potassium released is probably caused by an inhibition of the sodium-potassium pump of the muscle fibre sarcolemma. This could either be due directly to the increase in hydrogen ions (resulting from a build up of lactate and pyruvate) or, as this pump is an active process, to a decrease in the availability of ATP. The latter explanation may be of special importance at the highest workloads of this study, as decreases in ATP content within muscles has been shown to occur in the horse during exercise of high intensity and short duration (Snow, Harris and Gash, 1985).

2.4.8 Maximal Lactate Production in the Horse

In 1985, Saibene et al. investigated the maximal anaerobic capacity and power of the horse. This study was undertaken in an attempt to determine the maximal rate of lactate production of the horse in order to assess the contribution of anaerobic metabolism during exercise of maximal intensity and short duration in different types of horses. Sixteen horses were used for this study (3 thoroughbred, 7 standardbreds and 6 polo ponies). Blood lactate concentrations were determined before and 5 minutes after the horses galloped over 200, 300 and 400m at maximal speed. On examination of the results, the polo ponies attained the highest lactate levels for all distances covered, whereas the thoroughbreds were always the fastest. Within each individual group of horses the fastest horse always produced the most lactate. The polo ponies had the highest rate of lactate production for all distances covered (35 mmol/L), whereas in the thoroughbred there was a decrease in the rate of lactate production with increasing distance. In discussing their results, these authors (Saibene et al., 1985) conclude that the rates of lactate production in the horses tested were similar to those found in human athletes. In addition, they also suggest that the differences seen between groups of horses may be the result of differences in skeletal muscle fibre types. This suggestion is supported by Snow and Guy (1980) who reported a difference in muscle fibre composition between sprinters, middle distance and endurance horses. In this study the authors reported that type II fibres contain a high concentration of myosin ATPase and that the levels of this compound are directly proportional to the speed of sarcomere shortening in normal muscle. As a result, the greater the proportion of type II fibres, the greater the frequency of contraction and the greater the potential for the horse to run fast.

2.4.9 STUDIES INVOLVING SHOWJUMPING HORSES

Compared to the number of studies involving the thoroughbred racehorse there is a scarcity of data involving the exercise physiology of the showjumping horse. The few studies that have been conducted consist simply of single blood samples being taken following a competitive round. The authors of these studies have made little attempt to standardise the tests and as a result, these studies lack both depth and scope. Their results are discussed below:

A study by Art, Amory, Desmecht and Lekeux (1990a) examined heart rate, blood lactate and other plasma biochemical values of showjumping horses following competition. The study involved taking a blood sample before and after a showjumping competition during the Belgian Junior Championships. The objectives of this study were to improve the knowledge of exercise physiology in jumping horses by studying the metabolic changes induced by one round of competition. The results of the study showed that jumping induces significant changes in packed cell volume, lactate, total plasma protein, bicarbonate, sodium, chloride, calcium, plasma lactate dehydrogenase, creatine kinase and aspartate amino transferase. Mean resting values of lactate and heart rate were 0.53 mmol/L and 43.9 beats/min respectively. On completion of the competition these values rose to 9.04 mmol/L and 191.4 beats/min respectively. It was concluded that although the speed and duration of the showjumping competition are low, jumping requires severe exertion which necessitates the use of anaerobic metabolism. Further, these authors (Art, Amory, Desmecht and Lekeux, 1990a) also report a number of biochemical changes in the jumper during competition. However the experimental procedure of the study involved taking one blood sample 24 hours before

exercise and one blood sample 2 minutes after exercise. It would be expected that peak levels of the values being tested would vary with time and that several blood samples should have been taken in order to obtain true peak values of the biochemical variables being tested.

A separate investigation by Art, Amory Desmecht and Lekeux, (1990b) attempted to correlate biochemical constituents of the jumping horse with performance. This study involved taking blood samples from 8 horses over five rounds of the Belgium Junior Championships. Blood was analysed for a variety of constituents including packed cell volume, glucose, lactate, creatine kinase (CK), potassium, and other values. The authors concluded that the performance of the jumpers depends not only on the athletic ability of the horse but also on its neuromuscular coordination, experience, motivation and training. The results of the study showed that generally, the horses with the highest score over the five heats had the highest blood lactate levels. However, only one blood sample was taken following exercise. It was acknowledged by the authors that blood samples should be repeated following exercise and the horses should be treated individually and not compared to mean values. This study was conducted during regular rounds of the Belgian Junior Showjumping Championships. In Europe, showjumping is a major spectator sport. As a result, the horses tested in this study may have become nervous, or excited during the competition, causing elevations in lactate RBC counts, PCV and Hb. In addition, no reference was made by the authors of the study in relation to the type and intensity of the warm-up period for the various horses. Variations in warm-up procedures may have resulted in discrepancies in lactate production and hence this study would not provide a suitable reference of the changes in biochemical and haematological values induced by showjumping.

A study by Covalesky, Russoniello and Malinowski (1992) examined the effects of jumping on cortisol and lactate levels in horses at three levels of showjumping experience. It was shown that at a jumping competition, the most experienced horses produced more lactate than the inexperienced horses. In addition, a low positive correlation was found between heart rate and blood lactate levels following performance. These findings are in agreement with those found in the thoroughbred racehorse studies mentioned above. As a result, this information may be used by exercise physiologists to assess athletic potential in young horses.

To date there have been no investigations into the biochemical and haematological response of showjumping horses after a standardised incremental exercise test.

2.5 HAEMATOLOGY

Since the 1960s, evaluation of the haemogram (haematological profiles) has been a cornerstone in the assessment of the athletic horse (Rose and Hodgson, 1994 pg 63-78 pg 63-78). In more recent years, automated techniques have become available for the measurement of many haematological parameters. Individual haematological profiles can be used by the exercise physiologist to evaluate fitness and monitor adaptations to specific training programs. In addition, the clinician can use haemograms in an attempt to explain the loss of performance or ill health of a particular horse (Rossdale, Burguez and Cash, 1982; Carlson et al., 1983; Mason et al. 1989; Mason et al., 1990; Rose and Hodgson, 1994 pg 63-78 pg 63-78).

2.5.1 THE RESTING HAEMOGRAM

The resting haemogram is used by equine exercise physiologists attempting to detect abnormalities that are not obvious on clinical examination (Rossdale, Burguez and Cash, 1982; Mason, Watkins and Luk, 1989; Mason, Watkins and McNeil, 1990; Rose and Hodgson, 1994 pg 63-78 pg 63-78). In addition red cell indices can provide valuable information for the assessment of performance potential and fitness profiling. Table 2.1 illustrates some normal haematological variables as used by exercise physiologists. It can be noted from the table that while normal ranges for the horse are quite broad as can be seen from Table 2.1, the variations for individual animals are small (Stewart, Riddle and Salmon, 1977; Blackmore, 1983 pg 344-353; Rose and Hodgson, 1994 pg 63-78 pg 63-78).

Variable	Minimum Value	Maximum Value
*Hb (g/L)	100	180
HCT (L/L)	0.32	0.52
*RBC ($x10^{12}$/L)	6	12
MCV (fL)	34	58
MCH (pg)	13	19
MCHC (g/L)	310	370
WBC ($x10^{9}$/L)	5.5	12.5
Neutrophils (% of WBC)	30	65
Lymphocytes (% of WBC)	25	70
Eosinophils (% of WBC)	0	10
Monocytes (% of WBC)	0.5	7

Table 2.1 - Normal haematological values for adult horses (derived from Schalm, 1986).

* Normally lower in young animals (20-30 g/L Hb less) and in cold blood horses. Higher in stallions (10-20 g/L Hb more).

While the majority of adult athletic horses will show resting haemograms within the normal ranges (refer to table 2.1), these parameters can be affected by a variety of factors including time of day, the relationship to feeding, temperament during sample collection, and the relationship of sample time to exercise (Stewart, Riddle and Salmon, 1977; Revington, 1983a; King, Rose and Evans, 1994).

In 1977, Stewart, Riddle and Salmon investigated the effects of excitement on the resting haemogram of the thoroughbred racehorse. A total of 216 blood samples were

taken from 136 thoroughbreds in 17 stables. The temperament of the horse at the time of collection was assessed subjectively and divided into four categories termed, placid, timid, apprehensive and excited. All blood samples were analysed for red cell count (RBC), haemoglobin concentration (Hb), total white cell count (WBC), packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and total eosinophil count (TEC). The results showed that horses who were excited during sample collection had significantly higher RBC, Hb, PCV, WBC, and total eosinophil count. Those horses which were timid or apprehensive during sample collection did not show any significant increases in blood indices. In addition, the authors noted that if the blood sample was collected within) seconds of entering the stable and with no more than 2 people in the stable at any one time (one sample collector and one person holding the horse), the excitability of the horse was greatly reduced. It was concluded from this study that, if many samples are to be collected from a particular stable, the excitability of the individual horses will be kept to a minimum if the same person collects all the samples.

The effect of the temperament of the horse on the resting haemogram, as reported by Stewart, Riddle and Salmon (1977) has also been confirmed by Revington (1983a). It was noted in this study that horses in the presence of another horserace or exercise situation will display significant increases in their resting haemograms. It was concluded by Revington (1983a) that these psychogenic effects also extend to the anticipation of exercise or racing. As a result, it has been recommended that resting blood samples are taken in the morning, while the horse is standing quietly in its stable and before its morning feed. (Stewart, Riddle and Salmon, 1977; Revington, 1983a; Rose and Hodgson, 1994 pg 63-78).

2.5.2 Haematological Changes Associated with Exercise

The red cell pool of the horse is under the direct control of catecholamine concentrations (Rose and Hodgson, 1994 pg 63-78). Consequently, exercise can have a profound effect on red cell indices, depending on the intensity and duration of the exercise bout. The horse is capable of storing approximately 50% of its red cell pool in its spleen (Persson and Lydin, 1973). Under intense exercise or excitement, the red cells are ejected into the circulation to provide a greater oxygen carrying capacity (Evans and Rose, 1988). The splenic capacity for red cell storage and the subsequent release during exercise is related to the breed of horse, with draught horses having much lower splenic weights than thoroughbred racehorses (Rose and Hodgson, 1994 pg 63-78). As a result of the increasing red cell count during exercise, a concomitant rise is also seen in haematocrit (HCT). There is a linear increase in HCT with increasing exercise intensity, up to intensities of three quarter pace (defined as 90-100% of maximal oxygen consumption) (Rose and Allen, 1985). In addition to changes in RBC and HCT following exercise, decreases in mean corpuscular volume, increases in mean corpuscular haemoglobin and increases in mean corpuscular haemoglobin concentration have been reported (Rose and Hodgson, 1994 pg 63-78).

2.5.3 THE USE OF HAEMATOLOGY FOR PERFORMANCE PREDICTION

The practice of using haematological values as a guide to fitness in horses is well established in thoroughbred horses (Revington, 1983b). Because the aim of training thoroughbred racehorses is to win races, it is common for trainers to elevate red cell parameters in order to increase the animal's performance (Blackmore, 1983 pg 344-353; Revington, 1983b). As a result, research was conducted to evaluate the effects of red cell indices on performance in thoroughbred racehorses.

Between 1974 and 1981, Blackmore (1983) collected blood samples from over 600 thoroughbred racehorses in the United Kingdom. When individual blood haematological and biochemical profiles were compared to timeform ratings, it was apparent that a relationship existed between RBC count and performance. The authors concluded that due to the huge variations in individual blood profiles, repetitive sampling was required to establish the animal's normal values, which could then be used as an indicator of future performance. In addition, the observation that plasma sodium concentrations and red blood cell counts were related to inherent performance opened the door for research into identifying an indicator for lack of performance in situations where no pathological symptoms are apparent.

Revington (1983b) collected 816 blood samples from thoroughbred racehorses at the track 1-3 hours before racing, and subjected them to haematological examination. The author attempted to correlate the haemogram with subsequent performance. Comparisons were made between haemograms and racing performance for horses from different classes, with the final outcome being a complete lack of any relationship or

correlation. In addition, the haemograms of individual horses taken on different occasions were compared with performance and again no consistent or significant relationships were observed. The extent of changes in red and white cell parameters between horses at rest and immediately before racing were also examined as possible indicators of performance but did not yield any significance.

It is pertinent to note that the above study conducted by Revington (1983b) consisted of single samples collected at the track a few hours before racing. Possibly, if several samples were collected over a period of days and individual normal haemograms calculated, a relationship between racing performance and resting haemograms could emerge. In addition, the excitement of the horses being at the track may have resulted in elevations of red cell indices. This study would be strengthened by the additional collection of samples post exercise.

2.6 CREATINE KINASE

Muscle soreness, tenderness or pain a few days after exercise is a common occurrence in humans, and these subjective feelings are paralleled by simultaneous or delayed biochemical and physiological responses (Hortobagyi and Denahan, 1989). One particular physiological response to exercise is the elevated level of Creatine Kinase (CK) in the blood. It is generally accepted in the literature that an increased blood CK level is associated with disruption of the skeletal muscle cell membrane, and hence a marker of skeletal muscle damage or injury (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983; Hortobagyi and Denahan, 1989; Morris et al., 1991; Manfredi et al., 1991; Volfinger, Lassourd, Michaux, Braun

and Toutain, 1994; Rose and Hodgson, 1994 pg 63-78). Due to the paucity of literature regarding this topic in the equine literature the majority of this review will discuss the effects of exercise on CK levels in the human. However some authors (Rose and Hodgson, 1994 pg 63-78; Volfinger et al., 1994) have used elevated CK levels as an indicator of skeletal muscle injury in the horse following exercise and these will also be discussed.

Creatine kinase is a dimeric enzyme that catalyses the reversible phosphorylation of ADP by creatine phosphate to form ATP and free creatine. (Hortobagyi and Denahan, 1989; Guyton, 1991; Lehninger, Nelson and Cox, 1993). The CK molecule is a large molecule (80 000 Da) and therefore does not pass through the cell membrane and enter the blood directly (Hortobagyi and Denahan, 1989). With injury, the skeletal muscle cell membrane is damaged and CK leaks out of the cell, passes to the lymph via the interstitial fluid and finally empties into the blood stream. CK levels can therefore be measured in the general circulation.

2.6.1 The Effects of Exercise on CK Levels in Humans

It has been reported by many authors that exercise causes an increase in blood CK levels (Newham, Jones and Edwards, 1983; Schwayne et al., 1983; Hortobagyi and Denahan, 1989; Morris et al., 1991; Manfredi et al., 1991; Volfinger, Lassourd, Michaux, Braun and Toutain, 1994; Rose and Hodgson, 1994 pg 63-78). However, the type and intensity of the exercise will determine the levels of CK released into the blood and hence, the amount of muscle damage occurring during exercise can be measured.

It is generally agreed that during exercise the intensity produces a larger post exercise CK response than the duration does. For example, Tiidus and Ianuzzo (1983) designed a protocol of "intensive work", "duration work" and "constant work" and tested for changes in total CK after each mode of exercise. The results of this investigation showed that the "intensity" work group attained the highest levels of post exercise CK despite performing the least total work. It was therefore concluded that intensity of exercise is the key factor in producing large post exercise CK levels.

In a contradictory study, Astrand and Rodahl (1986), report a "distance threshold" for blood CK levels. Long distance or marathon running is a duration activity that occurs at 70-90% of maximal oxygen uptake. Blood CK levels remain relatively normal with only slight increases up to distances of approximately 21 km. However, after this distance and up to a distance of 42 km, blood CK levels increase sharply in a threshold type manner. After a distance of 42 km and up to a distance of 96 km blood CK levels increase no further. These results imply that the duration of exercise may be as important as the intensity of exercise in producing high CK levels, if the duration of exercise is longer than approximately 1-2 hours.

A relationship has been shown by Hortobagyi and Denahan (1989) to exist between the mode of muscle contraction and post exercise CK levels. The type of muscular contraction performed during exercise will significantly influence the time taken for peak blood CK activity to be reached (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983; Spitler, Alexander, Hoffler, Doerr and Buchanan, 1984; Hortobagyi and Denahan, 1989).

The above relationship was examined by Newham, Jones and Edwards (1983). Sixteen normal human subjects completed a 20 minute step test. During the stepping action, the quadriceps muscles contracted concentrically (a contraction of a muscle reducing its length) during the stepping up phase and the contralateral muscles contracted eccentrically (a contraction of a muscle increasing its length) during the stepping down phase. Blood samples were taken immediately after the exercise and at 24 hour intervals for 9 days.

On examination for muscle soreness, pain and tenderness was only experienced in those muscles that had worked eccentrically. The degree of pain varied amongst the subjects ranging from mild discomfort lasting 1-2 days to severe pain which lasted for several days. All subjects showed a small CK increase immediately after the exercise period and CK activity continued to rise during the next 24 hours, reaching 2-3 times the initial levels. After 24 hours, seven of the subjects blood CK levels returned to normal resting values. However, the remaining 5 subjects experienced an unexpected delayed rise in CK activity which was one to two orders of magnitude greater than the rise seen after the initial 24 hour period. This rise did not occur until the second or third day after exercise, peaked between 4-5 days after exercise and returned to normal levels 7-9 days after exercise. Those subjects who experienced a delayed rise in blood CK levels also experienced considerable muscle soreness. However, there were also subjects with considerable levels of discomfort with no large increases in blood CK levels.

In concluding, Newham et al. (1983) suggested that all eccentric muscle contractions involved in this type of exercise produce some degree of muscle damage as seen by

increases in CK levels. In susceptible subjects this exercise may initiate changes giving rise to a large delayed release of muscle enzymes.

Schwayne, Johnson, Vandenakker and Armstrong (1983) investigated the effects of eccentric muscular contraction on muscle damage and CK levels. All subjects completed three treadmill exercise tests including a VO_2 max test, a level run (involving mainly concentric muscle contractions) and a downhill run (involving mainly eccentric muscle contractions). The level and downhill tests involved the subjects running intermittently for a period of 45 minutes, with work periods lasting 5 minutes. Venous blood samples were collected at 0, 24, 48 and 72 hours after each test. Following analysis of blood CK levels, no significant increases in blood CK were observed for exercise consisting of concentric contractions. However, a marked increase was observed in CK levels following exercise involving eccentric muscle contractions. In addition, peak CK levels occurred 24 hours after the completion of the exercise. These results were in agreement with the conclusions of Newham, Jones and Edwards (1983)

In concluding their results, Schwayne, Johnson, Vandenakker and Armstrong (1983) reported that concentric exercise does not cause significant muscle damage, whereas exercise involving eccentric muscular contractions will cause significant structural changes in skeletal muscle tissue, resulting in elevated blood CK levels. In addition, this increase will occur 24 hours after the completion of the exercise period.

2.6.2 The Effects of Exercise on CK Levels in the Horse

Studies involving the effects of CK in the horse are not as conclusive as those involving human subjects. However, a relationship between exercise intensity, duration and blood CK levels has been reported (Snow, Mason, Ricketts and Douglas, 1983 pg 389-399; Rose and Hodgson, 1994 pg 63-78; Volfinger, Lassourd, Michaux, Braun and Toutain, 1994).

The study by Snow, Mason, Ricketts and Douglas (1983) investigated post-race blood biochemistry in thoroughbred racehorses. Post-race blood samples were collected from 45 thoroughbreds following races of distances between 1000 to 2400 metres. Despite the high intensities of exercise attained during racing, it was reported that only a small but gradual increase in blood CK levels was seen. Previous studies (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983; Hortobagyi and Denahan, 1989) using human subjects (as discussed previously) performing high intensity exercise resulted in large increases in post exercise blood CK levels. Therefore, it would be expected that thoroughbred racehorses, competing at maximal intensity, would show similar increases in post race blood CK levels. This discrepancy may be attributed to differences in post exercise blood sampling periods. In the present study, blood samples were collected 10 minutes after the completion of the race. However, studies involving human subjects showed peak blood CK levels do not occur until at least 24 hours after the completion of exercise (Newham et al., 1983; Schwayne, et al. 1983). It can therefore be suggested that if blood samples were taken 24 hours after the completion of exercise in the study by Snow et al. (1983), higher CK levels would have been observed.

It has been reported that both the type of exercise and its duration are important parameters influencing the increases occurring in blood CK levels during exercise in the horse (Rose and Hodgson, 1994 pg 63-78). In a study of endurance exercise (Kerr and Snow, 1983 pg 432-445), those horses who completed the course at an average speed of 234 m/min had mean blood CK levels that were double that for horses who completed the course at an average speed of 144 m/min. It was therefore concluded that both exercise duration and intensity will produce increases in blood CK concentrations. However, blood samples were collected immediately following the completion of exercise. As a result, the CK levels reported in this study may not reflect peak CK levels which have been reported (in humans) to occur between 24 and 96 hours post exercise, as mentioned above.

Studies involving the showjumping horse have shown that during a jumping competition, small but significant increases in blood CK levels occur (Art, Amory, Desmecht and Lekeux, 1990a; Art et al, Amory, Desmecht and Lekeux, 1990b; Lekeux, Art, Linden, Desmecht and Amory, 1991 pg 384-390). The increases in blood CK levels observed in the showjumping horse may be attributed to the high levels of muscular exertion required to jump a fence. In addition, the deceleration of the horse upon landing after jumping a fence would require large amounts of eccentric muscular contractions. This may account for the increases seen in blood CK levels as described in human studies as already discussed. As mentioned above, blood samples taken immediately following the completion of exercise does not indicate peak CK levels. The CK values reported in this study may therefore not reflect the peak levels obtained by the horses following exercise.

2.7 CONCLUSIONS

The metabolic and contractile properties of individual muscle fibres are the major parameters involved in the success of the equine athlete. The composition of fibre types within a particular muscle group has been shown to influence the type of exercise intensity for which a certain individual is genetically best suited to (Farrell, Wilmore, Coyle, Billing and Costill, 1979; Ivy, Withers, Van Handel, Elger and Costill, 1980; Jacobs and Kaiser, 1982; Ohkuwa, Kato, Katsumata, Nakao and Miyamura, 1984; Gollnick, Bayly and Hodgson, 1986; Jacobs, 1986). There have been relationships shown between speed of running over short distances and a high percentage of type II fibres and conversely, speed of running over long distances and a high percentage of type I fibres (Farrell et al. 1979; Ivy et al. 1980).

To determine the percentage of type I or type II fibres within a particular muscle, it is essential to take a muscle biopsy sample from the individual (Meyer and Terjung, 1979). However, it has been reported that lactate production in the type II fibre is double that of the type I fibre (Gollnick, Bayly and Hodgson, 1986). Therefore, blood lactate values can be used as an indirect measure of the percentage of fibre types within a muscle (Ivy, Withers, Van Handel, Elger and Costill, 1980; Ohkuwa, Kato, Katsumata, Nakao and Miyamura, 1984). With the use of an incremental or standardised exercise test, the rate of lactate production for a particular individual can be correlated to the percentage of type I or type II fibres. A high rate of lactate production is correlated to a high percentage of type II fibres (Ivy et al., 1980). In addition it has been found, both in humans and in horses, that the rate of lactate production is greater in those animals who compete successfully in events of short duration and high intensity (Ivy et al., 1980;

Bayly, Grant and Persson, 1987 pg 426-437; Okhuwa et al., 1984; Snow, Harris and Gash, 1985; Harris, Marlin and Snow, 1991 pg 173-178; Harkins, Beadle and Kamerling, 1993; Evans, Harris and Snow, 1993). It is therefore proposed that, because showjumping is a sport consisting of high intensity exercise over a short period, the type of animal best suited for this type of activity is a horse with a high percentage of type II fibres and hence a high rate of lactate production.

Haematological profiles have been used by equine exercise physiologists as a measure of fitness in horses (Rose and Hodgson, 1994 pg 63-78). The horse is capable of storing upto 50% of its red cell volume in the spleen (Persson and Lydin, 1973). During exercise these red cells are ejected into the circulation to provide a greater oxygen carrying capacity (Evans and Rose, 1988). The volume of red cells ejected during exercise is dependent on the intensity of exercise and the fitness of the horse (Evans and Rose, 1988). As a result, red cell counts and other haematological indices can be used by exercise physiologists to assess fitness and performance potential in horses (Blackmore, 1983 pg 344-353; Revington, 1983 a and b).

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The materials and methods used in this study can be divided into two phases. Phase 1 consisted of an incremental exercise test which was performed on subject animals at a public training track. The results of this test were used to prepare lactate performance curves which were then used as a measure of the lactate kinetics of the showjumping horse. The second phase of the study consisted of a jump test. The results of this test provided data concerning changes occurring within the working muscles of the showjumping horse. It is a longer term objective that these results may then be used as a guide for the development of training programs, or for the talent identification of young showjumping horses.

Ethical approval for all aspects of the study was granted by the Animal Ethics and Experimentation Committee, Edith Cowan University.

3.2 QUESTIONNAIRE

In an attempt to compare the overall status of each horse with other horses used in this study and explain any anomalies in data obtained, the rider of each horse tested was asked to complete a questionnaire (appendix 1). The survey asked for information such as the horse's age, sex, competition status, training methods, diet and temperament during the testing and blood sampling period.

The temperament of the horse at the time of testing and sampling periods was assessed subjectively into four categories as follows (Stewart, Riddle and Salmon, 1977):

Placid - The animal remained placid and still during the venipuncture and blood collection.

Timid - The animal remained still during venipuncture and blood collection, but a forceful jugular pulse with an increased rate was evident when the jugular vein was raised.

Apprehensive - The horse moved slightly during blood collection, either pulling back a little during venipuncture, or flinching when the jugular vein was raised.

Excited - The horse moved about forcefully and resisted venipuncture.

3.3 PHASE 1: INCREMENTAL EXERCISE TEST

The development of lactate performance curves was accomplished by the use of an incremental exercise test consisting of the horse cantering or galloping at four workloads for a period of 2 minutes on an all weather training track. Lactate concentrations (derived from venipuncture samples) were plotted against velocity to provide a lactate performance curve. Refer to protocol for details

3.3.1 BLOOD COLLECTION

Blood was collected from the jugular vein using a 20 gauge vacutainer needle (Vacutainer Systems, Franklin Lakes New Jersey) as shown in Figure 3.1. Blood was collected into two 5mL vacutainer tubes, the first containing ethylenediaminetetraacetic acid (EDTA) and the second containing lithium heparin. Blood in the lithium heparin tube was centrifuged at 3000 rpm for 10 minutes as soon as possible after collection and

the serum was removed and stored at 4 °C until later analysis. Blood in the tube containing EDTA was also stored at 4 °C until later analysis.

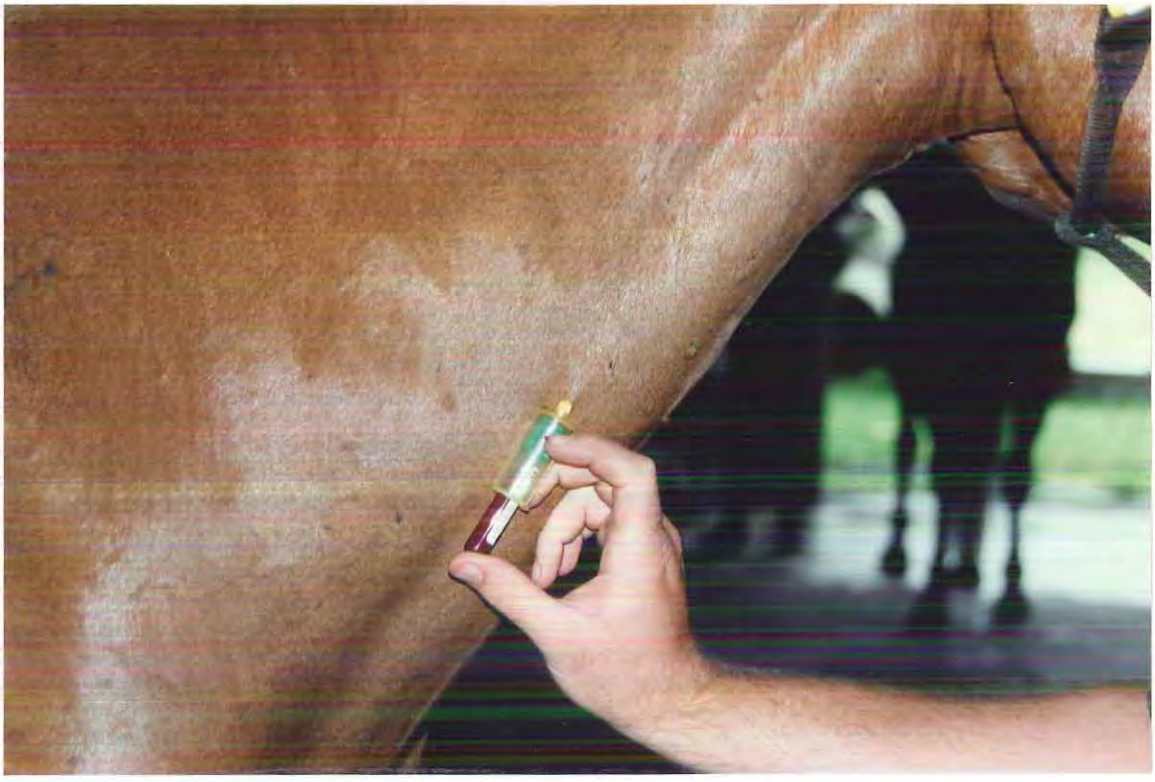

Figure 3.1 - Blood was collected from the jugular vein using a 20 gauge vacutainer needle (Vacutainer systems, Franklin Lakes, New Jersey). The temperament of the horse during blood sampling was observed and recorded.

It has been reported that the effects of transportation and storage of blood samples will cause alterations in blood indices (Stewart, Riddle and Salmon, 1977; King, Rose and Evans, 1994; Ferrante and Kronfield, 1994: Rose and Hodgson, 1994 pg 63-78). Following blood collection, blood smears for cytological examination were made as early as possible because the anti-coagulant, EDTA, causes the degradation of cellular material in approximately 4-6 hours if the blood is left at normal room temperature. If

blood was to be stored overnight it was stored at 4 °C and analysed within 24 hours of collection (Stewart et al., 1977). If blood was to be stored overnight a small increase (1-2%) in haematocrit, PCV, MCV, MCH, and MCHC was expected due to swelling and perhaps rupture of red blood cells during storage and transportation (Stewart et al., 1977: Rose and Hodgson, 1994 pg 63-78). Exposure of blood to extremes in temperature or sunlight may also result in some haemolysis, causing serum values to be falsely elevated. Therefore following collection, all blood samples were immediately placed on ice and kept away from direct sunlight (Ferrante and Kronfeld, 1994: King, Rose and Evans, 1994).

3.3.2 HORSES

Five horses were used for this part of the study. All horses were specifically trained for showjumping and were currently competing in the local Western Australian Professional Show Jumping Circuit. Table 3.1 illustrates the age, gender, competition status and breeds of the horses tested. All horses were considered injury free and competition fit by their trainers.

Horse	Age (years)	Sex	Breed	Competition Level	Total Points	Points per Year	Jockey's Weight (kg)
NTL	13	gelding	Th	B	55	11	69
WMC	8	gelding	Th	D	0	0	69
MSH	8	gelding	Th	D	1	1	69
CDB	7	gelding	WB	C	22	11	69
TVA	12	gelding	Th	D	0	0	79

Table 3.1 - Horses used in phase 1. Th - thoroughbred; WB - warm blood (the exact breeding of the warm blood horses were not known).

All testing was performed between 8 am and 10 am between the months of July and September. The weather on all test days was fine with temperature and humidity ranging from 16-21 °C and 78 to 96% respectively. On the day that horse TVA was tested it was raining prior to the test, however it remained fine for the duration of the test and the track was not affected by the rain. All horses were ridden by the same jockey with the exception of horse TVA (see table 3.1) where the usual jockey was unavailable due to prior commitments.

3.3.3 PROTOCOL

Resting heart rates and blood samples were taken on the morning of the test between the hours of 5 am and 7 am. Heart rate was measured using a stethoscope for a period of 30 seconds. The number obtained was then doubled to give a value in beats per minute.

The incremental exercise test was conducted at the Byford City Council horse training facility, Masters Rd, Byford. The training track used was 1300 meters in length and consisted of a sand-gravel surface which was firm and fast on all days of testing. This surface was selected due to its ability to remain firm and level under all weather conditions.

Prior to the commencement of the test, 20 markers were spaced equally around the track at 65 metres apart. For each individual workload, velocity, distance travelled and time to pass between markers was calculated as shown in table 3.2. Therefore, before the commencement of each workload, the rider was given specific information about how many seconds were required to pass between markers in order to maintain the required velocity. In addition, this information was used to instruct the rider as to what number marker to start the work period in order to finish as close to the sampling station as possible at the completion of the 2 minute period.

