METABOLIC RESPONSE TO HIGH-INTENSITY EXERCISE

IN THE THOROUGHBRED HORSE

by

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ABSTRACT

The Thoroughbred racehorse is capable of maintaining speeds of approximately 17 m/s for distances of over a mile. This represents an average speed and the Thoroughbred can reach speeds in excess of 20 m/s over short distances. The present series of studies was undertaken to investigate further the metabolic response to high-intensity exercise in the Thoroughbred racehorse.

Unlike man, high-intensity exercise in the horse results in an increase in packed cell volume. This in turn causes acute changes in the colligative properties of blood and plasma. The changes in these properties were investigated and the effect on calculation and distribution of metabolites in blood and muscle was determined.

The horse has a high capacity for lactate production compared to man and existing methodology for the assessment of muscle buffering capacity in biopsy samples was further developed and investigated. The horse was found to have a significantly higher muscle buffering capacity compared with man and it was calculated that this could be explained wholly on the basis of the higher muscle carnosine content in the horse.

The metabolic response to high-intensity exercise was investigated using several exercise models, including single and multiple field gallops and treadmill exercise. A consistent finding was that high-intensity exercise in the Thoroughbred racehorse was nearly always accompanied by a reduction in muscle ATP content. The nature of the ATP decrease was further investigated using a treadmill exercise model. Muscle ATP decrease was found to occur at a particular exercise intensity rather than show a gradual decrease with increasing intensity. The intensity at which muscle ATP content began to decline significantly varied between individual horses, but in each case appeared to coincide with muscle lactate contents of approximately 70 mmol/kg dry muscle. The significance of the decline in ATP is discussed.

KEYWORDS: Horse; Exercise; Muscle Metabolism; Fatigue

II

Harris, R.C., Snow, D.H. and Marlin, D.J. (1988) Muscle buffering capacity and dipeptide content in the Thoroughbred horse, Greyhound dog and man. Proc. 2nd Int. Congress. Comp. Physiol. Biochem., Louisiana State University, 820P.

Marlin, D.J., Harris, R.C., Gash, S.P. and Snow, D.H. (1989) Carnosine content of the middle gluteal muscle in Thoroughbred horses in relation to age, sex and training. Comp. Biochem. Physiol. 93(A), No.3., 629-632.

Marlin, D.J., Harris, R.C. and Snow, D.H. (1989) Influence of active and passive recovery on changes in muscle and blood metabolites following treadmill exercise in the horse. Equine Vet. J. (submitted).

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Table of Abbreviations

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
TAN	Total adenine nucleotide
IMP	Inosine monophosphate
PCr	Phosphocreatine
Cr	Creatine
Glucose 1-P	Glucose 1-phosphate
Glucose 6-P	Glucose 6-phosphate
Fructose 6-P	Fructose 6-phosphate
Glycerol 3-P	Glycerol 3-phosphate
FFA	Free fatty acids
βm	Muscle buffering capacity
H^+	Hydrogen ions
Pi	Inorganic phosphate
Ca^{2+}	Calcium ions
PCV	Packed cell volume
VO_2max	Maximal oxygen uptake
PFK	Phosphofructokinase
CPK	Creatine phosphokinase
LDH	Lactate dehydrogenase
OGDH	Oxoglutarate dehydrogenase
w.m.	Wet muscle
d.m.	Dry muscle

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NUMBERING

AS ORIGINAL

CHAPTER 1

GENERAL INTRODUCTION

"horse races and wild-goose chases, which are disports of greater men, and good in themselves, though many gentlemen by such means gallop themselves out of their fortunes."

Robert Burton, The Anatomy of Melancholy, 1621.

"whoever said that moving house and getting divorced are the most stressful things in life got it wrong - they obviously never wrote a Ph.D. thesis."

David Marlin, 1989.

INTRODUCTION

The history of racing

      Races involving horses formed part of Hittite, Assyrian, Uratian and Egyptian culture as long ago as the second millenium BC, although what form these early races took is uncertain. Two and four horse chariot racing was popular with the Greeks and the latter was introduced to the 25th Olympic games in 680 BC. Some of the earliest recorded ridden races almost certainly took place over 2700 years ago at the Greek Olympic games of 648 BC. The races were ridden bareback, although saddle-cloths with girths were used for hunting and war.

      Chariot racing was particularly popular with the Romans, the first race run in Rome taking place immediately after the city was founded and it was the Romans who introduced racing to ancient Britain. Race meetings were held at York and Chester and the 'racehorses' were hardy ponies bred in Scotland known as 'Galloways' or 'Running Horses'. However, although racing became popular there was little organization or attempt to improve the horses used. Horseracing continued in this form throughout the Middle-Ages, with Galloways and Hobbies, small horses bred in Ireland, being the 'racehorses' of this period. In England the origins of present day organized horseracing probably began with races held on the 'Roodee' or 'Roodeye' at Chester in 1540, where race meetings still take place today. The earliest known 'Rules of Racing' were drawn up nearly eighty years later in 1619 at Kiplingcotes in Yorkshire. However, the growth of racing suffered a setback following the English Civil War and the execution of Charles I by Oliver Cromwell. Cromwell not only proceeded to close the royal studs, dispersing the stallions and mares around England, but also outlawed horseracing.

      The monarchy was restored in 1660 under Charles II who took a great interest in racing. Under his patronage Newmarket became established as the centre for the sport and this early royal involvement is still retained as horse racing is also known by the euphemism "the sport of kings". However, there were only limited attempts at any form of organization until the late 18th Century, following the formation of the Jockey Club in 1752 "to promote good fellowship among racing and horse-breeding gentlemen from all over the country" (see Wright, 1986). As the members of the Jockey Club included some of the most influential men of that period, the Club quickly acquired prestige and authority and was soon involved in settling disputes concerning races, although its power was initially confined only to Newmarket. Over the next 100 years the Jockey Club became increasingly

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responsible for the organization of racing.

With encouragement from Charles II, the 17th and 18th Centuries saw the introduction to England of three horses that were destined to become the male-line ancestors of present day Thoroughbreds: the Byerley Turk, the Godolphin Arabian and the Darley Arabian. The most influential of these imported horses was the Darley Arabian, brought to England around the start of the 18th Century, which can be traced to be the male-line ancestor of 90% of modern Thoroughbreds. Initially, these horses were introduced to improve the stock of British horses, not only for racing, but also to satisfy the demand for hacks, hunters and light cavalry horses.

The term Thoroughbred was first used in 1713, although it was originally written Thro-bred. By 1740 horseracing was growing so rapidly in popularity that parliament passed a law outlawing nearly all races worth less than £50 in an effort to curb its growth. Obviously this met with limited success as 250 years later Thoroughbred racing and breeding in Britain is a multi-billion pound industry and in 1981 alone represented an income of over £100 million for the government in the form of betting tax revenue.

Thoroughbred racing in Britain

Official Thoroughbred racing in Britain today is still controlled by the Jockey Club and consists of 'Flat' and 'National Hunt' racing. Flat racing takes place over distances of between 5 and 21 furlongs (8f = 1 mile, 1f ≈ 200 metres) on a grass course with no obstacles, although around three hundred years ago races took place over distances of 3-4 miles (24-32 furlongs). The Flat season runs from the end of March to the middle of November and races are therefore run under a wide variety of environmental conditions . In spite of what might be inferred from the title, Flat racing often takes place on courses which are far from flat. For example, on the 12f Derby course at Epsom, perhaps one of the most famous courses in the world, the horses climb 150 ft in the first 4f of the race, then descend 100 ft over the next 6½f and finally climb uphill again in the last ½f to the finish.

National Hunt racing comprises 'Hurdle' races and 'Steeplechases', the shortest distance for both being 2 miles (16f, 3200m). In Hurdle races the jumps are made of lightly constructed wooden fencing and foliage are relatively narrow although they slope in the direction the horses run and are 3ft 6in high (1.1m). In Hurdle races there are on average 4 obstacles per mile, irrespective of the distance. The youngest age at which a horse

may start Hurdle racing is as a 3 year old.

'Steeplechasing' is so called as races initially took place across country between two landmarks, frequently the steeples of two churches. The shortest Steeplechases are also 2 miles, although in contrast to Hurdle races the obstacles are much larger. On average there are 6 fences per mile and these are approximately 4ft 6in high (1.4m), usually around 3-4ft wide and are relatively solid. The longest Steeplechase is The Grand National which is run over 4½ miles (7200m) and 30 fences, with some of the fences up to several metres wide. The greater demands imposed by Steeplechasing is reflected in the minimum age for horses competing of five. The oldest horse to win a Steeplechase in recent years was Sonny Somers who won twice at the age of 18 in 1980.

It is something of an irony that whilst man has attempted to produce faster and better horses, principally through selective breeding of the most successful horses, records in terms of absolute time tend to mean very little. This situation has arisen because of the way in which the majority of horse races are run, in that horses are raced to beat other horses and not to beat the clock. Races, therefore, tend to be run tactically, as opposed to flat-out. This has led to many so called 'elite' racehorses not having achieved record times.

Although the success of individual racehorses is not based on their speed, all races are timed, the majority of courses possessing electronic timing. The fastest recorded electronically timed race was run in England at Epsom in August 1983, when a four year old horse called Spark Chief covered 5f in a time of 53.7 seconds, representing an average speed of 42 mph (≈ 18.6m/s) and carrying 110 pounds in weight. The record times for various race distances between 5 and 20f are shown in Table 1.1. The domination of the records by horses in the USA can be explained firstly by the 'faster' nature of American tracks, as these often consist of dirt which is a hard surface compared to grass, and secondly, that racing in The States tends to be run at a much faster pace throughout, that is, there is generally less reliance on tactics. It is also interesting to note that all the records were set by horses of 4 years and over.

The earliest age at which a Thoroughbred may race is when it is classed as a 'two year old'. Foals are generally born between January and June. However, all Thoroughbreds are given the same birthday, on the 1st January each year, so on that date a horse born on the 1st January the previous year would indeed be one year old (termed a yearling), whilst a late foal born on the 1st July would in fact be only six months old,

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Table 1.1. Fastest Flat race times in the world for distances ranging from 5 to 20 furlongs.

Distance	Time min:sec	Name	Age yrs	Weight lbs	Course	Year
5f	53.7	Spark Chief	4	110	Epsom, England	1983
6f	1:07.2	Grey Papa	6	112	Longacres, USA	1972
8f	1:32.0	Royal Heroine	4	123	Hollywood Park, USA	1984
10f	1:57.4	Double Discount	4	113	Santa Anita, USA	1977
12f	2:23.0	Fiddle Isle	5	124	Santa Anita, USA	1970
12f	2:23.0	John Henry	5	126	Santa Anita, USA	1980
16f	3:16.8	Il Tempo	7	130	Trentham, NZ	1970
20f	4:14.6	Miss Grillo	6	118	Pimlico, USA	1948

From: Randall and Morris, 1985.
N.B. only times from electronically timed races are included.

although it would still be classed as a yearling. The following year, for a race taking place at the start of the flat season in April, one horse would be 2 years & 4 months old whilst the other would be only 1 year & 10 months old.

The majority of races with the highest prize money are for three year olds in flat racing and successful horses are often retired to stud at the end of their 2nd season (i.e. at three). Although there are a number of races each season for horses of 4 and over, it is very rare to find horses of over six years old taking part in Flat racing.

The success of a Thoroughbred racehorse in either Flat or National Hunt racing is denoted by its Timeform rating. Timeform was founded in 1947 by Phil Bull and is the ability of a racehorse expressed in pounds (lbs) on an unvarying scale, which allows horses from different years to be compared. Each horse that runs on the Flat or in National Hunt races in Great Britain is Timeform rated and the rating is based on the performance of each horse in relation to its contemporaries. The simplest explanation is that in theory, if a horse rated at 100 was placed in a race against a horse rated 80, then the former carrying 20 lbs more weight should ensure a close finish to the race. This is the aim of the 'Handicap' system. Table 1.2. lists the highest rated Timeform horses in both Flat and National Hunt racing since Timeform was initiated. It should be noted that it is not possible to compare the Timeform of Flat and National Hunt horses. The limitation of Timeform as an indicator of performance is that each horse is only rated for the distance over which it runs. This may not necessarily be its optimal distance.

Investigations into the response to exercise in the horse

Equine exercise physiology is a discipline that has expanded rapidly in the past 15-20 years, although some of the earliest recorded exercise studies focusing on gait, haemodynamics and respiratory gas exchange took place in the 17th, 18th and 19th Centuries, respectively (Cavendish, 1657; Hales, 1733; Hering, 1829; Zuntz, 1896). Several studies relating to the work capacity of the horse took place in the early part of this Century (e.g. Hall and Brody, 1934) but with the appearance of motorized vehicles for transport and farming the interest in the horse diminished. An increasing interest in equine exercise physiology began in the mid-sixties. Studies carried out around this time included Steel's work showing an association between heart size and racing performance (Steel, 1963), Persson's work on blood volume (Persson, 1967), investigations into the haematological response to endurance exercise (Carlsson, Fröberg and

Table 1.2 Highest Timeform (TF) rated horses in Flat and National Hunt racing.

FLAT RACERS

3 Year Old Colts and Older

	Year	TF
Sea-Bird	1965	145
Tudor Minstrel	1947	144
Brigadier Gerard	1972	144
Abernant	1950	142
Ribot	1956	142
Mill Reef	1971	141
	1972	
Vaguely Noble	1968	140
Shergar	1981	140

3 Year Old Fillies and Older

	Year	TF
Allez France	1974	136
Habibti	1983	136
Coronation	1949	135
Dahlia	1974	135
Petite Etoile	1959	134
	1960	
All Along	1983	134

STEEPLECHASERS

	Year	TF
Captain Christy	1975/76	182
Badsworth Boy	1982/83	179
Bregawn	1982/83	177
Little Owl	1980/81	176
Brown Lad	1975/76	175
Night Nurse	1980/81	175

HURDLERS

	Year	TF
Night Nurse	1976/77	182
Monksfield	1978/79	180
Birds Nest	1975/76	176
	1976/77	
Golden Cygnet	1977/78	176
Sea Pigeon	1976/77	175
	1977/78	
	1978/79	
	1979/80	
	1980/81	

Persson, 1965) and the monitoring of the ECG during exercise by telemetry (Banister and Purvis, 1968).

The introduction of the percutaneous needle biopsy to study muscle electrolytes in pathological conditions in man in the early sixties (Bergström, 1962) subsequently resulted in its wide application in the late sixties and early seventies to study the muscular response to exercise and training in normal human subjects. These early studies utilized the biopsy technique to investigate muscle glycogen depletion and repletion (Bergström and Hultman, 1966; Saltin and Hermanssen, 1967), muscle ATP and phosphocreatine (Hultman, Bergström and McLennan-Anderson, 1967), muscle pH (Hermanssen and Osnes, 1972) and muscle histochemistry (Brooke and Kaiser, 1970). The same approach was subsequently applied to the horse with histochemical and biochemical investigations of muscle at rest (Lindholm and Piehl, 1974; Snow and Guy, 1976), the effects of training (Guy and Snow, 1977) and following both endurance (Hodgson, Rose and Allen, 1983) and high-intensity exercise (Nimmo and Snow, 1983).

Adaptations to high-intensity exercise in the Thoroughbred horse

What adaptations have taken place in the Thoroughbred horse which may help to account for its athletic ability? In direct comparison with the cheetah, the fastest animal on land, the Thoroughbred may not seem particularly fast, however it is the only animal capable of carrying a man's weight at speeds in excess of 40 miles per hour (17.7 m/s). An insight into some of the adaptations shown by the Thoroughbred can be demonstrated by comparison to man (Tables 1.3 & 1.4).

VO_2max is considered to be a good indicator of aerobic capacity in man, with values around 30 to 40 ml/min/kg in untrained subjects (see Fox and Mathews, 1981) and up to 60 to 80 ml/min/kg in elite athletes (see Table 1.3). In contrast, the Thoroughbred horse has been shown to have a significantly higher VO_2max, in some cases in excess of 160 ml/min/kg (Evans and Rose, 1987). The greater muscle mass of the Thoroughbred compared to man cannot explain the difference in VO_2max as a large difference still exists even after recalculation to ml/min/kg muscle (Table 1.3). The higher VO_2max in the Thoroughbred is also matched by higher activities of oxidative enzymes in the muscles (Snow and Harris, 1985) indicating a higher density of mitochondria. Mitochondria from a variety of species have also been shown to consume O_2 at approximately the same rate at VO_2max ($\approx$ 4 ml O_2/min/cm^3) further supporting the hypothesis that the higher VO_2max in the Thoroughbred is a function of a higher mitochondrial density (Hoppeler et al, 1987a; Hoppeler et al, 1987b) rather than any

References

1	Gunn, 1987	13	Rose et al, 1988
2	Lamb, 1984	14	Parkhouse et al, 1983
3	Astrand & Rodahl, 1986	15	Iwaoka et al, 1988
4	Stadden, Weaver & Lunn, 1984	16	Högberg, 1952
5	Wilmore, Brown & Davis, 1977	17	Leach, 1987
6	Macková et al, 1986	18	Pratt, 1983
7	Judson & Mooney, 1983	19	Snow & Guy, 1981
8	Leonard, Leach & Rambaut, 1983	20	Lindholm et al, 1983
9	Kubo, Senta & Sugimoto, 1974	21	Mero, 1985
10	Rost, 1982	22	Guy, 1978
11	Evans & Rose, 1987	23	Snow, 1983
12	Bayly et al, 1987	24	Saltin & Gollnick, 1983
		25	see Rice et al, 1988
		26	Snow & Harris, 1985

Table 1.3. Comparison of morphometric and physiological characteristics of Thoroughbred horse and man. Values quoted are for trained individuals unless specified otherwise and for humans, where possible, for elite athletes.

	Thoroughbred	Man	Ref.
Weight (kg)	500	70	
% Muscle	53%	40%	1-3
% Bone	13%	15%	1-3
% Fat	1-5%	5-15%	1, 4, 5, 6
Total Body Water (1)	350	46	7, 8
Heart Wt:Body Wt	0.95-1.25%	0.6-0.8%	9, 10
VO_2max (ml/min/kg)	130-160	60-80	11-15
VO_2max (ml/min/kg musc)	245-300	150-200	
Max Stride Freq.(s/min)	≈165	230 [a]	16-18
Max Stride Length (m)	6-7.5	2.2 [a]	16-18
Max speed (m/s)	≈20	10	
% Type II Fibres			
Middle gluteal	80-90	–	6, 14, 19-22
Vastus Lateralis	90-95	40-85 [S]	
		20-40 [M]	
Fibre size (μm^2)			23, 24, 25
Type I	1500-3000	1700-10000 [St]	
		3400-6900 [E]	
Type II	3000-5000	5800-14500 [St]	
		2500-6900 [E]	
Enzyme activities[b]			26
CPK	4350	3220	
LDH	980	220	
OGDH	4.0	0.6	

[a] 800m runners at 8.3 m/s   [b] μmol/min/g wet muscle, 30°C
S   Sprinters   M   Marathon Runners
St   Sprint-trained athletes   E   Endurance trained athletes

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References

1 Keenan, 1979

2 Snow et al, 1983

3 Mason & Kwok, 1977

4 Judson, Frauenfelder & Mooney, 1983

5 Astrand & Rodahl, 1986

6 Stadden, Weaver and Lunn, 1984

7 Catling, 1978

8 Persson, 1968

9 Kubo et al, 1974

10 Ehrlein et al, 1973

11 Asheim et al, 1970

12 Astrand & Christensen, 1964

13 Thomas & Fregin, 1981

14 Smale, personal communication

15 Astrand et al, 1964

16 Manohar, 1987

17 Jorfeldt & Wahren, 1971

18 Clausen, 1973

19 Leach, 1987

20 Pratt, 1983

21 Woakes, Butler & Snow, 1987

22 Hörnicke, Weber & Schweiker, 1987

23 Bayly et al, 1987

24 Rose & Evans, 1987

25 Gregg & Nunn, 1973

Table 1.4. Comparison of physiological characteristics of Thoroughbred horse and man at rest and the changes with maximal exercise. Values quoted are for trained individuals and for humans, where possible, for elite athletes. Values are based on a 500kg horse and a 70kg man.

	Thoroughbred		Man		Ref.
	Rest	Ex.	Rest	Ex.	
PCV (1/1)	0.40	0.65	0.47	0.50	1-5
Hb (g/1)	140-150	230-245	158	168	2, 4, 5
TBV (1)	40	60-70	5-6	5-6	5-8
TBV (ml/kg)	80	120-140	70-85	70-85	
TBV (ml/kg muscle)	151	226-264	179-214	179-214	
HR (beats/min)	30	240	50	200	9-12
Cardiac Output (1/min)	66-75	240-280	4-6	25	13-15
Cardiac Output (ml/min/kg)	153-171	545-651	57-86	357	
Cardiac Output (ml/min/kg muscle)	249-283	905-1056	143-214	893	
Muscle blood flow [a] (ml/min/kg)	20-40	2400-2700	40-50	530-750	6, 16-18
Muscle blood flow (ml/min/kg muscle)	38-76	4500-5100	100-125	1325-1875	
Respiratory Freq. (breaths/min)	20	150-165	10-20	60	5, 19-22
Peak Flow Rates (1/sec)					21-23, 25
Inspiration	-	55-61	-	-	
Expiration	-	64-70	-	10-11 [b]	
Tidal Volume (1)	2.1	11-15	≈0.5	4-6	5, 21-24

a Non-Thoroughbreds
b Non-athletes

intrinsic species related differences in mitochondrial function.

The transport of oxygen from the terminal alveoli to the mitochondria may limit VO_2max (Di Prampero, 1986; Karas et al, 1987; Rose and Evans, 1987; Lindstedt et al, 1988) and the horse shows several adaptations for the maintenance of a high rate of oxygen delivery to the muscles. Firstly, the haematocrit of the horse can increase by as much as 60% from rest to exercise (Table 1.4) due to the release of red blood cells stored in the spleen at rest. This results in a large increase in the oxygen carrying capacity of the blood and also an increase in blood volume. Secondly, the horses heart weight to body weight ratio is just over twice that in man and in combination with the high maximal heart rate facilitates a high cardiac output during high-intensity exercise. However, when expressed per kg of muscle, the cardiac output of the horse is only around 10-15% higher than that in man. However, the increase in blood volume possibly allows a much greater proportion of the blood flow to be directed towards the active muscles. This is supported by the approximately 2½ fold higher muscle blood flow in the horse compared to man (expressed per kg of muscle, see Table 1.4). Therefore, the horse exhibits a high VO_2max due to the high muscle oxidative capacity as a result of a higher mitochondrial density and this is supported by cardiovascular adaptations which facilitate a high rate and volume of oxygen delivery.

The high athletic ability of the Thoroughbred may also be related to its body composition. In the Thoroughbred horse, the total muscle mass represents 53% of the liveweight (Gunn, 1987; see Table 1.3) compared to only around 40% in man (Lamb, 1984). In the canine world, the muscle mass of the Greyhound also represents a very high proportion of its liveweight, 57% compared to 44% in less athletic breeds (Gunn, 1978). A similar difference also exists between Thoroughbreds (muscle ≈ 53% liveweight) and less athletic breeds of horse (muscle ≈ 44% liveweight; Gunn, 1987).

The Thoroughbred horse also has a very high capacity for lactate production from glycogenolysis during high-intensity exercise (Keenan, 1979; Kubo et al, 1984; Bayly, Grant and Pearson, 1987) compared to man (Sahlin et al, 1976; Gass, Rogers and Mitchell, 1981; Jacobs et al, 1982; Cheetham et al, 1986), although rates comparable to those seen in the horse have been reported in man following electrical stimulation (Harris and Hultman, 1985; Spriet et al, 1987). The higher capacity for energy production from anaerobic glycolysis in the Thoroughbred can be inferred from the higher activities of phosphorylase, PFK and LDH in the horse compared to man. It has been suggested that the higher glycolytic capacity in the Thoroughbred may be a function of the higher proportion of type II

fibres (Snow and Harris, 1985).

During high-intensity exercise a high lactate accumulation will result in acidosis. The extent of the acidosis within the muscle will depend on both the efflux of H^+ from the muscle and on the intracellular muscle buffering capacity. Castellini and Somero (1981) found that amongst vertebrates the highest muscle buffering capacities were observed in muscles of species capable of either intense, short-duration glycolytic activity or prolonged, low-intensity anaerobic activity. In the horse, the few measurements available suggest that the high glycolytic capacity is also associated with a high muscle buffering capacity (Fox et al, 1987; McCutcheon et al, 1987). Furthermore, this appears to be higher than in man (Cheetham, 1988).

It is suggested that the high athletic ability of the Thoroughbred horse is a result of high capacities for both aerobic and anaerobic energy production, the former demonstrated by the high mitochondrial density or aerobic enzyme activities relative to less athletic species (Hoppeler, 1986; Essén-Gustavsson, 1986) and the latter by the high activities of glycolytic enzymes (Snow and Harris, 1985). Furthermore, the increased aerobic capacity of the muscle appears to have been matched by adaptations in the cardiovascular and respiratory systems.

Limitations to performance in the Thoroughbred horse

Many factors may interact to determine athletic ability in the Thoroughbred horse, including conformation, cardiovascular and respiratory capacity, muscle composition, neurological coordination, environment, training, health and mental attitude. Although for many of these factors the capacity or nature will be genetically predetermined, training, nutrition, health and environment are factors over which a great deal of control can be exercised and will inevitably effect the extent to which an individuals genetic potential is realized.

Muscle has been shown to consist essentially of two distinct fibre types which may be distinguished according to the activity of their myosin ATPase at pH 9.4 (Padyluka and Herman, 1955). Those showing low activity are termed type I and those showing high activity, type II. Type I fibres have been shown to have slower contraction and relaxation times compared to type II fibres within the same muscle in both human and cat gastrocnemius (Garnett et al, 1978; Burke et al, 1973). Further subdivision of type II fibres into type IIA, IIB and IIC following incubation at pH 4 to 5 was demonstrated by Brooke and Kaiser (1970). Alternatively, fibres may be

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classified according to their oxidative capacity based on histochemical staining for aerobic enzymes such as succinate dehydrogenase or NADH-diaphorase. This has been used to classify fibres into ST (slow-twitch), FT (fast-twitch) and FTH (fast-twitch, high oxidative, Lindholm and Piehl, 1974; Snow and Guy, 1976). For a more detailed review of fibre classification in the horse see Snow (1983).

Consistent with classification of fibres according to their speed of contraction is the observation that the proportion of type II or FT fibres is higher in the faster or more athletic breeds of horse (Table 1.5). This has led to the hypothesis that within a breed the faster horse may be the one possessing the highest proportion of type II fibres. In man, the proportion of type II fibres in the vastus lateralis has been found to be higher in sprinters compared to endurance athletes (Gollnick et al, 1972; Costill et al, 1976; Parkhouse et al, 1985). Compared to man, the variation in fibre composition of individual muscles between Thoroughbred horses appears to be much less, although there are differences between muscles of the same individual (Guy, 1978). The relative homogeneity is most likely a reflection of the selective breeding practices that have been taking place within the Thoroughbred breed over the past 300 years. Possibly the mistake in comparing variation in the Thoroughbred horse with variation in man is that in the former to a certain extent we are already dealing with an "elite" population. It is quite likely that examination of muscle characteristics of populations of elite marathon runners or elite sprinters would reveal a much smaller degree of variation within each population compared to athletes in general (see Saltin and Gollnick, 1983; Macková et al, 1986). However, it would seem that even within the Thoroughbred population, the importance of the correct genetic endowment cannot be ignored.