Velocity (m/sec)	**Time Period Between Markers (sec)**	**Distance Travelled per Workload (m)**
6	11	720
8	8	960
10	6.5	1200
12	5.5	1440

Table 3.2 - Time period between markers and distance travelled for each velocity.

On arrival at the track, the heart rate monitor (Polar PE4000 Sports Tester, Polar Electro Oy, Finland) was attached to the horse, as shown in Figure 3.2 and Figure 3.3, and 3.4

and the digital timer on the receiver was activated. The horse was saddled and the rider was instructed to warm up the horse by completing one lap of the track at a trotting pace (approximately 3-4 minutes duration). At the completion of the warm up period, the rider was instructed to canter the horse around the course at workloads of 6, 8, 10 and 12 metres per second. These velocities were chosen from previous studies (Harris and Snow, 1988; Lekeux, Art, Linden, Desmecht and Amory, 1991 pg 384-390) to provide a range of work intensities varying from light exercise to near maximal exercise. Each workload period was maintained for two minutes. This was considered to be the minimum time necessary to produce a physiological response in the test animals (Harris and Snow, 1988)

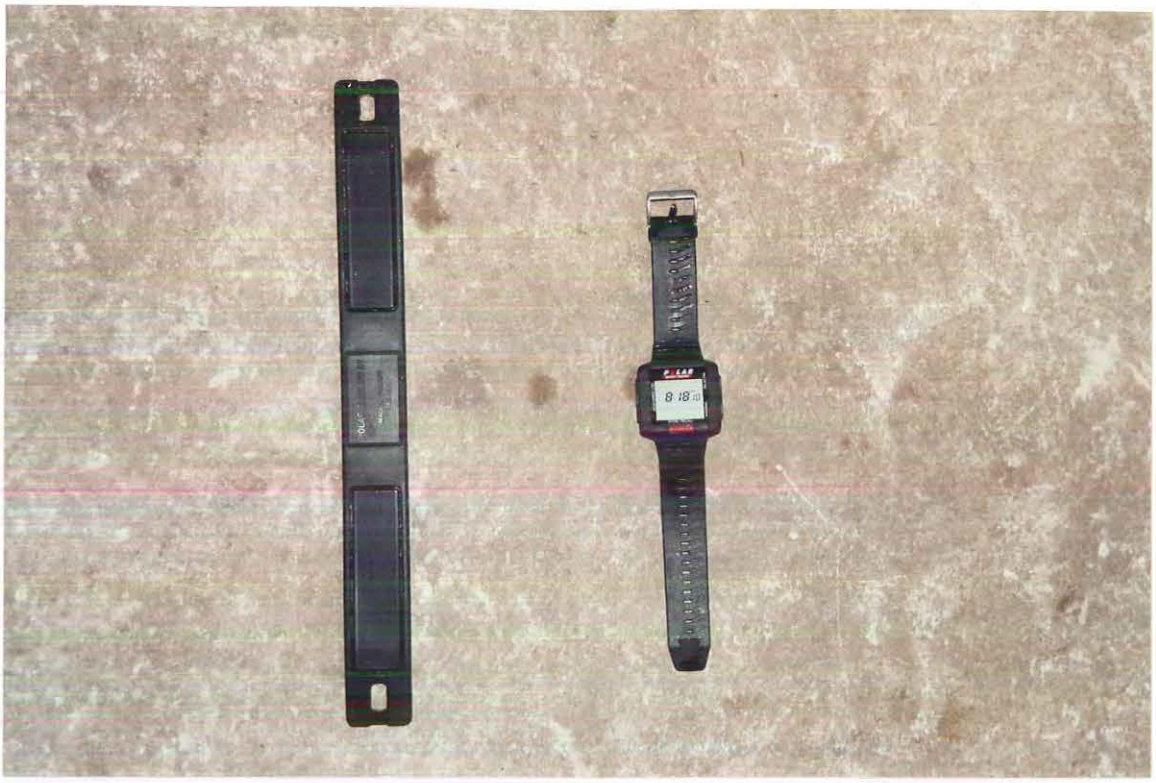

Figure 3.2 - The Polar PE4000 Sports Tester (Polar Electro Oy, Finland) This device, originally designed for human use, was adapted to suit the horse. The accuracy of this device was checked manually with a stethoscope. The transmitter, on the left is held firmly over the heart of the horse by a strap (not shown here). The receiver (on the right) is connected to the girth of the saddle.

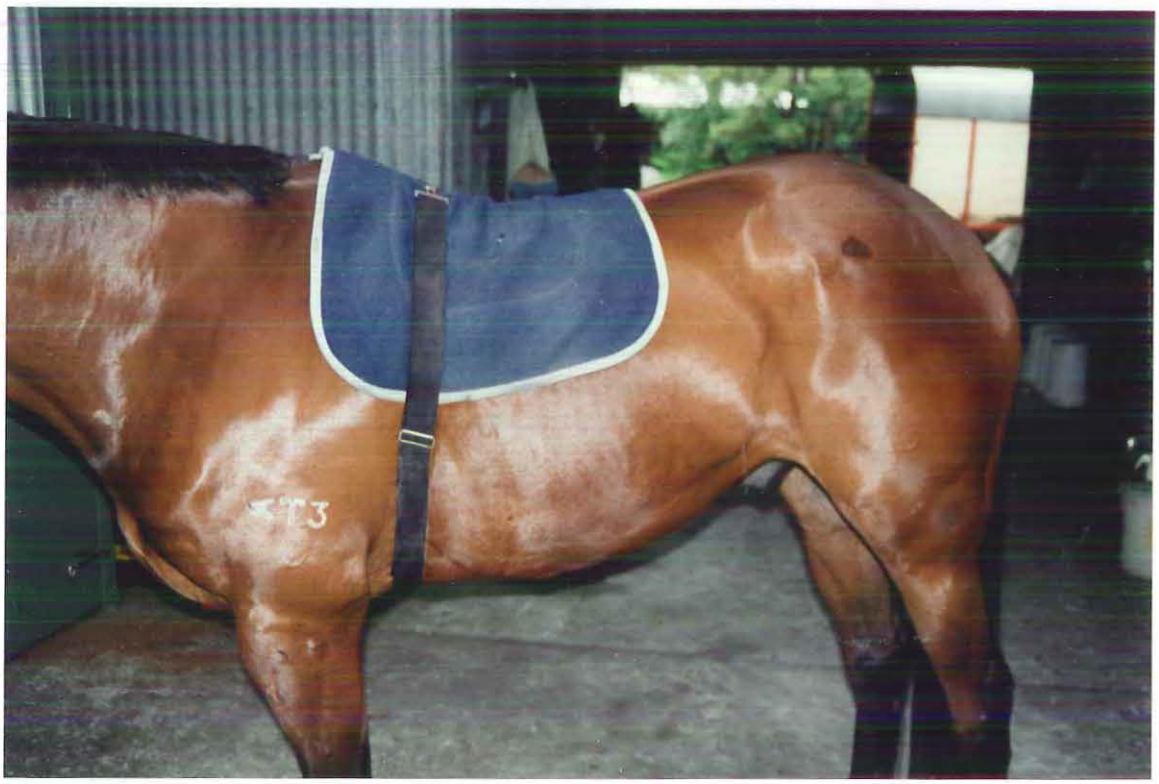

Figure 3.3 - The transmitter is placed over the heart of the horse and is held firmly in position by a strap (the black strap in the figure). Electrode gel is placed onto the face of the transmitter to aid in electrical conduction. The horse in this figure is horse NTL used in this study.

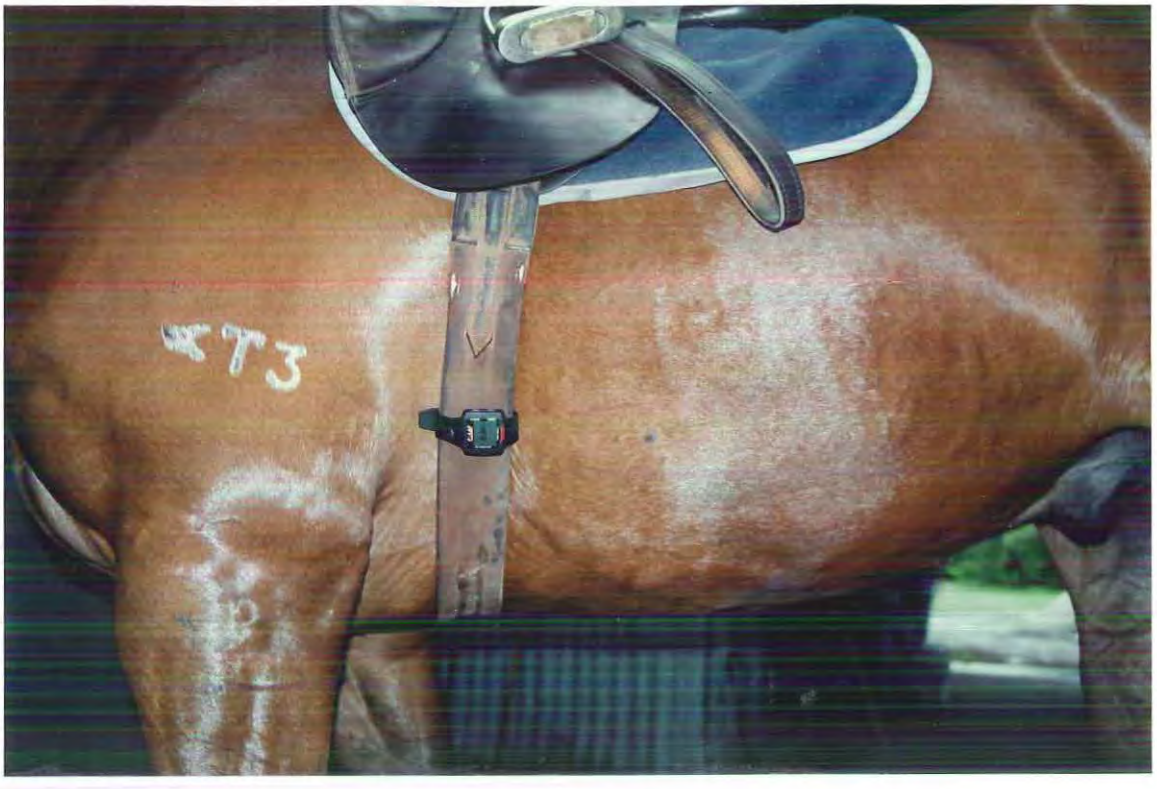

Figure 3.4 - The saddle is placed over the heart rate monitor and the receiver is attached to the girth, as shown. The girth also helps to hold the transmitter firmly in position.

At the completion of each workload, the rider was instructed to slow and stop the horse as quickly as possible without injuring the animal. Blood samples were then taken at 0, 3 and 5 minutes post exercise. During the rest periods (blood sampling time) the horses were continuously walked for 10 minutes. At the completion of the blood sampling times, the next workload was commenced. Exercise intensities of the test were increased from the slowest velocity (6 m/sec) to the fastest velocity (12m/sec). Heart rate was recorded from the beginning of the "warm-up" until completion of the "warm-down", a period of approximately 60 minutes.

At the completion of the test, the horses were trotted for 1 lap of the track and then walked for a further lap of the track. A further blood sample was taken 24 hours after the completion of the test to measure peak creatine kinase (CK) levels (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983) and additionally, to ensure a return to baseline levels for other haematological parameters.

At the completion of each work period, the rider was asked to rank the horse's temperament during the test by selecting one of the choices below:

1: The horse struggled to keep pace with the required workload and the rider had to continually push the horse forward.

2: The horse initially maintained the required workload, however struggled towards the end of the workload.

3: The horse easily maintained the required velocity with no encouragement from the rider.

4: The rider initially fought to control the horse, however, as the workload progressed, the horse settled and maintained the required workload.

5 : The horse was very eager and the rider had to continually fight with the horse to slow it down to the required velocity.

Rehearsals of this test were performed prior to the testing, on a separate day in order to accustom the horses and rider to the testing methodology.

3.4 PHASE 2: JUMP TEST

In order to identify the major physiological changes occurring to the showjumping horse during competition, a standard jump course was designed and the horses were tested on their ability to complete this course. This test was designed in two stages. (refer to protocol).

3.4.1 HORSES

Eight horses from 3 independent commercial showjumping stables were used in this study. The horses tested were specifically trained for showjumping and were currently competing in the local Western Australian Professional Showjumping Circuit. Table 3.3 lists the age, gender, competition status and breeds of the horses tested. Horses TVA, NTL, CDB and MSH also participated in the first phase of this study. All horses were stabled, fed and considered injury free and competition fit by their trainers. As the horses used in this study were supplied by different trainers, feed constituents could not be standardised. However, feeding routines were documented and no major discrepancies were observed.

Horse	Age (years)	Sex	Breed	Comp. Level	Total Points	Points per year	Jockey's Weight (kg)
NTL	13	gelding	Th	B	55	11	69
MSH	8	gelding	Th	D	1	1	57
CDB	7	gelding	WB	C	22	11	95
FK	16	gelding	Th	B	42	6	79
TVA	11	gelding	Th	D	0	0	79
AJS	6	stallion	WB	C	27	13	95
JMB	6	gelding	WB	D	9	9	95
CZ	9	gelding	Th	B	48	9	95

Table 3.3 - Horses used in phase 2. Th - thoroughbred, WB - warm blood (the specific breeding of the warmblooded horses was unavailable. However, horse AJS was a Hanouvarian, and horse CDB was part Percheron..

All jump testing was performed between the months of September and October. Weather conditions on all test days were fine and sunny with temperatures ranging from 18-24 °C. The arena surface was dry and firm for all horses. It was planned that all testing would be conducted at a similar time of day, however, due to prior commitments of the individual riders, the testing times ranged from 8 am to 7 pm, however, 5 of the horses were tested between 1 and 3 pm.

3.4.2 PROTOCOL

Resting heart rate and blood samples were taken on the morning of the test as previously described. Heart rate was measured using a stethoscope for a period of 30 seconds. The number obtained was then doubled to give a value in beats per minute.

This jumping test was conducted at Argyle Showjumping Stables, Wanneroo, Western Australia. The jumping test was conducted in a 65m x 65m sand-woodchip surfaced arena. This surface was selected as it was similar to those used in competition and also providing sufficient cushioning on landing to prevent injury.

A competition standard jumping course was designed by Mr Jarrod Hall (an internationally recognised course designer) as shown in Figure 3.5. Jump heights were divided into two classes as shown in Table 3.4. Those horses competing at C and D grades would jump the first height and those horses competing at A and B grades would jump the second (greater) height. In Western Australia showjumping is divided into four major levels. Horses begin their jumping careers in D grade and gradually move through the grades, via an accumulation of points obtained by placing in competitions, until they reach A grade which is the top grade. During competition, A and B grade horses are grouped and compete together. The course was specifically designed to test the horse's physical ability whilst minimising the demand for technical skills.

COURSE DISTANCE = 438 metres

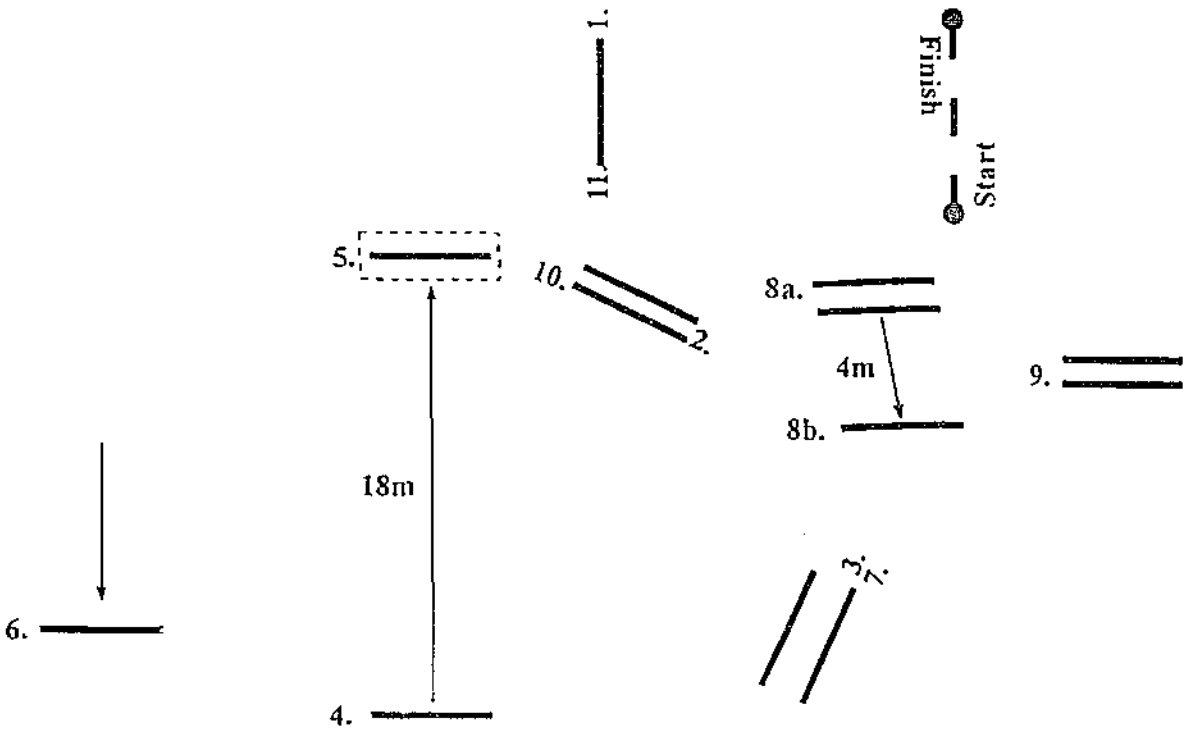

Figure 3.5. Jump course used in the Jump Test. The course was completed with the number of the jump on the right hand side of the rider.

Fence Number	Fence Height and Width (metres)		Fence Type
	C and D Grade	A and B Grade	
1	1.23	1.35	Vertical
2	1.10/1.00	1.20/1.10	Oxer - Parallel
3	1.05/1.00	1.30/1.30	Oxer - Rising
4	1.15	1.30	Style
5	1.15	1.25	Water - Vertical
6	1.10	1.30	Vertical
7	1.05/1.00	1.30/1.30	Oxer - Rising
8a	1.05/1.00	1.25/1.15	Oxer - Rising
8b	1.10	1.35	Vertical
9	1.15/1.10	1.35/1.25	Oxer - Rising
10	1.10/1.00	1.20/1.10	Oxer - Parallel
11	1.23	1.35	Vertical

Table 3.4 Fence height and style.

On arrival at the stables, the heart rate monitor (Polar PE4000 Sports Tester. Polar Electro Oy, Finland) was attached and the timer activated as previously described. The horse was saddled and the rider instructed to "warm up" the horse by completing 5 minutes of trotting and 5 minutes of cantering. The rider was free to implement circle work or changes of direction at random. Heart rate was recorded from the beginning of the first warm up until the completion of the second stage of the test, a period of approximately 35-40 minutes.

In the first stage of this test (the control stage), the jump course was assembled without any jump poles in position. The rider was asked to complete the course at a pace similar to that required assuming the jumps were placed in position, and at a speed that is similar to that performed in a formal jump off or speed competition. At the completion of the course blood samples were collected at 1, 3, and 5 minutes post exercise.

The second stage of this test was performed 10 minutes after the completion of the first stage to allow the horses to return to resting conditions. During this rest period, the horses were walked with the rider dismounted. During the rest period, the course was now set with the jumps in position.

Upon completion of the rest period, the rider was instructed to warm up the horse using the same procedure as undertaken in the first stage. However, during the 5 minutes of cantering, the rider was permitted to jump 5 practice fences with the last two jumping efforts being equal to competition height. At the completion of the warm up period, the

rider was then instructed to complete the course in the fastest possible time. During the test, competition conditions were maintained with 4 penalty points being awarded for any fences knocked down. If a horse refused to jump a fence, the rider was instructed to circle, attempt to jump the fence a second time and continue with the course. To compensate time lost for a refusal, 10 seconds were deducted from the total time for each occasion when an animal did not perform a jump. Blood samples were taken at 1, 3, and 5 minutes post-test. A further blood sample was taken 24 hours after the completion of the test to measure peak creatine kinase levels (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983) and additionally, to ensure a return to baseline levels for other haematological parameters.

3.5 Analysis of Data

3.5.1 Heart Rate

Heart rate was recorded using a POLAR PE4000 Sports Tester (Polar Electro Oy, Finland). This device, originally designed for human use, was adapted for the equine animal and its accuracy was checked by the use of a stethoscope. The transmitter was placed over the heart of the horse and held firmly in position by an elastic strap as shown in Figure 3.1 and Figure 3.2. To aid in electrical conduction, water soluble electrode gel was placed both on the horse and the electrodes of the transmitter before attachment to the animal. The heart rate receiver was attached to the girth of the saddle, no more than 1 metre away from the

transmitter as shown in Figure 3.2. Heart rate was measured by the receiver at 5 second intervals and stored. The data were then transferred to a microprocessor for later analysis.

3.5.2 BLOOD ANALYSIS

Blood plasma was assayed for lactate using the Boehringer Mannheim Accusport (Boehringer Mannheim GmbH, West Germany) This device determines the concentration of lactate in blood or plasma by means of reflectance photometry. This machine is capable of accurately measuring lactate at concentrations within the range of 0.7 to 26.0 mmol/L.

Plasma glucose, potassium and creatine kinase were analysed using the Boehringer Mannheim Reflotron® (Boehringer Mannheim GmbH, West Germany). This device measures blood or plasma concentrations of various biochemical parameters by means of reflectance photometry. This machine has an accuracy of ± 0.5% of the reflectance with respect to the mean of the instruments measured. The precision of this machine is S≤0.2% of the reflectance.

Whole blood was analysed for red cell volume, white cell volume, haematocrit, haemoglobin, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration by Murdoch University Veterinary Clinic, Department of Clinical Pathology. A white cell differential count was performed by the author using standard haematological techniques.

All blood was analysed within 5 hours of collection to avoid the degradation of plasma proteins and cellular constituents. Whole blood was analysed within 24 hours of collection.

3.5.3 STATISTICAL ANALYSIS OF DATA

Data obtained from this study was analysed using one way analysis of variance (ANOVA) and a Student t-test using Minitab V8 and V10 software on a P.C. microprocessor.

CHAPTER 4

RESULTS

4.1 PHASE 1 - INCREMENTAL EXERCISE TEST

To evaluate the lactate kinetics of the showjumping horse, an incremental exercise test was conducted to develop lactate performance curves and investigate the response of other biochemical and physiological variables to exercise of relatively high intensity.

All horses were transported by horse float to the Byford Training Track.. Horses NTL and WMC were the least travelled (approximately 15 minutes) whereas horses MSH and CDB had the longest travel time (60-70 minutes). Horse TVA had a travel time of approximately 30 minutes. Although travel time was variable, all animals were well accustomed to being transported in the horse float and therefore remained relaxed throughout the journey. However, it must be noted that longer travel times may have caused some degree of fatigue. Horses FK, CZ, AJS and JMB, who participated in phase 2 of this study, could not complete this test due to injuries obtained during regular competition.

Whilst testing horse MSH, horse CDB (who was tied to the float) became very excited requiring placement into the horse float for approximately 15 minutes. This was to minimise any psychogenic effects triggered by observing another horse performing exercise causing elevations in red cell indices (Stewart, Riddle and Salmon, 1977: Revington 1983a).

As tests were conducted on a public horse training track, during some tests the track had to be shared with other trainers. However, the maximum number of horses on the track at one time was never above four and in these instances, the other trainers were informed that testing was in progress and most were willing to give way to our horses. During the testing of horse WMC, one particular trainer chose not to give way during the 10 m/sec workload. As a result horse WMC could not pass and had to slow down considerably for approximately 10-20 seconds before passing the other trainer. The required velocity was then re-established and the workload was completed. The rider therefore rated this workload as 1.5 (as shown in Table 4.1) as she had to encourage the horse to move past the other trainer. Rider's ranking's and temperament of all horses tested are shown in Table 4.1.

	Velocity							(m/sec)
Horse	**6**		**8**		**10**		**12**	
	R.R.	**Temp**	**R.R.**	**Temp**	**R.R.**	**Temp**	**R.R.**	**Temp**
NTL	3	placid	3	placid	3	placid	3	placid
WMC	3	placid	3	placid	1.5	timid	4	placid
MSH	3	excited	3	excited	3	placid	3	placid
CDB	3	placid	3	placid	3	timid	2	timid
TVA	4	apprehensive	2.5	excited	2	excited	1	excited

Table 4.1 - Rider's ranking of the horse for each workload and the horse's temperament during sampling periods. R.R. - rider's ranking; Temp - temperament of horse during sampling period.

It can be seen from Table 4.1 that the majority of horses were placid during sampling periods. Although horse MSH was defined as being excited during the first two blood collections, it was found that if this horse was positioned next to horse CDB during collection he would stand quietly and could therefore be defined as placid. Horse TVA was very excited during blood collection, resisted sampling and had to be restrained.

On examination of blood samples taken from horses WMC, NTL and TVA, it became evident that peak lactate values occurred at 0 minutes post exercise as shown in Table 4.2. It was also observed that peak levels for most other variables (with the exception of white cell differential values) also occurred at this sample time. Therefore, to reduce the number of blood samples taken from each horse, lessening the chances of injury and reducing stress on animals, samples were taken at 0 minutes post workload only. This reduced the number of samples from 14 to 6 per horse.

Workload m/sec	Sample Time	RBC $x10^{12}$/L	WBC $x10^{9}$/L	HCT L/L	Hb g/L	Neutro % of WBC	Lympho % of WBC	Eosino % of WBC	Mono % of WBC	Glucose mmol/L	CK U/L/L	K^{+} mmol/L	Lactate mmol/L
Resting		11.13	8.2	0.59	189	60	35	4	1	5.58	85.8	>12.0	<0.7
6	0	11.23	8.6	0.58	191	54	43	1	2	6.48	127	3.89	<0.7
6	3	10.73	8.2	0.56	184	56	40	3	1	6.13	94.8	3.42	<0.7
6	5	10.36	8.5	0.54	179	52	47	1	0	6.14	96.4	3.69	<0.7
8	0	11.73	8.6	0.61	200	56	40	3	1	4.82	99.1	4.02	1
8	3	11.16	8.2	0.58	193	53	47	0	0	5.86	90.5	3.77	<0.7
8	5	10.83	7.9	0.57	187	54	40	5	1	5.61	125	3.59	<0.7
10	0	12.27	9.1	0.64	212	69	30	1	0	6.52	123	4.89	8.7
10	3	12.24	8.8	0.64	211	64	33	3	0	6.55	136	3.7	6.9
10	5	11.53	8.8	0.60	197	52	47	1	0	6.21	110	3.96	6.0
12	0	13.49	10.5	0.70	237	59	37	4	0	7.68	1170	4.44	15.3
12	3	12.2	8.9	0.63	210	58	38	4	0	7.33	119	3.67	15.2
12	5	11.8	8.5	0.61	205	59	39	2	0	7.05	128	3.54	14.9
24 hr		8.76	10.7	0.46	150	72	24.5	0	2	4.16	172	>12.0	<0.7

Table 4.2 - Raw data for horse WMC. Sample times are given in minutes post exercise.
Peak blood levels for most variables occurred at 0 minutes post exercise.

The incremental exercise test required the horses to work at a high intensity of exercise. Consequently all horses tested (except horse MSH) showed some signs of lameness and tenderness the day following the test. This is not untypical, however horse WMC severely injured his right front fetlock joint and was required to be spelled for 6 months. The post-exercise symptoms in all other horses ranged from foot soreness to inflammation of the legs which healed within 2-3 days.

4.1.2 HEART RATE

As discussed earlier in materials and methods, heart rate was recorded using a Polar PE4000 Sports Tester. Information obtained from this device was down-loaded onto a computer and displayed as shown in Figure 4.1.

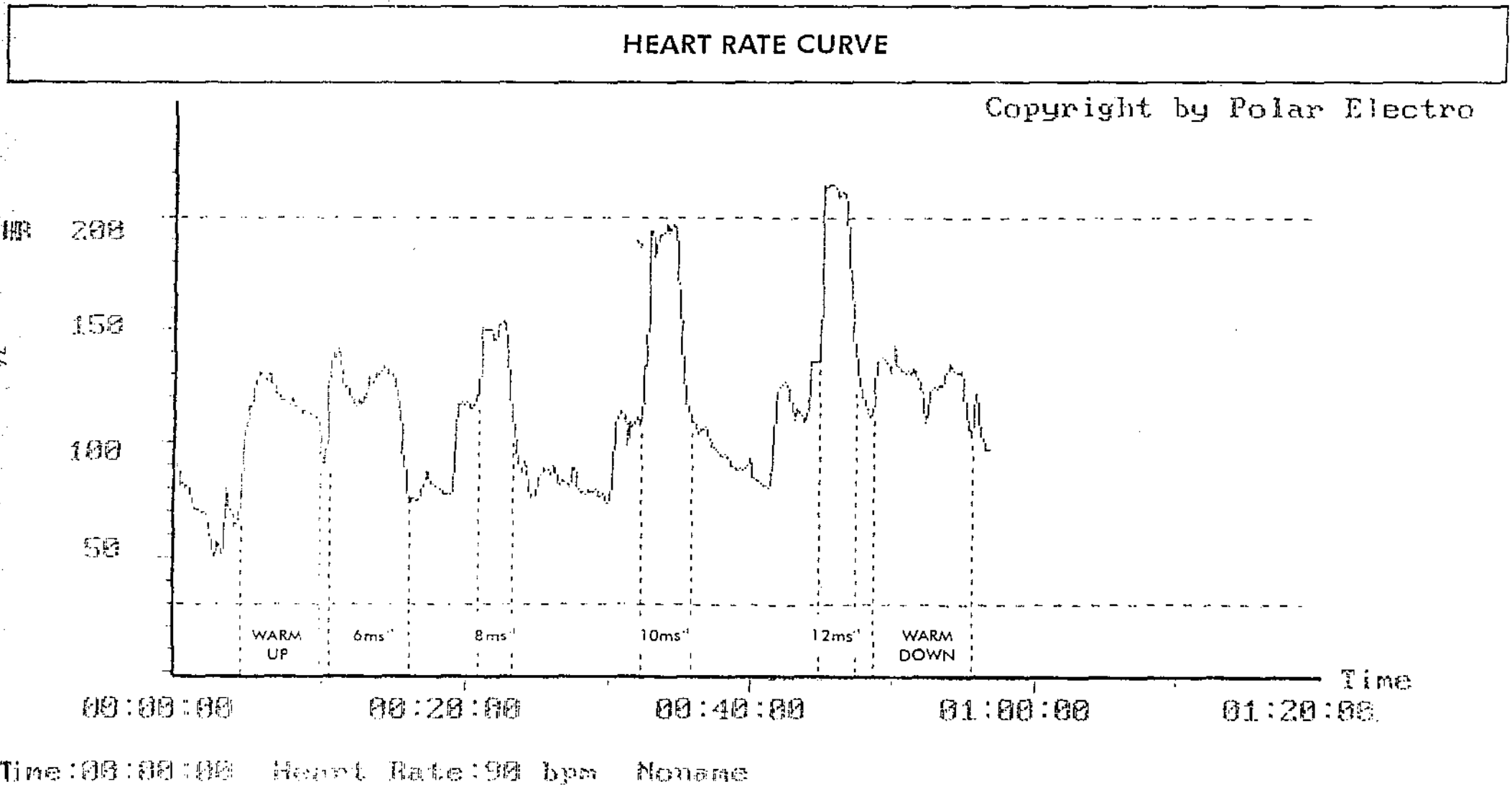

Figure 4.1 - A sample of the original PC Microprocessor output recording for horse CDB during an incremental exercise text.

Resting heart rates for the horses tested in this study were in the range of 30 to 40 beats per minute as shown in table 4.3 with a mean value of 34.6 b/min. During the first work period heart rate values increased rapidly from resting values with a mean value of 149.2 b/min. (Figure 4.2a). For each successive work period, heart rates increased linearly, with maximal heart rates occurring during the 12 m/sec workload with a mean value of 200.4 b/min being observed (Figure 4.2a). Horses NTL and WMC achieved heart rates of over 200 b/min during the 10 m/sec workload and horse CDB achieved a heart rate of over 200 b/min during the 12 m/sec workload, as shown in table 4.3. During each blood sampling period heart rates returned to near resting levels as shown in Figure 4.1. All horses showed a similar pattern in heart rate values for this test, with the exception of horse TVA whose heart rate values remained high during sampling periods. However, this animal was very excited during sampling periods, as shown in Table 4.1, causing the elevated heart rate observed during this period.