In a healthy horse, with the necessary genetic endowment and correct training, what factors may limit racing performance? As mentioned previously, poor performance has been attributed to both cardiovascular (Rose and Davis, 1977; Stewart et al, 1983) and respiratory disease (Gillespie, 1975; Mumford and Rossdale, 1980). In the healthy horse, Steel (1963) demonstrated an association between heart size (estimated from the QRS duration) and performance and studies by Persson (1967) on blood volume in the horse also indicated that the cardiovascular system may limit performance. With reference to the respiratory system of the horse, hypoventilation at moderate running speeds (Woakes, Butler and Snow, 1987), arterial hypoxemia (Bayly, 1984) and the apparent lack of response of the respiratory system to training (Rose and Evans, 1987) have

Table 1.5. Fibre composition of the middle gluteal of different breeds of horse.

	% Type I	% Type II
Quarter horse [1]	9	91
Thoroughbred [1]	11	89
[2]	12	88
Standardbred [3]	24	76
[4]	20	80
Pony [1]	23	77
Heavy Hunter [1]	31	69

[1] Snow and Guy, 1981

[2] Lindholm et al, 1983

[3] Lindholm and Piehl, 1974

[4] Essén-Gustavsson et al, 1983

all been taken as indications that limitations to performance may occur as a result of respiratory insufficiency.

The running speed of an animal is a function of stride length and stride frequency. Stride length in horses increases linearly with speed (Deuel and Lawrence, 1985; Ratzlaff et al, 1985). Stride frequency in the galloping horse also demonstrates an approximately linear increase with increasing velocity (Leach and Cymbaluk, 1986). However, at near maximal speeds, the relationship may become non-linear (Dusek et al, 1970). Bayer (1973) concluded that many of the higher class trotting horses had a higher maximal stride frequency compared to less successful contemporaries.

The studies of Bayer (1973) and Dusek et al (1970) both indicate that maximal speed in the horse may be limited by stride frequency, as has been intimated for human sprinters (Mero et al, 1981). Stride frequency itself may be limited by the swing time of the limbs (i.e. the movement of the limbs through the air), the extent to which the elastic recoil of the limbs can be utilized, nerve conduction velocities and the proportion of type II fibres (Leach, 1987; Chapman and Caldwell, 1983; Mero and Komi, 1985). In spite of a high heritability component, these factors, and hence stride frequency, may be influenced by training (Leach, 1987; Dusek et al, 1970).

The motivational aspect of equine performance remains a poorly understood area. It could be speculated that the competitive instinct is inate, but to what extent does it vary between horses? Furthermore, is the horse in a race striving to reach the front because it is competitive or is it running through fear (Schäfer, 1975). The former may be illustrated by the horse which reaches the front in a race, but then refuses to pass the other horses and the latter by the horse which reaches the front and then wins by a large margin.

The extent to which early development and training may influence the desire to compete is not known, although foals in paddocks can be observed to take part in competitive play (Schäfer, 1975; Kiley-Worthington, 1987). Also, it is not known whether training and racing serve to increase or decrease the competitive urge. However, it would seem reasonable to assume that both fear and interindividual competition may be involved in equine performance and to a certain extent this will be dependent on the temperament of each individual.

The limitation to performance in the neuromuscular system is more usually referred to as fatigue and has recently been defined as " a loss of force output leading to a reduced performance of a given task " (Fitts and Metzger, 1988). The causes of fatigue depend on factors such as the nature

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of the exercise being undertaken, preceding exercise, the fitness and nutritional status of the athlete and genetic endowment. Throughout this thesis, unless specified otherwise, fatigue from high-intensity exercise only will be discussed. For a horse in a race, fatigue is expressed as either failure to accelerate or maintain its running speed. Fatigue is also essentially a protective phenomenon which functions to prevent either physical damage to the contractile machinery or death of individual fibres due to depletion of energy stores (Edwards, 1981; Wilkie, 1980; Harris, 1985). Furthermore, the extent of fatigue will depend on factors such as intensity and nature of exercise, muscle fibre composition, level of fitness and environmental factors (Fitz and Metzger, 1988).

Initiation of muscle contraction begins in the higher centres of the brain and ends at the level of actin-myosin cross-bridge formation (Edwards, 1981). Fatigue may therefore occur at one or more of the various different levels between the brain and the site of force production. At the highest level, failure of central nervous command has been termed central fatigue (Bigland-Ritchie et al, 1978), fatigue at lower levels being termed peripheral fatigue.

Evidence for central fatigue during high-intensity exercise has been presented by Bigland-Ritchie, Jones and Woods (1979) who demonstrated that motor nerve firing decreases during continuous contractile activity. However, this has been suggested to occur due to feedback from muscle afferents (Fitts and Metzger, 1988) as a reduced firing rate persists when fatigued muscle is kept ischemic (Bigland-Ritchie et al, 1986). Several studies have also demonstrated that maximum voluntary force and the force produced by direct stimulation of human quadriceps muscle decline in parallel (Bigland-Ritchie, Furbush and Woods, 1986; Vøllestad et al, 1988). Substantial evidence therefore exists to suggest that during high-intensity exercise the main sites of fatigue are within the muscle and do not involve the brain, peripheral nerves or neuromuscular junction under most conditions (Edwards, 1981; Fitts and Holloszy, 1977; Fitts and Metzger, 1988; Vøllestad and Sejersted, 1988).

Providing there is no reduction in firing rate of the motorneurones innervating the muscle, the next level at which fatigue may occur is excitation-contraction coupling. Excitation-contraction coupling involves propagation of the action potential across the sarcolemma and down into the t-tubular system where the signal is communicated to the sarcoplasmic reticulum (SR), by a process not completely understood, which results in the release of calcium, cross-bridge formation and hence, force production. As propagation of the action potential is an electrical phenomenon,

disruption of the ionic balance between interstitial, t-tubular and cytoplasmic compartments will alter the concentration gradients between these compartments and may impair transmission of the action potential (Sjøgaard, 1983; Sejersted et al, 1984; Sahlin, 1986). Many of the metabolic changes occurring during exercise may also affect excitation-contraction coupling.

High-intensity exercise results in a high rate of glycogen utilization through the transformation of phosphorylase b to a, a decrease in the muscle contents of ATP and phosphocreatine (PCr) and increases in ADP, lactic acid and inorganic phosphate (Pi). Of these changes, the formation of lactic acid and the relationship between depressed muscle pH and fatigue has probably been the most widely investigated. The majority of evidence seems to indicate indirect effects of increases in H^+ ion concentration.

Decreased muscle pH has been shown to have many effects on the contractile mechanism including decreases in maximal force, velocity of shortening and myosin ATPase activity (Schädler, 1967; Donaldson et al, 1978; Donaldson, 1983; Chase and Kushmerick, 1988), an increase in the amount of calcium required to produce a given tension (Fabiato and Fabiato, 1978; Ricciardi, Bucx and Terkeurs, 1986) and increased binding of calcium within the SR (Nakamura and Schwartz, 1972; Fabiato and Fabiato, 1978). It has also been suggested that the fall in muscle pH with intense exercise may limit glycolytic ATP supply and therefore cause a drop in force production (Hermanssen, 1981; Sahlin, 1983). This is in part based on the identification of PFK as a key regulatory enzyme in glycolysis (Lowry et al, 1964) and the report of complete inhibition of phosphofructokinase activity at pH 6.4 in vitro (Danforth, 1965). However, Spriet et al (1987) have more recently demonstrated that there is little change in glycolytic activity from pH 7.1 to 6.5 in electrically stimulated muscle, probably due to the effects of H^+ on PFK being counteracted by increases in activators such as AMP, ADP, fructose 6-phosphate, fructose 1,6-diphosphate, Pi and NH_4^+ and a decrease in the muscle ATP content. These observations are also in agreement with the study by Dobson, Yamamoto and Hochachka (1986).

The reduction in muscle PCr content during high-intensity exercise has two important consequences. Firstly, a reduction in the rate of ADP rephosphorylation via the PCr-ATP shuttle, which has been suggested to participate in the movement of ATP from the mitochondria to the cross-bridges (Bessman and Geiger, 1981; Meyer, Sweeney and Kushmerick, 1984), leading to a deficit of ATP at the cross-bridges (Savabi, Geiger and Bessman, 1984) and secondly, an increase in intracellular Pi. Several studies have demonstrated a close relationship between Pi and force

production in frog skeletal muscle (Dawson, Smith and Wilkie, 1986; Wilkie, 1986), skinned rabbit muscle fibres (Nosek, Fender and Godt, 1987) and most recently in human subjects using NMR (Wilson et al, 1988).

A decrease in muscle ATP content has been shown to be associated with a reduction in force output in both man (Hultman, Bergström and McLennan-Anderson, 1967) and the horse (Snow, Harris and Gash, 1985), although other studies have shown little or no relationship either in vivo (Karlsson and Saltin, 1970) or in vitro (Fitts and Holloszy, 1976; Fitts and Holloszy, 1978). However, the use of the muscle biopsy technique indicates an average ATP content for each fibre and the possibility exists that some fibres may have fatigued due to a highly reduced or complete loss of ATP. Although Janssen et al (1986) found no evidence of complete ATP depletion in single fibres from biopsies taken following high intensity exercise in man, Foster et al (1986) found a proportion of single fibres with no ATP following high-intensity exercise in the horse. These authors also found that instead of a continuous spread of ATP concentrations, the highest concentration above 0 was around 6 mmol/kg d.m. This may indicate that there is a minimum ATP content, below which the fibre becomes necrotic due to insufficient ATP to maintain the internal environment of the cell, principally through the ATPase supported transport processes.

Reduction in ATP and PCr and increased H^+ will combine to cause transient rises in ADP. Although in vitro studies have not demonstrated a reduction in maximal force with increased ADP concentration (Kawai et al, 1987), a decreased rate of cross-bridge cycling has been reported (Cooke and Pate, 1985). However, evidence from studies on patients with AMP deaminase deficiency, where fatigue occurs in the absence of increases in H^+ and Pi, suggests that elevated ADP may be involved (Sabina et al, 1984b; Sahlin and Katz, 1988). ADP increase has also been shown to correlate to decreased force output in stimulated frog muscle (Dawson et al, 1978).

In horse races on the flat lasting for several minutes, glycogenolysis will make a significant contribution to total energy production (Nimmo and Snow, 1983; Hultman and Harris, 1988) although the rate of glycogen utilization has been shown to be reduced markedly at distances over 1600 metres (Nimmo and Snow, 1983). As the horse has high resting levels of glycogen (Nimmo and Snow, 1983; Snow, Harris and Gash, 1985) it may seem unlikely that substrate availability at the whole muscle level could be a cause of fatigue in this type of exercise, although it may be more relevant during endurance exercise (Hodgson, Rose and Allen, 1983). However, in man, both Piehl (1974) and Thomson et al (1979) observed a consistent proportion of FT (type IIB) fibres that had apparently become completely depleted of

glycogen following supramaximal exercise. More recently, White and Snow (1987) investigated glycogen depletion patterns in the Thoroughbred following maximal exercise and found the greatest glycogen depletion, assessed by quantitative histochemistry, to occur in type IIB fibres. This amounted to approximately 50% of the resting content in these fibres, although the resting muscle glycogen content is higher in type II compared to type I fibres in both man (Essén, 1978) and the horse (Snow, Baxter and Rose, 1981; Hodgson et al, 1984; White and Snow, 1987). Therefore, fatigue during high intensity exercise due to complete glycogen depletion in highly recruited type IIB fibres must remain a possibility (Tesch, 1980). Another effect of reduced glycogen levels, rather than complete depletion, may be to reduce the conversion of phosphorylase b to a (Constable, Favier and Holloszy, 1986). This may well be related to the observation that high glycogen levels enhance glycogen breakdown in isolated muscle (Richter and Galbo, 1986).

There are a large number of factors which may limit the ability of an individual horse. At the muscle level, during high-intensity exercise, a large number of metabolic changes occur which may have an effect on performance or induce fatigue.

The present series of studies were undertaken to investigate:

(I)   The nature of the metabolic changes in muscle and blood associated with single and multiple bouts of high-intensity exercise, with special reference to changes in ATP and lactate.

(II)   Factors affecting post-exercise recovery in muscle and blood metabolites.

(III)   Validation of motor driven treadmills for investigations into the response to exercise in the Thoroughbred horse.

(IV)   The identification of muscle characteristics or metabolic changes with exercise of use in determining individual exercise capacity.

These are the main studies undertaken, however, the first two chapters describe the investigation and development of essential methodology.

CHAPTER 2

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

Blood sampling and analytical procedures

Sample collection: Blood samples were collected either by venepuncture of the left or right jugular vein or via an indwelling catheter.

Catheterization procedure: A small area of skin over the left or right jugular vein was shaved and disinfected. Local anaesthesia was achieved by injecting 3 x 0.5ml, 2% Lignocaine (1:200,000 adrenaline) subcutaneously. An incision was made in the skin over the vein and an intravenous catheter (Becton-Dickinson, 14g with 17g x 8in inner needle) was introduced. The catheter was sutured to the skin. For exercise studies a 100cm extension line was attached (Lectroflex, PVC, Vygon UK, 2ml capacity) which was filled with saline. Patency was maintained by regular flushing with heparinized saline.

Sample treatment: Venous blood samples collected either by venepuncture or via an indwelling catheter were immediately divided as follows: 10ml into lithium heparin; 5ml into EDTA and 2-3ml into pre-weighed tubes containing 5ml of 1 mol/l perchloric acid (PCA). Samples for plasma glucose were collected into tubes containing fluoride and oxalate. All samples were stored on ice. Samples for plasma pH analysis were drawn into 2ml syringes with the deadspace filled with sodium-heparin (5000u/ml) and containing a stainless steel washer to aid in mixing. Bubbles were removed and the sample capped and placed in a plastic container filled with ice and water to prevent freezing of the sample.