Horse	Resting	6 m/sec	8 m/sec	10 m/sec	12 m/sec
MSH	40	158	155	179	188
NTL	35	152	184	202	210
CDB	30	142	154	199	216
WMC	33	156	168	202	207
TVA	35	138	148	179	181
Mean ± SEM	34.6 ± 1.63	149.2 ± 3.93	161.8 ± 6.43	192.2 ± 5.41	200.4 ± 6.74

Table 4.3 - Peak heart rates (b/min) during each workload of the test. Mean values are given ± Standard Error of the Mean (SEM). n=5.

4.1.3 BIOCHEMICAL ANALYSIS

Table 4.4 illustrates mean biochemical variables for the values obtained from this test.

Workload (m/sec)	Lactate (mmol/L)	Glucose (mmol/L)	CK (U/L)	Potassium (mmol/L)
Resting	0.75 ± 0.03	5.882 ± 0.12	88.1 ± 20.65	7.75 ± 2.46
6	0.86 ± 0.06	4.898 ± 0.77	94.0 ± 20.48	3.87 ± 0.13
8	1.1 ± 0.09	4.808 ± 0.61	125.6 ± 21.68	3.93 ± 0.16
10	6.76 ± 2.08	6.184 ± 0.27	118.8 ± 21.7	5.78 ± 1.56
12	14.62 ± 2.68	7.160 ± 0.37	357.7 ± 13.68	5.54 ± 0.45
24 hr	0.98 ± 0.08	5.108 ± 0.27	157.6 ± 33.63	8.43 ± 2.19

Table 4.4 - Biochemical variables. Mean values ± SEM (n=5) are given.

4.1.3.a PLASMA LACTATE

All horses showed a marked increase in plasma lactate from resting values. Plasma lactate was analysed using the Boehringer Mannheim Accusport (as discussed in Materials and Methods). The lower detection limit of this instrument was 0.7 mmol/L. Resting values for most horses in this study were below the detection limit of the machine. Therefore, all lactate values under the detection limit of the machine are presented as 0.7 mmol/L. However it must be noted that resting values were probably less than this value.

As can be seen from Figures 4.2a and 4.2b, plasma lactate values remained close to resting for the workloads of 6 and 8 m/sec. However after this point, lactate concentration increased in an exponential fashion, peaking at 14.6 mmol/L (mean values) at a velocity of 12 m/sec.

Plasma lactate values varied greatly between individual horses as shown in Figure 4.2b. All horses produced similar amounts of lactate for the workloads of 6 and 8 m/sec. However lactate production increased at varying rates for velocities of 10 and 12 m/sec. Horse TVA produced the most lactate with a maximal value of 21.1 mmol/L for the 12 m/sec workload. Horse MSH produced very little lactate compared to the other horses tested. In addition, this horse did not show any signs of physical fatigue at the completion of the test. Horses MSH and CDB have similar training programs and were considered to have equal levels of fitness by their trainer. However, the resulting lactate performance curve for horse CDB was to the left and above the curve for horse MSH indicating that at the same work intensities, horse CDB will produce more lactate than horse MSH.

Lactate curves for horses NTL and CDB were very similar. These two horses (the highest graded horses tested) had the highest rates of lactate production of all horses tested for velocities between 10 and 12 m/sec.

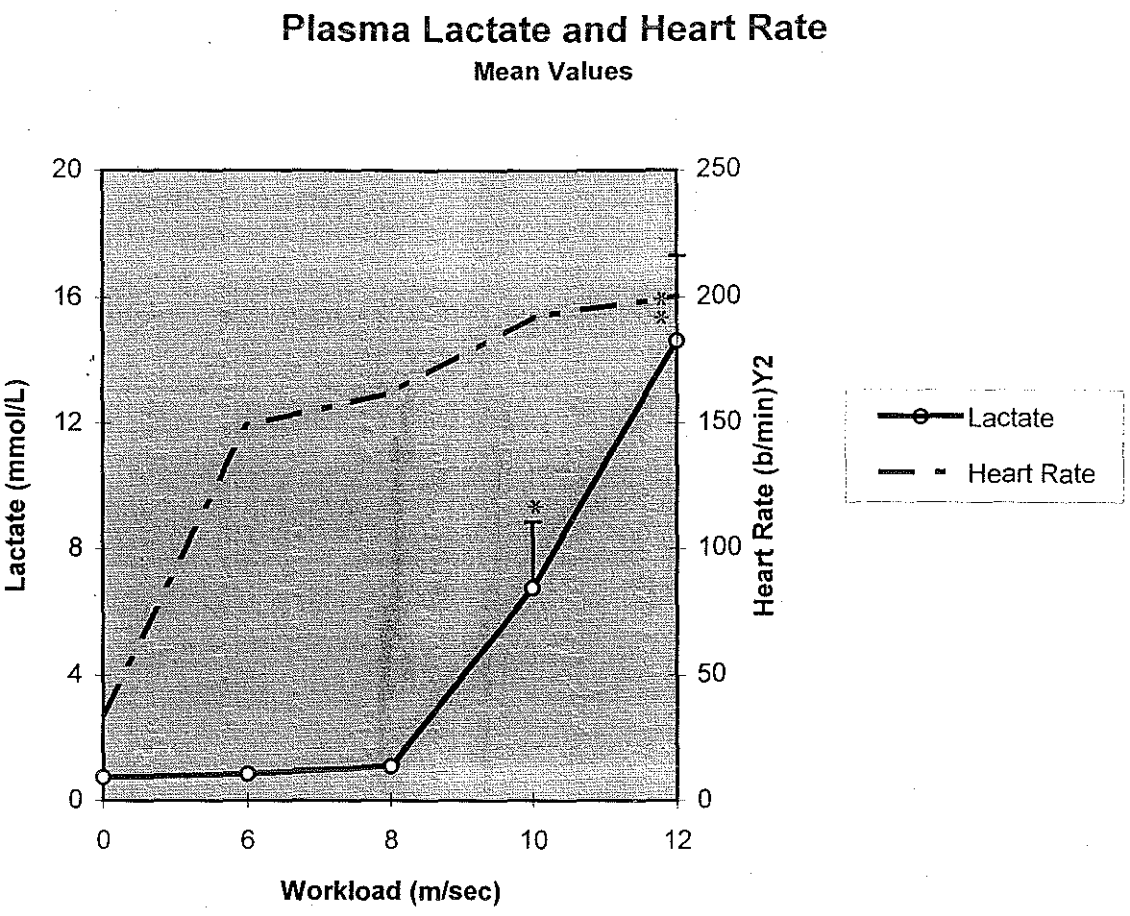

Figure 4.2a - Plasma lactate and heart rate, mean values (± SEM) n=5. n=4 for the resting sample. *significantly different from resting values ($P<0.05$) ** significantly different from resting values ($P<0.01$).

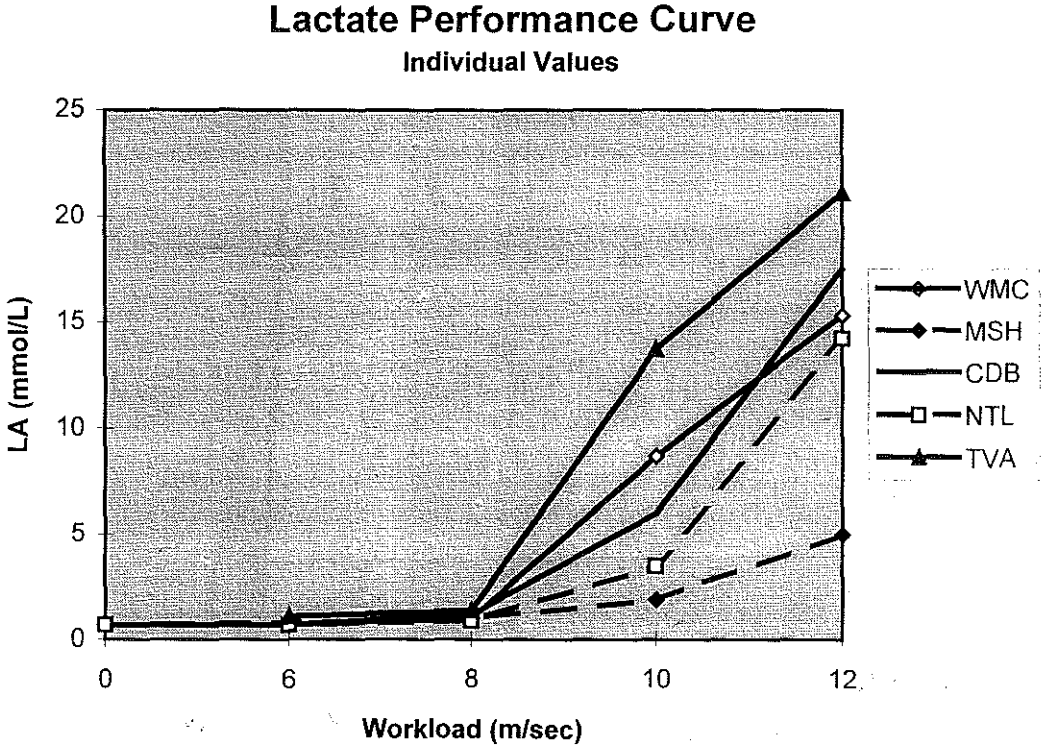

Figure 4.2b - Individual Lactate Performance Curves for 5 horses subjected to an incremental exercise test.

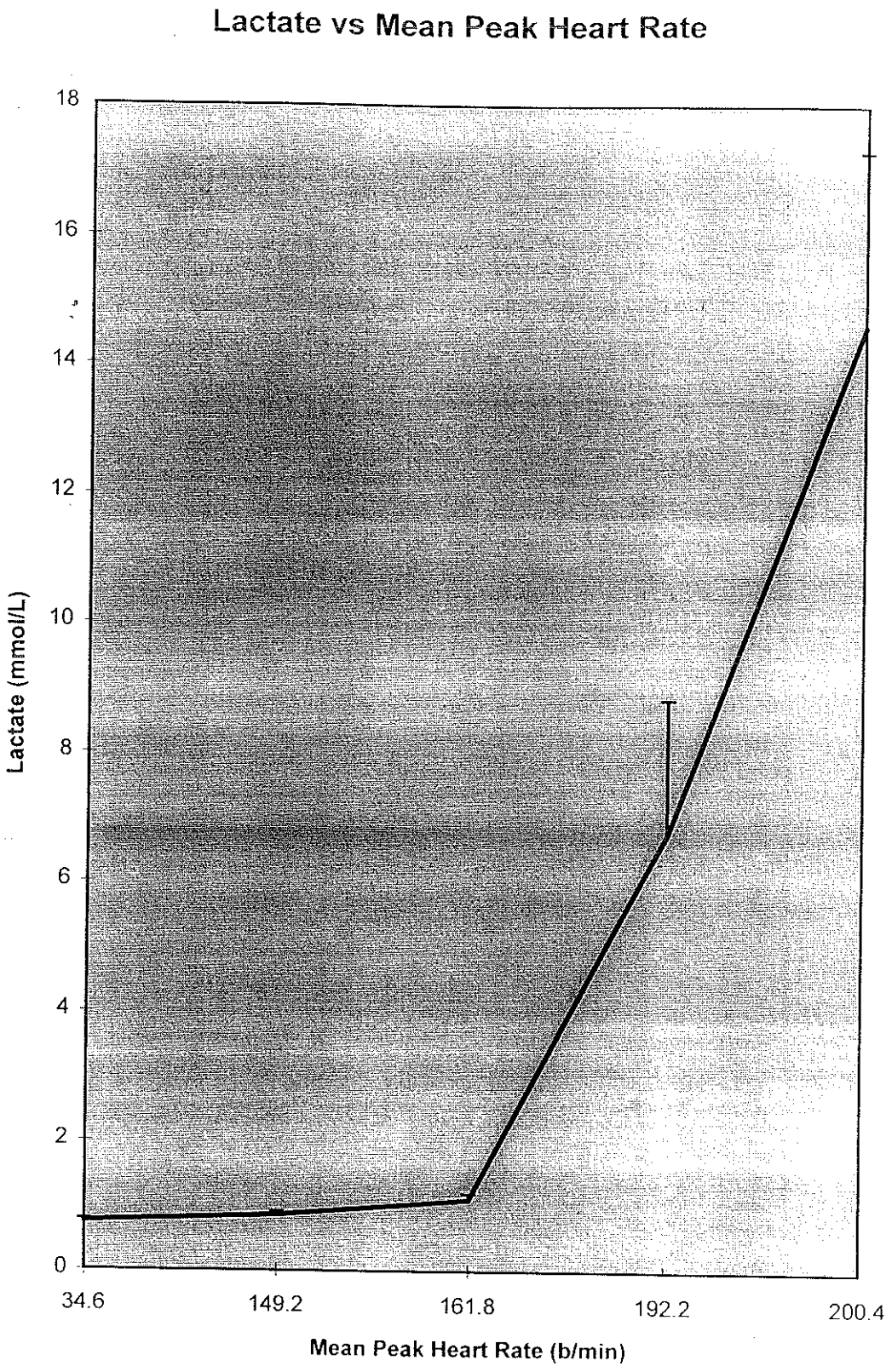

Figure 4.3 - The relationship between lactate and mean peak heart rate during the incremental exercise test. Mean values are given, ± SEM. n=5.

4.1.3.b PLASMA GLUCOSE

The mean resting plasma glucose level was 5.88 mmol/L as shown in figure 4.4. Plasma glucose decreased below resting levels during work intensities of 6 and 8 m/sec, with mean values decreasing to 4.81 mmol/L during the 8 m/sec workload. However, as the intensity of exercise was raised, plasma glucose levels increased. Mean glucose values for 10 m/sec second were 6.18 mmol/L rising to 7.16 mmol/L at 12 m/sec, (the highest intensity of exercise). Glucose values returned to resting levels 24 hours after exercise.

On examination of individual values (Figure 4.5) a large variation between horses was observed. Horse TVA had the lowest plasma glucose concentration during the first workload (2.14 mmol/L). However as the exercise intensity increased, this animal showed a marked increase in blood glucose levels as can be seen from figure 4.5. The highest individual glucose values were obtained by horse NTL. This horse's plasma glucose levels remained close to resting for the first two workloads and then increased significantly to 7.88 mmol/L during the final workload. All horses tested showed an increase in plasma glucose levels between resting and a work intensity of 12 m/sec.

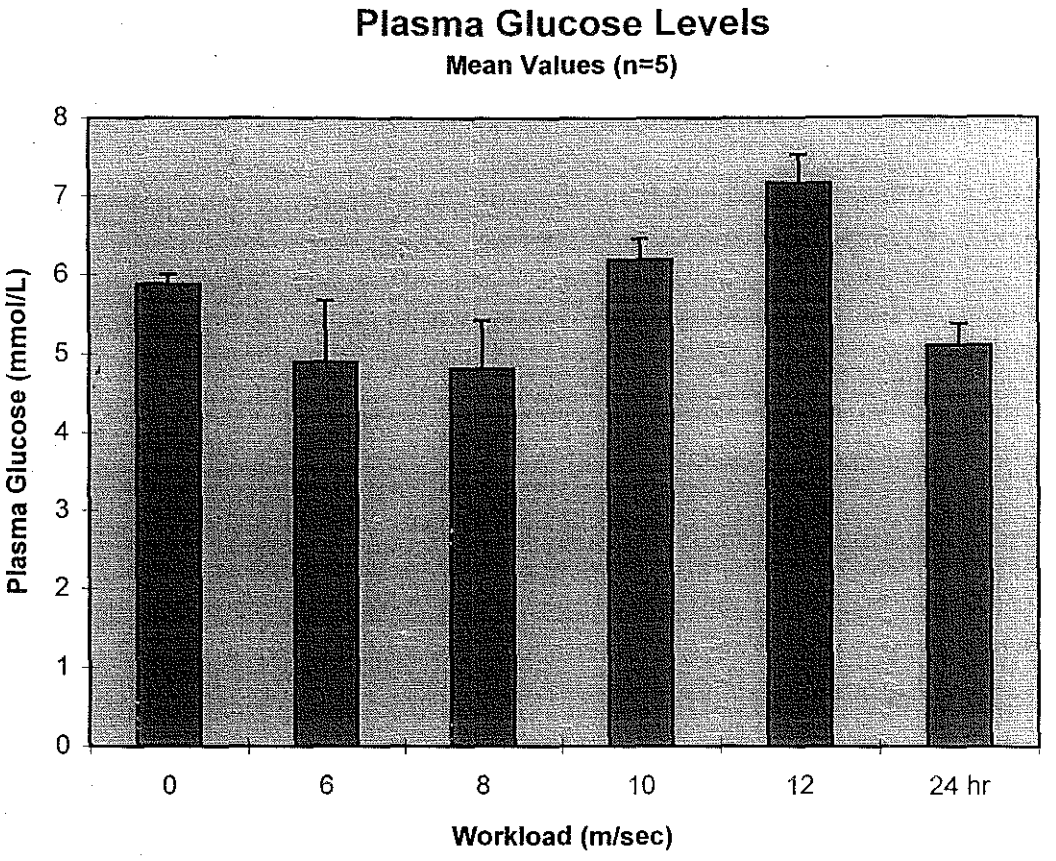

Figure 4.4 - Mean (± SEM) plasma glucose values for 5 horses subjected to an incremental exercise test. n=4 for the resting sample.

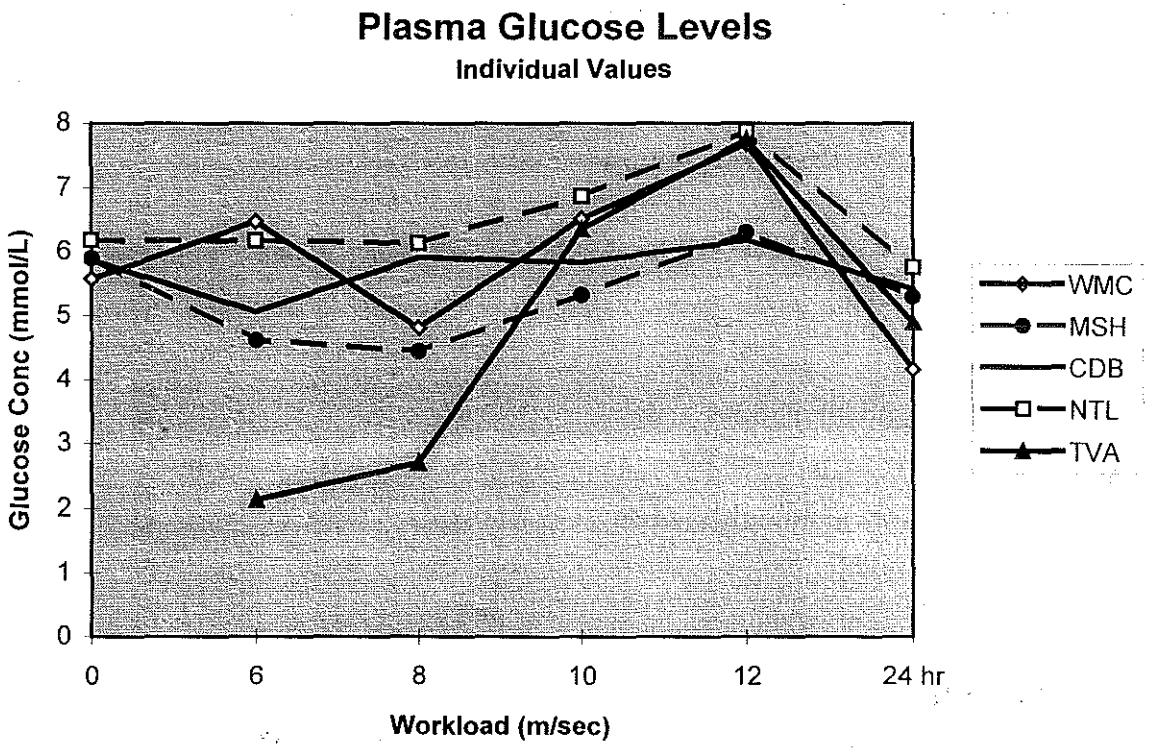

Figure 4.5 - Individual plasma glucose values for 5 horses subjected to an incremental exercise test.

4.1.3.c PLASMA CREATINE KINASE

Mean plasma creatine kinase levels remained unchanged during this exercise test for workloads of 6, 8 and 10 m/sec (Figure 4.6) However, a significant increase was noted in plasma CK levels during the 12m/sec workload. Mean plasma CK levels for the 6, 8 and 10 m/sec workloads were 94.0, 125.6, and 118.8 U/L respectively. However as can be seen from Figure 4.6 the mean plasma CK value for the final workload was 357.0 U/L. CK levels 24 hours after exercise were not significantly higher than resting values.

On examination of individual CK values (Figure 4.7) a large increase was noted for horse WMC during the 12 m/sec workload (1170 U/L). This horse fractured its right front fetlock joint during this test. It can be postulated that this injury was the cause of the large increase in CK levels observed for this workload. If this value is excluded from the data, the resulting mean is 153.75 U/L for the 12 m/sec workload. It can therefore be reported that no increase in plasma CK levels occurred during this test.

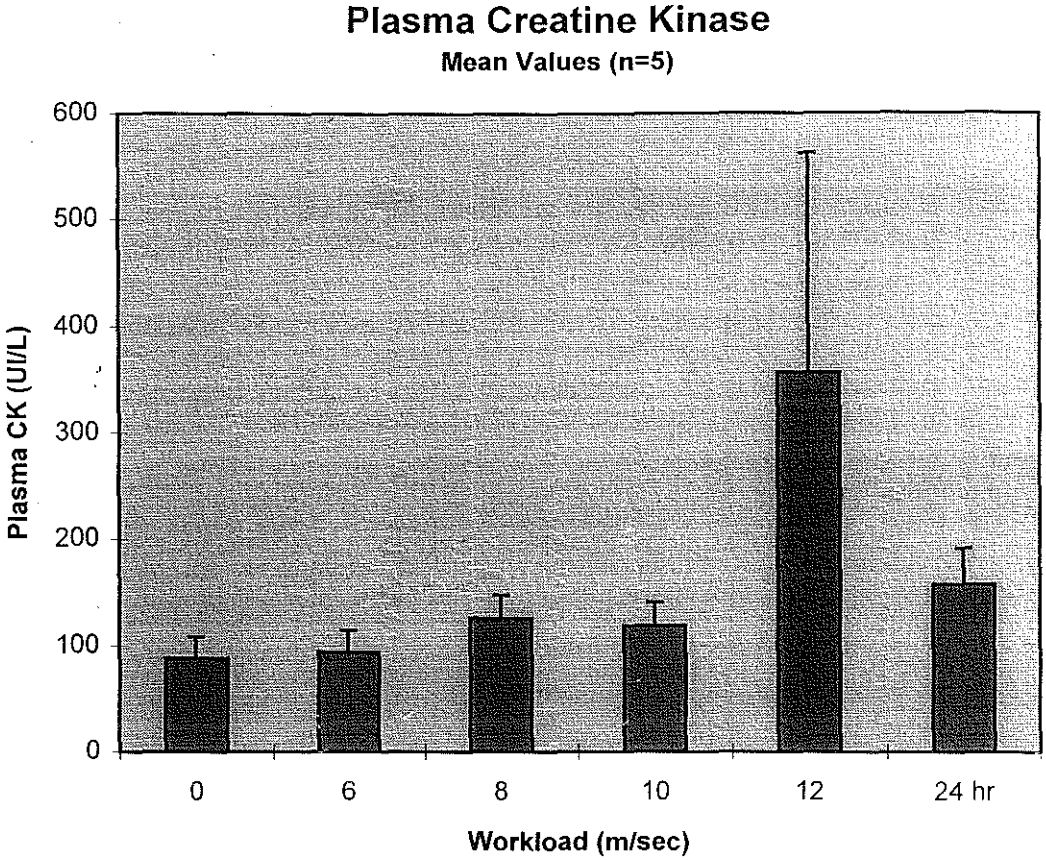

Figure 4.6 - Mean (± SEM) plasma creatine kinase values for 5 horses subjected to an incremental exercise test. n=4 for the resting sample.

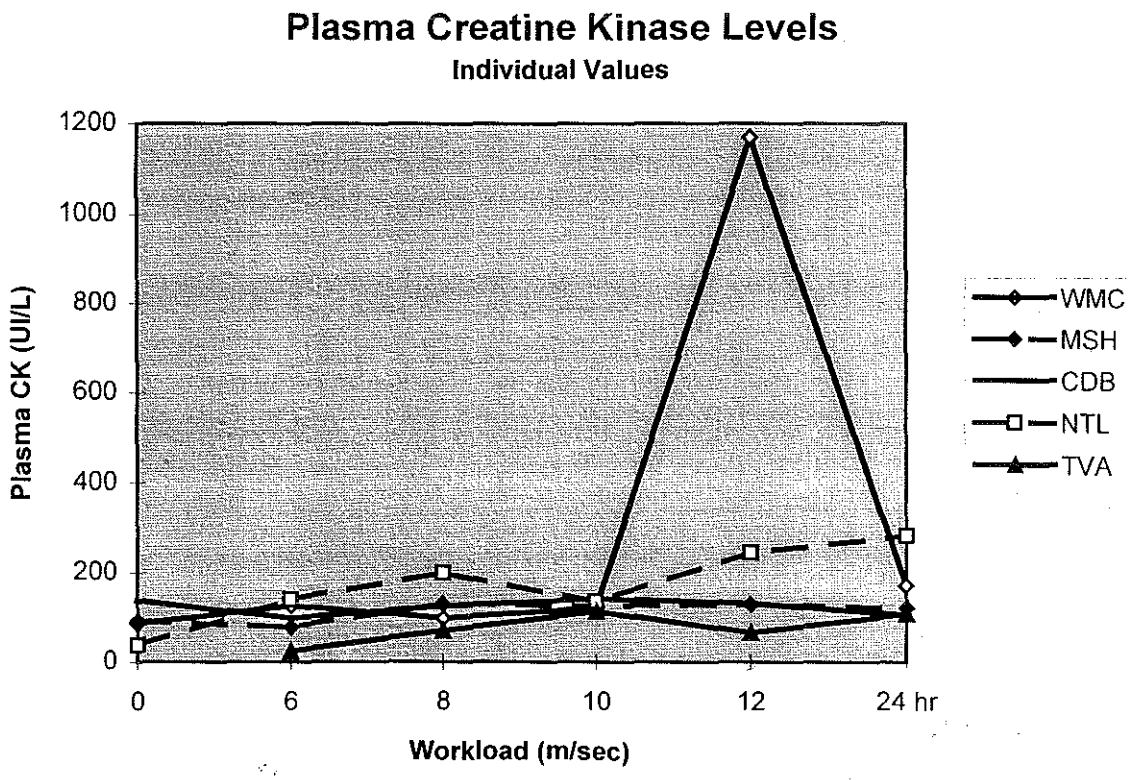

Figure 4.7 - Individual plasma creatine kinase values for 5 horses subjected to an incremental exercise test.

4.1.3.d PLASMA POTASSIUM LEVELS

Plasma potassium was analysed using the Boehringer Mannheim Reflotron (as discussed in Materials and Methods). This machine cannot measure plasma potassium levels above 12.0 mmol/L. As a result, any values above this level are defined by the Reflotron as >12.0 mmol/L. For statistical analysis of data, values lying above the detection limit of the machine, were standardised as 12.0 mmol/L. However, it must be noted that these values of 12.0 mmol/L may actually be in excess of this figure.

Horses NTL and WMC had resting potassium values of greater than 12.0 mmol/L. These horses were receiving high electrolyte supplements during the testing period, which may account for the high resting values obtained. The normal range for plasma potassium levels in the horse is 3.2 to 4.2 mmol/L (Rose and Hodgson, 1994 pg 63-78). These horses showed values much higher than the normal levels.

As can be seen from figure 4.9, horse TVA showed a marked increase in potassium levels during the 8 m/sec workload. This increase continued throughout the test and levels remained above 12.0 mmol/L for the 24 hr blood sample. There were no significant changes observed in plasma potassium levels for the other horses tested with the exception of horses NTL and WMC whose potassium levels increased above 12.0 mmol/L 24 hours after the test.

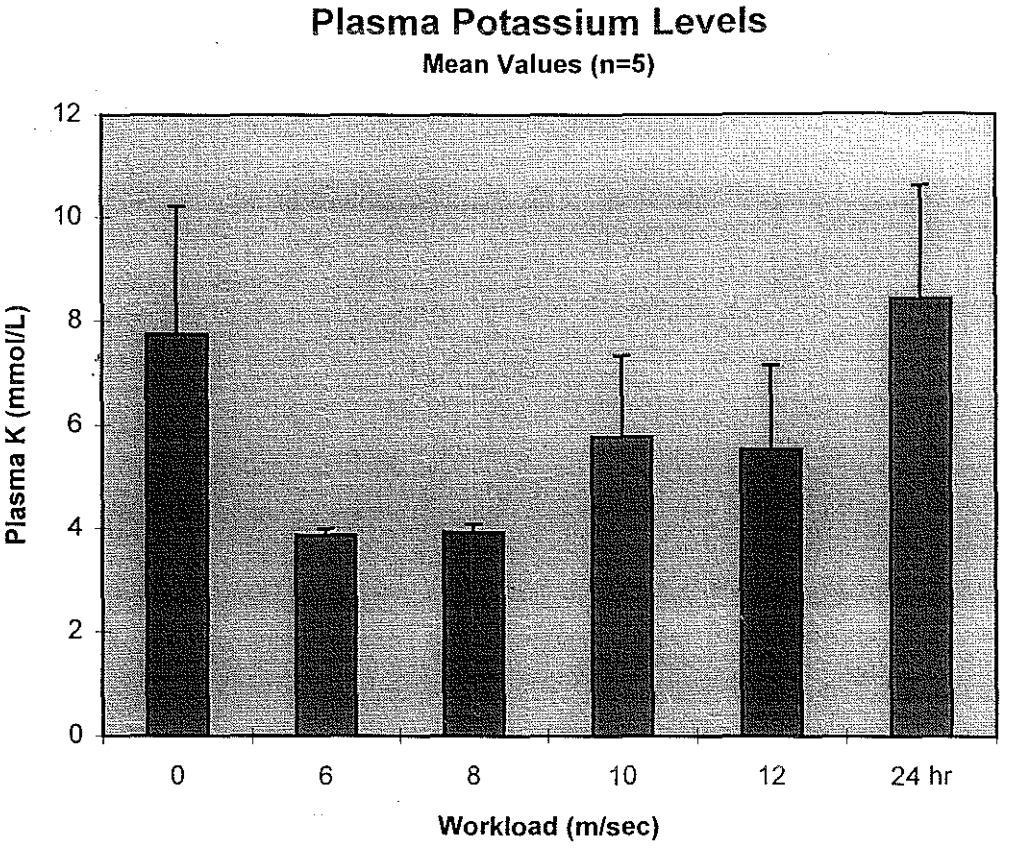

Figure 4.8 - Mean (± SEM) plasma potassium values for 5 horses subjected to an incremental exercise test.n=4 for the resting sample.