Blood analysis: Analysis was carried out on the samples preserved or treated as shown in Table 2.1. Lactate was determined enzymically on PCA extracts of either whole blood or plasma by the method of Hohorst (1963). Glucose in PCA extracts of whole blood or plasma was determined enzymically using hexokinase and glucose 6-phosphate dehydrogenase. Packed cell volume (PCV) was determined on the EDTA sample by a standard microhaematocrit method. Free fatty acids (FFA) were also analysed on the EDTA sample using an enzymic, colourimetric method (Wako Chemicals, GmbH). Blood pH· analysis was performed on the blood gas sample within 2 hours of collection on either a Corning 166 Micro/pH blood gas analyser or on a Radiometer ABL3 blood gas analyser. Plasma ammonia analysis was performed on an aliquot of the lithium-heparin sample stored in liquid nitrogen and using a kit from Sigma Diagnostics adapted for use on a Multistat III centrifugal analyser (Instrumentation Laboratories). Plasma uric acid was determined colourimetrically using a kit from Sigma diagnostics. The procedure was

Table 2.1. Sample treatment.

Lithium-heparin (plasma)	PCA (whole blood)	EDTA (whole blood)
lactate	lactate	PCV
ammonia	glucose	
uric acid	glycerol	
AST		
CPK		
TP		

Fluoride/oxalate (plasma)	Blood gas (whole blood)	EDTA (plasma)
glucose	Plasma pH	FFA

modified by boiling the reagent to denature the uricase before mixing the sample and reagent in cuvettes. This allowed an initial absorbance reading to be taken, after which uricase was added to each cuvette and the reaction allowed to reach an endpoint. Plasma hypoxanthine and xanthine were analysed by HPLC (see Harkness, 1988). AST (Aspartate amino transferase) and CPK (creatine phosphokinase) were analysed on a centrifugal analyser using an optimized method (Beckman Dri-Stat reagent). Total Protein (TP) was also determined on a centrifugal analyser by the Biuret method.

Muscle sampling and analytical procedures

Carnosine analysis: Carnosine in neutralized PCA extracts of plasma or whole blood were analysed by an automated HPLC method.

Apparatus: The chromatography system consisted of Constametric I and IIG pumps regulated by an MP3000 programmable chromatography controller. Mixing of mobile phases was via a dynamic mixer. Derivatized amino acids and dipeptides were analysed using a Fluoromonitor III detector equipped with an excitation monochromator of 365nm and a broad spectrum emmision filter of 400-700nm. Peaks were integrated by the MP3000 and visualized on a printer-plotter. Chromatography was carried out on a 3μm Hypersil ODS II column (150 x 4.6mm) preceded by a guard column containing Lichrosorb ODS 30-40μm packing. Delivery of the samples to the column was via a Waters Wisp 712 autosampler equipped with an auto-addition accessory.

Preparation of mobile phases: Solvent A consisted of 0.1M sodium acetate buffer, methanol and tetrahydrafuran in the ratio 900:95:5 and was delivered by pump A. Pump B delivered methanol. Solvents A and methanol were filtered through 0.22μm HVLP filters (Millipore, UK) and degassed with helium. A gradient was applied to the column over 55 min (Figure 2.1) at a flow rate of 1.2ml/min.

Derivatization of samples: Neutralized PCA extracts or carnosine standards (20μl) were mixed with 0.4M borate buffer, pH 9.65 (100μl) in the autosampler vials. An aliquot (20μl) of the sample/borate mixture was derivatised with O-phthaladehyde reagent (10μl) in the autosampler syringe for 60 seconds, following which the whole mixture was injected onto the column. The derivatisation time was pre-programmed into the autosampler via the auto-addition accessory.

Chemicals: Crystalline L-carnosine hydrochloride was purchased from Sigma, UK. Individual stock standards were prepared in 0.5M PCA neutralized with 2.1M potassium bicarbonate and stored at -70°C. Methanol was HPLC grade. Tetrahydrafuran was purchased from Sigma, UK. Sodium acetate, acetic acid,

Figure 2.1. Elution profile and gradient used to seperate carnosine.

ELUENT COMPOSITION (% METHANOL)   CARNOSINE

40 ─

30 ─

20 ─

10 ─

        4   8   12   16   20   24   28   32

                ELAPSED TIME (MIN)
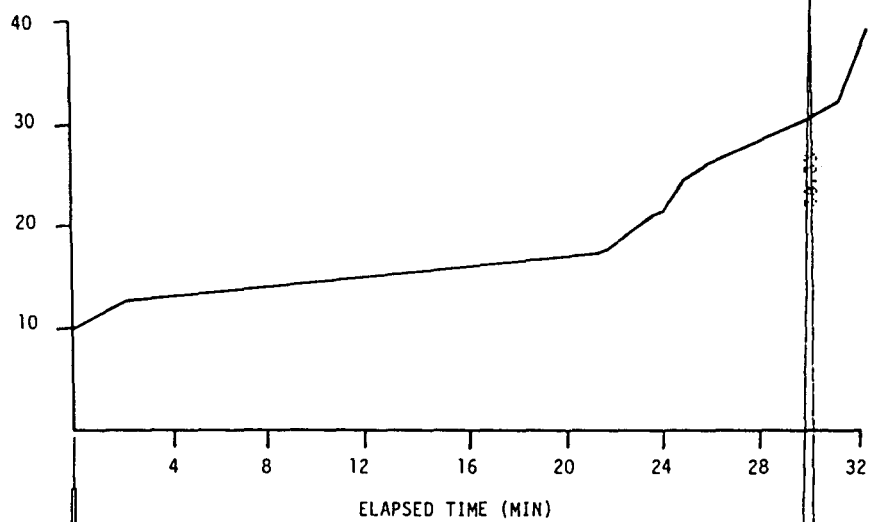
INJECTION

sodium hydroxide and boric acid were Analar grade and purchased from Anglia biochemicals. Water used for the preparation of buffer and standards was glass distilled and deionized through an Elgastat deionizer. It was further purified by passing through an Elgastat activated charcoal column to remove organics and an Elgastat SCII ion-exchange column. O-phthaladehyde reagent was purchased from Pierce, UK and stored at 4°C. The reagent in the autosampler was replaced at 12 hourly intervals.

Limits of detection, linearity and precision: For muscle extracts, the assay was linear up to a content of 150 mmol/kg dry muscle (d.m.), with a limit of detection of 0.5 mmol/kg d.m. The coefficient of variation of the carnosine assay in either muscle or blood extracts or standard solutions was 6.1-6.9%. For muscle, the coefficient of variation for the total procedures, including muscle extraction, was 7.8%.

Muscle biopsying: An area of skin over the left or right middle gluteal muscle was shaved and disinfected with surgical spirit. The site chosen for biopsying was determined to be one third of the distance along a line running from the tuber coxae to the tail-head. This procedure was adopted to try to ensure the same relative sampling site in different horses. Following anaesthesia of the skin (2ml 2% Lignocaine, 1:200,000 adrenaline, subcutaneously) an incision 1cm long, was made with a sterile surgical blade for each biopsy to be taken. Muscle samples were obtained at a depth of 6cm with 5mm Bergström-Stille biopsy needles.

Sample treatment: Muscle tissue for LDH Isoenzyme analysis was placed in a capped tube, stored on ice and analysed within 2 hours of collection. Samples for histochemistry were placed on filter paper with the fibres orientated at 90° to the surface of the paper. They were covered in talcum powder and frozen and stored in liquid nitrogen. Samples for muscle metabolite, muscle pH or enzyme analysis were rapidly frozen and stored in liquid nitrogen.

Muscle metabolite analysis: ATP, ADP, AMP, phosphocreatine (PCr), creatine (Cr), hexosemonophosphate (HMP), free glucose, glycerol 3-phosphate, glycerol , pyruvate, lactate and alanine were analysed in neutralized PCA extracts prepared from 5-10mg of freeze-dried muscle powder rendered as free as possible of visible blood and connective tissue as described by Harris, Hultman and Nordesjö (1974). Total creatine (TCr) is the sum of PCr and Cr and total adenine nucleotide (TAN) is the sum of ATP, ADP and AMP. Except where indicated otherwise, muscle metabolite contents have been

corrected to the resting TCr content with the exception of muscle lactate and glucose (see chapter 3). Glycogen was determined in 0.5-4.0mg of freeze dried muscle powder according to Snow, Harris and Gash (1985). Muscle carnosine analysis was carried out on neutralized PCA extracts of muscle as described for plasma.

Muscle pH: Muscle pH was determined in freeze-dried samples (Harris, Katz, Sahlin and Snow, 1988) using the homogenate method of Sahlin, Harris and Hultman (1975). Approximately 4 mg of freeze-dried powder was homogenized at a concentration of 20 mg/ml in an ice cold solution of 145 mmol/l KCl, 10 mmol/l NaCl, 5 mmol/l iodoacetic acid (sodium salt), pH 7.0, in a 1 ml all glass homogenizer for 1 min. The homogenate was equilibrated to 37°C and the pH measured with an MI-410 combination microelectrode (Microelectrodes Inc.) with a corning 150 pH/Ion meter.

Muscle buffering capacity (βm): Freeze-dried muscle powder was homogenized at a concentration of 10mg/ml in the same solution as used for muscle pH for 1 min with a Polytron homogenizer set at speed 7. Homogenates were equilibrated to 37°C for 5 min and the initial pH recorded. If this was less than pH 7.1, it was adjusted upwards with 50 mmol/l NaOH. Homogenates were titrated against 20μl aliquots of a 10 mmol/l HCl standard added with a 25μl Hamilton syringe. Homogenates were maintained at 37°C and stirred continuously during the titration. Homogenate pH was measured as for muscle pH. βm is expressed as the μmol H^+ required to change the pH of 1g of freeze dried muscle from 7.1 to 6.5. Further aspects of muscle buffering capacity are dealt with in Chapter 4.

LDH Isoenzymes: Analysis of muscle LDH isoenzyme patterns were performed on a portion of fresh, unfrozen muscle kept on ice, within two hours of collection. The sample was washed in buffer (100 mmol/l TRIS, 10 mmol/l EDTA, pH 7.6) and blotted on filter paper to remove any contaminating blood, the latter being high in LDH-1 and LDH-2. The sample was then homogenized in fresh buffer at a concentration of 4mg/ml for 20 sec on a Polytron homogenizer at speed 10. homogenates were then centrifuged at 10,000g for 20 min in a refrigerated centrifuge at +4°C. Samples were subjected to electropheresis for 15 min at 300V on cellulose acetate plates with Tris-Barbital buffer, pH 8.6. Bands were visualized with Helena LD-VIS isoenzyme reagent (Helena laboratories) and scanned at 570nm on an ACD-2020 scanning densitometer (Gelman Sciences Inc.). The accuracy for each of the isoenzymes was determined on one sample run 8 times. Reliability expressed as the mean and standard deviation of the percentage for each isoenzyme was as follows: LDH5-68.0±4.5%; LDH4-20.6±3.0%; LDH3-5.2±1.2%; LDH2-2.7±0.7%; LDH1-2.6±0.5%.

Histochemistry: Muscle samples for histochemistry were frozen and stored in liquid nitrogen. Samples were mounted in OCT (Tissue Tek II, Miles Labs. Inc.) and 10μm sections were cut on a Bright OFT motor-driven cryostat. Serial sections were stained for myofibrillar actomyosin ATPase (mATPase) after preincubation at pH 4.4-4.6 and for succinate dehydrogenase (SDH) as described by Brooke and Kaiser (1970) and Nachlas, Walker and Seligman (1957), respectively. All stained sections were mounted in Kaiser's glycerine jelly. The advantage of mounting the mATPase sections in glycerine rather than dehydrating through alcohol is that fibre area measurements can be made directly on the mATPase sections. The disadvantage is that the stain fades more rapidly and hence, the samples were stored in light tight boxes at -20°C.

Counts of fibres for calculation of fibre frequency and measurements of fibre area were performed on a Magiscan I television image analysis system (Joyce Loebl) coupled to a Leitz Dialux 20 microscope. For determining the relative percentage frequency for type I, IIA and IIB fibres, in the mATPase sections and the percentage high and low staining fibres in the SDH sections, a minimum of 300 fibres were counted. Mean fibre area for type I, IIA and IIB fibres was calculated by measuring a minimum of 40 fibres of each fibre-type selected randomly. The relative cross-sectional area that each fibre-type occupied in the biopsy was calculated according to Sullivan and Armstrong (1978).

Enzyme activities: The activities of oxoglutarate dehydrogenase (OGDH), phosphofructokinase (PFK), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were assayed at 25°C on freeze-dried muscle by following the oxidation or reduction of NADH on a Vitatron filter photometer at 334nm. A slit width of 2 mm was used as this reduced interference from small particles of homogenate entering the beam and gave better traces. Homogenization was performed on a Polytron homogenizer set at speed 10 for 30 sec. Homogenization and assay conditions were as follows:

OGDH: Homogenization: 10 mg/ml in a solution containing 50 mmol/l Tris, 1 mmol/l EDTA, 5 mmol/l Mg(Ac)$_2$, 1000 mmol/l sucrose, 20 mmol/l mercaptoethanol, pH 8.2.

Assay medium: 50 mmol/l MOPS, 5 mmol/l Mg(Ac)$_2$, 1 mmol/l mercaptoethanol, 2 mmol/l NAD, 0.4 mmol/l Coenzyme A, 8mmol/l oxoglutarate, pH 7.4.

PFK, CPK & LDH: Homogenization: 1 mg/ml in a solution containing 50 mmol/l KH$_2$PO$_4$-K$_2$HPO$_4$, pH 7.5. Final concentrations were: PFK - 1 mg/ml, LDH - 0.1 mg/ml and CPK - 0.01 mg/ml.

<u>Assay medium - PFK:</u> 100 mmol/l Tris, 1 mmol/l ATP, 0.18 mmol/l NADH, 2 mmol/l AMP, 5 mmol/l Mg(Ac)$_2$, 3 mmol/l fructose 6-phosphate, 1u aldolase, 25u triose-phosphate isomerase, 2u glycerol 3-phosphate dehydrogenase, pH 8.2.

<u>Assay medium - LDH:</u> 50 mmol/l KH_2PO_4-K_2HPO_4, 0.6 mmol/l EDTA, 0.18 mmol/l NADH, 1 mmol/l pyruvate, pH 7.5.

<u>Assay medium - CPK:</u> 80 mmol/l Triethanolamine, 1 mmol/l NAD, 1 mmol/l ADP, 20 mmol/l glucose, 10 mmol/l AMP, 1 mmol/l mercaptoethanol, 35 mmol/l phosphocreatine, 10 mmol/l Mg(Ac)$_2$, 1.5u hexokinase, 4u glucose 6-phosphate dehydrogenase, pH 7.0.

Activities are expressed as μmol substrate converted per g of dry muscle (d.m.) per minute at 25°C (μmol/g d.m./min 25°C).

<u>Precision:</u> The precision and coefficient of variation for analyses carried out in the present series of studies are shown in Table 2.2.

<u>Heart rate:</u> Heart rate was monitored with an EQB Equistat heart rate computer in the studies in Chapter 6, section (5) and Chapter 7, section (2). In all other studies, heart rate was monitored with a Hippocard PE2000 telemetric heart rate computer, averaging heart rate over 5 second intervals.

<u>Stride frequency:</u> Stride frequency during treadmill exercise was measured by recording the exercise on a video tape and subsequently measuring the time taken to complete 20 strides. Each section was counted 3 times and a mean taken.

<u>Statistical analysis</u>

Where appropriate, initial statistical evaluation was carried out by analysis of variance or analysis of variance for repeated measures. When a statistically significant effect was indicated, where applicable, this was located using Student's t-test for paired data. Alternatively, Student's t-tests for paired or unpaired data were used where appropriate. Simple linear regression (least squares method) and correlation (Pearson's method) were also performed. Precision (S) was calculated as the square root of the sum of the squared differences between duplicate determinations over 2n (S = $\sqrt{\Sigma d^2/2n}$). Coefficient of variation (CV, %) was calculated from S.100/X. Significance level was set at P<0.05 and interpreted as follows: P<0.05 -

probably significant; P<0.01 - significant; P<0.001 - highly significant. Unless otherwise stated, all values in the text are presented as mean and standard deviation (mean ± sd) and as mean and standard error of the mean (mean ± se) in figures.

Table 2.2. Precision (s) and coefficient of variation (CV, %) for analyses carried out in the present series of studies. Each estimate is based on a minimum of 10 paired determinations on the same sample.

	Precision (s)	Mean of material	CV (%)
Muscle (mmol/kg d.m.)			
ATP	0.72	20.93	3.4
ADP	0.22	2.55	8.6
AMP	0.05	0.45	11.1
PCr	3.29	51.00	6.5
Cr	3.86	75.77	5.1
Glycogen	10	470	2.1
HMP	0.33	9.95	3.3
Glycerol 3-P	0.25	7.89	3.2
Glycerol	0.47	5.36	8.8
Pyruvate	0.06	0.59	10.2
Lactate	2.66	74.90	3.6
Carnosine	6.7	110.5	6.1
βm (μmol/g d.m.)	4.1	101.3	4.0
Enzyme activities (μmol/g d.m./min)			
OGDH	0.32 [a]	2.41	13.3
CPK	50	2108	2.4
PFK	1.7	40.0	4.3
LDH	16	702	2.3
Blood			
Lactate (mmol/l)	0.5	17.8	2.8
Plasma			
Lactate (mmol/l)	0.5	19.8	2.5
Ammonia (μmol/l)	10	152	6.6
Uric acid (μmol/l)	5	133	3.8
pH	0.003	7.206	1.5

a   estimated between different homogenates

CHAPTER 3

ACUTE CHANGES IN THE WATER CONTENT AND DENSITY OF BLOOD AND PLASMA IN THE THOROUGHBRED HORSE: RELEVANCE TO THE CALCULATION OF METABOLITE CONCENTRATIONS IN BLOOD AND MUSCLE.

<u>INTRODUCTION</u>

In contrast to man where intense exercise does not result in any significant increase in the circulating red blood cell volume (CRCV), maximal exercise in the horse results in a marked output of red blood cells from the spleen, with a doubling in haematocrit (Catling, 1978; Snow et al, 1983). Intense exercise has also been shown to result in increases in plasma protein both in the horse (Pösö, Soveri and Oksanen, 1983; Snow et al, 1983) and in man (Delanne, Barnes and Brouha, 1958; Joyce and Poortmans, 1970). Changes in both the blood packed cell volume (B-PCV) and plasma total proteins (P-TP) will alter the water content and density of both blood (B-Vw and B-D, respectively) and plasma (P-Vw and P-D). These values are used in the calculation of metabolite concentrations when blood is deproteinized in pre-weighed tubes containing a known volume of perchloric acid (PCA). Following the addition of approximately 2-3 ml of blood, the tube is re-weighed to obtain the volume of blood added. The dilution factor ('F') can be calculated according to the equation E(1):

E(1)   'F' = B-Vw + (V-PCA.B-D) / (W_2-W_1)

where V-PCA is the volume of PCA in ml, W_1 is the weight of the tube and perchloric acid and W_2 is the weight of the tube following addition of blood.

This method of deproteinization is particularly convenient under field conditions or where a large number of samples are being collected in a short period of time. The alternative procedure of pipetting a measured volume of blood accurately into a known volume of PCA and then calculation of the dilution factor according to equation E(2) is a less satisfactory approach in these situations.

E(2)   'F' = [(VB.B-Vw) + V-PCA] / (VB.B-Vw)

where VB is the volume of blood added in ml. From equation E(2) it can be seen that alterations in the blood water content (B-Vw) will affect the dilution factor and in equation E(1), blood water content and blood density (B-D) will both exert an effect. Previously, in the absence of specific data on the horse, values from man for B-Vw and B-D of 0.800 1/1 and 1.04 g/ml respectively have been used (see Bergmeyer, 1970). Furthermore, these have been assumed to remain constant during exercise.

This investigation was undertaken to obtain specific data on the changes in water content and density of horse blood and plasma during short

term, high-intensity exercise and to determine what effect, if any, these changes have on the calculation of metabolite contents in plasma and blood. An extension has been made to illustrate the use of these values in the correction of metabolite contents in muscle biopsies contaminated with blood, a problem frequently encountered in the horse due to high post-exercise muscle hypereamia.

With information on the changes in the water spaces of plasma and red blood cells, it is possible to calculate the distribution of metabolites between these compartments. The changes in water content and density may then be used to investigate the spatial distribution of metabolites, such as lactate, between plasma and red blood cell compartments. This is discussed in terms of the relevance of measuring plasma or whole blood lactate. Finally, temporal and spatial distribution of lactate between plasma and red blood cells is considered as this could have important implications for the treatment of samples for plasma lactate analysis.

MATERIALS AND METHODS

Experimental protocol: The findings in this study were obtained from four Thoroughbred geldings (SM, HR, JW & SL). Each horse participated in three experimental sessions, at least one week being allowed between sessions. Each horse was catheterized and prepared for muscle biopsies as described in Chapter 2. Exercise consisted of 4 minutes walking at 1.6 m/s, 4 minutes trotting at 3.2 m/s and then acceleration (40 sec) to 12 m/s which was maintained for 2 minutes. All exercise was on a 5° incline. The two minute gallop was followed by one of three recovery modes: (T) - 30 minutes trotting (3.2 m/s) and 40 minutes walking (1.6 m/s); (W) - 70 minutes walking (1.6 m/s); (S) - 70 minutes standing. All recovery exercise was carried out at 0° incline.

Blood sampling: Ten ml samples were collected into lithium-heparin tubes from which plasma was obtained after centrifugation. Blood samples were collected before and during exercise and at regular intervals during recovery. For determination of density (B-D & P-D, g/ml) and volume water content (B-Vw & P-Vw, 1/1), 2 x 250 µl aliquots of well mixed blood or plasma at room temperature were pipetted into weighed 1.5 ml polypropylene tubes using a calibrated Gilson Microman positive displacement pipette. Tubes were then reweighed to 0.1 mg and from the difference in weights, P-D and B-D were calculated. The 0.5 ml aliquots of blood or plasma were then freeze-dried to constant weight. Complete removal of water was checked by further drying of samples in an oven set at 40°C and re-weighing to check for constant weight. B-Vw and P-Vw were calculated from the reduction in

weight, assuming a density of water of 1 g/ml.

Lactate in blood and plasma, ATP, PCr, Cr and lactate in muscle, packed cell volume (B-PCV, 1/1) and plasma total protein (P-TP, g/1) were determined as described in Chapter 2.

In vitro addition of lactate to blood samples: Venous blood samples (50 ml) were drawn by venepuncture at rest from each of 3 horses. Each sample was placed in a beaker containing a stirring bar in a water bath at 37°C and allowed to equilibrate to temperature. A pre sample was taken and then two ml of a 1M solution of lactate standard was added and two 1 ml samples removed at 20 sec and at 5, 10, 15, 20, 25 and 30 minutes. Of the two samples removed at each time point, the first was immediately centrifuged in a high-speed centrifuge for 1 min at 10,000g and 250 μl of the plasma obtained deproteinized with 750 μl of 1M PCA. The second 1 ml sample was deproteinized with 3 ml 1M PCA. Lactate was analysed in the PCA extracts of both plasma and whole blood.

Estimation of resting muscle blood content: As values for muscle blood content at rest in the horse are not available, muscle blood contamination was estimated by measurement of the 2,3 diphosphoglycerate (2,3 DPG) content of muscle biopsies, the majority of which comes from contaminating blood. By measuring 2,3 DPG in paired muscle and blood samples, it is then possible to calculate the muscle blood contamination in terms of kg of dried blood per kg of dried muscle according to equation E(3).

E(3)   Muscle blood contamination = [2,3 DPG-M.B-D.(1 - B-Vw)] / 2,3 DPG-B
         (kg dry blood/kg d.m.)

where 2,3 DPG-M is the concentration of 2,3 DPG in muscle in mmol/kg d.m. 2,3 DPG-B is the concentration of 2,3 DPG in blood in mmol/1, B-D is blood density in g/ml and B-Vw is blood volume water in 1/1.

Nine muscle biopsies of the middle gluteal were collected from different horses at rest as described in Chapter 2. Venous blood samples were collected into PCA. 2,3 DPG in blood and muscle was analysed by an adaptation of the method of Ericson and de Verdier (1972) as follows:

Blood 2,3 DPG: 25μl of PCA extract of whole blood was added to a reagent volume of 750μl in a semi-micro uv cuvette. The reagent consisted of triethanolamine buffer pH 7.6 (150 mmol/1), Mg(Acetate)$_2$.4H$_2$O (9 mmol/1), EDTA (1.5 mmol/1), ATP (3 mmol/1), NADH (0.16 mmol/1), dithiothreitol (2 mmol/1), glycerol 3-phosphate dehydrogenase (7 units), triosephosphate isomerase (13 units), glyceraldehyde 3-phosphate dehydrogenase (2 units),

3-phosphoglycerate kinase (6 units), phosphoglycerate mutase (1 unit) and lactate dehydrogenase (3 units). All concentrations and amounts refer to final concentrations in the cuvette. Changes in absorbance at 334nm were monitored using a Vitatron filter photometer and a chart recorder. As the trace was not linear, either before or at the end of the reaction, absorbance readings were made by extrapolation both forwards, to obtain the initial absorbance reading and back, to obtain the final absorbance reading. The reaction was started by the addition of 20 μl per cuvette of a 1:1 mixture of phosphoglycerate mutase (1700 units per ml) and 2-phosphoglycolate (100 mmol/l). The values obtained are divided by two, as two NADH are consumed for every 2,3 DPG (refer to reaction sequence) and expressed as mmol/l.

Muscle 2,3 DPG: Freeze-dried muscle samples were extracted as described in Chapter 2. The reagent final concentrations in the cuvette are as described for blood, with the exception that adjustment was made to allow a smaller reagent to sample ratio (250 μl of reagent to 50 μl of sample) due to the low level of 2,3 DPG from blood in muscle extracts. The reaction was started with 10 μl of a 1:1 mixture of phosphoglycerate mutase (1700 units/ml) and 2-phosphoglycolate (100 mmol/l). Results were divided by two and are expressed as mmol/kg d.m..

Statistics: For the purpose of this study, observations were made on HR on all 3 sessions, on SM during (S) and (T), on SL during (S) and (W) and from JW during (S) recovery. Changes in variables with exercise were assessed using Student's t-test for paired data. Where more than one set of data within an individual horse were available, the mean change for that horse was used. Values are presented as mean ± standard deviation.

In the comparison of P-D and P-Vw with P-TP and B-D and B-Vw with B-PCV, only the data obtained in the (S) recovery sessions were used. Data from the four horses were analysed together by simple linear regression analysis, with the assumption that the between-horse variance in the relationship was minimal, as indicated by comparison of within-horse regressions. In certain of the comparisons, the data was characterized by a high degree of clustering of values within-horses, which precluded the use of other analyses, such as multiple linear regression analysis with a dummy variable for horse.

Abbreviations: A full list of the abbreviations relative to this chapter is given in Table 3.1.

Table 3.1. Abbreviations relevant to Chapter 3.

Abbreviation	Description	Units
D	Density	g/ml
Vw	Volume water	1/1
B-D	Blood density	g/ml
P-D	Plasma density	g/ml
B-Vw	Blood volume water	1/1
P-Vw	Plasma volume water	1/1
B-PCV	Blood packed cell volume	1/1
P-TP	Plasma total protein	g/1
VB	Volume of blood	ml
VP	Volume of plasma	ml
V-PCA	Volume of perchloric acid	ml
V-EXT	Volume of acid extract	ml
PW	Plasma water compartment	1/1
PS	Plasma solids	1/1
RBCW	Red blood cell water compartment	1/1
RBCS	Red blood cell solids	1/1
RBC	Red blood cells	1/1

RESULTS

Changes in B-Vw, P-Vw, B-D and P-D: The changes in P-TP, Vw and D of plasma and B-PCV, Vw and D of whole blood during exercise and recovery were similar in all 4 horses. The results from two of the horses (HR and SM) with both (S) and (T) recovery are shown in Figures 3.1 and 3.2. As far as could be interpreted, there were no differences in the rates of recovery in any of the parameters with (S), (W) or (T) recovery. With the exception of P-D, maximum changes from rest in each of the parameters was recorded with the immediate post-exercise sample. In the case of P-D, maximum change was seen after 4 minutes of recovery.