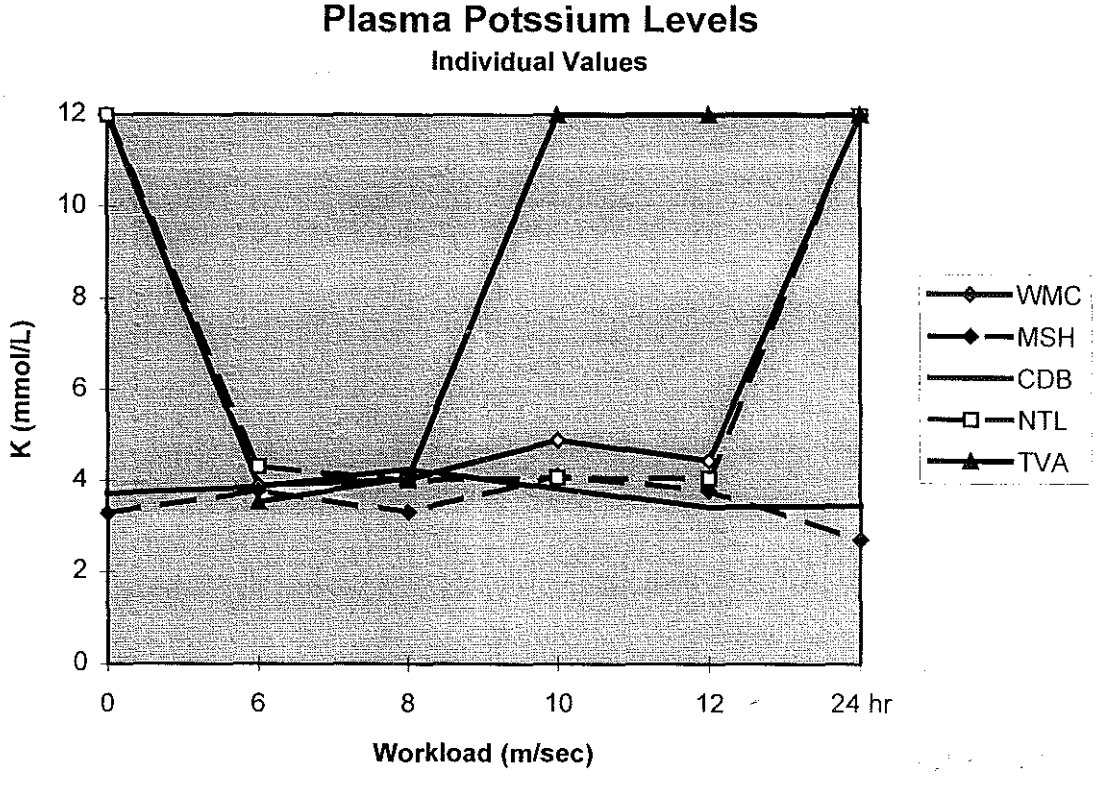

Figure 4.9 - Individual plasma potassium levels for 5 horses subjected to an incremental exercise test.

Mean values show an initial decrease from resting plasma potassium levels during the 6 and 8 m/sec workloads (Figure 4.8), with a slight increase then being noted during the more intense workloads, (10 and 12 m/sec) and a larger increase was observed 24 hours post test. Mean potassium values are shown in Table 4.4. It can therefore be concluded from examination of the mean values that this test caused an increase in plasma potassium levels during incremental exercise. However, if horse TVA is excluded from the mean values, no increases in plasma potassium levels were observed.

4.1.4 HAEMATOLOGICAL VARIABLES

Table 4.5 illustrates the mean values of haematological variables obtained from all horses subjected to this test.

Workload (m/sec)	RBC ($x10^{12}$/L)	HCT L/L	Hb g/L	WBC ($x10^{9}$/L)	Neutro (% of WBC)	Lymph (% of WBC)	Eosino (% of WBC)	Mono (% of WBC)
Resting	9.3 ± 0.77	0.49 ± 0.04	162.5 ± 12.1	8.3 ± 0.09	51.2 ± 4.72	44.8 ± 4.9	2.8 ± 0.75	1.25 ± 0.6
6	10.7 ± 0.50	0.56 ± 0.02	187.2 ± 5.71	8.7 ± 0.38	57.4 ± 4.0	38.8 ± 4.4	2.4 ± 0.6	1.6 ± 0.51
8	11.0 ± 0.51	0.58 ± 0.02	193.6 ± 6.07	9.1 ± 0.31	55.6 ± 2.25	40.6 ± 3.7	3.4 ± 1.52	0.6 ± 0.24
10	11.5 ± 0.54	0.59 ± 0.02	203.0 ± 6.13	8.6 ± 0.23	56.2 ± 4.96	39.6 ± 4.2	4.0 ± 1.14	0.2 ± 0.2
12	12.2 ± 0.64	0.63 ± 0.02	218.2 ± 8.97	9.1 ± 0.46	58.0 ± 4.16	39.6 ± 4.1	2.4 ± 0.5	0
24 hr	8.6 ± 0.27	0.46 ± 0.01	151.6 ± 3.5	10.9 ± 0.56	66.0 ± 3.20	28.2 ± 2.6	1.3 ± 0.88	2.0 ± 0.89

Table 4.5 - Haematological variables for incremental exercise tests. Mean values ± SEM

4.1.4.a RED CELL INDICES

An increase was observed in all red cell indices tested in this study from resting to the 12 m/sec workload. Mean RBC counts ranged from 9.3 $x10^{12}$/L at rest to 12.2 $x10^{12}$/L during the 12 m/sec workload (refer to Figure 4.10).

As can be seen in Figure 4.11 individual values for each horse are quite varied, although the rate of RBC increase was similar for all horses. Horse TVA showed the largest number of red cells with values peaking at 14.01 $x10^{12}$/L, however, this horse was very excited during sample collection (refer to Table 4.1) and this may have caused an elevation in RBC numbers. Horse MSH showed a significant increase in RBC values from resting to the 6 m/sec workload, however these values did not increase during the incremental exercise. This horse was also excited during sample collection which may have caused an elevation in RBC numbers.

Mean haematocrit values increased from 0.49 L/L (litres per litre) at rest to 0.64 L/L during exercise at 12 m/sec as shown in Figure 4.12. This increase was very similar to that observed in RBC values. In addition, increases seen in individual haematocrit values were similar to those seen in individual RBC values for all horses (see Figure 4.13).

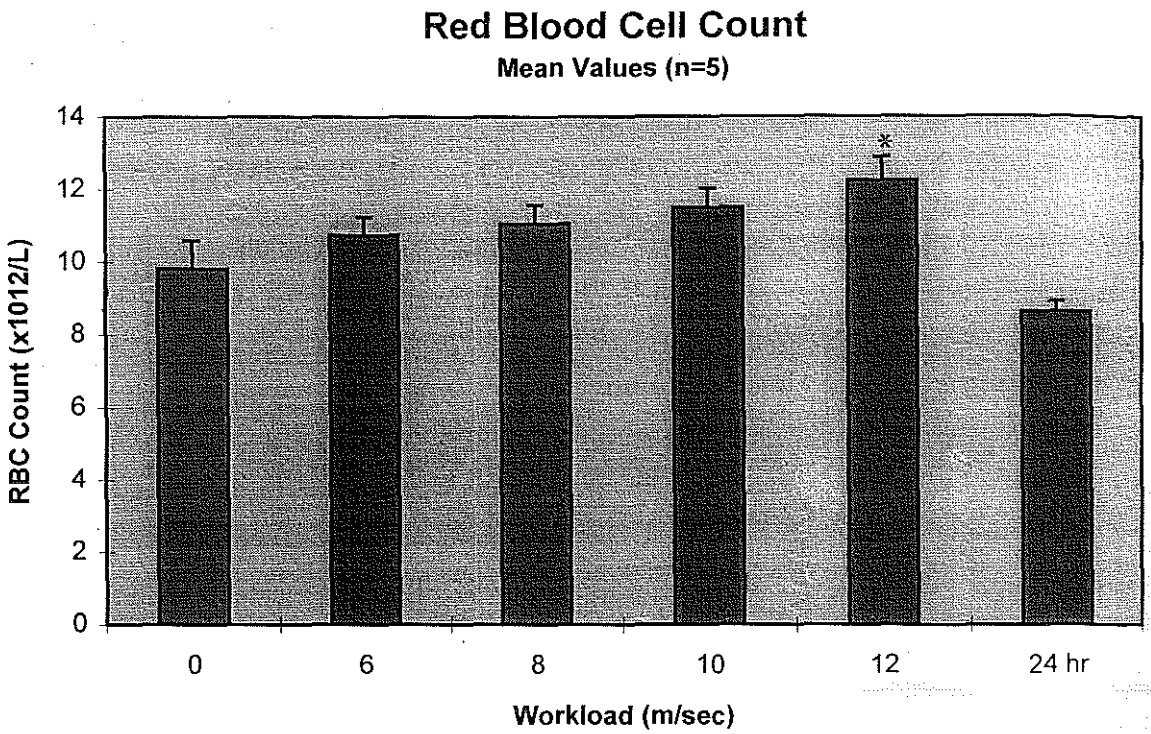

Figure 4.10 - Mean (± SEM) RBC values for 5 horses subjected to an incremental exercise test. A resting sample was unavailable for horse TVA. * significantly different from resting values ($P<0.05$)

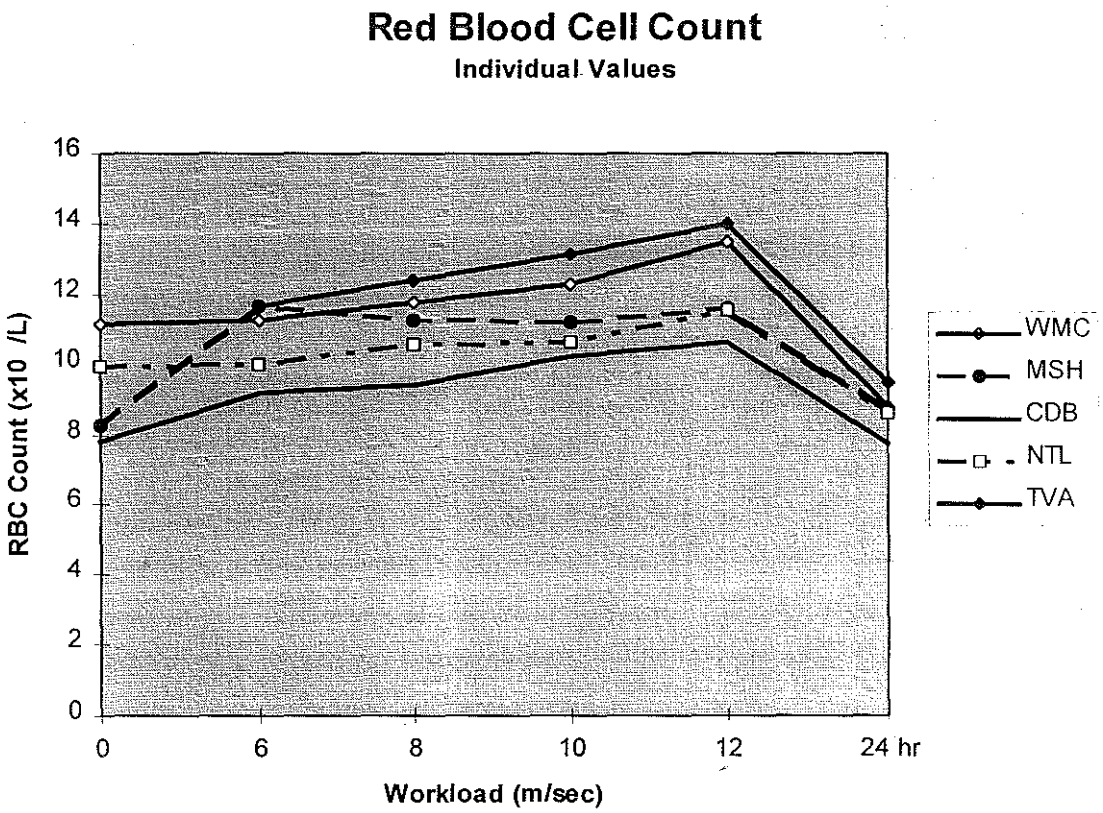

Figure 4.11 - Individual RBC values for 5 horses subjected to an incremental exercise test. 0 m/sec is the resting value, 24 hr denotes a 24 hr post exercise blood sample.

Mean haemoglobin increased from 162.5 g/L at rest to 218.2 g/L during the final workload as shown in Figure 4.14. In addition, haemoglobin values for individual horses (as shown in Figure 4.15) increased in a similar manner to both RBC and haematocrit.

4.1.4.b WHITE CELL COUNT

During the testing period there was no significant increase in white blood cell counts. However a significant increase was observed in 24 hour post exercise blood samples as shown in Figure 4.16. Mean resting values were 8.3 $x10^9$/L and this value increased to 10.9 $x10^9$/L 24 hours after completion of the test. There was apparent deviation observed between horses for individual WBC values (Figure 4.17). Horse NTL showed the highest post exercise WBC values. This horse also had a large degree of inflammation in the legs, 24 hours after the test.

Figure 4.18 shows mean white cell differential count values. As with WBC values, there was no significant change in differential white cells during the test. An increase in the percentage of neutrophils was observed 24 hours after the completion of the test. Although this increase was not statistically significant, some degree of change was observed as seen in Figure 4.18. This therefore caused a decrease in the percentage of lymphocytes in the differential count. No basophils were detected in any sample.

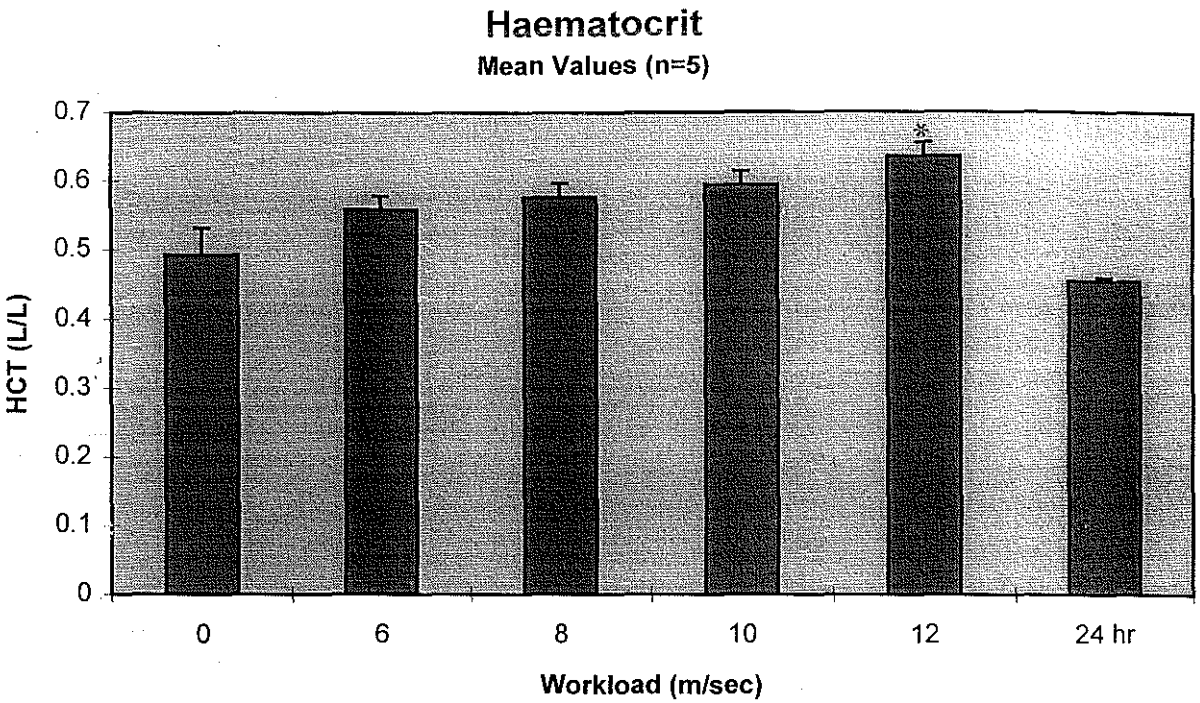

Figure 4.12 - Mean (± SEM) haematocrit values for 5 horses subjected to an incremental exercise test. For the resting blood sample (0 m/sec) n=4.
* statistically significant from resting values ($P<0.05$).

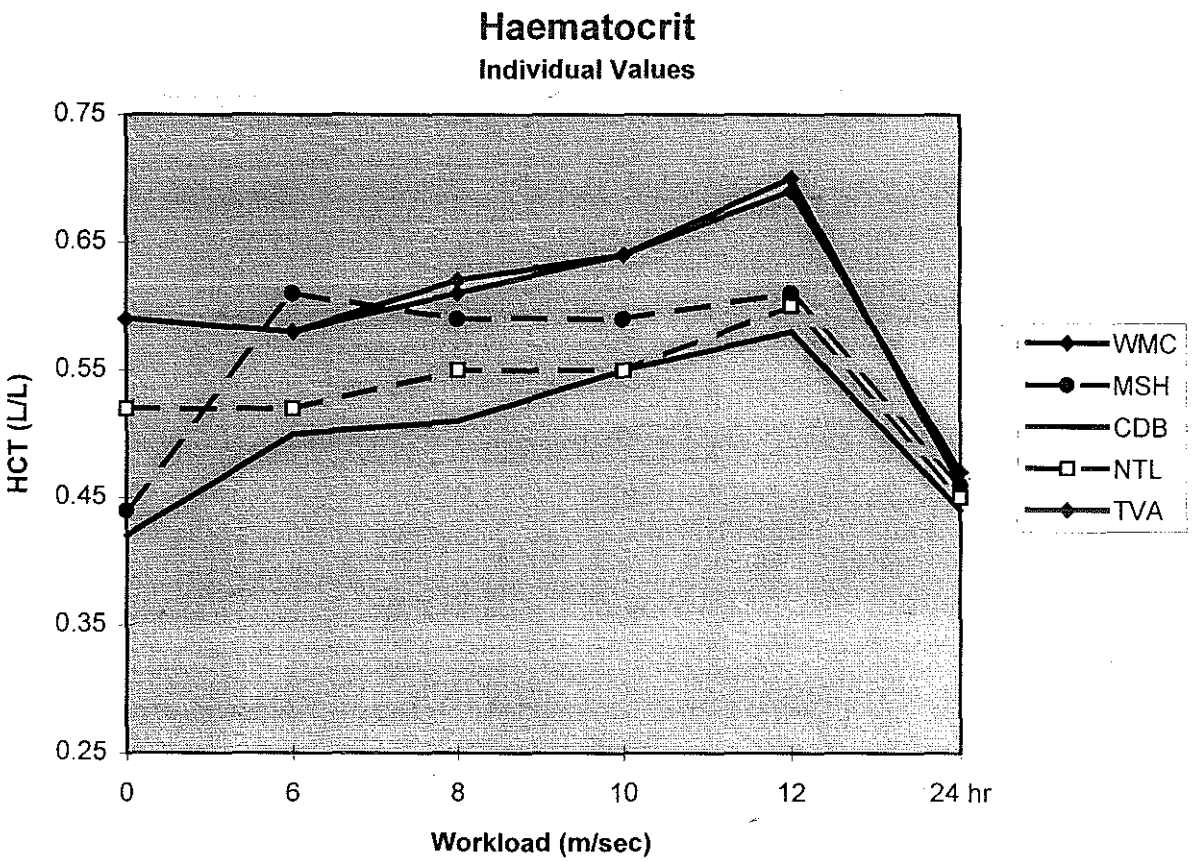

Figure 4.13- Individual haematocrit values for 5 horses subjected to an incremental exercise test.

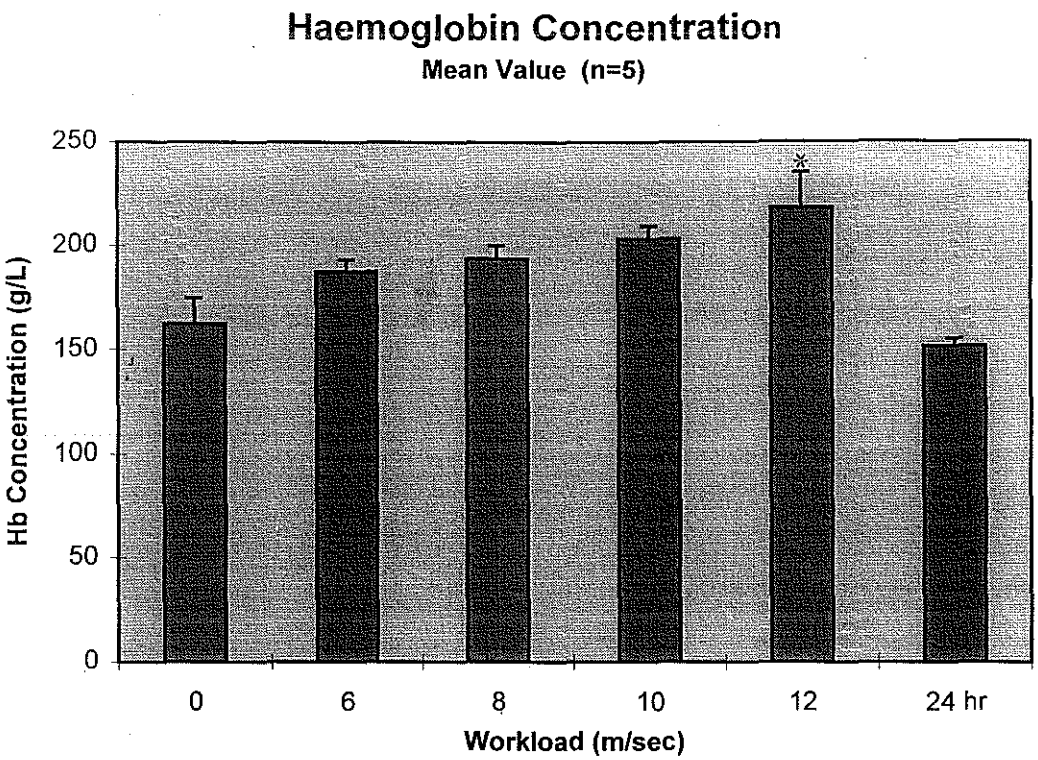

Figure 4.14 - Mean (+SEM) haemoglobin values for 5 horses subjected to an incremental exercise test. For the resting sample (0 m/sec) n=4. * significantly different from resting values.

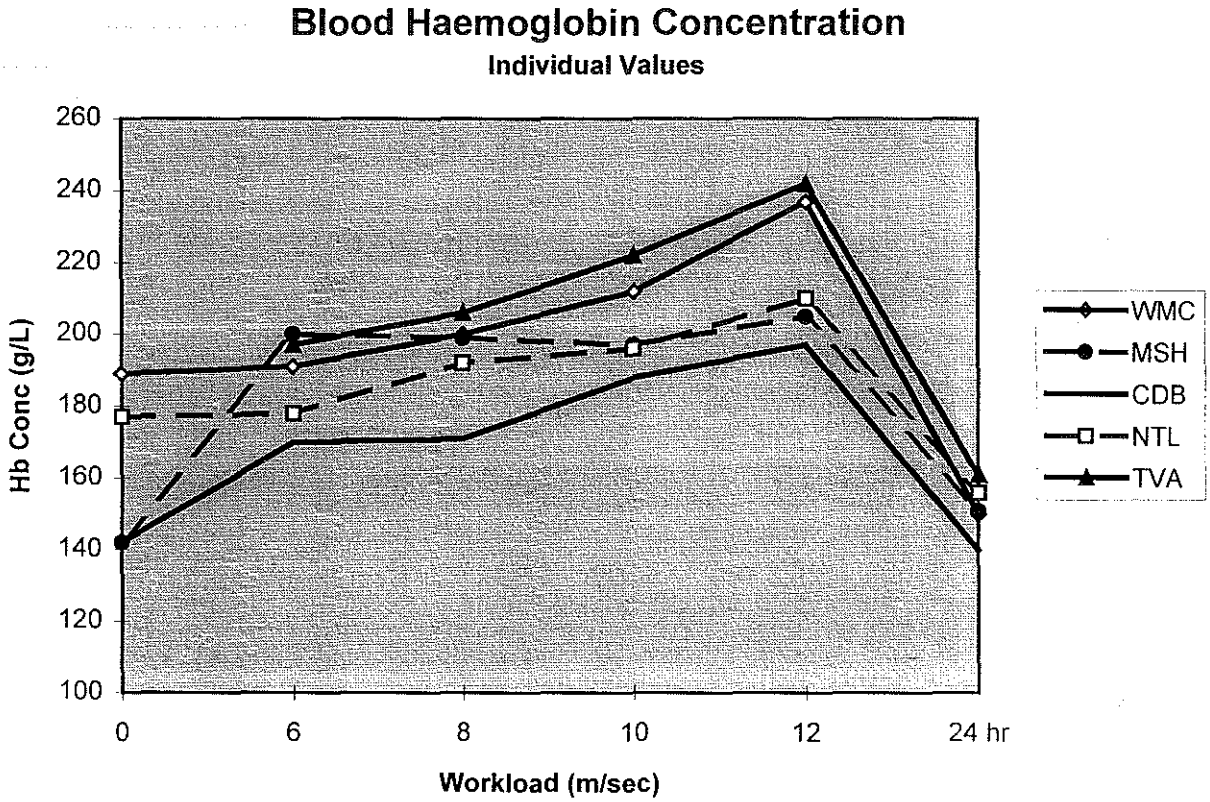

Figure 4.15 - Individual haemoglobin values for 5 horses subjected to an incremental exercise test.

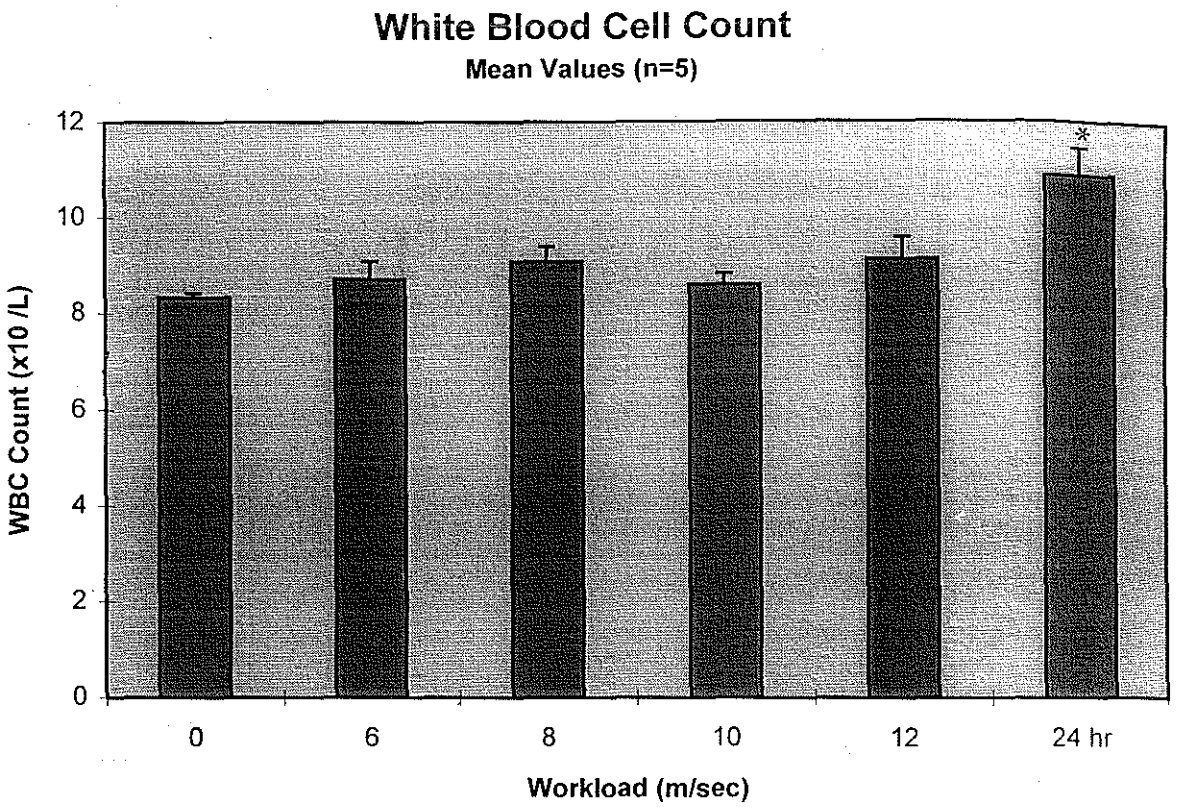

Figure 4.16 - Mean (+SEM) WBC values for 5 horses subjected to an incremental exercise test. For the resting sample n=4. * significantly different from resting values ($P<0.05$).

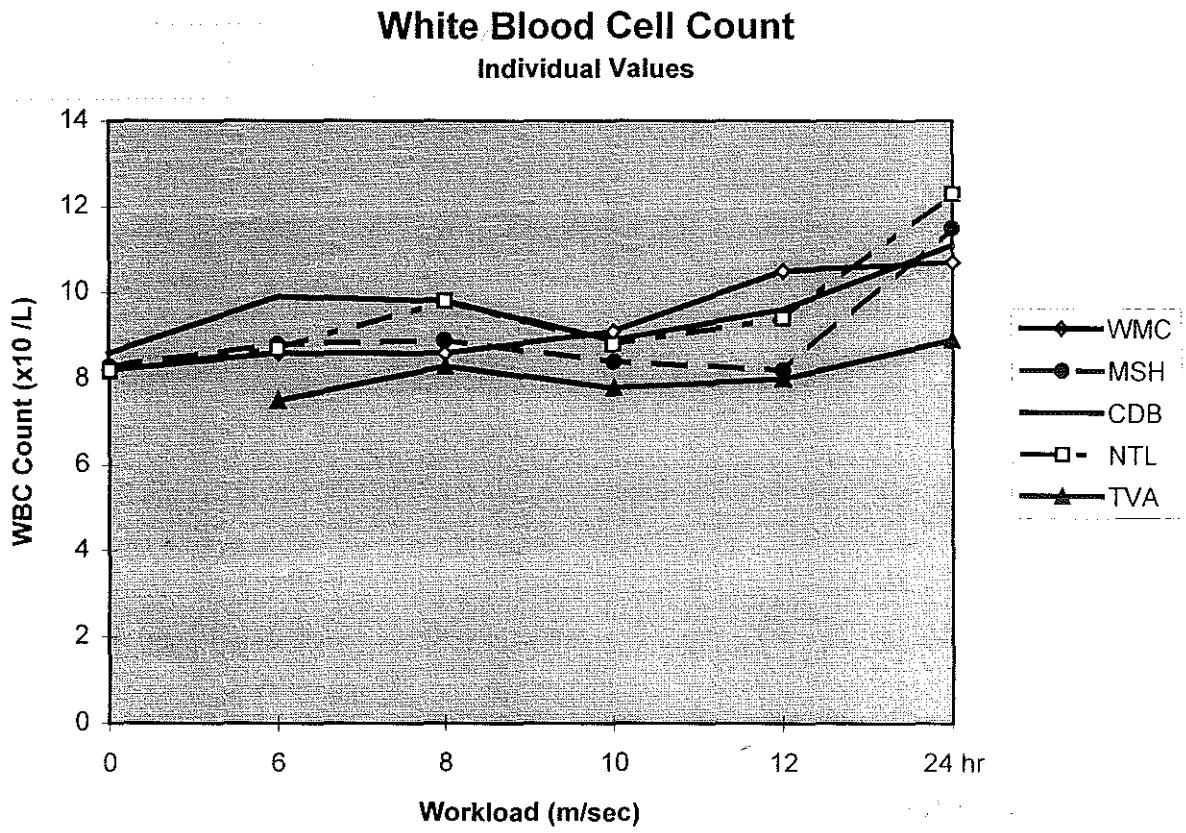

Figure 4.17 - Individual WBC values for 5 horses subjected to an incremental exercise test.