Based on all four horses, mean P-TP increased 10.98 ± 3.45 g/l ($P < 0.01$) with exercise from a mean content at rest of 61.29 ± 3.88 g/l. Recovery of P-TP back to resting values took approximately 50 minutes. In comparison to the changes in P-TP, the changes in both P-Vw and P-D were small. P-Vw decreased 0.015 ± 0.002 1/l ($P < 0.01$) from a mean at rest of 0.943 ± 0.004 1/l (approximately -1.6%), whilst P-D showed a non-significant increase of 0.00022 ± 0.0050 g/ml from a mean at rest of 1.02478 ± 0.00029 g/ml. Both P-D and P-Vw were significantly correlated to P-TP (Figure 3.3, E(4), E(5)):

E(4)   P-D = 1.00103 + 0.00036 . [P-TP]
         n = 64, r = 0.74

E(5)   P-Vw = 0.99717 - 0.000921 . [P-TP]
         n = 64, r = 0.90

Mean B-PCV showed an increase of 0.208 ± 0.059 1/l ($P < 0.01$) by the end of the 2 minute gallop from a mean at rest of 0.426 ± 0.065 1/l. Recovery in B-PCV to resting values took 50-60 minutes. Changes in B-Vw and B-D of blood were large in comparison to the changes of P-Vw and P-D in plasma. B-Vw decreased 0.061 ± 0.14 1/l ($P < 0.01$) from a mean at rest of 0.832 ± 0.081 1/l, whilst B-D increased 0.01723 ± 0.00542 g/ml ($P < 0.01$) from a mean of 1.05714 ± 0.00564 g/ml. Both B-D and B-Vw were significantly correlated to B-PCV (Figure 3.4, E(6), E(7)):

E(6)   B-D = 1.01926 + 0.08717 . [B-PCV]
         n = 67, r = 0.96

E(7)   B-Vw = 0.942 - 0.260 . [B-PCV]
         n = 67, r = 0.98

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Figure 3.1 Horse HR: Plasma total protein (P-TP), volume water content (P-Vw) and density (P-D),   and blood packed cell volume (B-PCV),   volume water content   (B-Vw) and density (B-D),   before exercise (Pre),   after 4 minutes walking   (4'W) and trotting (4'T) and during 70 minutes recovery from   a   2 minute gallop at 12 m/s.   Dashed line - (T) recovery, Continuous line - (S) recovery.

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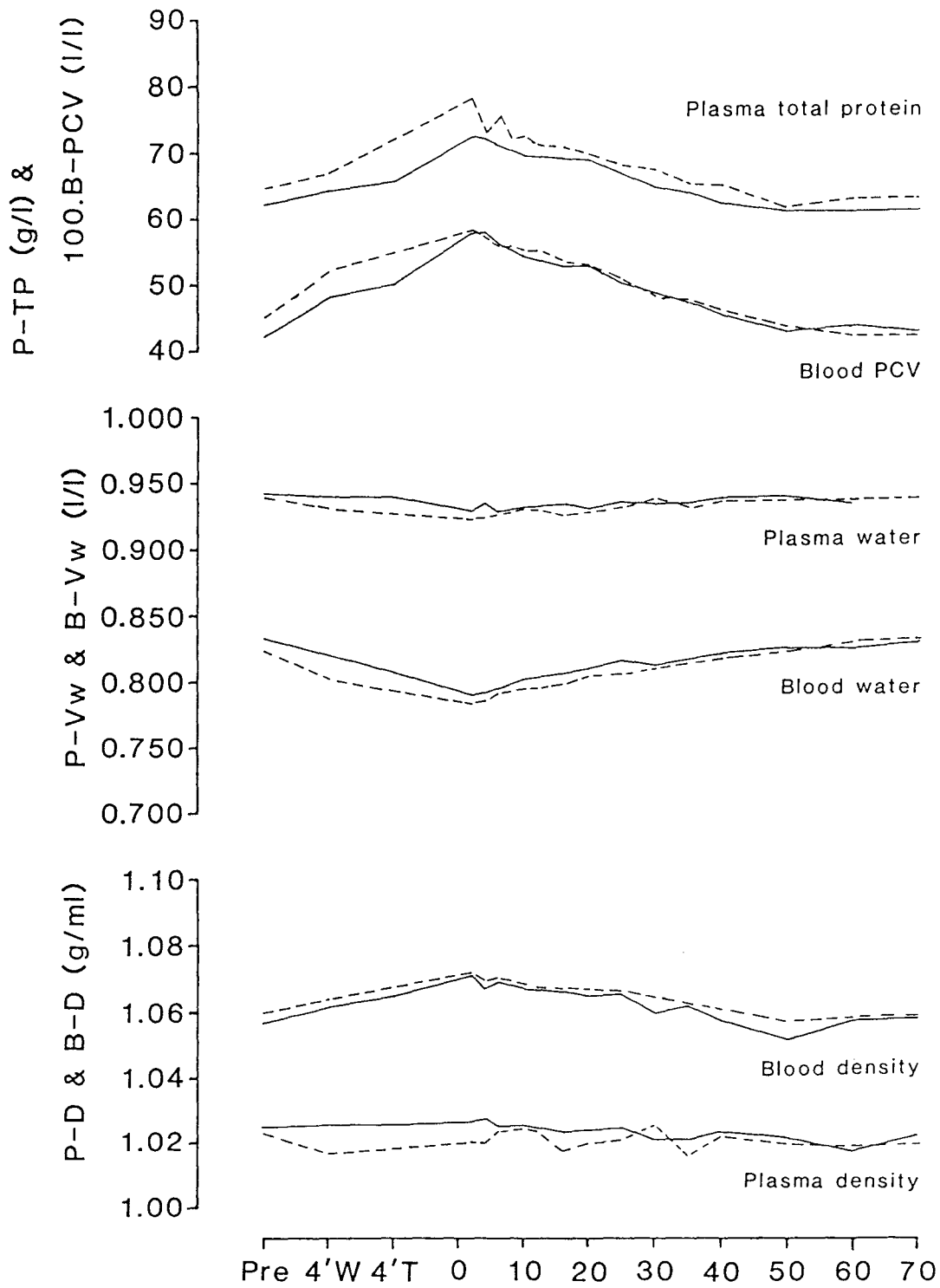
Figure 3.2 Horse SM: Plasma total protein (P-TP), volume water content (P-Vw) and density (P-D), and blood packed cell volume (B-PCV), volume water content (B-Vw) and density (B-D), before exercise (Pre), after 4 minutes walking (4'W) and trotting (4'T) and during 70 minutes recovery from a 2 minute gallop at 12 m/s. Dashed line - (T) recovery, Continuous line - (S) recovery.
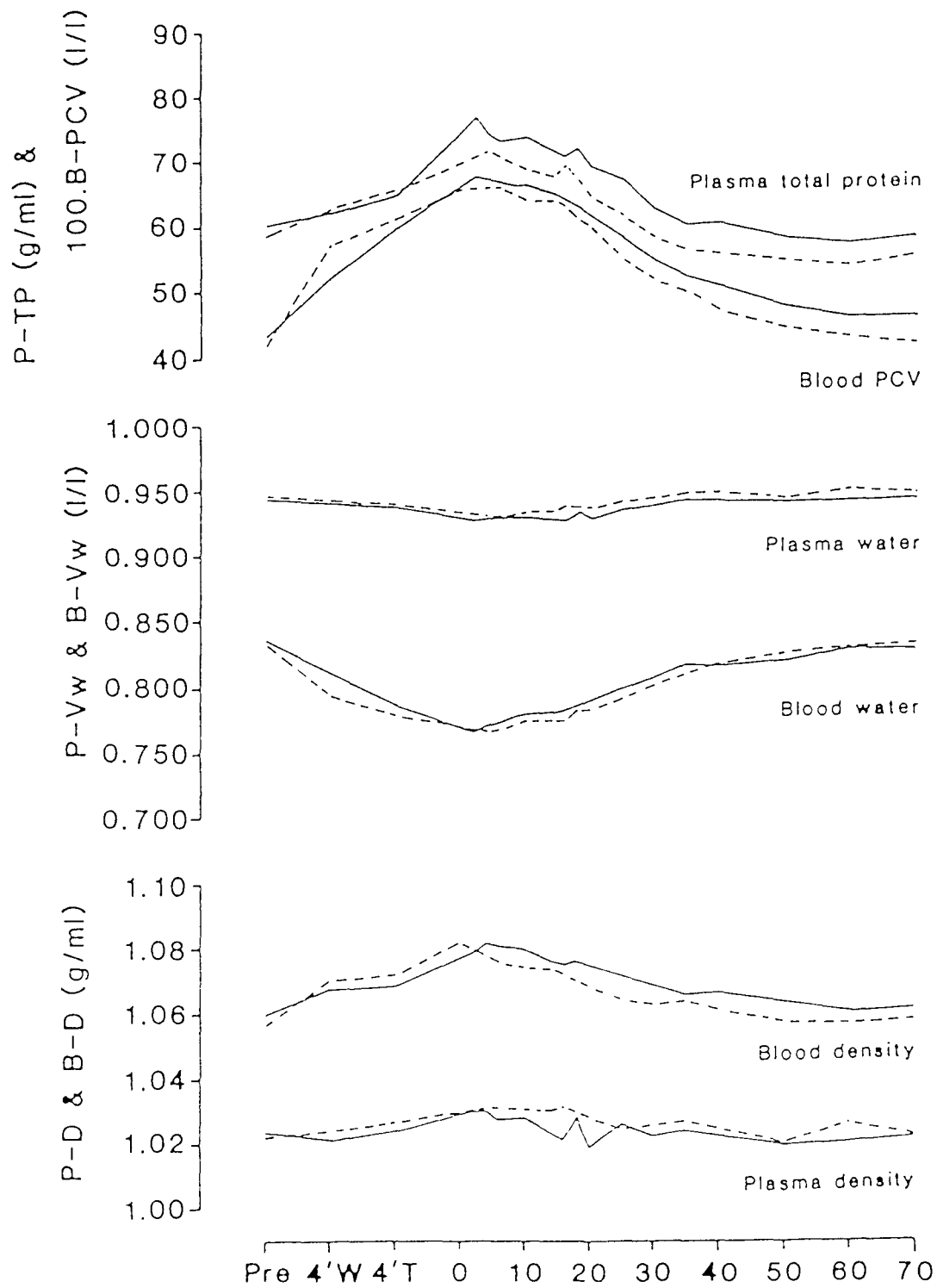
Figure 3.3 Comparison of plasma density (P-D) and volume water content (P-Vw) to plasma total protein (P-TP). Only four sets of data obtained with (S) recovery are included in the figure. Data from one horse (SL) are shown as open circles to illustrate the degree of clustering, which was particularly marked in the comparison of P-D with P-TP. The linear regression lines were calculated from E(4) and E(5) in the text. The dashed line shows the relationship between P-Vw and P-TP reported in Carlson and Harrold (1977).
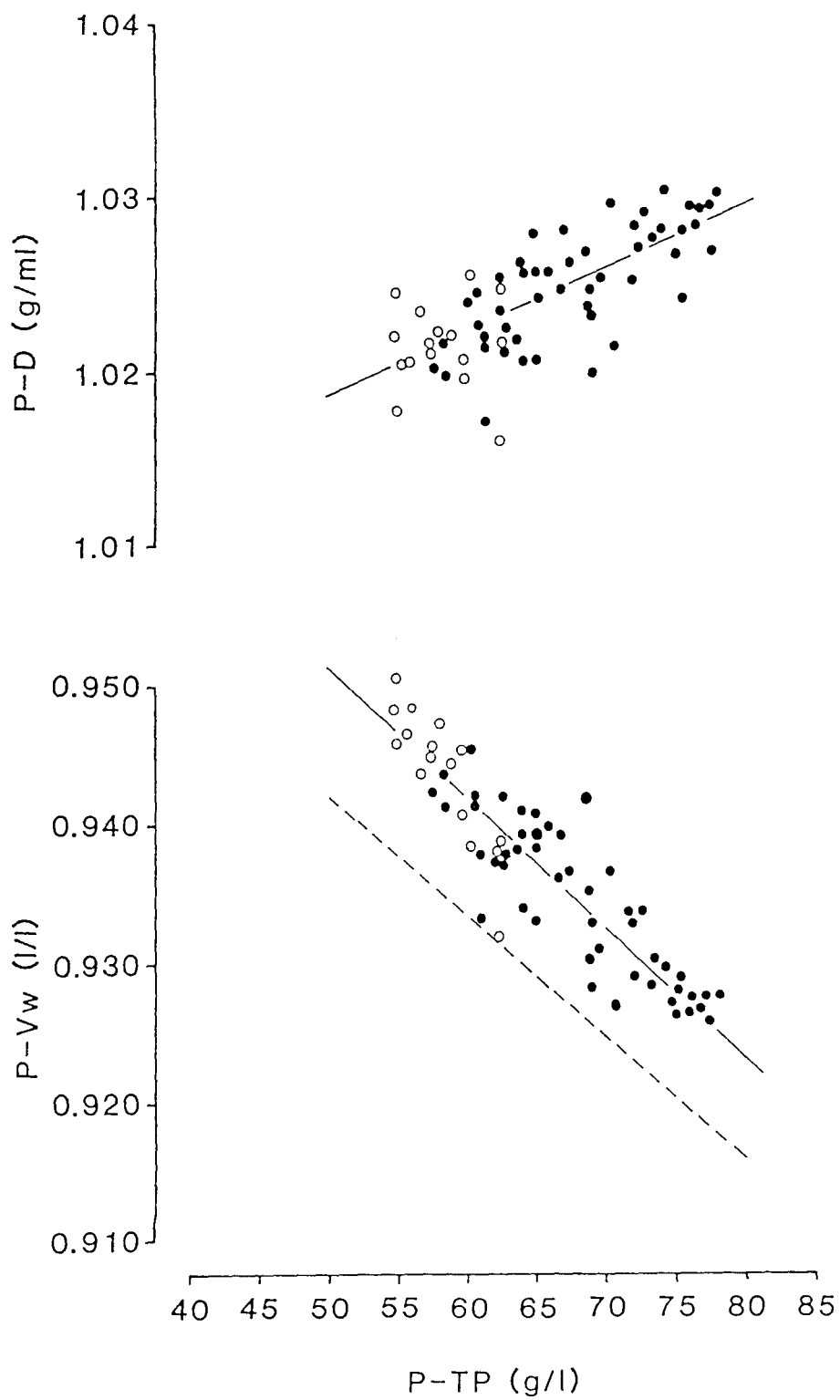
Figure 3.4. Comparison of blood density (B-D) and volume water (B-Vw) to packed cell volume (B-PCV). Only four sets of data obtained with (S) recovery are included in the figure. Data from one horse (SL) are shown as open circles. The linear regression lines were calculated from E(6) and E(7) in the text.
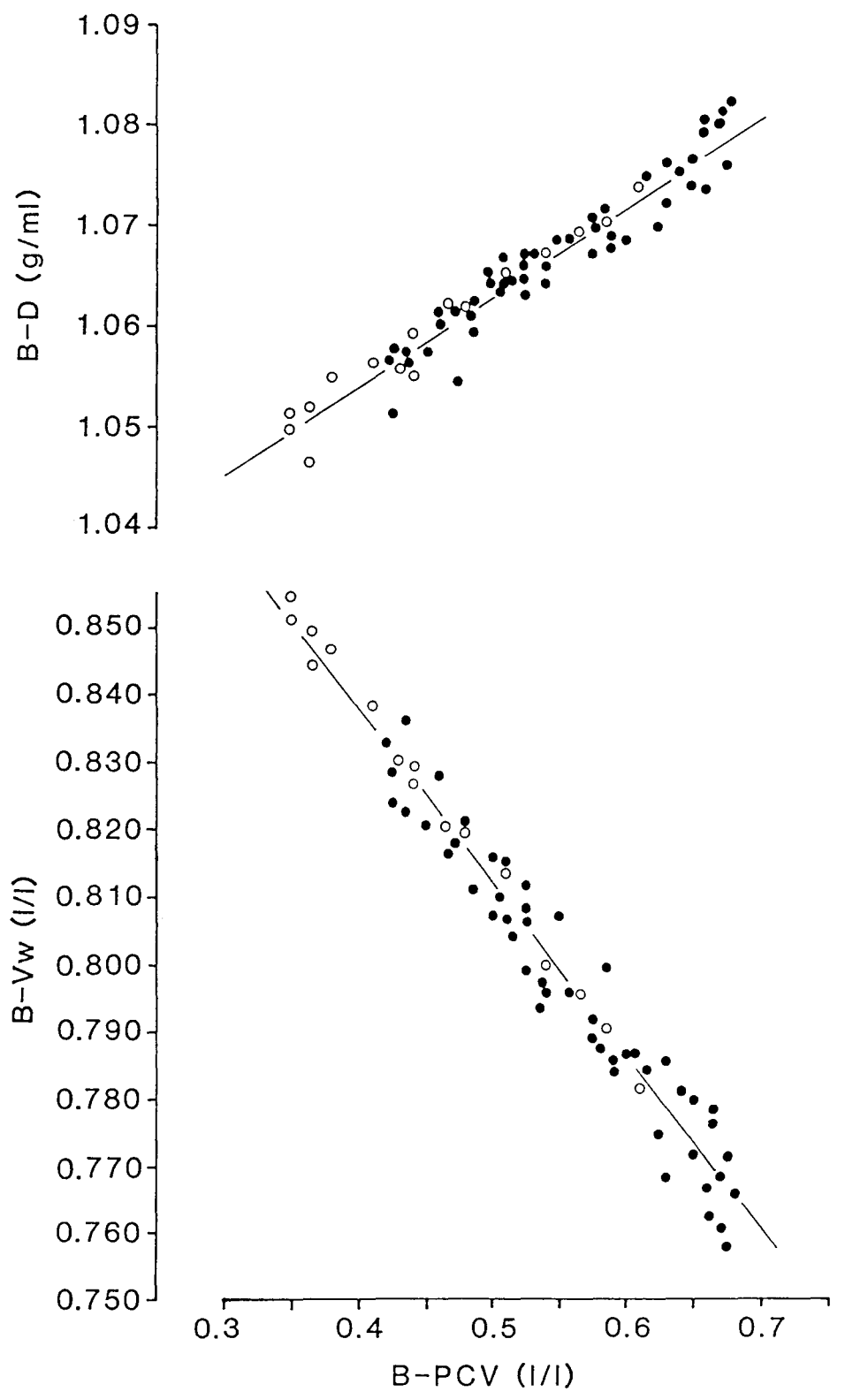
<u>Determination</u> <u>of</u> <u>blood</u> <u>and</u> <u>plasma</u> <u>metabolite</u> <u>concentrations:</u> A procedure commonly employed in the assay of blood samples is to add an approximate volume of blood to a pre-weighed tube containing a known volume of perchloric acid (V-PCA). The tube is then re-weighed and the volume of blood (VB, ml) added is calculated according to:

E(8) $\qquad$ $VB = (W_2 - W_1) / B-D$

The volume of acid extract (V-EXT) obtained after centrifugation is calculated from:

E(9) $\qquad$ $V-EXT = (VB.B-Vw) / \qquad V-PCA$

Combining equations E(8) and E(9) the dilution factor ('F') for use in the calculation is:

E(10) $\qquad$ $'F' = B-Vw + (V-PCA.B-D) / (W_2 - W_1)$

In the past, values of 1.04 g/ml for B-D and 0.800 l/l for B-Vw based on human data, and cited in Bergmeyer (1970), have been used. In Table 3.2, concentrations of lactate in blood calculated using these estimates for B-D and B-Vw (row a) are shown together with concentrations calculated using values based on the changes in B-PCV (row b). A similar approach may be adopted in the calculation of plasma lactate concentrations, based on the assay of perchloric acid extracts. However, the practice employed to date has been to pipette a measured volume of plasma into a measured volume of PCA, and thus, only P-Vw is required for the calculation of volume of acid extract (V-EXT). In Table 3.2, row c, plasma lactate concentrations based on a P-Vw of 0.935 l/l are shown, whilst in row d, concentrations are based on P-Vw calculated from P-TP. Table 3.3 shows mean percentage differences between corrected and uncorrected whole blood and plasma lactate concentrations.

In all cases, blood lactate calculated using the assumed values of 1.04 g/ml for B-D and 0.800 l/l for B-Vw resulted in a small, but consistent underestimation of whole blood lactate concentration. Corrected whole blood lactate concentrations ranged from 1.7±0.2 to 2.2±0.3 % greater than the uncorrected values. The use of a fixed P-Vw value of 0.935 l/l resulted in a smaller error in the estimation of plasma lactate, although this was not always in the same direction (i.e. there was both under-and over-estimation). This error ranged from -1.4% underestimation to +1.2% overestimation.

Table 3.2. Blood and plasma lactate concentrations before (PRE) and after 2 minutes treadmill exercise at 12 m/s with 5° incline and during 70 minutes standing recovery (S) in horses SM, SL, JW & HR.

| | Recovery time (min) | | | | |
	Pre	0	10	30	70
Horse: SM					
A) Blood	0.7	18.1	24.9	20.1	7.1
B) Blood (correct)	0.7	18.3	25.2	20.5	7.2
C) Plasma	0.9	31.8	39.0	27.3	10.7
D) Plasma (correct)	0.9	31.6	38.8	27.5	10.8
Horse: SL					
A) Blood	0.7	10.9	13.8	9.0	2.3
B) Blood (correct)	0.7	11.1	14.0	9.2	2.4
C) Plasma	0.7	16.5	21.1	11.4	3.0
D) Plasma (correct)	0.7	16.6	21.2	11.5	3.0
Horse: JW					
A) Blood	0.5	15.8	21.5	19.1	8.0
B) Blood (correct)	0.5	16.1	21.9	19.4	8.1
C) Plasma	0.5	26.9	35.7	28.0	12.0
D) Plasma (correct)	0.5	26.7	35.3	27.8	12.1
Horse: HR					
A) Blood	0.6	16.7	23.2	18.4	5.4
B) Blood (correct)	0.6	17.0	23.6	18.8	5.5
C) Plasma	0.7	25.0	33.9	25.2	7.4
D) Plasma (correct)	0.7	24.9	33.8	25.3	7.4

Footnotes: A) B-D and B-Vw taken as 1.04 g/ml and 0.800 1/1, respectively.
B) B-D and B-Vw estimated from changes in B-PCV, E(6) and E(7).
C) P-Vw taken as 0.935 1/1.
D) P-Vw estimated from changes in P-TP according to E(5).

Table 3.3. Mean percentage differences between blood lactate and blood lactate (corrected) and plasma lactate and plasma lactate (corrected).

Recovery time (min)

% Difference B v A	Pre	0	10	30	70
Mean	+1.8	+1.8	+1.7	+2.0	+2.2
sd	±0.3	±0.1	±0.2	±0.2	±0.3
n = 4					

% Difference D v C	Pre	0	10	30	70
Mean	+0.1	-0.4	-0.3	+0.3	+0.9
sd	±1.1	±0.7	±0.6	±0.7	±0.4
n = 4					

Footnote: A = Blood lactate, mmol/l

B = Blood lactate, mmol/l, corrected

C = Plasma lactate, mmol/l

D = Plasma lactate, mmol/l, corrected

Distribution of lactate between plasma and red blood cell compartments:
From the values of P-D, P-Vw, B-D and B-Vw calculated from equations E(4)
to E(7), the compartments of plasma water (PW), plasma solids (PS), red
blood cell water (RBCW) and red blood cell solids (RBCS) have been
estimated for the four horses in this study during (S) recovery and are
shown in Tables 3.4 to 3.7. The relationships pertaining to the calculation
of the compartments within blood samples and the distribution of lactate
between these compartments has been calculated according to the
relationships given in Table 3.8. An example of lactate concentrations and
contents for the various whole blood compartments for horse SM is shown in
Figures 3.5 - 3.7.

Recalculation of the plasma lactate concentration in terms of mmol/l
PW resulted in mean increases in concentration ranging from 6.1 ± 0.3 to
7.3 ± 0.7 % . The effect of recalculation to mmol/l PW was greatest on the 0
and 10 minute samples.

In spite of large differences in plasma and whole blood lactate
concentrations, in terms of the lactate content of the plasma and red cell
compartments, the difference was much less, although plasma content was
always greater than red cell content. The smallest difference was at rest
$(0.18\pm0.15$ mmol) and the greatest at 30 minutes recovery $(4.62\pm1.8$ mmol).

Lactate gradients between plasma and red cell compartments: The
concentration gradient between plasma lactate (mmol/l PW) and red blood
cell lactate (mmol/l RBCW) averaged 0.99 ± 0.33 at rest and increased to
1.75 ± 0.15 (P<0.05) at the end of exercise (0 min recovery). At all times
during recovery, the mean gradient was significantly elevated above unity
(0 min - P<0.002; 10 min - P<0.002; 30 min - P<0.01; 70 min - P>0.01).

Plasma and whole blood lactate disappearance rates: Mean half-times for
plasma (mmol/l) and whole blood lactate (mmol/l) were 29.7 ± 7.4 and 32.8 ± 8.1
min respectively and did not differ significantly (P>0.05). Mean half-times
for plasma and RBC lactate concentrations were 29.7 ± 7.4 and 26.1 ± 5.3 min,
respectively and were not significantly different (P>0.05). For the
different blood compartments, half-times for plasma content (mmol) and RBC
content (mmol) were 34.1 ± 9.4 and 21.7 ± 4.5 min, respectively and differed
significantly (P<0.05).

Temporal distribution of lactate added to blood samples in vitro:
Individual changes in the lactate content of plasma and red cell
compartments were calculated according to the relationships shown in Table

Table 3.4. Lactate concentrations and contents in plasma and red blood cell compartments before (PRE) and at 0, 10, 30 and 70 min following 2 min treadmill exercise at 12 m/s and 5° incline in horse SM.

	PRE	0	10	30	70
Plasma					
TP, 1/1	60.1	76.7	73.8	62.7	58.1
P-Vw, 1/1[a]	0.942	0.927	0.929	0.939	0.944
PW, 1	0.532	0.297	0.311	0.423	0.510
PS, 1	0.033	0.023	0.024	0.027	0.030
Plasma lactate, mmol/l[c]	0.9	31.6	38.8	27.5	10.8
Plasma lactate content, mmol	0.5	10.1	13.0	12.4	5.8
Plasma lactate, mmol/l PW	0.9	34.1	41.7	29.2	11.4
Red blood cells (RBC)					
Whole blood lactate, mmol/l[c]	0.7	18.3	25.2	20.5	7.2
B-PCV, 1/1	0.435	0.680	0.665	0.550	0.460
B-Vw, 1/1[b]	0.829	0.765	0.769	0.799	0.822
B-D, g/1[b]	1.057	1.079	1.077	1.067	1.059
RBCW, 1	0.297	0.468	0.458	0.376	0.312
RBCS, 1	0.138	0.212	0.207	0.174	0.148
RBC lactate, mmol/l	0.5	12.1	18.4	14.9	3.1
RBC lactate content, mmol	0.2	8.2	12.2	8.2	1.4
RBC lactate, mmol/l RBCW	0.8	17.6	26.7	21.8	4.6

a Values for P-Vw calculated from regression equation E(5).

b Values for B-Vw and B-D calculated from regression equations E(7) & E(6)

c Lactate values corrected using P-Vw for plasma and B-Vw and B-D for whole blood.