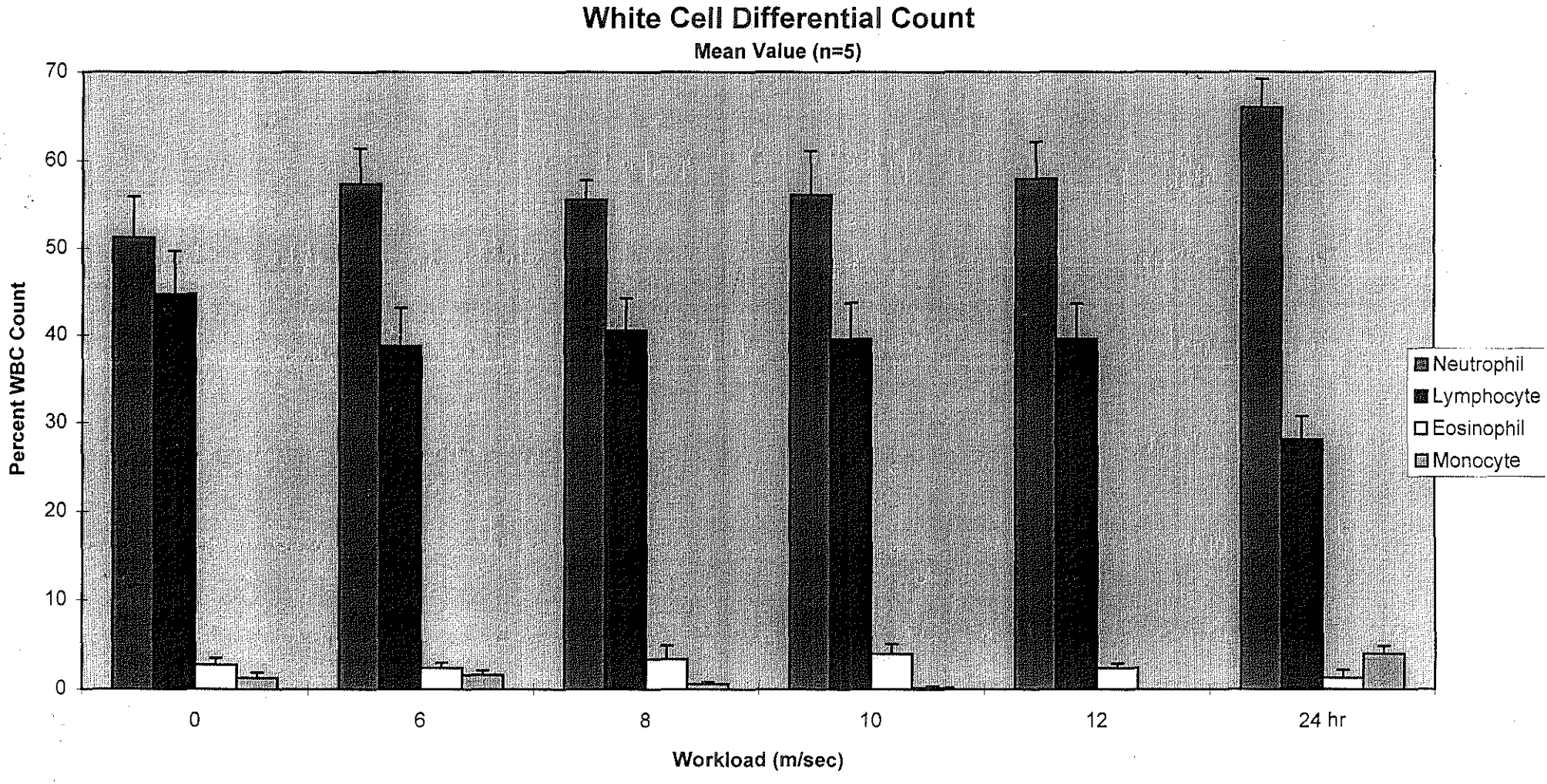

Figure 4.18 - White cell differential count (mean values +SEM) for 5 horses subjected to an incremental exercise test. For the resting sample n=4. * significantly different from resting values ($P<0.05$) .

4.2 PHASE 2 - JUMP TEST

This test was designed to evaluate the biochemical and haematological responses of showjumping horses to a competition standard jump course. As a control, horses were cantered around the course without any jump poles in position (as described in Methods). The horses were then required to complete the course with jumps placed into position, thus the physiological demands of the jumping action could be isolated.

This test was conducted at Argyle Showjumping Stables, Wanneroo, Western Australia. Horses MSH, CDB, CZ, AJS and JMB, were stabled at this complex and therefore did not have to travel to the venue. Horse NTL was required to be transported in a horse float from home to this venue. Travel time for this horse was approximately 1 hour. Although this horse is well accustomed to travelling and competing at many different venues, it had never visited this venue before and therefore the effects of excitement or anxiety brought about by strange surroundings cannot be ignored. However, this horse remained very calm during the test and sampling periods as shown by Table 4.7.

Horses FK and TVA were stabled approximately 100 to 120 km from the testing venue. Due to the long travel time required to transport these horses to the testing venue the trainer of these animals found it very difficult to make time for the journey. As a result, horses FK and TVA were tested at their trainer's place of residence, a separate location to the other horses. However the arena surface and size was very similar to that used at Argyle Stables and the jump course was identical.

Due to an injury sustained during phase 1 of this study, horse WMC was unable to complete this jump test.

Table 4.6 illustrates the times taken to complete each jump course and penalty points awarded for each horse. (refer to Methods section) It can be seen from this table that there was little difference in the times taken to complete each course, with the exception of horse TVA. From all horses tested, horse AJS completed course 2 in the fastest time of 65 seconds whereas the slowest horse was horse FK, who also knocked down 6 fences.

If the horses are grouped according to their level of competition and the height of the fences on the course, for the C and D grade horses, horse AJS (a C grade horse) completed the course in the fastest time (65 seconds) and horses TVA and MSH completed the course in the slowest time (72 seconds). Of the B grade horses, horse CZ obtained the fastest time of 67 seconds, with horse FK having the slowest time of 80 seconds.

It must also be noted that horses CDB, AJS, CZ and JMB were ridden by the same jockey. It would therefore be expected that this rider could complete the course faster than any of the other riders due to a training effect.

Horse	Time to Complete Course 1	Time to Complete Course 2	Fences Knocked Down	Refusals
MSH	70	72		
AJS	66	65	-	-
NTL	74	70	1	2
FK	82	80	6	-
TVA	90	72	2	-
CZ	64	67	-	1
JMB	72	71		
CDB	78	68	-	-

Table 4.6 - Course times and penalties for horses completing the jump test. Times are given in seconds. Times for completion of course 2 have been corrected for refusals of jumps, as discussed in Methods.

	Temperament of Horse			
Horse	Resting	Course 1	Course 2	24 Hours
NTL	placid	placid	placid	placid*
CDB	apprehensive	placid	placid	-
MSH	placid	timid	apprehensive	-
AJS	placid	placid	apprehensive	timid**
CZ	placid	placid	placid	placid
FK	placid	placid	placid	-
TVA	timid	placid	timid	-
JMB	placid	placid	timid	placid

Table 4.7 - Temperament of horses during blood collection (scale described in methods).
* - Horse had been lightly exercised 10 minutes prior to sample collection
** - Although timid during sample collection, this horse refused to be caught in the paddock and ran wildly for approximately 5 minutes before collection.

Blood samples were collected at rest and at 1, 3 and 5 minutes after each stage of this test and then analysed for biochemical and haematological variables (as described in Methods). The results obtained were stored on computer as shown in Table 4.8. As can be seen from the table, peak values for most variables tested (with the exception of white cell differential counts) occurred at 1 minute post-exercise. Therefore, after testing horses NTL, CDB and MSH, it was confirmed that peak blood values occurred at 1 minute post-exercise. As a result for the remaining horses, blood samples were taken at resting and at 1 minute post-exercise for each jump course. This reduced the number of blood samples from 8 to 4 per horse. Horses FK, TVA, MSH and CDB were unavailable for 24 hour post-exercise blood sampling due to prior commitments of the trainers.

	RBC $x10^{12}$/L	WBC $x10^{9}$/L	Hb (g/L)	HCT L/L	MCV FL	MCH pg	MCHC g/L	Neutro % of WBC	Lymph % of WBC	Eosino % of WBC	Mono % of WBC	Glucose mmol/L	Lactate mmol/L	CK U/L	K^+ mmol/L
Resting	7.82	8.6	142	0.42	54	18	336	40	58	1	1	5.87	<0.7	138	3.71
Course 1															
1 min	9.54	10.2	176	0.52	54	18	340	40	59	1	0	5.15	<0.7	111	4.23
3 min	9.02	9.7	165	0.49	54	18	338	43	53	4	0	3.29	<0.7	53.9	4.11
5 min	8.73	9.4	159	0.47	54	18	336	45	51	4	0	5.85	<0.7	105	4.2
Course 2															
1 min	10.1	9.5	185	0.54	54	18	340	40	59	1	0	5.49	3.4	134	3.95
3 min	9.57	9.2	176	0.52	54	18	340	42	53	3	1	6.23	2.8	80.3	3.43
5 min	8.95	8.9	165	0.49	55	18	338	53	45	2	0	5.06	2.4	27.7	3.64

Table 4.8 - Raw data for horse CDB. Times are given in minutes post exercise. Peak values occurred at 1 minute post exercise for most variables.

4.2.1 HEART RATE

As mentioned in Materials and Methods, heart rate was recorded from before the first warm up period until the completion of course 2. Heart rates were then down-loaded onto computer and the resulting print out is shown in Figure 4.19.

Heart rates for all horses increased with exercise, as shown in Table 4.9. The greatest increase in heart rate was shown by horse CDB who obtained a peak heart rate of 203 b/min. All horses obtained peak heart rates whilst jumping course 2, with the exception of horse MSH, whose heart rate remained relatively constant throughout the entire test.

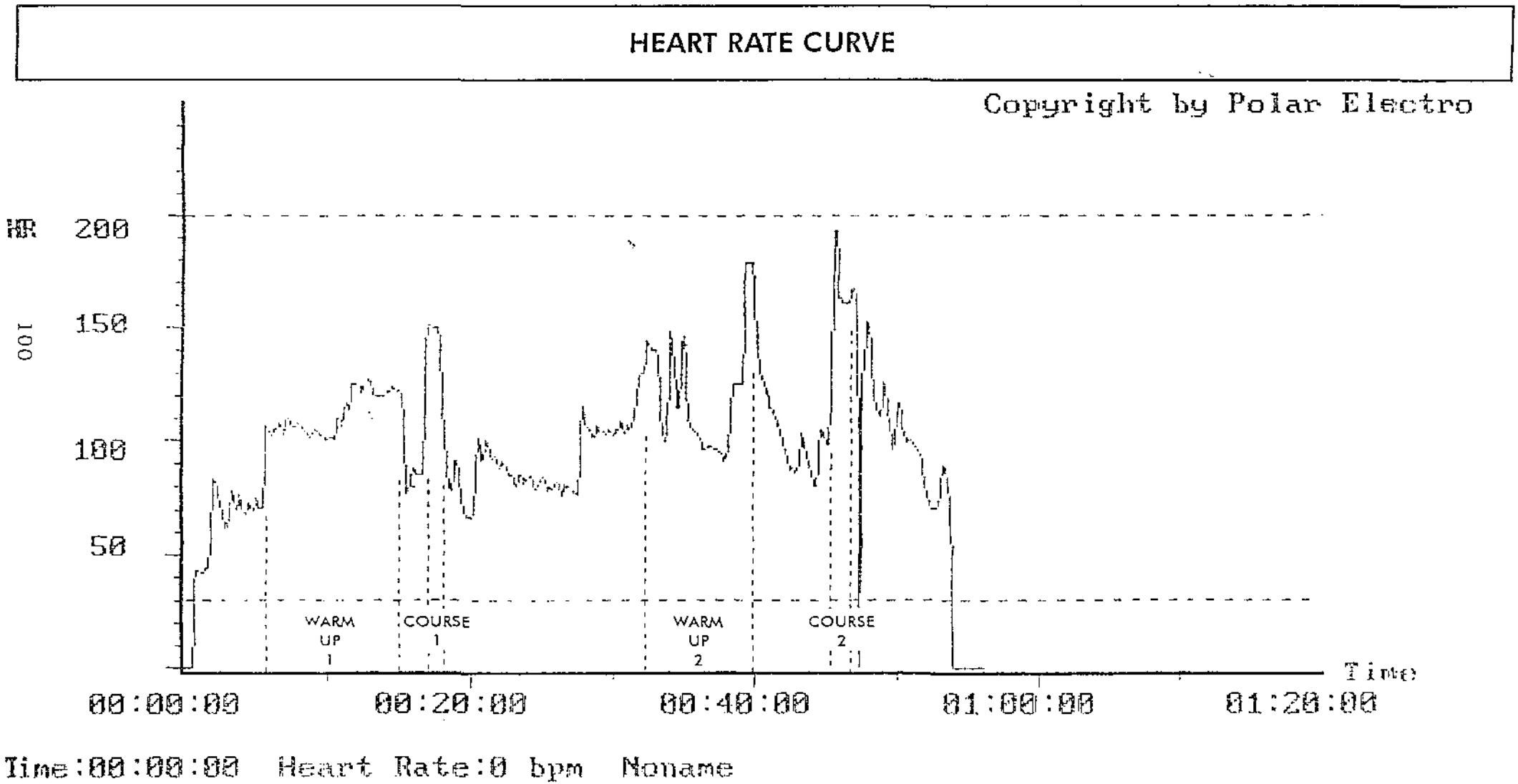

Figure 4.19 - A sample of the original PC Microprocessor output recording for horse FK during the Jump Test.

Horse	Resting	Warm-Up 1	Course 1	Warm-Up 2	Course 2
TVA	37	119	146	155	164
FK	41	124	151	179	193
NTL	-	-	-	-	-
CDB	35	142	170	164	203
MSH	42	133	158	158	157
CZ	32	144	162	163	176
JMB	31	132	163	148	182
AJS	34	117	146	120	199

Table 4.9 - Peak heart rates (b/min) during the jump test for all horses. Data for horse NTL was unavailable due to technical problems. Heart rates are given in beats/minute.

Mean heart rate values are presented in Figure 4.20. The mean resting heart rate for this test was 36.37 b/min. During the first warm up period, this increased to 130.4 b/min. During the first jump course, the mean heart rate value was 156.6 b/min. With the introduction of jumping efforts in the second warm-up period, the horses' heart rates increased slightly to a mean value of 155.3 and then peaked at 182 b/min during the second jumping course. It can therefore be observed from Figure 4.20 that heart rate increased with increasing exercise intensity.

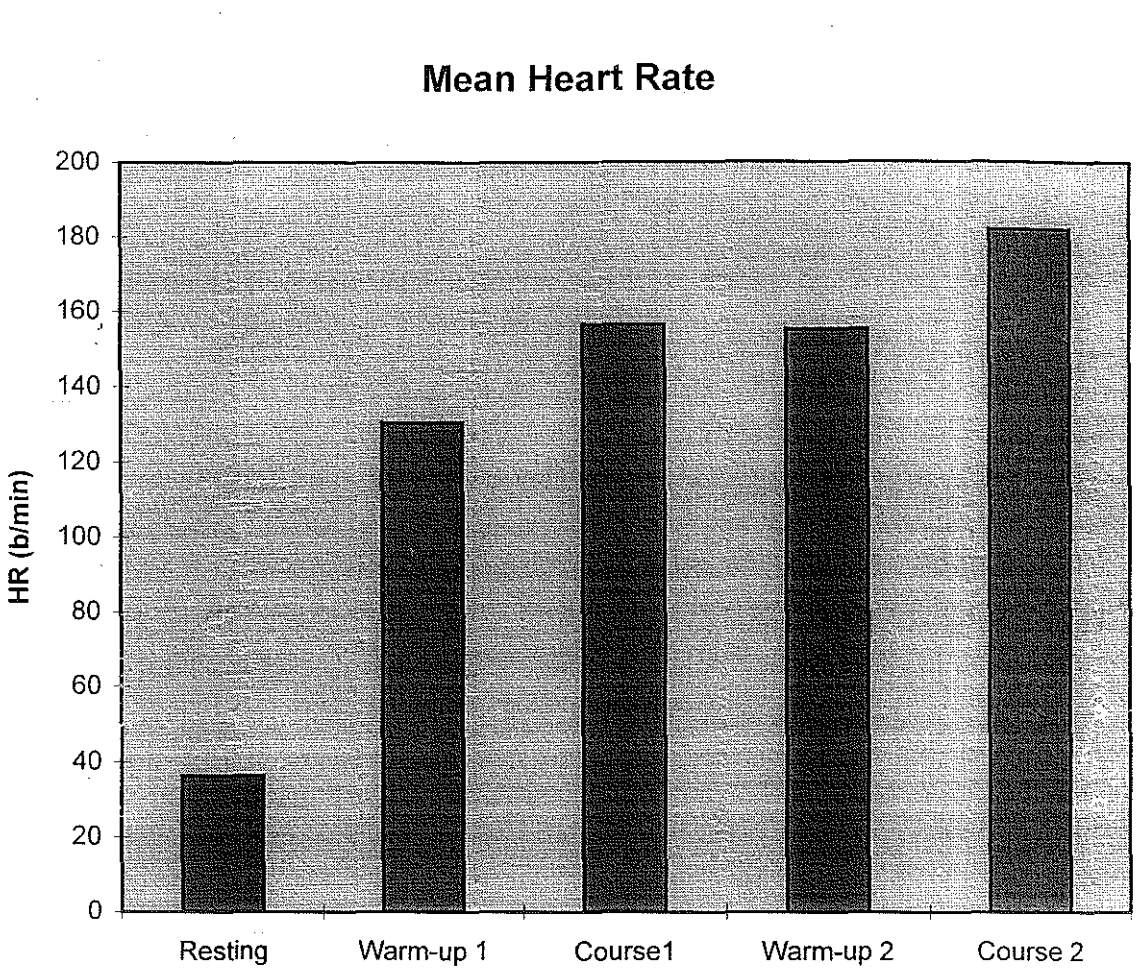

Figure 4.20 - Mean heart rates for eight horses completing the jump test.

No heart rate could be recorded from horse NTL due to technical difficulties with the transmitter. During this horse's test, the transmitter lost contact with the animal's skin and therefore no data were recorded.

4.2.2 BIOCHEMICAL VARIABLES

Blood samples were obtained from all horses and analysed for various biochemical constituents. The mean values for these constituents are shown in table 4.10 below. In addition, individual values for all horses tested are shown in Table 4.11.

	Lactate (mmol/L)	Glucose (mmol/L)	CK U/L	Potassium (mmol/L)
Resting	0.7 ± 0.026	5.93 ± 0.08	85.70 ± 14.4	3.53 ± 0.057
Course 1	0.75 ± 0.052	5.36 ± 0.18	108.75 ± 25.4	3.92 ± 0.2
Course 2	2.30 ± 0.53	5.80 ± 0.15	108.81 ± 9.57	3.85 ± 0.14
24 hr	0.73 ± 0.08	5.97 ± 0.15	102.57 ± 23.43	3.06 ± 0.23

Table 4.10 - Mean (+ SEM) biochemical values for the jumping test. For the 24 hr sample (n=4) samples from horses TVA, FK, CDB and MSH were unavailable.

4.2.2.a PLASMA LACTATE

All horses had similar resting plasma lactate concentrations (0.7 mmol/L mean value). During course 1 this value did not increase above resting levels as shown in Figure 4.21. However, at the completion of jump course 2 the mean plasma lactate value had increased significantly ($p=0.001$) above resting and post-course 1 levels as shown in Figure 4.21. As can be noted from table 4.11, individual plasma lactate values, after completion of course 2, ranged from 0.8 mmol/L (horse CZ) to 5.0 mmol/L (horse TVA).

There was no relationship observed between plasma lactate levels and the times taken to complete the jump courses for individual horses.

Horse	Glucose (mmol/L)	Creatine Kinase (UI/L)	Potassium (mmol/L)	Lactate (mmol/L)
Resting				
NTL	6.18	37.0	>12.0	<0.7
CDB	5.87	138	3.71	<0.7
MSH	5.9	91.6	3.28	<0.7
FK	5.55	84.9	3.46	<0.7
AJS	6.1	146	3.47	<0.7
JMB	5.76	34.7	3.49	<0.7
CZ	6.21	70.7	3.66	<0.7
TVA	5.85	82.7	3.65	0.8
Course 1				
NTL	5.82	88.0	2.71	0.8
CDB	5.15	111	4.23	<0.7
MSH	5.97	34.2	3.73	0.8
FK	5.69	66.2	4.23	0.7
AJS	5.00	270	4.03	<0.7
JMB	4.36	80.9	3.78	<0.7
CZ	5.58	81.7	4.09	<0.7
TVA	5.35	138	4.61	1.00
Course 2				
NTL	-	116	3.28	2.6
CDB	5.49	134	3.95	3.4
MSH	6.51	134	3.43	0.8
FK	5.94	111	3.95	3.00
AJS	5.33	135	>12.0	1.8
JMB	5.46	69.3	4.24	1.00
CZ	5.97	70.2	3.89	0.8
TVA	5.90	101	4.18	5.00
24 hr				
NTL	6.02	91.2	2.38	1.4
CDB	-	-	-	-
MSH	-	-	-	-
FK	-	-	-	-
AJS	5.62	148	3.35	0.7
JMB	6.34	89.8	3.22	<0.7
CZ	5.89	69.9	3.3	0.9
TVA	-	-	-	-

Table 4.11 - Individual biochemical values. 24 hour samples for horses CDB, MSH, FK and TVA were unavailable. The glucose sample for horse NTL following completion of the second jump course was also unavailable.

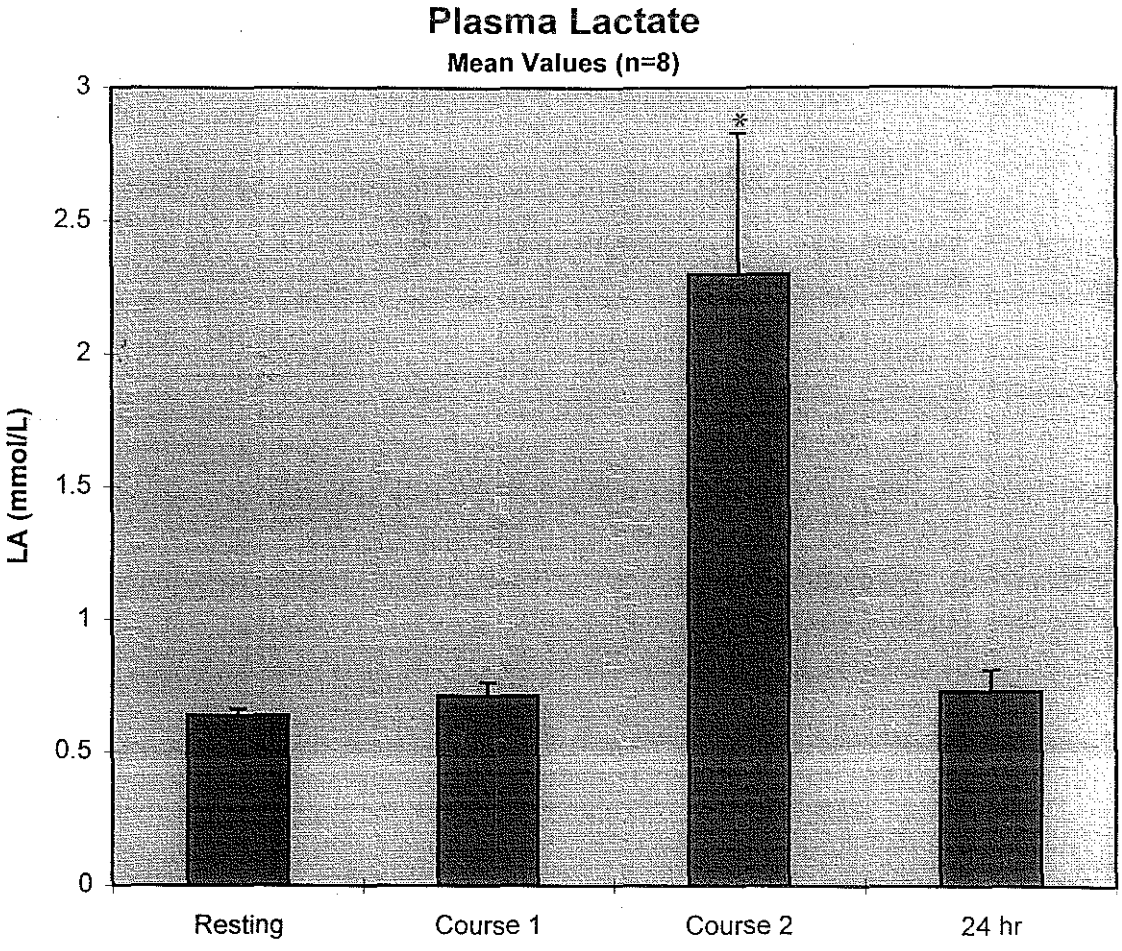

Figure 4.21 - Mean (+SEM) plasma lactate levels for 8 horses subjected to a jump test. n=4 for the 24 hr sample. * significantly different from resting values ($P<0.05$).

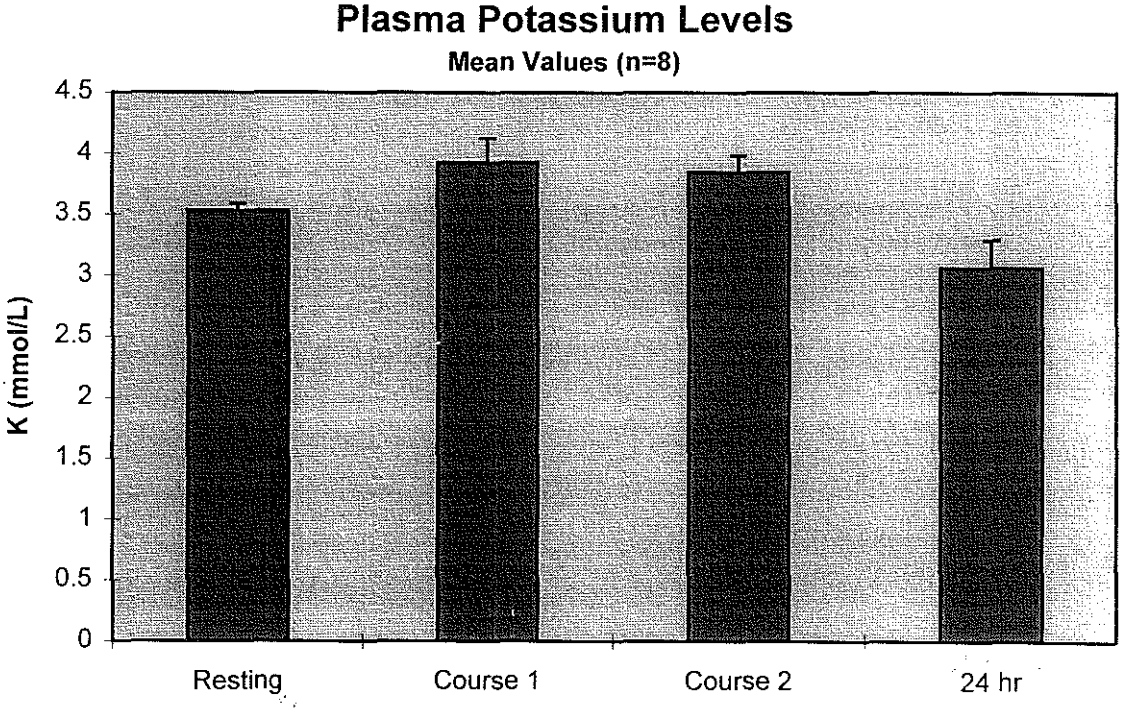

Figure 4.22 - Mean (± SEM) plasma potassium levels for 8 horses subjected to a jump test. n=4 for the 24 hr sample.

4.2.2.b PLASMA POTASSIUM

A small but statistically insignificant increase in plasma potassium concentrations was observed between resting and post-course 1 values as shown in figure 4.22. Plasma potassium levels also remained slightly elevated during course 2 and had returned to normal resting levels 24 hours after the test.

On observation of individual horse values (Table 4.11) horse AJS showed a marked increase in potassium levels following the completion of course 2, an increase of more than 8 mmol/L from post-course 1 values. The normal ranges for plasma potassium concentrations in the horse are between 3.2 and 4.2 mmol/L (Rose and Hodgson, 1994 pg 63-78) and this value of greater than 12 mmol/L for horse AJS is well above the normal range. However, after 24 hours, this animal's plasma potassium concentrations had returned to resting levels (3.35 mmol/L).

4.2.2.c PLASMA GLUCOSE

Mean resting plasma glucose concentrations were 5.93 mmol/L as seen from table 4.10 and figure 4.23. A decrease from resting levels was noted after the completion of course 1 as seen from figure 4.23. At the completion of jump course 2, plasma glucose values had returned to near resting levels (5.80 mmol/L, mean value). Plasma glucose levels had returned to normal resting values 24 hours after completion of the test (5.97 mmol/L mean value).

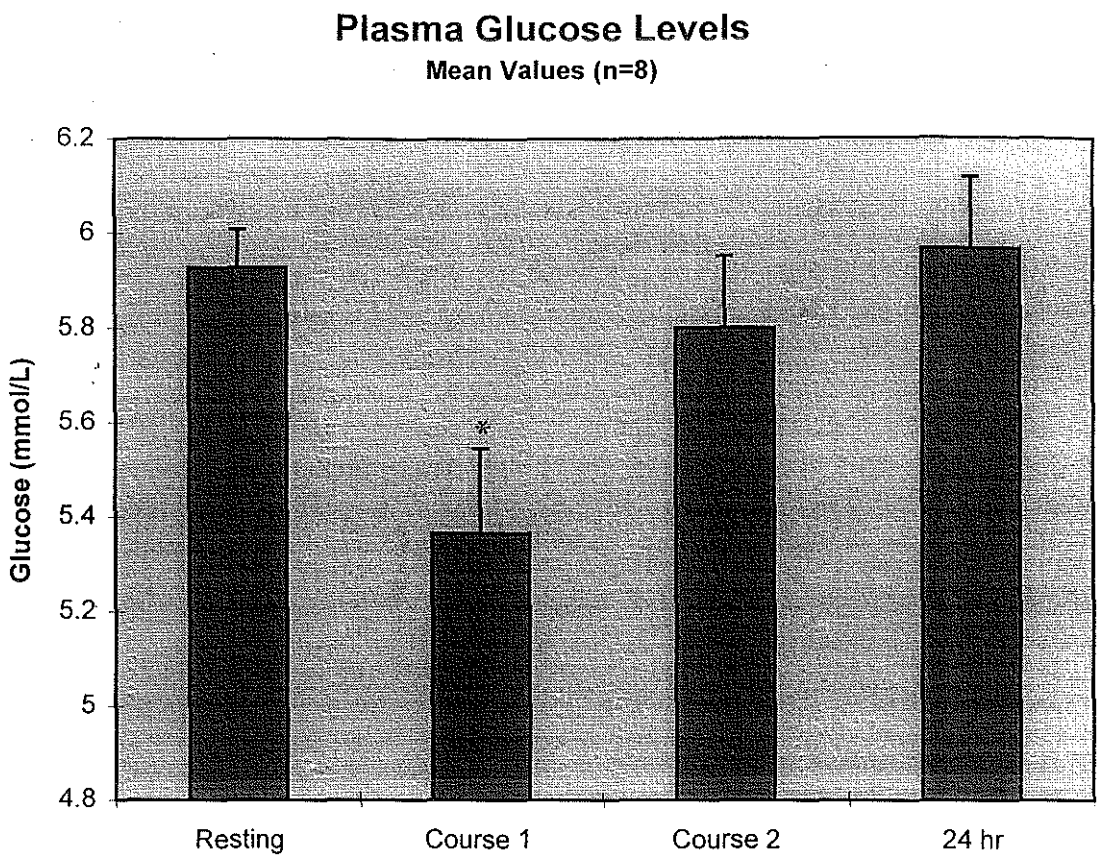

Figure 4.23 - Mean (± SEM) plasma glucose values for 8 horses subjected to a jump test n=4 for the 24 hr sample * significantly different from resting values ($P<0.05$).

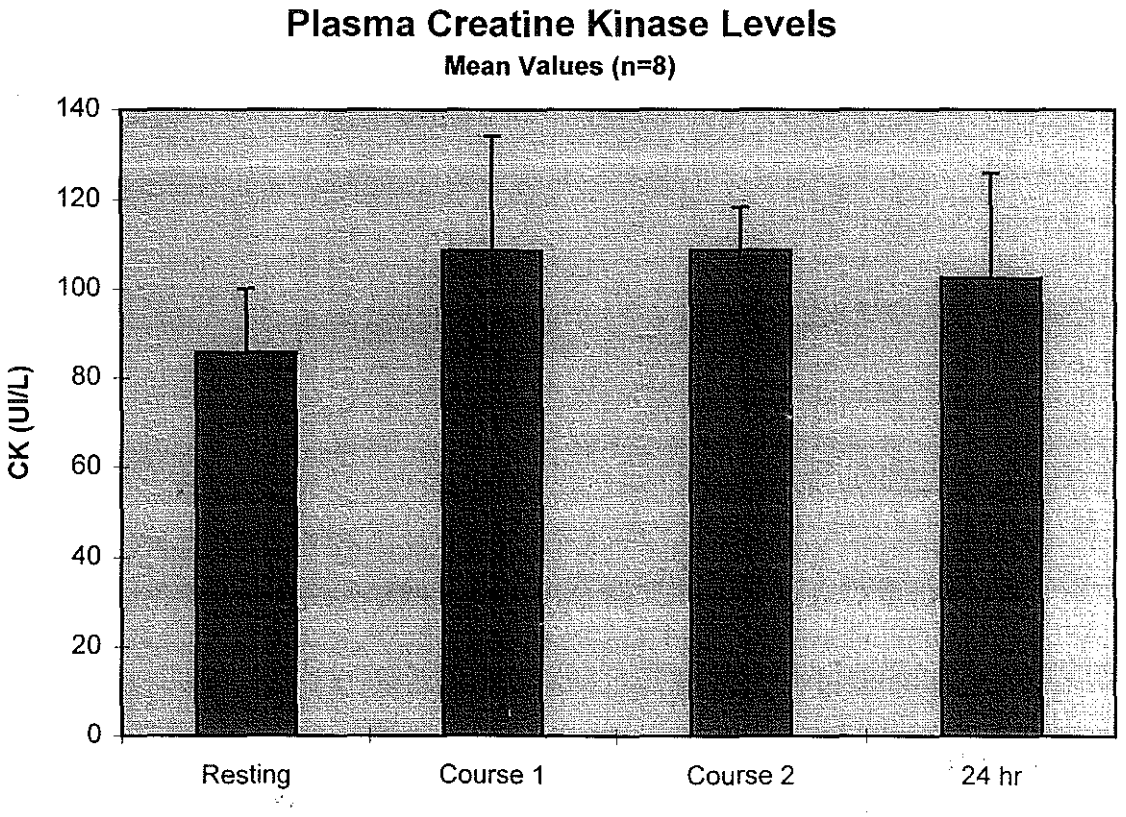

Figure 4.24 - Mean (± SEM) plasma CK values for 8 horses subjected to a jump test. n=4 for the 24 hr sample.

4.2.2.d PLASMA CREATINE KINASE

There was no significant difference in plasma creatine kinase levels between groups of horses in this study (Figure 4.24). A small increase in CK values was observed between resting levels to the completion of course 1, however, this was not statistically significant. On observation of individual horses (table 4.11) a large increase in CK levels for horse AJS was observed at the completion of course 1 (270 U/L) indicating some degree of skeletal muscle damage. However, at the completion of course 2 this horse's CK levels had returned to near resting levels (135 U/L).

4.2.3 HAEMATOLOGICAL VARIABLES

Haematological variables examined for this test were RBC, HCT, Hb, MCV, MCH, MCHC, WBC and a white cell differential count. Mean red cell indices are presented in table 4.12. Mean white cell counts and white cell differential counts are presented in table 4.13. Individual values for all horses tested are presented in table 4.14.

	RBC ($x10^{12}$/L)	HCT (L/L)	Hb (g/L)	MCV (FL)	MCH (pg)	MCHC (g/L)
Resting	8.22 ± 0.4	0.42 ± 0.2	145.25 ± 5.4	51.14 ± 0.4	17.57 ± 0.2	346.4 ± 6.4
Course 1	10.37 ± 0.6	0.52 ± 0.03	184.37 ± 11.5	57.88 ± 7.3	17.88 ± 0.2	345.2 ± 4.2
Course 2	10.28 ± 0.5	0.51 ± 0.02	180.86 ± 9.5	58.57 ± 6.4	17.71 ± 0.2	346 ± 3.2
24 hr	7.06 ± 0.2	0.37 ± 0.01	124.75 ± 5.2	52.00 ± 1.0	17.35 ± 0.4	339.5 ± 6.2

Table 4.12 - Red cell indices for all horses tested (mean values ± SEM) n=8 for all values except 24 hr samples where n=4.

		White Cell Differential Count			
	WBC ($x10^9$/L)	Neutrophils (% of WBC)	Lymphocytes (% of WBC)	Eosinophils (% of WBC)	Monocytes (% of WBC)
Resting	7.23 ± 0.4	54.25 ± 3.5	41.88 ± 3.4	2.62 ± 0.5	1.5 ± 0.5
Course 1	8.58 ± 0.6	55.38 ± 2.9	41.88 ± 3.1	1.5 ± 0.6	1.25 ± 0.4
Course 2	8.42 ± 0.6	54.0 ± 3.0	42.0 ± 3.1	2.5 ± 0.9	1.38 ± 0.5
24 hr	7.15 ± 0.6	57.5 ± 2.8	34.5 ± 3.0	3.75 ± 0.4	3.75 ± 1.2

Table 4.13 - White blood cell indices. Mean values ± SEM. n=8 for all values except 24 hr samples where n=4.

Horse	RBC $x10^{12}/L$	HCT L/L	Hb g/L	MCV FL	MCH pg	MCHC g/L	WBC $x10^{9}/L$	Neutro % of WBC	Lympho % of WBC	Eosino % of WBC	Mono % of WBC
Resting											
NTL	-	-	-	-	-	-	-	-	-	-	-
CDB	7.82	0.42	142	54	18	336	8.6	40	58	1	1
MSH	8.24	0.44	142	53	17	325	8.3	58	40	2	0
AJS	7.79	0.42	140	54	18	335	6.1	70	27	0	3
CZ	7.16	0.35	132	49	18	377	5.6	43	50	4	3
JMB	7.4	0.38	132	51	18	349	6.8	60	35	4	1
TVA	9.51	0.45	159	47	17	355	7.1	53	44	3	0
FK	7.92	0.4	138	50	17	348	5.6	61	35	3	1
Course 1											
NTL	10.3	0.54	184	53	18	338	10.5	54	41	5	0
CDB	9.54	0.52	176	54	18	340	10.2	40	59	1	0
MSH	10.7	0.56	188	52	18	335	10.3	59	40	1	0
AJS	13.4	0.69	253	52	19	364	7.8	63	33	2	2
CZ	9.56	0.5	172	53	18	344	10.4	52	46	0	2
JMB	8.08	0.41	136	102	18	332	5.7	64	35	0	2
TVA	11.26	0.53	191	47	17	355	8.0	49	47	2	2
FK	10.13	0.5	175	50	17	348	6.6	62	34	2	2
Course 2											
NTL	10.8	0.56	195	52	18	345	11.3	66	34	0	0
CDB	10.1	0.54	185	54	18	340	9.5	40	59	1	0
MSH	10.73	0.56	191	53	18	338	9.5	60	36	4	0
AJS	-	-	-	-	-	-	-	58	38	1	3
CZ	7.16	0.38	126	52	18	332	7.4	50	49	1	0
JMB	10.44	0.51	178	102	17	346	7.0	60	34	4	2
TVA	11.97	0.57	200	47	17	353	7.8	53	41	3	3
FK	10.76	0.54	191	50	18	357	6.7	45	45	7	3
24 hr											
NTL	7.56	0.4	134	53	18	335	8.9	58	32	5	4
CDB	-	-	-	-	-	-	-	-	-	-	-
MSH	-	-	-	-	-	-	-	-	-	-	-
AJS	7.1	0.36	126	51	17	350	6.4	65	29.5	3	2
CZ	7.11	0.37	129	52	18	349	7.2	51.5	43	3.5	2
JMB	6.48	0.34	110	52	16.4	324	6.2	55.5	33.5	3.6	7
TVA	-	-	-	-	-	-	-	-	-	-	-
FK	-	-	-	-	-	-	-	-	-	-	-

Table 4.14 - Individual haematological variables for all horses tested. Resting sample for horse NTL and 24 hour samples from horses CDB, MSH, FK and TVA were unavailable. Red cell indices and WBC counts for horse AJS could not be calculated due to a mishap with the sample.

4.2.3.a RED CELL INDICES

The mean resting RBC count was 8.22 $x10^{12}$/L as shown in Table 4.12. After completion of the first jump course a significant (p=0.001) increase in mean RBC values was noted as shown by Figure 4.25. Red cell counts remained at this level at the completion of the second jump course with a mean value of 10.28 $x10^{12}$/L. Twenty four hours after the jump test RBC values had returned to normal resting values for all horses tested.

From Figure 4.25 it can be observed that mean RBC values were marginally higher for course 1 than for course 2. However, on examination of individual results (Table 4.14) the majority of horses (with the exception of horse CZ) obtained higher RBC values after completing course 2. Horse AJS had the highest RBC count of all horses at the completion of course 1 (13.4 $x10^{12}$/L). However, red blood cell counts for course 2 were unavailable for this horse due to a mishap with the sample. As a result, the high value obtained for this horse, for course 1, increased the mean value above that obtained for the second jump course. If a sample was available for this horse, the mean value may have been higher.

On examination of the results for horse CZ, it can be noted (Table 4.14) that this horse had RBC values very close to resting levels. In addition, this horse had lower RBC values for the second jump course when compared to results obtained from course 1. Due to the higher intensity of exercise of the second jump course, it was expected that RBC counts would be higher after the completion of this course, as observed in the other horses tested.

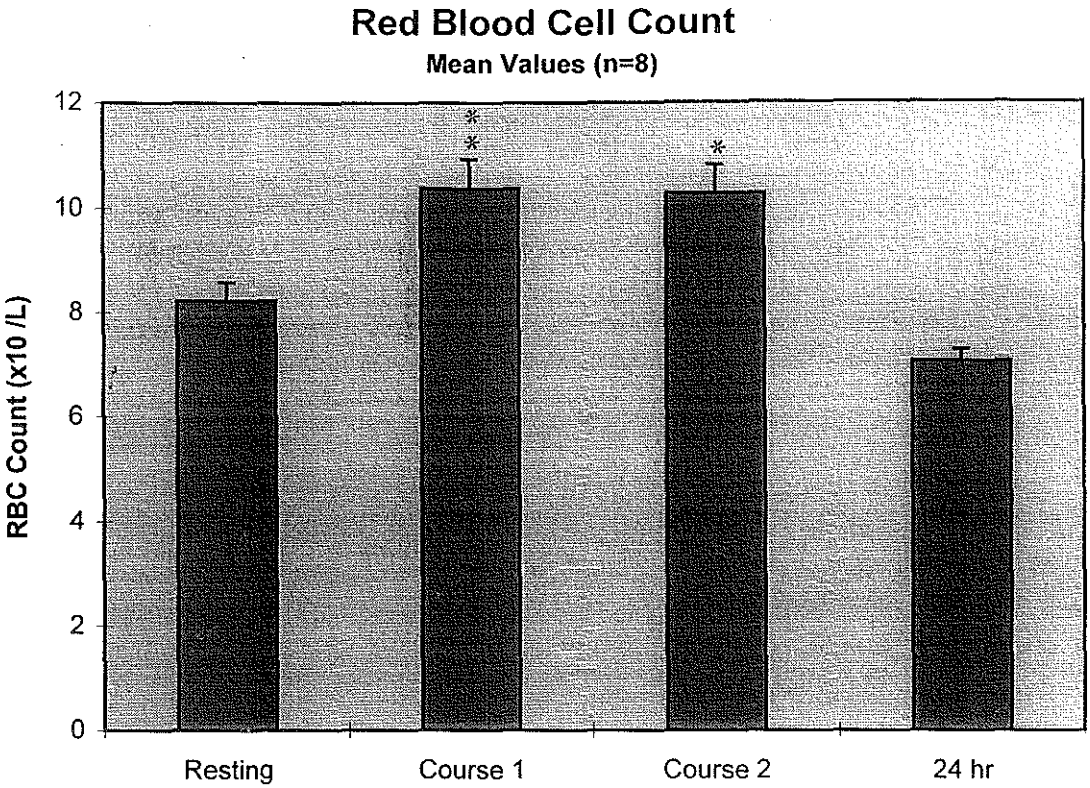

Figure 4.25 - Mean (± SEM) RBC values for 8 horses subjected to a jump test. n=4 for 24 hr sample. * significantly different from resting values ($P<0.05$) ** significantly different from resting values ($P<0.01$).

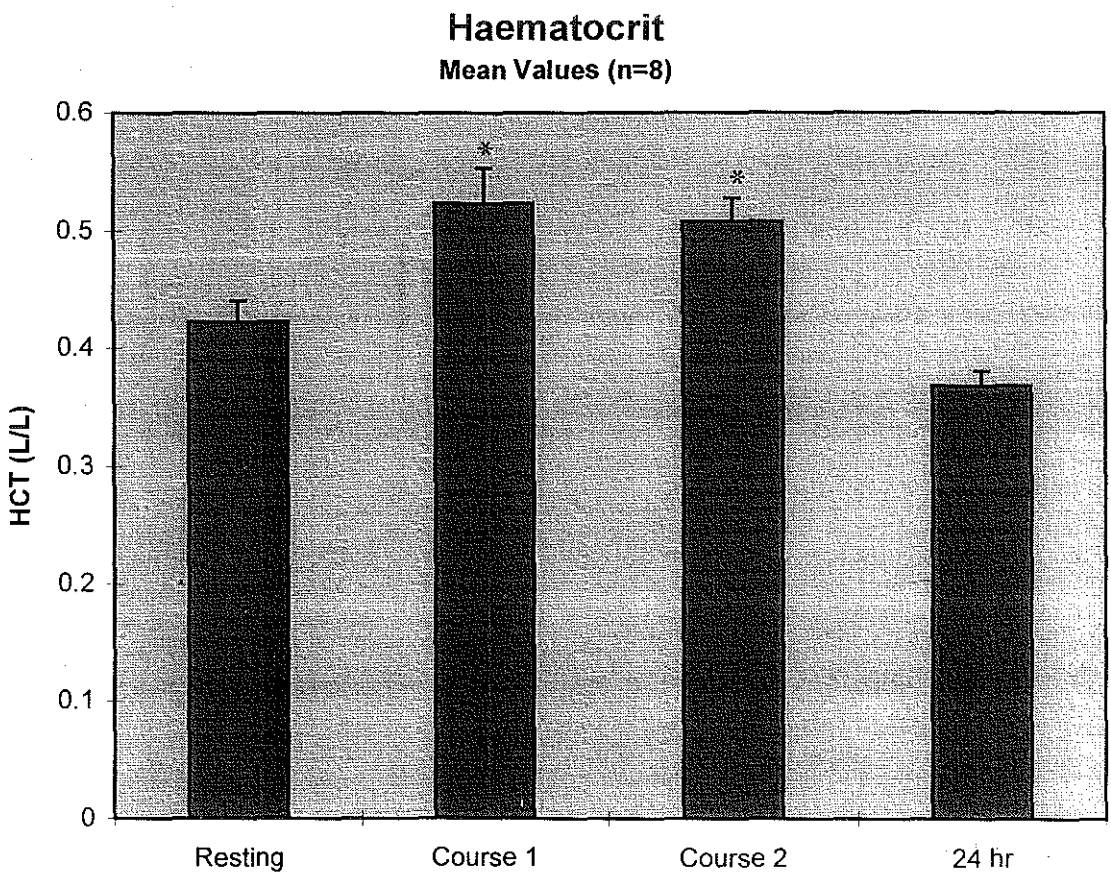

Figure 4.26 - Mean (± SEM) haematocrit values for 8 horses subjected to a jumping test. n=4 for the 24 hr sample. * significantly different from resting values ($P<0.05$).

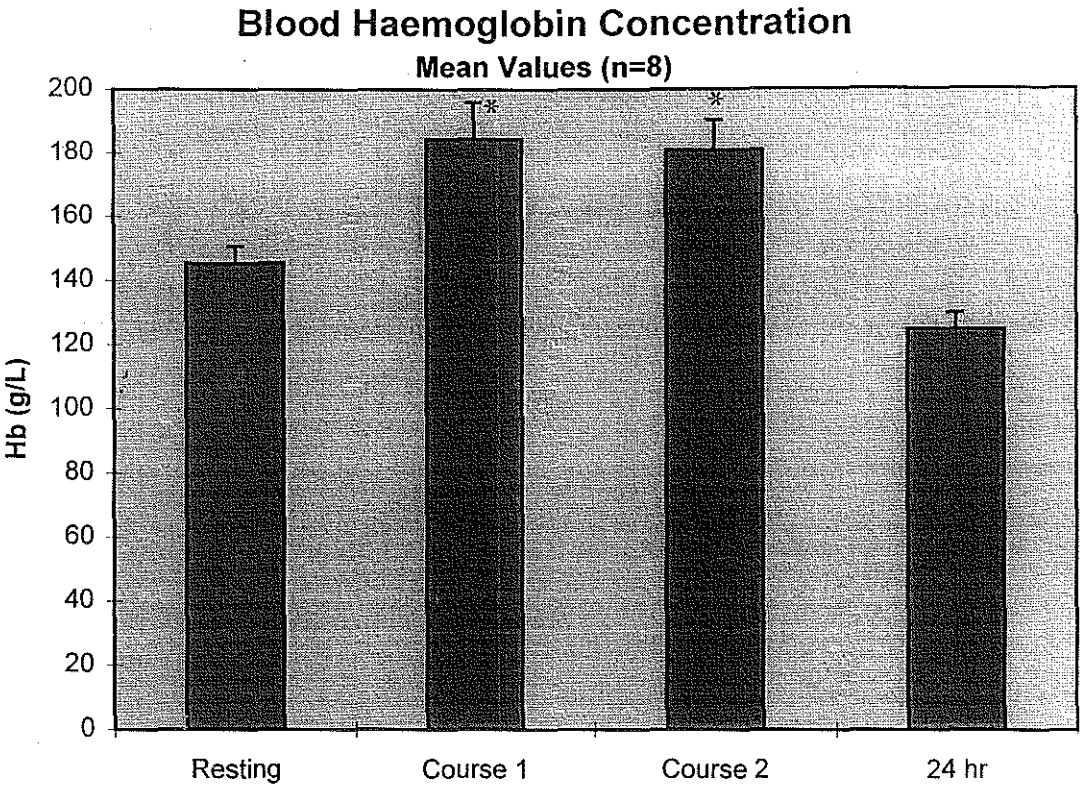

Figure 4.27 - Mean (± SEM) haemoglobin concentration for 8 horses subjected to a jump test. n=4 for 24 hr samples. * significantly different from resting values ($P<0.05$).

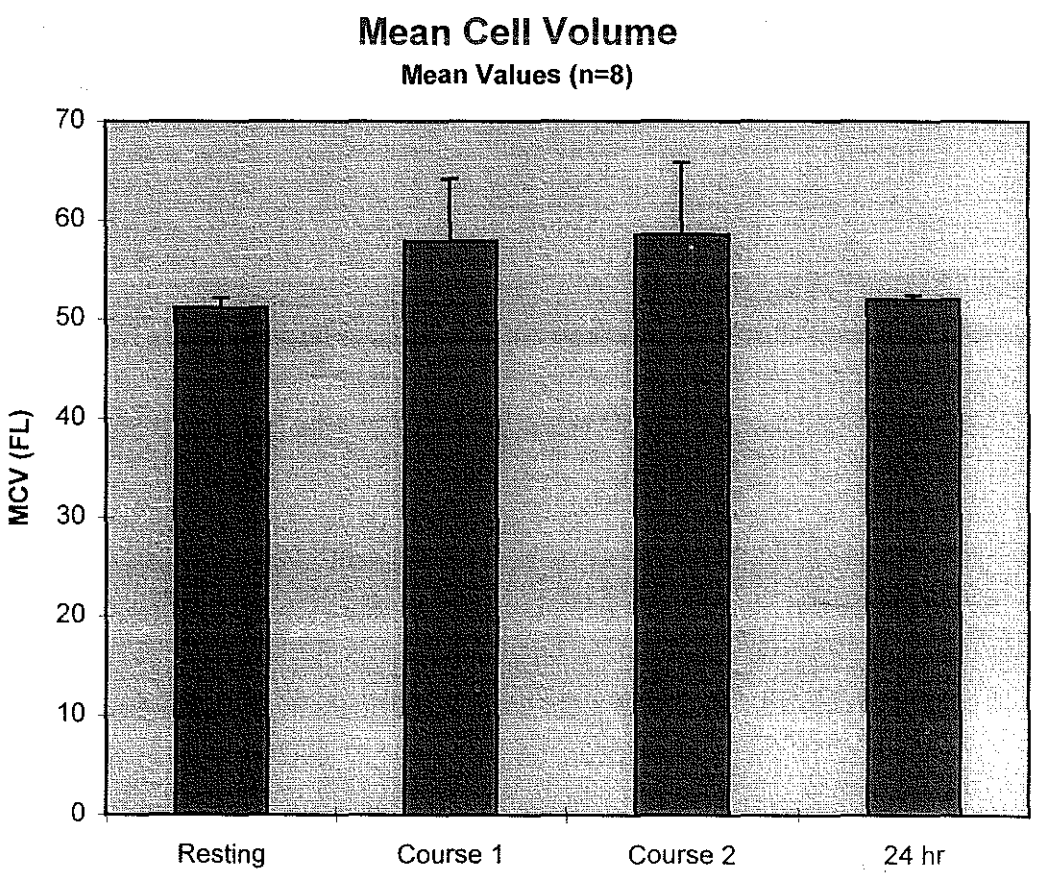

Figure 4.28 - Mean (± SEM) MCV values for 8 horses subjected to a jump test. n=4 for the 24 hr sample.

Increases in haematocrit and blood haemoglobin concentration showed a similar increase to those observed in RBC counts, as shown in Figure 4.26 and 4.27. The greater increase seen after completion of the first jump course was due to the reasons described above.

From Figure 4.28, it can be observed that on completion of both jump courses a slight but non-significant increase in MCV occurred. On examination of individual values, horse JMB showed a marked increase from resting values in MCV after completion of both jump courses. Resting MCV for this horse was 51 FL. After completion of jump course 1 this value increased to 102 FL and remained at this level at the completion of course 2. The maximal normal value for MCV as reported by Schalm (1986) is 58 FL. Therefore, the value of 102 FL obtained by this horse exceeds the normal ranges by 44 FL. The MCV values for this horse had returned to normal 24 hours after completion of the test.

There was no significant change in MCH or MCHC values for all horses tested. As can be seen from Figure 4.29, a slight increase in MCH values occurred at the completion of course 1 however this increase was not statistically significant.

Figure 4.30 illustrates MCHC values for this jump test. As can be seen from the figure there was no change in these values throughout the test. However, a small decrease in MCHC occurred 24 hours after completion of the test.

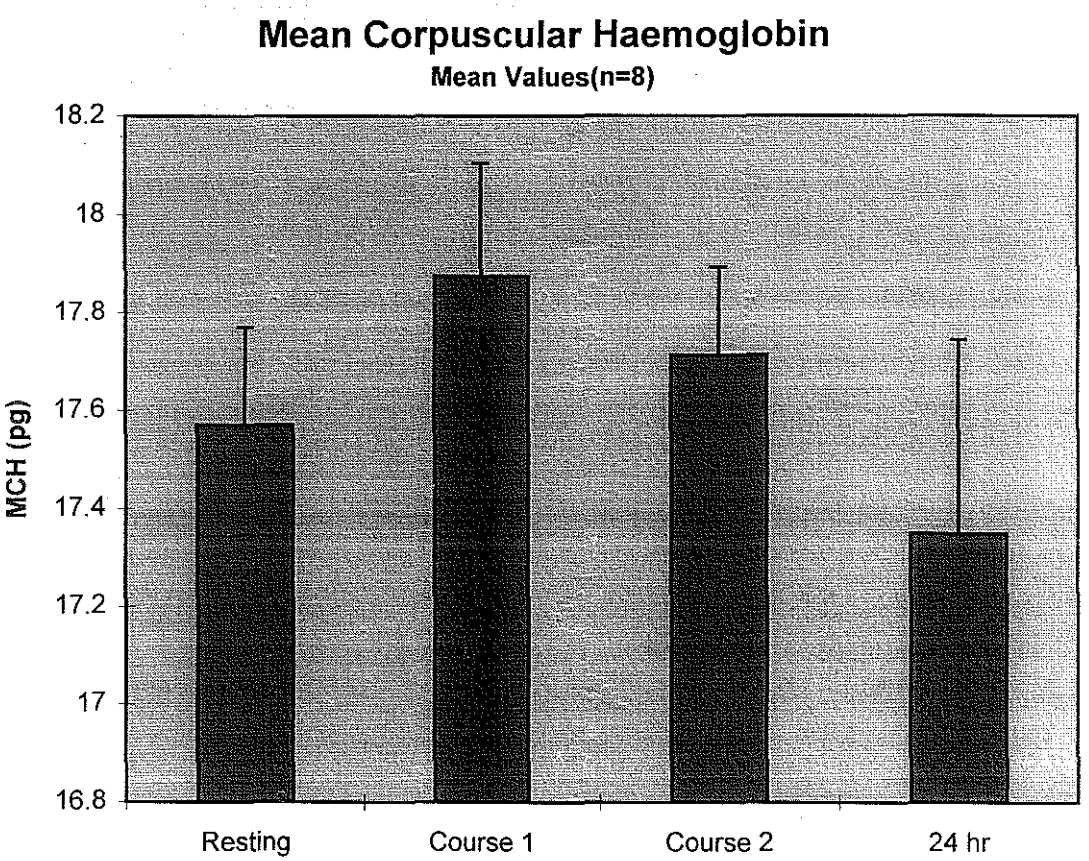

Figure 4.29 - Mean corpuscular haemoglobin (mean values $\pm$ SEM) n=8 for all stages except the 24 hr sample where n=4.

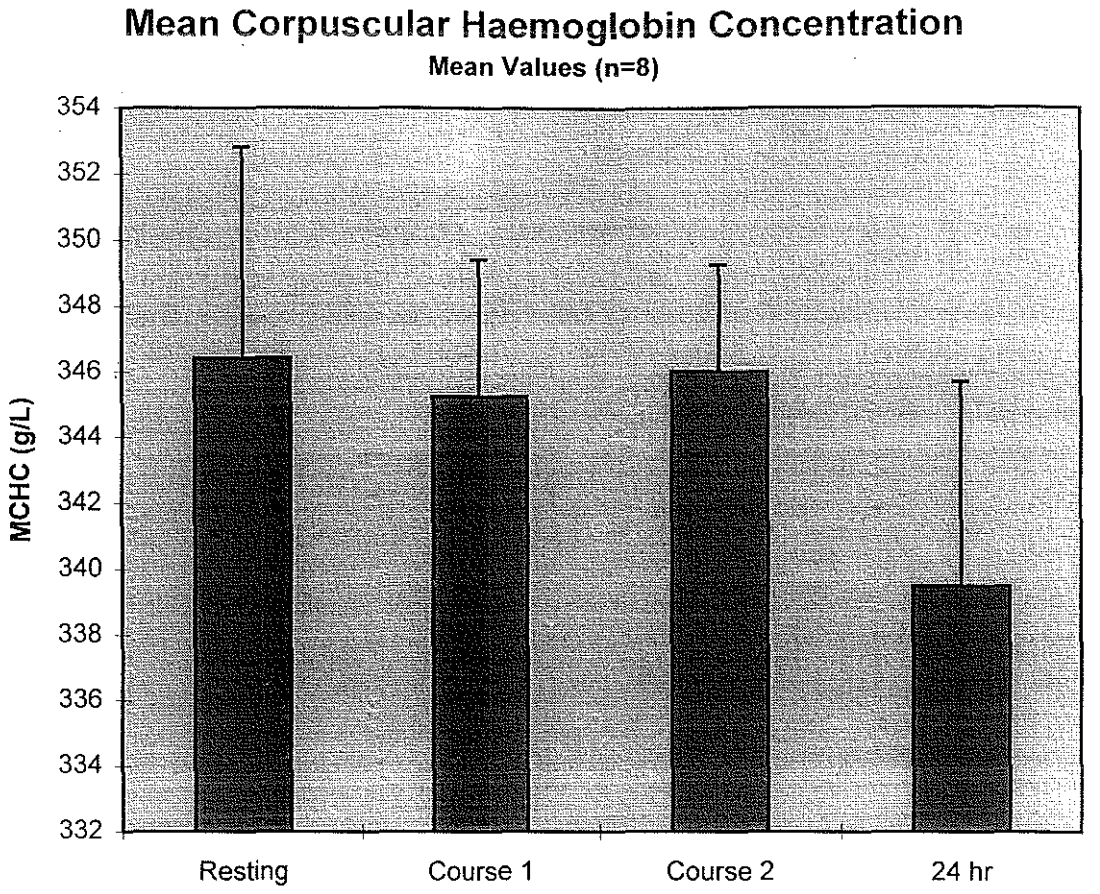

Figure 4.30 - Mean corpuscular haemoglobin concentration (mean values ± SEM) n=8 for all tests except the 24 hr sample where n=4.

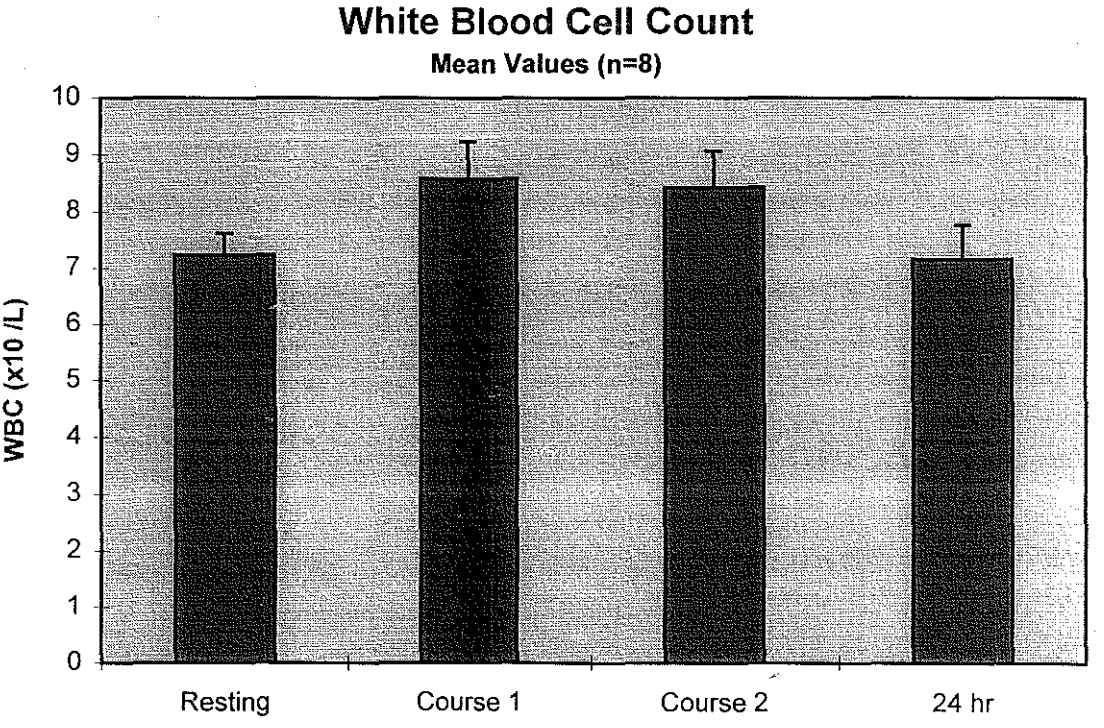

Figure 4.31 - Mean (± SEM) WBC values for 8 horses subjected to a jumping test. n=4 for the 24 hr sample.

4.2.3.b WHITE CELL INDICES

The mean resting WBC value for the horses in this test was 7.2 $x10^9$/L. At the completion of the first jump course, this value increased to 8.58 $x10^9$/L as shown in Figure 4.31, however, this increase was not statistical significant. WBC values remained at a mean value of 8.423 $x10^9$/L (Table 4.13) at the completion the second jump course. These values returned to normal resting values 24 hours after the test. Also, there were no significant changes observed in the white cell differential counts as shown in Figure 4.32.

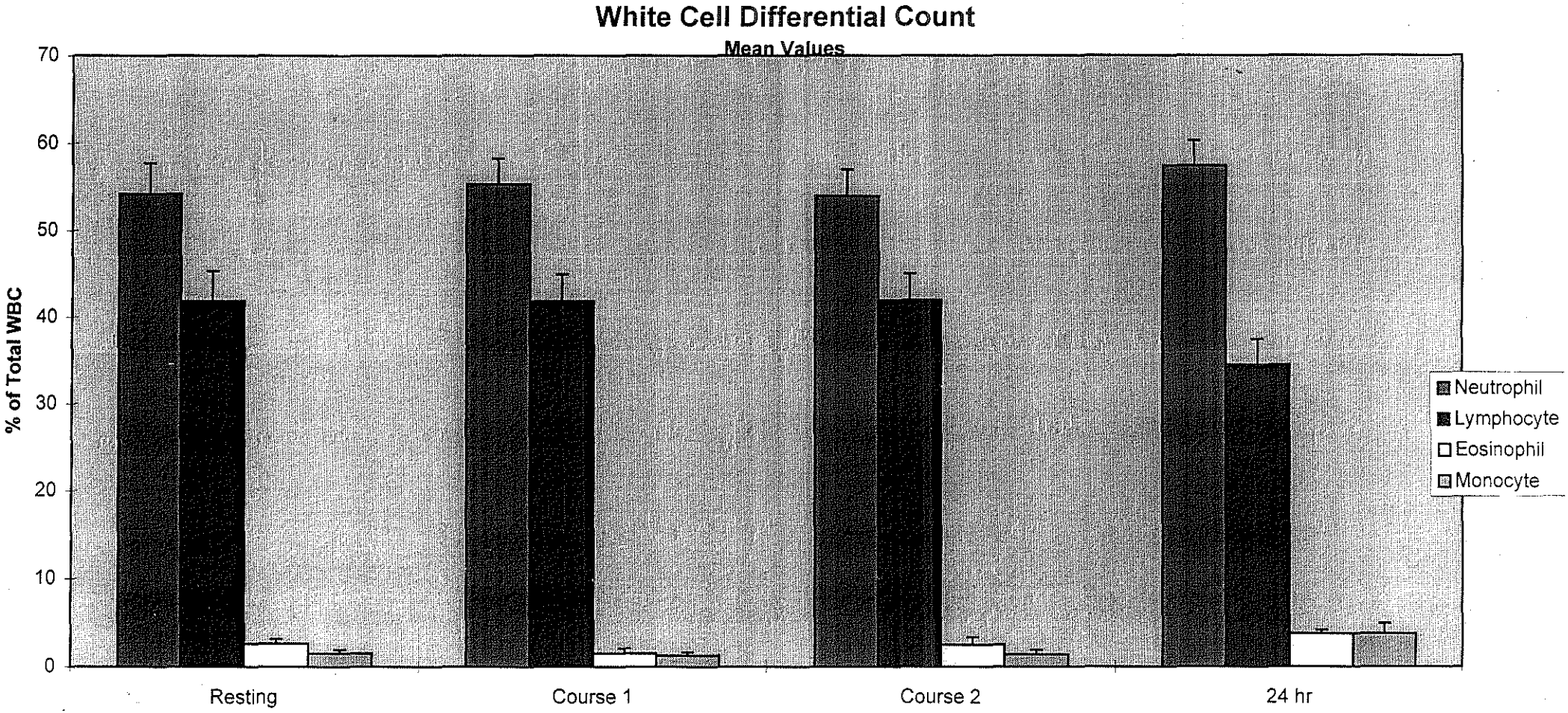

Figure 4.32 - White cell differential count (mean values ± SEM) n=8 for all tests except the 24 hr sample where n=4.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 INTRODUCTION

This research project was undertaken to examine certain biochemical and haematological variables in relation to performance in the showjumping arena as a gauge of athletic potential in horses while also addressing the paucity of scientific literature in this area. Further, it was expected that this work would provide a foundation to develop a regime that can be used for screening animals for potential excellence in showjumping.

To achieve these objectives, 5 to 8 showjumping horses, currently competing in the Western Australian Professional Show Jumping Circuit were subjected to 2 forms of exercise of varying intensity. Blood samples were analysed for designated biochemical and haematological variables in order to investigate the performance potential and physical fitness of these animals.

To determine precisely how well the measured variables correlated with performance in the showjumping arena, it was necessary to study horses competing at all levels of competition. Show jumping in Australia is divided into four classes. Horses begin their jumping career in D grade and are awarded points for winning or placing in events. As the horse's points accumulate the horse is then promoted to the next grade. The highest grade of competition in Australia is A grade, however during normal competition A and B grade horses compete equally, and horses from both of these grades are permitted to compete in the major competitions such as world cup events. The horses used in this study covered a wide range of competition levels, consisting of older animals who have

been competing in D grade for many years with little success, to younger horses who are currently progressing through the grades, to horses competing at world cup level.

5.2 LIMITATIONS OF THE STUDY

The major limitation of this study was the attainment of horses. Due to the nature of the testing protocol many owners and trainers were initially sceptical of the testing procedures. Showjumping horses are very valuable and the owners of these horses did not want to risk injuring the animals during the testing procedure or blood sampling periods. Despite this, 34 horses were initially recruited for this study. Before any horses could be tested 14 of the 34 horses were taken to the Eastern states by their owner, reducing the number to 20. Four of these horses were withdrawn from the study by their owners due to time constraints and during the showjumping season a further 8 of the remaining horses sustained injuries during competition, thus excluding them from the study. Of the remaining 8 horses, 3 sustained small injuries, excluding them from phase 1 of the study, although these injuries healed in time for the commencement of phase 2. Horse WMC injured its front right fetlock joint during phase 1 of the study, excluding it from the jump test. As a result, 5 horses were subjected to phase 1 and 8 horses completed phase 2 of the study. These horses were supplied by three commercial showjumping stables within the Perth metropolitan area.

The incremental exercise test was conducted on an all weather (self-draining) training track. When a track is used in exercise testing, the value of the results is limited by the difficulties in standardising the conditions and speeds of the test. However if the track is firm and well drained, the same jockey is used and extremes in weather conditions are

avoided, then it has been shown that reliable results can be obtained (Thornton, 1985). It would have been preferable to perform this test on a high speed treadmill as this enables the strict control of the speed of exercise, environmental conditions and running surface (Thornton, 1985). However, there are only 2 high speed treadmills operating in Western Australia and both of these were unavailable for the test. In addition, horses must be pre-trained to run on the treadmill and none of the trainers who participated in this study would have been able to spare their horses for this additional time.

Ideally, to confirm the reliability of this study, the track test and the jump test should have been repeated by at least 3 horses. If the two tests conducted in this study indicated the true physiological responses of showjumping horses to exercise, then a repeat of the tests should have caused similar changes in biochemical and haematological values. This relationship would only occur if the environmental conditions of the repeat tests were kept as close as possible to the initial tests. Due to the prior commitments of the trainers, no repeat testing could be conducted.

The horses used in this study were supplied by three commercial show jumping stables. To reduce variations in resting biochemical and haematological values between individual horses, all animals ideally should have followed a standardised feeding and exercise program for a period of at least 2 weeks prior to the testing. This would reduce the variations in observed in resting blood indices which may have been caused by differences in feed constituents with particular reference to vitamin, mineral or electrolyte supplements. However, the stabling and training of horses is very expensive and not practical for the purposes of this study. In addition, a number of resting blood samples should have been collected from the horses participating in this test in order to

determine the true resting biochemical and haematological values of these animals (Blackmore, 1983 pg 344-353). As these horses were currently competing in the Western Australian Professional Showjumping Circuit, the trainers were reluctant to change the training programs and feeding patterns of the horses in fear of affecting the animal's performance in upcoming competitions. In addition, due to the busy routines of the trainers, limited access to the horses was available and therefore only 1 resting blood sample per horse could be collected. In some cases, 24 hour blood samples could not be collected due to prior commitments of the trainers.

To identify differences in training programs and feeding patterns between horses the trainers were asked to complete a questionnaire, detailing a general 2 week training program and daily food intake for their horses (Appendix 2). This revealed that there were no major differences in the general feeding and training programs between horses. However, specific training programs for the horses tested were not available as the trainers were reluctant to divulge this information.

The workloads for this test were chosen to provide exercise intensities ranging from easy to near maximal to examine the full range of each horse's physiological response. The effects of biological variability should be noted, such that while the horses were galloping at the same velocity they may not have been exercising at precisely the same intensity. For example, horse MSH showed a very natural and rhythmic galloping motion and was able to complete the 12 m/sec workload with little physical stress as can be seen from this horses biochemical and haematological values. However horses CDB, TVA and NTL had poor galloping styles and therefore the 12 m/sec workload was more difficult for these horses to maintain. Therefore, although all horses were travelling at

the same speed, horses CDB, TVA and NTL were exercising at a higher intensity than horses MSH and WMC due to their poor galloping styles.

A more accurate method, to ensure that all horses were exercising at similar intensities, would have been to use specific workloads for each horse based on their maximal exercise capacities. For example, instead of using workloads of 6, 8, 10 and 12 m/sec, workloads of 50%, 60%, 70% and 80% of the animals maximum exercise capacity could have been used. Although using this method is a more efficient means of standardising the test, an initial exercise test is required to calculate the animal's maximal capacity. For this study, this was not possible because the trainers were unwilling to allow their horses to undergo this test.

5.3 PLASMA LACTATE AND HEART RATE

Plasma lactate for all horses increased in an exponential fashion during phase 1 of this test, as shown in figures 4.2 and 4.3. It was anticipated that plasma lactate values for the showjumping horse would be higher than lactate values recorded in the thoroughbred racehorse for exercise of similar intensities. However, from the results of this study, it was apparent that mean plasma lactate values for the showjumping horse are lower than previously recorded values in the thoroughbred racehorse during comparable exercise (Snow, Harris and Gash, 1985; Harris and Snow, 1988; Valette, Barrey and Wolter, 1991 pg 337-342). During an incremental exercise test involving 5 well trained thoroughbred racehorses Harris and Snow (1988) reported lactate values significantly higher than those observed in this study. At a velocity of 8 m/sec, lactate values for the showjumping horses did not increase above 1 mmol/L. However Harris and Snow

(1988) reported lactate values between 4.2 and 8.3 mmol/L in their thoroughbred racehorses. Similarly mean lactate values for the showjumping horses tested in this study at an exercise intensity of 12 m/sec were 14.62 mmol/L, whereas Harris and Snow (1988) report lactate concentrations ranging from 16.5 to 25.6 mmol/L following exercise of the same intensity.

On examination of individual training programs for the horses used in this study (Appendix 3) it can be noted that most training schedules of the animals studied consist of a large proportion of long duration, low intensity type exercise, with short term high intensity exercise being undertaken approximately 2 days per week. In addition, these horses rarely exercise at maximal intensities. When training programs of thoroughbred racehorses are examined (Snow and Mackenzie, 1977; Gollnick, Bayly and Hodgson, 1986; Lovell and Rose, 1991) it is apparent that these animals regularly perform maximal exercise of short duration and high intensity (approximately 3 times per week) with the remainder of their training programme consisting of exercise of medium intensity and duration (Lovell and Rose, 1991). Therefore, the type and intensity of training programmes for showjumping horses and thoroughbred racehorses are significantly different. If a training programme consists only of high intensity exercise, then skeletal muscle fibre types of the type II variety will be recruited and developed, resulting in an increase in lactic acid production (Hermansen and Stensvold, 1972; Kindermann et al., 1979; Jacobs, 1986) as seen in the thoroughbred racehorses. Conversely, training programs consisting of large amounts of long duration, low intensity exercise will result in the development of type I muscle fibres causing a decrease in the rate of lactate production in the working skeletal muscles.

It can be suggested that the differences observed between lactate production in the showjumping horses, used in this study, and lactate production in the thoroughbred racehorses can be attributed to the variations in training programs between the two types of animals. The high intensity training of the thoroughbred racehorse engenders the recruitment of more type II muscle fibres than the training programme of the showjumper. As a result, during an incremental exercise test, it is expected that the thoroughbred racehorse will produce lactate at a greater rate than the showjumper at the same exercise intensity (Hermansen and Stensvold, 1972; Kindermann et al., 1979; Jacobs, 1986). In addition, it can be postulated that showjumping horses and thoroughbred racehorses are selected on the basis of their fibre type characteristics, thus effecting the rate of lactate production. However, there has been little work completed in this area regarding the thoroughbred racehorse and no work regarding the showjumper. Therefore, further studies in this area are required to investigate this theory.

On examination of individual lactate curves, it was observed that horses NTL and CDB (the highest graded horses participating in this test) produced very similar amounts of lactate in response to the incremental exercise test. Although these horses did not produce the greatest amounts of lactate in total, it can be seen from Figure 4.2b that the differences between plasma lactate concentrations measured following 10 and 12 m/sec workloads were the greatest of all the test animals. Of the horses who completed both the track test and the jump test, these two horses (CDB and NTL) completed the jump course in the fastest times. It is tempting to speculate that a correlation exists between the change in lactate levels in response to incremental exercise, and performance in the showjumping horse. This might imply that optimal performance in the showjumping arena is related to a specific ratio of type I to type II fibre types.

It would be premature to conclude that this relationship between lactate performance curves and showjumping performance exists based on the data from two horses. To investigate this relationship in more detail a large number of showjumping horses must be tested and the reliability of the incremental exercise test used in this study must be confirmed.

A linear relationship was observed between heart rate and increasing work intensity. Heart rate values obtained from this test are in agreement with those reported in the literature. The relationship between heart rate and blood lactate concentrations can be important indicators of the horse's response to the test (Thornton, 1985). In Figure 4.3, the data support a linear relationship between plasma lactate levels and heart rate at exercise levels from 8m/sec onwards. The relationship is strikingly similar to that noted between plasma lactate and workload (refer to Figure 4.2a). These relationships illustrate that as the workload of the test increases, the metabolic demands of the animals are raised and an increase is observed in both heart rate and blood lactate levels.

Phase 2 of this study was divided into two stages. The first stage of this test was the control stage. By having the horses complete the jump course without the jumps placed into position the biochemical and haematological responses of the horse to the actual jumping action could be isolated during phase 2 of the test. Plasma lactate values did not increase beyond resting values after the completion of the first jumping course. This suggests that despite the tight cornering required from the animal to complete the course, the major energy system used by the animal was aerobic. However, completion of the second jump course bought about a significant increase ($p=0.001$) in plasma

lactate values with a mean of 2.30 mmol/L. It can therefore be suggested that the jumping effort of the horse requires the working muscles to obtain energy through the use of anaerobic pathways, resulting in an increase in plasma lactate concentrations.

It has been reported in the literature that although the average speed required to complete a showjumping course is approximately 350 m/min, the metabolic demands of the entire showjumping competition are equal to a run on the track at 600m/min (10m/sec) (Lekeux, Art, Linden, Desmecht and Amory, 1991 pg 384-390). However from the results of this study, it has been shown that the metabolic demands of Australian show jumping horses are equivalent to a run on the track at a speed of approximately 8.5 m/sec.

Heart rates observed during the jump test suggested that jumping requires an increase in the metabolic demands of the working muscles. During the first jump course, peak heart rates ranged from 120 b/min to 179 b/min. During the second stage of this test, the increasing energy demands of the working muscles caused an increase in peak heart rates with values ranging from 164 b/min to 203 b/min.

Heart rates obtained during the second stage of this test were in agreement with those reported by Art, Amory, Desmecht and Lekeux (1990a) and Lekeux, Art, Linden, Desmecht and Amory (1991) suggesting that the work intensity between studies was similar (although heart rate can increase due to stress or excitement, and this must be noted when using heart rate as an indicator of exercise intensity in the horse). The jump courses used for these studies were slightly larger and longer than those in the present study (fence heights ranged from 1.4m to 1.5 m). However, blood lactate concentrations

reported by the above authors were 9.04 mmol/L and 8.7 mmol/L respectively. These values are much higher than those observed in the present study indicating that for similar work intensities, the European showjumping horses produced up to 4.5 times the amount of lactic acid than the Australian horses.

The breeds of horses used by Art, Amory, Desmecht and Lekeux. (1990a) and Lekeux, Art, Linden, Desmecht and Amory (1991) were Belgian and French saddlebred horses respectively. Whereas the horses used in the present study were either thoroughbreds or Australian warmbloods. The higher lactate concentrations reported by Art et al. (1990a) and Lekeux (1991) following showjumping competition may be attributed to the different breeds of horses examined. Snow and Guy (1980) report a variation in the percentage of type I and type II skeletal muscle fibre types among breeds of horses. The authors of this study conclude that the ratio of fibre types within various muscles is different among breeds of horses. The discrepancy in lactate values observed between Australian and European showjumping horses might be due to variations in the percentage of fibre types among different breeds of horses. In addition, methods of training horses may differ throughout the world. Clayton (1994) has suggested that to develop the type II fibres of the showjumping horse, an exercise program consisting largely of high intensity short duration exercise should be employed by trainers. However, as mentioned in this study, some Australian show jumping trainers incorporate a large amount of low intensity long duration exercise into their training programs.

The discrepancies observed in lactate values between the European and Australian showjumping horses may also be due variations in the testing protocol of this study and

of those conducted in Europe. Blood samples for the European studies (Art, Amory, Desmecht and Lekeux, 1990 a & b; Lekeux, Art, Lindon, Desmecht and Amory, 1991) were collected following a round of normal competition, whereas, this study involved sample collection during a non-competition round. In Europe showjumping is a major spectator sport and the excitement of the horses caused by large crowds, may have resulted in a higher lactate production than the horses used in this study, who remained very relaxed during testing. In addition, the warm-up procedures for the studies of Art et al. (1990 a&b) and Lekeux et al. (1991) were not documented. If the horses used in these studies were subjected to a strenuous warm up procedure, plasma lactate levels would be increased. It can also be suggested that sample collection times between the European studies and this study would be the cause of the discrepancies observed in lactate levels. However, Art et al. (1990 a&b) and Lekeux et al. (1991) collected blood at 2 minutes post-exercise. As shown by the present study, blood lactate concentrations decrease following the completion of exercise. Therefore if blood sampling times were the cause of the discrepancy, the difference would be decreased, not increased.

There were no relationships observed between showjumping performance and plasma lactate concentrations. However, it would be interesting to speculate that if horses AJS, CZ and JMB were to complete an incremental exercise test, lactate values similar to horses NTL and CDB might be observed, suggesting a link between plasma lactate values and performance in the showjumping horse. There is minimal evidence to support this notion and it should be confirmed by further research.

The variations seen in lactate production among breeds of showjumping horses may be due to the percentage of type II fibres within the working muscles or to differences in

training programs around the world. To investigate this area in more detail, further studies must be conducted.

5.4 PLASMA GLUCOSE

Plasma glucose concentrations have been well studied in the horse (Bergstrom, Guarnieri and Hultman, 1971; Anderson, 1975; Snow, Harris and Gash, 1985). During exercise of high intensity there is an increase in the concentrations of lactate and pyruvate in the blood. In addition a decrease has been noted in blood glucose levels during exercise of long duration (Anderson, 1975). As a result, a negative correlation has been shown between blood glucose and blood lactate levels during exercise (Rose and Hodgson, 1994 pg 63-78). Plasma glucose levels were investigated in this study as a measure of the rate of anaerobic glycolysis occurring within the working muscles during exercise.

As shown in figure 4.4 mean plasma glucose levels decreased during the first two workloads of the track test. During this period the glucose in the blood taken into the cells, broken down and used for energy. However, as the intensity of exercise increased, blood glucose levels also increased, peaking during the 12 m/sec workload. It was anticipated at the beginning of this study that a decrease in plasma glucose concentrations would be observed as the intensity of exercise increased due to an increase in the rate of anaerobic glycolysis (Anderson, 1975).

The increase in plasma glucose levels observed in this study during exercise intensities of 10 and 12 m/sec are the result of an increased rate of glycogenolysis. As the intensity

of exercise is increased, the release of insulin from the pancreas is suppressed by the sympathetic nervous system. As a result, blood glucagon levels increase, causing an increased rate in the breakdown of glycogen into glucose (Rose and Hodgson, 1994 pg 63-78). Glucose is then released into the blood stream causing the elevation in plasma glucose levels observed in this study. This effect is observed during exercise of short duration and high intensity. During the initial stages of the test, the workloads were relatively low and plasma glucose levels decreased. However as the test progressed, the intensity of exercise increased and glycogen stores within the muscle and liver were released, possibly as a result of stimulation of the sympathetic nervous system (Astrand and Rodahl, 1986 pg 556-562; Guyton, 1991 pg 862). If the work periods of this test were of longer duration, a decrease in plasma glucose levels may have been observed.

Plasma glucose levels during the jump test of this study showed a similar response to exercise as observed during the track test. During course 1, a low intensity exercise, plasma glucose levels decreased. However after completion of the second jump course plasma glucose levels had risen to near resting levels as shown by figure 4.23. The decrease in plasma glucose concentrations observed in this study are supported by the findings of Art, Amory, Desmecht and Lekeux (1990b) and Lekeux, Art, Linden, Desmecht and Amory (1991), however, these authors report on resting and post-jumping values only. The changes observed in plasma glucose concentrations observed are due to stimulation of the sympathetic nervous system, as described above.

5.5 CREATINE KINASE

Creatine kinase (CK) was used for the purposes of this study as an indicator of skeletal muscle damage following exercise (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983; Hortobagyi and Denahan, 1989; Morris et al, 1991; Manfredi et al, 1991; Volfinger, Lassourd, Michaux, Braun and Toutain, 1994; Rose and Hodgson, 1994 pg 63-78). It has been reported that during exercise of short duration and high intensity, significant muscle damage occurs resulting in increased plasma creatine kinase values (Tiidus and Ianuzzo, 1983). It has also been shown that exercise involving a large proportion of eccentric muscle contractions will cause greater elevations in CK levels than exercise involving concentric muscle contractions (Newham, Jones and Edwards 1983).

On observation of the heart rates and blood lactate concentrations for all horses used in this study, it can be suggested that the final workload of 12 m/sec for the track test required the horses to exercise at near maximal intensity. Despite the high intensity of exercise performed by these horses, no significant increases were observed in plasma creatine kinase concentrations immediately following exercise or 24 hours post-exercise. These results were in agreement with Snow, Mason, Ricketts and Douglas (1983) who reported a small but non-significant increase in plasma CK levels immediately following racing in the thoroughbred. It has also been shown that peak CK levels do not occur until 24 hours after the completion of exercise (Newham, Jones and Edwards, 1983; Schwayne, Johnson, Vandenakker and Armstrong, 1983) of short duration and high intensity. However, no significant increase in CK levels were observed 24 hours post-exercise in this study.

It was anticipated that the incremental exercise test used in this study would result in large increases in plasma CK levels due to the high intensity of exercise required to complete the 12 m/sec workload. It has been reported that concentric muscle contractions do not cause large amounts of muscle damage when compared to the muscle damage caused by eccentric contractions (Newham, Jones and Edwards 1983). Based on observations of exercising animals, it is proposed that the major muscles involved at full gallop are largely undergoing concentric contractions. This might explain the low CK levels generated in this study. However, confirmation of this theory would require further research. The CK data reported in this study suggest that while this type of exercise is metabolically demanding on the horse (as seen from high heart rates and blood lactate concentrations) it is not physically injurious to the animal's skeletal muscles (Newham, Jones and Edwards, 1983).

As shown by Figure 4.7, horse WMC produced a large increase in plasma CK levels at the completion of the 12 m/sec workload. At the completion of the 2 minute period, the jockey forced this horse to decelerate and stop very rapidly. It is proposed that this sudden, maximal eccentric contraction may have caused a large amount of muscle damage, resulting in the observed elevated CK levels. However, the CK levels for this horse returned to normal 24 hours after the test indicating no signs of long term muscular damage. The day after the test this horse had large amounts of swelling in the right front leg. Following examination by a vet it was concluded that the horse had fractured its right front fetlock joint during the test. It is probable that this injury occurred as a result of the sudden deceleration of the animal by the jockey.

Significant increases in plasma CK levels following showjumping have been reported (Art, Amory, Desmecht and Lekeux, 1990 a, b; Lekeux, Art, Linden, Desmecht and Amory, 1991 pg 384-390) indicating that showjumping causes a significant degree of muscle damage. These studies documented a change in plasma CK values from approximately 50 U/L at rest to 75 U/L following jumping ($p<0.001$, $n=16$), (Lekeux et al. 1991) ($p<0.05$ $n=8$), (Art et al. 1990 a, b). Similar increases in blood CK levels were observed in this study, however these results were not statistically significant. As shown by Table 4.11 and Figure 4.24 a large variation between individual animals was noted in both resting and post-exercise plasma CK levels, resulting in a large standard error of the mean. Although no major differences were observed in the general training programs of the horses, the specific training programs of the animals could not be standardised. The large variation observed may be due to the level of exercise the animals were subjected to before the testing sessions. For example, if a horse was subjected to a large amount of exercise 2 to 3 days before the test, the plasma CK levels of this horse would be higher than those of a horse that has been resting in its stable. Ideally, all horses should have been rested or lightly exercised for 7-9 days before the testing sessions in order to ensure baseline CK levels (Newham, Jones and Edwards, 1983). If the large variation between horses was reduced, the statistical significance of the changes observed in plasma CK levels may have been increased, as reported by Art et al (1990 a, b) and Lekeux et al. (1991).

It was anticipated at the beginning of this study that showjumping would cause significant increases in plasma CK levels. It has been noted that eccentric muscular contraction will cause a larger amount of damage than will concentric muscle contraction. During the action of jumping, the horse must quickly accelerate, power

over the fence and then quickly decelerate on landing in order to prepare for the next jump. It is proposed that upon landing, the skeletal muscles of the horse are contracting primarily eccentrically to stabilise and decelerate the animal. It is therefore surprising that as a consequence of executing a jump round, higher CK values were not noted, although it is recognised that peak CK levels may take 1-3 days to develop post-injury (Schwayne, Johnson, Vandenakker and Armstrong, 1983).

Although the increases in plasma CK levels reported by Art, Amory, Desmecht and Lekeux (1990 a, b) and Lekeux, Art, Linden, Desmecht and Amory (1991) show statistical significance, it is doubtful that CK values of this magnitude (50-75 U/L) represent muscle damage severe enough to effect the animal's performance. The normal ranges for resting plasma CK levels in the horse are between 100 and 300 U/L (Rose and Hodgson, 1994 pg 63-78). The normal ranges for plasma CK are large because the levels of this enzyme in the blood are quite variable and large increases do not necessarily indicate a severe muscle disorder. Horse AJS in this study obtained a plasma CK value of 270 U/L during course 1 of the jump course. Although this level is higher than those observed for the other animals, this horse completed jump course 2 faster than any of the remaining horses. It is worth noting that Kerr and Snow (1983) reported plasma CK levels of greater than 30 000 U/L in an endurance horse with no clinical signs of a muscle disorder. Therefore the increases in plasma CK levels reported by Art et al. (1990 a, b) and Lekeux et al. (1991) may be statistically significant, however the increases observed were small and it would be expected (as shown by the variations in resting values of the horses tested in this study) that the normal resting values of these horses would show a larger variation than was reported in these studies.

It has been shown that following strenuous exercise, an initial increase in plasma CK levels occurs at 24 hours post-exercise, with CK levels returning to normal shortly after this period (Schwayne, Johnson, Vandenakker and Armstrong, 1983; Newham, Jones and Edwards, 1983). However, some individuals may experience delayed onset muscle soreness following strenuous exercise, with peak CK levels occurring at up to 4-7 days following exercise (Schwayne et al. 1983; Newham et al. 1983). There are no current data in the literature stating the time taken for CK to reach peak levels in the horse. For this study, the time period of 24 hours post-exercise was chosen as the optimal sample time for peak CK levels, as shown by Schwayne et al. (1983) and Newham et al. (1983) (excluding delayed onset muscle soreness). To establish the true time taken for peak CK levels to occur following exercise, blood samples should have been collected at regular time intervals following the completion of exercise for a period of 7-9 days. However, due to the nature of this study and the difficulty in obtaining horses for sampling, this was not possible. Future research is needed in this area to establish the patterns of CK release from muscle following strenuous exercise in the horse.

5.6 PLASMA POTASSIUM

As well as lactate, potassium changes have been suggested to play an important role in muscular fatigue (Harris and Snow, 1988). In has also been shown that exercise of high intensity results in increased plasma potassium concentrations which may lead to electrocardiographic T-wave abnormalities and poor performance (Harris and Snow, 1988). There have been very few studies involving plasma potassium responses to high intensity exercise in the horse, however Harris and Snow (1988) investigated the relationship with relation to thoroughbred racehorses. This study involved galloping the

horses on a treadmill at the same velocities as used in this study for the same amounts of time (2 minutes). The authors of this study reported a high correlation (r=0.923) between plasma potassium and plasma lactate concentrations, with peak plasma potassium concentrations of 8.7 mmol/L occurring at a workload of 12 m/sec. In contrast to the study by Harris and Snow (1988), no changes in plasma potassium were noted in this study. It has been suggested that the accumulation of lactate and hydrogen ions in the muscle, decreases the osmolarity of the muscle cell, and this may retard the re-uptake of potassium released during muscular contraction (Harris and Snow, 1988). This failure to re-uptake the released potassium is probably caused by the inhibition of the sodium-potassium pump of the muscle cell membrane. This could be due to the increase in hydrogen ions or to a decrease in the availability of ATP (Harris and Snow, 1988). Lactate concentrations reported by Harris and Snow (1988) were in excess of 25 mmol/L at a velocity of 12 m/sec. However the mean plasma lactate values observed in this study were significantly less (14.62 mmol/L). It can therefore be suggested that inhibition of the sodium-potassium pump, as described by Harris and Snow (1988), occurs only when lactate concentrations are very high. Horse TVA, in the present study produced 21.1 mmol/L of lactate during the highest intensity of exercise (Table 4.11) and a plasma potassium concentration above 12.0 mmol/L. This horse was the only animal to show an increase in plasma potassium concentrations during the exercise test. More studies are required to investigate the relationship between plasma potassium and plasma lactate levels.

There were no significant increases observed in plasma potassium concentrations during showjumping in this study. As suggested above, plasma potassium concentrations will begin to increase when the rate of lactate production is so fast that it accumulates in the

muscle cell, disrupts the sodium-potassium exchange pump and causes an increase in extracellular potassium concentrations. Plasma lactate during jumping in this study was minimal compared to the levels of lactate required to cause an increase in plasma potassium levels. Therefore, no increase in potassium was observed during the jump test.

5.7 RED BLOOD CELL INDICES

As mentioned earlier, the horse has the ability to store up to 50 % of its red blood cells (RBC) in the spleen (Persson and Lydin, 1973). During periods of stress (physical and emotional) these red cells are ejected into the circulation, providing the animal with an increased ability to transport oxygen to the skeletal muscles, and hence providing the animal with a greater aerobic capacity (Persson and Lydin, 1973; Rose and Hodgson, 1994 pg 63-78). The type and intensity of exercise is the major factor governing the release of red blood cells into the circulation. Exercise of high intensity and short duration will stimulate a higher rate of splenic contraction and release of RBCs into the circulation than will exercise of low intensity and long duration. (Persson and Lydin, 1973; Evans and Rose, 1988; Rose and Hodgson, 1994 pg 63-78). As a result, the rate of RBC entering the circulation during exercise is used by equine exercise physiologists as a measure of the animal's physical fitness.

A significant increase ($p<0.001$) in RBC counts was observed during the incremental exercise test indicating that the intensity of exercise performed was quite high (Figures 4.10 and 4.11). On observation of individual RBC values (Figure 4.11) horses WMC and TVA recorded the highest RBC values. These horses also recorded the greatest rate

of increase in RBC for workloads of 10 and 12 m/sec. It is interesting to note that the lower graded horses recorded the highest RBC values and rates of increase of RBC levels, whereas horses CDB and NTL (the top two graded horses participating in this test) recorded the lowest RBC counts and had similar increases in these numbers as the intensity of exercise became more difficult. These results are indicative of successful showjumping horses having a lower red blood cell response to exercise than unsuccessful showjumping horses. However, due to the small size of the test group no conclusions can be made regarding this relationship. Horses CDB and NTL may have a larger aerobic capacity than the other horses, resulting in fewer red cells being ejected into the circulation by the spleen. Therefore further studies must be conducted involving many horses of similar physical fitness to determine if a relationship exists between RBC values and performance in showjumping horses.

Horse MSH recorded a large increase in RBC from resting at the completion of the first workload. This horse's RBC values then remained constant throughout the test until returning to normal resting levels 24 hours after the completion of the exercise. As this horse had the lowest heart rate and plasma lactate values it can be postulated that the increases in RBC counts observed for this horse were due to the psychogenic effects of anticipation of exercise on the red cell pool rather than the intensity of exercise (Blackmore, 1983 pg 344-353; Revington, 1983a).

During strenuous exercise it has been reported that, in response to catecholamine concentrations, the stored red blood cells are ejected into the circulation to increase the oxygen carrying capacity of the blood (Persson and Lydin, 1973; Evans and Rose, 1988; Rose and Hodgson, 1994 pg 63-78). During the incremental exercise test employed in

this study, the greatest values of red blood cells observed were for the 12m/sec workload. These values were only approximately 30% greater than resting values despite the near maximal nature of this exercise. Currently there is no information regarding the exercise intensities that would result in ejection of 100% of all red blood cells into the circulation. As a result, future research is recommended into this area to determine what exercise intensities require the cardiovascular system of the horse to function at maximal capacity. In addition, if the rates of release of red cell from the spleen are further investigated, a form of performance profiling may be developed. For example, in a showjumping competition or a race, the horse who can eject the greatest number of red cells into the circulation may have an advantage over other horses.

A significant increase ($p<0.05$) from resting values was observed in the jump phase of this study. RBC counts increased from resting values of 8.22 $x10^{12}$/L to 10.37 x 10^{12}/L during the first jump stage. There were no further increases in RBC counts during completion of the second jump course. The effects of showjumping on red blood cell counts have not been directly investigated by previous studies. However, Art, Amory, Desmecht and Lekeux (1990 a, b) and Lekeux, Art, Linden, Desmecht and Amory (1991) report a similar increase in packed cell volume.

The data obtained from the present study (heart rates and lactate) indicate that the intensity of exercise required to complete course 1 of the jump test was similar to that required to run at 6 m/sec on the track. However, during the first stage of the jump test, RBC values increased by 25 % from resting levels (figure 4.25). To obtain this degree of increase whilst running on the track, a velocity of 10 m/sec would have to be achieved. Therefore, despite the low exercise intensity required to complete course 1 of

the jump test, a large increase in RBC counts was observed. It is probable that this observation is the result of psychogenic effects on the red cell pool associated with the anticipation of exercise. Red cell numbers remained elevated during the second stage of the jump test and returned to normal resting levels 24 hours following the test. Therefore, showjumping resulted in an elevation in RBC counts, although it is difficult to conclude whether this increase was caused by exercise or the excitement of the horse anticipating exercise. There were no relationships observed between red cell indices and performance in all horses tested.

Mean haematocrit and haemoglobin concentrations showed a similar increase to RBC values. Haematocrit is defined as the volume of red cells, in one litre of blood. It would therefore be expected that an increase in the number of red blood cells in the circulation would be directly proportional to haematocrit values. Similarly, haemoglobin is contained within the red blood cell. If the numbers of red cells in the circulation are increased then a concomitant rise in haemoglobin concentrations will be observed (Astrand and Rodahl, 1986; Guyton, 1991).

On examination of individual red blood cell constituents no significant increases were observed in mean corpuscular volume (MCV), mean cell haemoglobin (MCH) or mean cell haemoglobin concentration (MCHC). At the completion of both jump courses, horse JMB showed a MCV value of 102 FL (femto litres). The maximal normal range for MCV as reported by Schalm (1986) is 58 FL. The MCV value recorded for this horse is approximately twice the resting value, indicating that the red blood cells of this horse doubled in size (volume) whilst completing the jump test. In explanation of this result it can be suggested that the anticoagulant EDTA contained in the blood collection

tube caused the red cells to swell, dramatically increasing the volume (Stewart, Riddle and Salmon (1977). However this sample was stored under the same conditions as blood samples from horses AJS and CZ and these values are normal. Therefore it is unknown why this horse produced such a large increase in MCV.

5.8 White Cell Indices

It has been well documented in human subjects that exercise causes a marked increase in circulating white blood cells, with the extent being proportional to the intensity of exercise (Snow, Ricketts and Mason, 1983). In the horse a similar relationship has been shown with significant increases in the circulating WBCs following exercise of high intensity such as racing (Revington, 1983a; Snow, Ricketts and Mason, 1983). In addition a non-significant increase has been reported following exercise of light intensity and longer duration (Rossdale, Burguez and Cash, 1982; Snow, Ricketts and Mason, 1983). With reference to specific cells, the above authors report a large increase in circulating lymphocytes. The spleen is an important production site for lymphocytes and during exercise as the spleen contracts, releasing red cells into the circulation, a large number of lymphocytes are released as well.

In this study, a significant ($P<0.01$) increase in white blood cells occurred 24 hours after completion of the track test. However, no increases in white cell counts were observed during the test. These findings were contradictory to those reported by Rossdale, Burgeuz and Cash (1982) and Snow, Ricketts and Mason (1983) who found that exercise of high intensity and short duration causes an increase in white cell indices within 4 hours of the completion of exercise. In addition, these authors report that blood

white cell values return to normal resting levels within 6 to 10 hours after exercise. In explanation of their results, the authors of these studies suggest that exercise of high intensity and short duration causes a rise in blood cortisol levels, whereas exercise of low intensity and longer duration does not. Cortisol causes a decrease in the migration of neutrophils from their intravascular sites and also stimulates their production in the bone marrow (Snow, Ricketts and Mason, 1983). Therefore, during intense exercise neutrophils are produced and released into the circulation.

During this study the increases observed in WBC values occurred over a longer time period than that reported by the above authors. It is therefore doubtful that this rise is due to an increase in plasma cortisol levels. On examination of white cell differential counts it was evident that the rise in WBC counts was due to an increase in neutrophil numbers. Absolute white cell counts increased from mean resting values of 8.3 $x10^9$/L (with 51.2 % of all white cells being neutrophils) to 24 hr post exercise values of 10.9 $x10^9$/L (Table 4.5). Sixty six percent of these white cells were neutrophils and 28.2 % were lymphocytes. It must be noted that absolute values of individual white cells were not documented during this study, it is therefore difficult to determine whether this increase was the result of a decrease in lymphocytes or due to an increase in neutrophils.

All horses tested in this study (with the exception of horse MSH) showed some degree of inflammation in the legs 24 hours after the completion of the test. As no significant increases in CK levels were observed, it can be postulated that during the exercise test, the firmness of the track caused a high degree of physical stress on the joints and tendons (as opposed to the musculature), of the lower legs, which may have resulted in some degree of connective tissue damage which initiated the inflammatory response.

During the first 24 hours of the inflammatory response neutrophils migrate to the injury site, through the blood stream, where they remove dead or damaged tissue (Robbins, Cotran and Kumar, 1991 pg 15-16). Therefore, as the increases in WBC counts were observed 24 hours after the completion of the exercise, it can be suggested that high neutrophil numbers were present in the blood in response to local tissue injury. If peak WBC counts were observed 4-6 hours following the completion of exercise then it could be suggested that this occurred as a result of cortisol stimulation.

During the jump test of this study a small but non-significant increase was observed in WBC values following the completion of course 1 and course 2, with values returning to normal levels 24 hours after the test.

5.9 CONCLUSION

The purposes of this study were to determine how well biochemical and haematological variables compared with performance in the showjumping arena as a gauge of athletic potential in horses, thus addressing the paucity of scientific literature in this area. Further, it was hoped that this work would provide a foundation to develop a regime that can be used for screening potential animals for showjumping.

Although this study was limited by a small test group of 8 horses, a wealth of information was gained regarding the exercise physiology of the showjumping horse relevant to Australian conditions.

By observing the lactate performance curves developed during this study it was evident that plasma lactate production is affected by methods of training. Those horses that undertake a large amount of high intensity exercise will recruit, develop and condition primarily skeletal muscle fibres of the type II variety. However, long duration, low intensity exercise causes the recruitment and development of the type I muscle fibre. As a result those animals who participate in a large amount of high intensity exercise will produce greater concentrations of lactate than those who train using low intensity exercise at similar work intensities. The show jumping horses investigated in this study produced notably lower concentrations of lactate than did the thoroughbred racehorses examined by Harris and Snow (1988).

This study hypothesised that methods of training showjumping horses does effect showjumping performance. However, the general training programs of all horses

examined in this study were similar. As a result, this hypothesis could not be thoroughly investigated and a more detailed study into this area is required involving showjumping horses undertaking various training programmes.

The Australian showjumping horses investigated in this study produced significantly lower concentrations of plasma lactate than the European horses competing at a similar level of competition. The variations observed in the rates of lactate production between the two classes of horses may be the result of differences in training methods between the two countries. In addition, this discrepancy might be the result of differences in the animals' skeletal muscle fibre composition. Snow and Guy (1980) reported such variations within the percentage of fibre types among different breeds of horses. Therefore, the European breeds of horses may have higher percentages of type II fibres within their skeletal muscles than do the Australian horses. This would explain the variations observed in lactate production during exercise among these horses. The European countries dominate the world of showjumping, consequently, it is interesting to speculate that the high lactate production demonstrated by European horses may be a desirable attribute of a successful showjumper. Further studies are needed in this area in order to examine this relationship in more detail.

Within the limitations of the study, the biochemical and haematological variables investigated did not correlate in any simple fashion with performance in the showjumping arena, with no obvious relationships between levels of competition being detected. However, during the incremental exercise test it was evident that the two most successful showjumping horses had the lowest rate of increase in red blood cell values as the intensity of exercise increased. In addition, the two horses with the lowest

professional competitive success rates (horses TVA and WMC) produced the greatest increases in red cell indices during the incremental exercise test. Although the sample size of the test group is small and only limited conclusions can be made, it is suggested that a relationship may exist between red blood cell indices and performance in the showjumping horse. However more horses will have to be tested to investigate this trend in more detail.

The development of lactate performance curves provides the equine exercise physiologist with valuable information regarding the muscle physiology of the performance horse. When the lactate curves of the horses tested in this study were compared with performance it was evident that horses CDB and NTL (the two highest graded horses of the study) produced lactate curves qualitatively similar to each other. In addition, when compared with the animals who participated in both phases of this study, these two horses demonstrated the shortest times over the test jump course. Although the sample size of the study was very small compared to the sample sizes used in human exercise physiology research, a trend has emerged by noting showjumping performance (as measured by the time to complete the standardised jump test), and plasma lactate levels during incremental exercise. If horses AJS and CZ had participated in the incremental exercise test it is speculated that these horses would have produced similar trends in plasma lactate levels. However, for this test to become a valid indicator of performance in the showjumping arena more horses from all levels of competition must be tested and a data base established documenting the changes in lactate production (with respect to exercise intensity and time), among different breeds of horses, at varying levels of competition and from alternative sporting disciplines.

This study is innovative as it introduces the concept and methodology of incremental exercise testing into the show jumping industry. The information gained from this study permits showjumping trainers to maximise the performance potential of their animals by developing training programs based on each horse's individual skeletal muscle fibre type ratio. The trends demonstrated in this thesis between plasma lactate levels and measured showjumping performance open the door for the development of an evaluation regime that can be used for the early identification of performance potential in young showjumping horses.

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QUESTIONNAIRE

EDITH COWAN UNIVERSITY DEPARTMENT OF HUMAN BIOLOGY

Sample No._____

Name of Horse:_____ Breed:_____

Sex:_____ Age:_____

Competition Level:_____ How Long at That Level:_____

Rider's Name:_____ Rider's Weight:_____

Trainer of Horse:_____ Time of Test:_____

Time of Last Feed:_____ Temperature:_____

Barometric Pressure._____ Humidity:_____

Comments:_____

APPENDIX 1

QUESTIONNAIRE

The following biochemical and haematological variables will be tested for:

Lactic Acid
Glucose
Potassium
Creatine Kinase
Packed Cell Volume
Red Cell Volume
White Cell Differential
Total Haemoglobin

No other biochemical or haematological constituents will be tested for and ALL information recieved during the testing or at any other times will remain strictly confidential..

Name of Tester:_____

Signature of Tester._____

Date: _____

I have had all procedures and methods fully explained to me and I give my consent to participate in this research project.

Name:_____

Signature._____

QUESTIONNAIRE

Nutrition

Daily food intake for horses competing at competition level.

	Feed Description
Morning	
Midday	
Evening	
Other	

*** Feeding times and descriptions of feed components.

QUESTIONNAIRE

Training

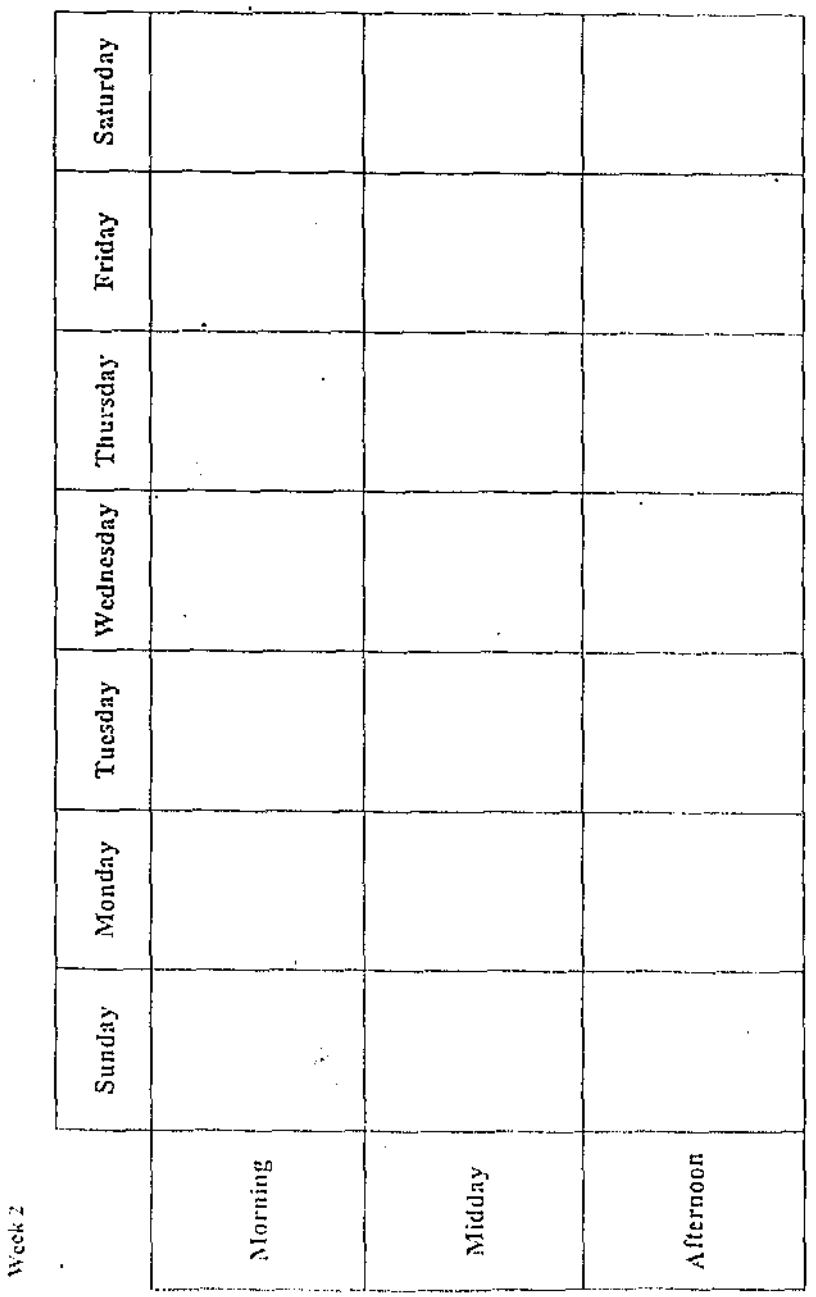
Week 1

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning							
Midday							
Afternoon							

***Description of a general two week training programme. Times and descriptions of work done are noted.

APPENDIX 1

QUESTIONNAIRE



Week 2

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning							
Midday							
Afternoon							

APPENDIX 2

NUTRITION

Daily food intake for horses participating in this study.

Feeding times and descriptions of feed components are recorded.

	Feed Description
Morning	Pellets ½ - 1 kg Oats ½ - 1½ kgs Chaff 1 - 2 kgs Horse FK received the above food constituents with the addition of 1kg rolled barley and ½ kg lucerne chaff.
Midday	All the horses received grass with the exception of Horse FK. Horse FK received 1 biscuit of oaten hay.
Evening	Pellets ½ - 1 kg Oats 1 - 1½ kgs Chaff 1 - 2 kgs Corn ¼ kg All horses were receiving vitamin and mineral supplements. Horse FK received the above food with the addition of 1 kg rolled barley, ½ kg lucerne chaff and 1 biscuit hay.
Other	Horse CZ received a biscuit of hay whilst standing in his stable.

TRAINING

Description of general two week training programme for all horses participating in this study. Times and descriptions of work done are noted.

Week 1	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning	Show Day	Day Off	CDB - Lunge MSH - Lunge JMB - Heavy Flat CZ - Day off AJS - Lunge	CDB - Hack out MSH - Hack out JMB - Hack CZ - Lunge AJS - Hack out	CDB - Heavy flat and small gallop MSH - Heavy flat work JMB - Heavy flat CZ - Light flat AJS - Heavy flat and small gallop	CDB - Jump MSH - Jump JMB - Jump CZ - Jump AJS - Jump	CDB - Light flat work MSH - Light flat work JMB - flat CZ - Flat or day off AJS - very light flat FK - 5km walk and trot roadwork
Midday							
Afternoon			FK - 20 min lunge NTL - Hack out	FK - 30 min flat work NTL - 30 min flat work	FK - 20 min luge NTL - Jumpig exercises	FK - 30 min flat work and jumping exercises NTL - Flat work	FK - 15-20 min jumping NTL - day off

TRAINING

Description of general two week training programme for all horses participating in this study. Times and descriptions of work done are noted.

Week 2	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning	Show Day	Day Off	CDB - Lunge and possible jump MSH - Lunge or day off JMB - Heavy Flat CZ - Day off AJS - Lunge or jump depending on performance at show	CDB - Hack MSH - Hack or jump JMB - Jump CZ - Lunge AJS - Hack	CDB - Heavy flat MSH - Heavy flat JMB - Hack CZ - Flat AJS - Heavy flat	CDB - Jump MSH - Jump JMB - Flat CZ - Lunge over jump AJS - Jump	CDB - Light flat MSH - Light flat JMB - flat CZ - Flat AJS - very light flat FK - 5km walk and trot
Midday							
Afternoon			FK - 20 min lunge NTL - Hack out	FK - 15 min roadwork, 20 min flat work. NTL - 30 min flat work	FK - 30 min luge NTL - Jumping	FK - 15 min roadwork, 20-30 min jumping NTL - flat work	FK - 15-20 min jumping exercises NTL - day off

TVA - (Anton Kulatz)				Data Summary											
	RBC	WBC	Hb	HCT	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	Lactate	CK	Potassium
Lactate Test 7/9/96															
6 m/sec 0 min	11.63	7.5	197	0.58	50	17	340	67	28	3	3	2.14	1.1	<24.4	3.52
6 m/sec 3 min	11.5	7.2	190	0.57	50	16	332	59	35	5	1	4.53	0.9	84.4	3.07
8 m/sec 0 min	12.37	8.3	206	0.62	50	17	334	54	45	1	0	2.71	1.4	71	4.06
8 m/sec 3 min	12.03	8.1	202	0.59	50	17	337	53	38	8	1	4.09	1.2	114	>12.0
10 m/sec 0 min	13.15	7.8	222	0.64	49	17	344	53	45	2	0	6.36	13.7	65.9	>12.0
12 m/sec 0 min	14.01	8	242	0.69	49	17	349	60	38	2	0	7.75	21.1	108	>12.0
24 hr	9.48	8.9	161	0.47	49	17	343	NR	NR	NR	NR	4.9	1.2	105	>12.0
Jump Test 9/10/96															
Resting	9.51	7.1	159	0.45	47	17	355	53	44	3	0	5.85	0.8	82.7	3.65
Course with no Jumps															
1 minute post	11.26	8	191	0.53	47	17	361	49	47	2	2		1	138	4.61
Course with jumps															
1 minute post	11.97	7.8	200	0.57	47	17	353	53	41	3	3	4.34	5	101	4.18

MSH - (David Dobson)															
	RBC	WBC	Hb	Hct	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	Lactate	CK	Potassium
Resting 18/9/96	8.24	8.3	142	0.44	53	17	325	58	40	2	0	5.9	<0.7	91.6	3.28
Lactate Test 11/9/96															
6 m/sec 0min	11.66	8.8	2	0.61	52	18	334	67	29	3	1	4.62	0.8	78.9	3.77
8 m/sec 0 min	11.23	8.9	199	0.59	53	18	337	63	27	10	0	4.45	1	131	3.3
10 m/sec 0 min	11.2	8.4	197	0.59	53	18	333	65	31	4	0	5.33	1.9	126	4.1
12 m/sec 0 min	11.6	8.2	205	0.61	52	18	336	70	29	1	0	6.31	5	130	3.78
24 hr	8.66	11.5	151	0.46	53	17	331	68	23	3	6	5.3	1.1	122	2.7
Jump Test 18/9/96															
Course with no jumps															
1 min post	10.7	10.3	188	0.56	52	18	335	59	40	1	0	5.97	0.8	34.2	3.73
3 min post	10.07	9.4	178	0.53	53	18	334	57	40	3	0	3.59	0.7	121	3.88
5 min post	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Course with jumps															
1 min post	10.73	9.5	191	0.56	53	18	338	60	36	4	0	6.51	0.8	134	3.43
3 min post	10.4	9.4	180	0.55	52	17	329	64	33	3	0	6.46	0.7	133	3.31
5 min post	9.64	9.1	172	0.51	53	18	337	65	30	5	0	6.13	0.7	85.1	3.33

FK - (Anton Kulatz)				Data Summary											
	RBC	WBC	Hb	HCT	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	Lactate	CK	Potassium
Jump Test															
Resting 9/10/96	7.92	5.6	138	0.4	50	17	348	61	35	3	1	5.55	0.7	84.9	3.46
Course with no jumps															
1 minute post	10.13	6.6	175	0.5	50	17	348	32	34	2	2		0.7	66.2	4.23
Course with jumps															
1 minute post	10.76	6.7	191	0.54	50	18	357	45	45	7	3		3	111	3.95

JMB - (David Dobson)							Data Summary								
Date of Test	RBC	WBC	HCT	Hb	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	CK	Potassium	Lactate
22/7/96 - Resting	7.82	7.2	0.4	132				59.5	36	2.5	2	6.2	84		
16/10/96 - Resting	7.4	6.8	0.38	132	51	18	349	60	35	4	1	5.76	34.7	3.49	<0.7
Jump Test															
Course 1	8.08	5.7	0.41	136	102	18	332	64	35	0	2	4.36	80.9	3.78	<0.7
Course 2	10.44	7	0.51	178	102	17	346	60	34	4	2	5.46	69.3	4.24	1
24 hr	6.48	6.2	0.34	110	52	16.4	324	55.5	33.5	3.5	7	6.34	89.8	3.22	<0.7

AJS - (David Dobson)					Data Summary										
Date of Test	**RBC**	**WBC**	**Hct**	**Hb**	**MCV**	**MCH**	**MCHC**	**Neutro**	**Lympho**	**Eosino**	**Mono**	**Glucose**	**CK**	**Potassium**	**Lactate**
16/10/96 - Resting	7.79	6.1	0.42	140	54	78	335	70	27	0	3	6.1	146	3.47	<0.7
Jump Test															
Course 1	13.36	7.8	0.69	253	52	19	364	63	33	2	2	5	270	4.03	<0.7
Course 2								58	38	1	3	5.33	135	>12.0	1.8
24 hrs	7.1	6.4	0.36	126	51	17	350	65	29.5	3	2	5.62	148	3.35	0.7

CZ - (David Dobson)	Data Summary														
Date of Test	RBC	WBC	Hct	Hb	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	CK	Potassium	Lactate
22/7/96 - Resting	8.79	7.4	0.46	154				50	57.5	2.5	-	8.26	75		
16/10/96 - Resting	7.16	5.6	0.35	132	49	18	377	43	50	4	3	6.21	70.7	3.66	<0.7
Jump Test															
Course 1	9.56	10.4	0.5	172	53	18	344	52	46	0	2	5.58	81.7	4.09	<0.7
Course 2	7.16	7.4	0.38	126	52	18	332	50	49	1	0	5.97	70.2	3.89	0.8
24 hrs	7.11	7.2	0.37	129	52	18	349	51.5	43	3.5	2	5.89	69.9	3.3	0.9

NTL - (Jay Reynolds)					Data Summary											
Date of Test	RBC	WBC	Hct	Hb	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	CK	Potassium	Lactate	
Course 2																
1 min post	10.8	11.3	0.56	195	52	18	345	66	34	0	0	1.81	116	3.28	2.6	
3 min post	10.5	10.6	0.53	180	53	18	340	54	44	1	2	6.98	51.2	2.52	2	
5 min post	10.03	10.8	0.53	180	53	18	340	64	33	2	1	3.51	85.2	3	1.5	
24 hr	7.56	8.9	0.4	134	53	18	335	58	32	5	4	6.02	91.2	2.38	1.4	
*** 24 hr sample taken after flat work																

WMC - (Jay Reynolds)					Data Summary							
Date of Test	RBC	WBC	Hct	Hb	Neutro	Lympho	Eosino	Mono	Glucose	CK	Potassium	Lactate
9/7/96 - Resting	8.33	8.5	0.42	141	60	35.5	1.5	2	7.61	<24.4	<2	
7/8/96 - Resting	11.13	8.2	0.59	189	60	35	4	1	5.58	85.8	>12.0	<0.7
Lactate Test 1												
6 m/s - 0 min	11.23	8.6	0.58	191	54	43	1	2	6.48	127	3.89	<0.7
6 m/s - 3 min	10.73	8.2	0.56	184	56	40	3	1	6.13	94.8	3.42	<0.7
6 m/s - 5 min	10.36	8.5	0.54	179	52	47	1	0	6.14	96.4	3.69	<0.7
8 m/s - 0 min	11.73	8.6	0.61	200	56	40	3	1	4.82	99.1	4.02	1
8 m/s - 3 min	11.16	8.2	0.58	193	53	47	0	0	5.86	90.5	3.77	<0.7
8 m/s - 5 min	10.83	7.9	0.57	187	54	40	5	1	5.61	125	3.59	<0.7
10 m/s - 0 min	12.27	9.1	0.64	212	69	30	1	0	6.52	123	4.89	8.7
10 m/s - 3 min	12.24	8.8	0.64	211	64	33	3	0	6.55	136	3.7	6.9
10 m/s - 5 min	11.53	8.8	0.6	197	52	47	1	0	6.21	110	3.96	6
12 m/s - 0 min	13.49	10.5	0.7	237	59	37	4	0	7.68	1170	4.44	15.3
12 m/s - 3 min	12.2	8.9	0.63	210	58	38	4	0	7.33	119	3.67	15.2
12 m/s - 5 min	11.8	8.5	0.61	205	59	39	2	0	7.05	128	3.54	14.9
5 hours post	8.76	10.7	0.46	150	72	24.5	0	2	4.16	172	>12.0	<0.7

NTL - (Jay Reynolds)						Data Summary										
Date of Test	RBC	WBC	Hct	Hb	MCV	MCH	MCHC	Neutro	Lympho	Eosino	Mono	Glucose	CK	Potassium	Lactate	
9/7/96 - Restin	8.66	9.2	0.47	159				55	41	2	2	4.29	92.4	<2		
30/7/96 - Resti	9.93	8.2	0.52	177				47	46	4	3	6.18	37.0	>12.0	<0.7	
Lactate Test 1 30/7/96																
6 m/s - 0 min	9.97	8.7	0.52	178				49	50	1	0	6.18	141	4.31	<0.7	
6 m/s - 3 min	9.41	8.5	0.49	169				52	46	2	0	5.68	175	4.06	<0.7	
6 m/s - 5 min	9.38	8.8	0.50	166				58	38	4	0	6.4	144	4.01	<0.7	
8 m/s - 0 min	10.53	9.8	0.55	192				56	42	1	1	6.14	201	4	0.9	
8 m/s - 3 min	9.74	8.7	0.51	179				55	43	2	0	6.32	216	4.23	<0.7	
8 m/s - 5 min	9.34	8.8	0.49	169				55	41	4	0	6.46	143	3.8	<0.7	
10 m/s - 0 min	10.60	8.8	0.55	196				53	40	6	1	6.87	236	4.07	3.5	
10 m/s - 3 min	10.17	8.2	0.53	184				53	41	4	2	6.87	229	3.44	2.4	
10 m/s - 5 min	9.70	8.5	0.51	176				57	41	2	0	6.63	223	3.52	1.7	
12 m/s - 0 min	11.53	9.4	0.60	210				57	40	3	0	7.88	245	4.05	14.2	
12 m/s - 3 min	11.10	8.8	0.58	202				58	39	2	1	8.16	178	3.38	16.8	
12 m/s - 5 min	11.03	8.9	0.58	200				59	39	2	0	7.98	183	3.26	16.8	
5 hours post	8.59	12.3	0.45	156								5.76	283	>12.0	<0.7	
Jump Test 19/9/96																
Course 1																
1 min post	10.3	10.5	0.54	184	53	18	338	54	41	5	0	5.82	88	2.71	0.8	
3 min post	9.67	10.4	0.51	173	53	18	339	54	45	1	0	5.89	71.4	3.35	<0.7	
5 min post	8.89	9.5	0.47	161	53	18	343	56	43	0	1	5.99	76.4	3.52	<0.7	

- STATISTICAL ANALYSIS

Shown here are samples of the information received from the statistical package Minitab. Values for plasma lacate are presented.

JUMP TEST

One-Way Analysis of Variance

```
Analysis of Variance
Source   DF      SS      MS      F      p
Factor    3  14.729   4.910   7.17  0.001
Error    23  15.754   0.685
Total    26  30.483
                          Individual 95% CIs For Mean
                          Based on Pooled StDev
 Level     N    Mean   StDev ---+---------+---------+---------+---
RESTING    8  0.6375  0.0744    (-----*-----)
JI         8  0.7125  0.1458    (-----*-----)
JII        8  2.3000  1.4890               (-----*-----)
24 HR      3  0.7333  0.1528  (---------*---------)
                          ---+---------+---------+---------+---
Pooled StDev =  0.8276          0.0       1.0       2.0       3.0
MTB > twosample c1:c3
```

Two Sample T-Test and Confidence Interval

```
Twosample T for RESTING vs JII
          N     Mean    StDev   SE Mean
RESTING   8   0.6375   0.0744    0.026
JII       8     2.30     1.49     0.53

95% C.I. for mu RESTING - mu JII: ( -2.909, -0.42)
T-Test mu RESTING = mu JII (vs not =): T= -3.15  P=0.016  DF=  7

MTB > twosample c2:c3
```

Two Sample T-Test and Confidence Interval

```
Twosample T for JI vs JII
     N     Mean    StDev   SE Mean
```

JI 8 0.713 0.146 0.052
JII 8 2.30 1.49 0.53

95% C.I. for mu JI - mu JII: (-2.839, -0.34)
T-Test mu JI = mu JII (vs not =): T= -3.00 P=0.020 DF= 7

MTB > twosamplec2:c4

Two Sample T-Test and Confidence Interval

Twosample T for JI vs 24 HR
N Mean StDev SE Mean
JI 8 0.713 0.146 0.052
24 HR 3 0.733 0.153 0.088

95% C.I. for mu JI - mu 24 HR: (-0.346, 0.304)
T-Test mu JI = mu 24 HR (vs not =): T= -0.20 P=0.85 DF= 3

MTB > twosample c3:c4

Two Sample T-Test and Confidence Interval

Twosample T for JII vs 24 HR
N Mean StDev SE Mean
JII 8 2.30 1.49 0.53
24 HR 3 0.733 0.153 0.088

95% C.I. for mu JII - mu 24 HR: (0.30, 2.829)
T-Test mu JII = mu 24 HR (vs not =): T= 2.94 P=0.022 DF= 7

INCREMENTAL EXERCISE TEST

One-Way Analysis of Variance

Analysis of Variance
Source DF SS MS F p
Factor 5 778.7 155.7 15.53 0.000
Error 23 230.6 10.0
Total 28 1009.2
Individual 95% CIs For Mean
Based on Pooled StDev
Level N Mean StDev ----+---------+---------+---------+-
Resting 4 0.750 0.058 (----*-----)

```
6 m/s     5   0.860   0.134  (---*----)
8 m/s     5   1.100   0.200  (----*----)
10 m/s    5   6.760   4.657         (----*----)
12 m/s    5  14.620   5.989                 (----*----)
24 hr     5   0.980   0.179  (----*----)
                        -----+---------+---------+---------+-
Pooled StDev =   3.166           0.0       6.0      12.0      18.0
MTB > twosample c1:c4
```

Two Sample T-Test and Confidence Interval

```
Twosample T for Resting vs 10 m/s
          N     Mean    StDev  SE Mean
Resting   4   0.7500   0.0577    0.029
10 m/s    5     6.76     4.66       2.1

95% C.I. for mu Resting - mu 10 m/s: ( -11.795,  -0.2)
T-Test mu Resting = mu 10 m/s (vs not =): T= -2.89  P=0.045  DF=  4

MTB > twosample c1:c5
```

Two Sample T-Test and Confidence Interval

```
Twosample T for Resting vs 12 m/s
          N     Mean    StDev  SE Mean
Resting   4   0.7500   0.0577    0.029
12 m/s    5    14.62     5.99       2.7

95% C.I. for mu Resting - mu 12 m/s: ( -21.309,  -6.4)
T-Test mu Resting = mu 12 m/s (vs not =): T= -5.18  P=0.0066  DF=  4

MTB > twosample c4:c6
```

Two Sample T-Test and Confidence Interval

```
Twosample T for 10 m/s vs 24 hr
          N     Mean    StDev  SE Mean
10 m/s    5     6.76     4.66       2.1
24 hr     5    0.980    0.179     0.080

95% C.I. for mu 10 m/s - mu 24 hr: ( -0.0,  11.568)
T-Test mu 10 m/s = mu 24 hr (vs not =): T= 2.77  P=0.050  DF=  4
```