PW   Plasma water
PS   Plasma solids
RBC   Red blood cells
RBCW Red blood cell water
RBCS Red blood cell solids

Table **3.5.** Lactate concentrations and contents in plasma and red blood cell compartments before (PRE) and at 0, 10, 30 and 70 min following 2 min treadmill exercise at 12 m/s and 5° incline in horse SL.

	PRE	0	10	30	70
Plasma					
TP, 1/1	57.8	60.0	62.1	56.3	54.5
P-Vw, 1/1[a]	0.944	0.942	0.940	0.945	0.947
PW, 1	0.614	0.367	0.420	0.529	0.601
PS, 1	0.036	0.023	0.027	0.031	0.034
Plasma lactate, mmol/1[c]	0.7	16.6	21.1	11.5	3.0
Plasma lactate content, mmol	0.5	6.5	9.5	6.5	1.9
Plasma lactate, mmol/1 PW	0.7	17.7	22.6	12.2	3.2
Red blood cells (RBC)					
Whole blood lactate, mmol/1[c]	0.7	11.1	14.0	9.2	2.4
B-PCV, 1/1	0.350	0.610	0.553	0.440	0.365
B-Vw, 1/1[b]	0.851	0.783	0.798	0.828	0.847
B-D, g/1[b]	1.050	1.072	1.068	1.058	1.051
RBCW, 1	0.237	0.416	0.378	0.299	0.246
RBCS, 1	0.113	0.194	0.175	0.141	0.119
RBC lactate, mmol/1	0.6	7.5	8.3	6.3	1.2
RBC lactate content, mmol	0.2	4.6	4.6	2.8	0.5
RBC lactate, mmol/1 RBCW	0.9	11.0	12.1	9.2	1.8

a Values for P-Vw calculated from regression equation E(5).

b Values for B-Vw and B-D calculated from regression equations E(7) & E(6)

c Lactate values corrected using P-Vw for plasma and B-Vw and B-D for whole blood.

PW   Plasma water
PS   Plasma solids
RBC   Red blood cells
RBCW Red blood cell water
RBCS Red blood cell solids

Table 3.6. Lactate concentrations and contents in plasma and red blood cell compartments before (PRE) and at 0, 10, 30 and 70 min following 2 min treadmill exercise at 12 m/s and 5° incline in horse JW.

	PRE	0	10	30	70
Plasma					
TP, 1/1	65.8	76.5	77.3	72.5	63.3
P-Vw, 1/1[a]	0.937	0.927	0.926	0.930	0.939
PW, 1	0.464	0.301	0.315	0.381	0.460
PS, 1	0.031	0.024	0.025	0.029	0.030
Plasma lactate, mmol/1[c]	0.5	26.7	35.3	27.8	12.1
Plasma lactate content, mmol	0.2	8.7	12.0	11.4	5.9
Plasma lactate, mmol/1 PW	0.5	28.8	38.2	29.9	12.9
Red blood cells (RBC)					
Whole blood lactate, mmol/1[c]	0.5	16.1	21.9	19.4	8.1
B-PCV, 1/1	0.505	0.675	0.660	0.590	0.510
B-Vw, 1/1[b]	0.811	0.767	0.770	0.789	0.809
B-D, g/1[b]	1.063	1.078	1.077	1.071	1.064
RBCW, 1	0.347	0.466	0.455	0.408	0.349
RBCS, 1	0.158	0.209	0.205	0.182	0.161
RBC lactate, mmol/1	0.5	11.0	14.9	13.6	4.4
RBC lactate content, mmol	0.3	7.4	9.8	8.0	2.2
RBC lactate, mmol/1 RBCW	0.8	15.9	21.6	19.7	6.4

a Values for P-Vw calculated from regression equation E(5).

b Values for B-Vw and B-D calculated from regression equations E(7) & E(6)

c Lactate values corrected using P-Vw for plasma and B-Vw and B-D for whole blood.

PW   Plasma water
PS   Plasma solids
RBC   Red blood cells
RBCW Red blood cell water
RBCS Red blood cell solids

Table 3.7. Lactate concentrations and contents in plasma and red blood cell compartments before (PRE) and at 0, 10, 30 and 70 min following 2 min treadmill exercise at 12 m/s and 5° incline in horse HR.

	PRE	0	10	30	70
Plasma					
TP, 1/1	62.2	72.1	69.4	64.7	61.1
P-Vw, 1/1[a]	0.940	0.931	0.933	0.938	0.941
PW, 1	0.545	0.396	0.429	0.483	0.541
PS, 1	0.035	0.029	0.031	0.032	0.034
Plasma lactate, mmol/1[c]	0.7	24.9	33.8	25.3	7.4
Plasma lactate content, mmol	0.4	10.6	15.5	13.0	4.3
Plasma lactate, mmol/1 PW	0.8	26.7	36.2	26.9	7.9
Red blood cells (RBC)					
Whole blood lactate, mmol/1[c]	0.6	17.0	23.6	18.8	5.5
B-PCV, 1/1	0.420	0.575	0.540	0.485	0.425
B-Vw, 1/1[b]	0.833	0.793	0.802	0.816	0.832
B-D, g/1[b]	1.056	1.069	1.066	1.062	1.056
RBCW, 1	0.288	0.397	0.372	0.333	0.291
RBCS, 1	0.132	0.178	0.167	0.152	0.134
RBC lactate, mmol/1	0.4	11.2	14.9	11.8	3.0
RBC lactate content, mmol	0.2	6.4	8.1	5.7	1.3
RBC lactate, mmol/1 RBCW	0.6	16.2	21.7	17.2	4.4

a Values for P-Vw calculated from regression equation E(5).

b Values for B-Vw and B-D calculated from regression equations E(7) & E(6)

c Lactate values corrected using P-Vw for plasma and B-Vw and B-D for whole blood.

PW Plasma water
PS Plasma solids
RBC Red blood cells
RBCW Red blood cell water
RBCS Red blood cell solids

Table 3.8. Equations used in the present study for the calculation of RBC
and plasma compartments and lactate contents and concentrations within
these compartments.

PW = (1 - B-PCV).P-Vw

PS = (1 - B-PCV) - PW

RBCW = B-Vw - PW

RBCS = B-PCV - RBCW

Plasma lactate content (mmol) = (1 - B-PCV).Plasma lactate conc.(mmol/l)

Plasma lactate conc.(mmol/l PW) = Plasma lactate conc.(mmol/l) / P-Vw

RBC lactate content (mmol) = (Blood Lac. conc.(mmol/l) - Plasma content (mmol)

RBC lactate conc. = RBC lactate content (mmol) / B-PCV

RBC lactate conc.(mmol/l RBCW) = RBC lactate content (mmol) / RBCW

Figure 3.5. Lactate contents and concentrations in whole blood, RBC and plasma and volume of the different blood compartments before (PRE) and immediately after 2 min treadmill exercise at 12 m/s with 5° incline in horse SM. Abbreviations: PV - plasma volume; PW - plasma water; PS - plasma solids; PCV - packed cell volume; RBCW - red blood cell water; RBCS - red blood cell solids.

PRE

Plasma
lactate
content
0.5 mmol

Whole blood
lactate conc.
0.7 mmol/l

Red
blood
cell
lactate
content
0.2 mmol

Plasma
lactate
0.9
mmol/l PW

RBC
lactate
0.8
mmol/l RBCW

PV
0.565 l

PW
0.532 l

PS 0.033 l

PCV
0.435 l

RBCW
0.297 l

RBCS
0.138 l

O'R

Plasma
lactate
content
10.1 mmol

Whole blood
lactate conc.
18.3 mmol/l

Red
blood
cell
lactate
content
8.2 mmol

Plasma
lactate
34.1
mmol/l PW

RBC
lactate
17.6
mmol/l RBCW

PV
0.320 l

PW
0.297 l

PS
0.023 l
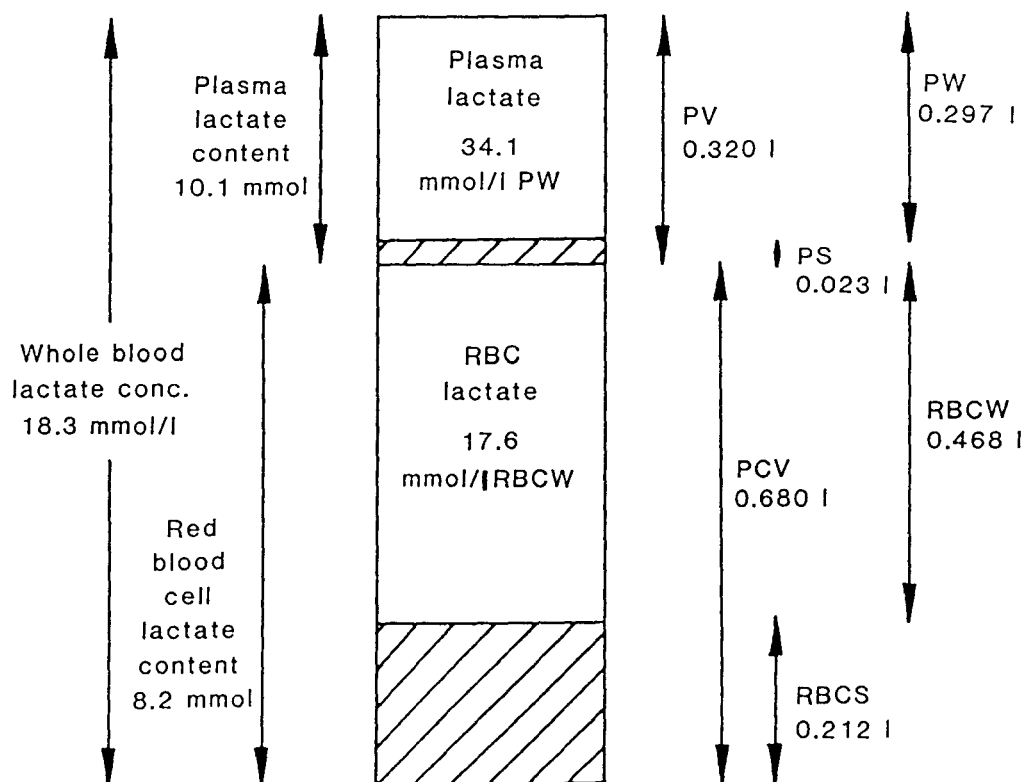
PCV
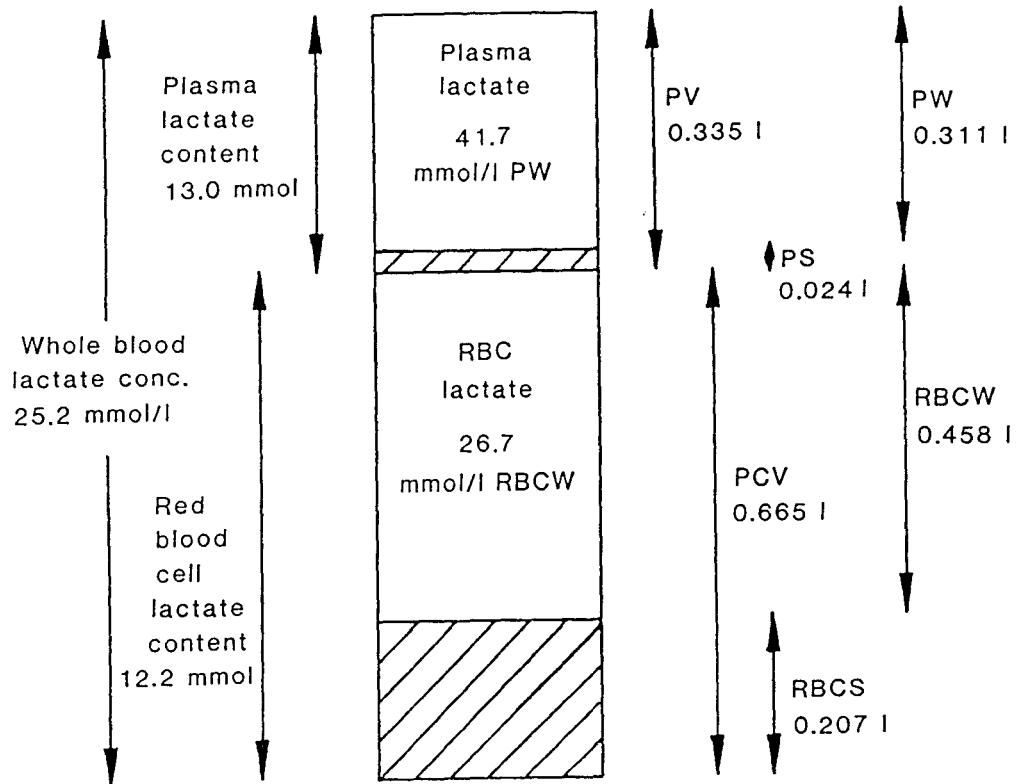0.680 l

RBCW
0.468 l

RBCS
0.212 l

51

Figure 3.6. Lactate contents and concentrations in whole blood, RBC and plasma and volume of the different blood compartments at 10 and 30 min recovery following 2 min treadmill exercise at 12 m/s with 5° incline in horse SM. Abbreviations: PV — plasma volume; PW — plasma water; PS — plasma solids; PCV — packed cell volume; RBCW — red blood cell water; RBCS — red blood cell solids.

10'R

Plasma lactate content 13.0 mmol

Whole blood lactate conc. 25.2 mmol/l

Red blood cell lactate content 12.2 mmol

Plasma lactate
41.7 mmol/l PW

RBC lactate
26.7 mmol/l RBCW

PV 0.335 l

PW 0.311 l

PS 0.024 l

RBCW 0.458 l

PCV 0.665 l

RBCS 0.207 l

30'R

Plasma lactate content 12.4 mmol

Whole blood lactate conc. 20.5 mmol/l

Red blood cell lactate content 8.2 mmol

Plasma lactate
29.2 mmol/l PW

RBC lactate
21.8 mmol/l RBCW

PV 0.450 l

PW 0.423 l

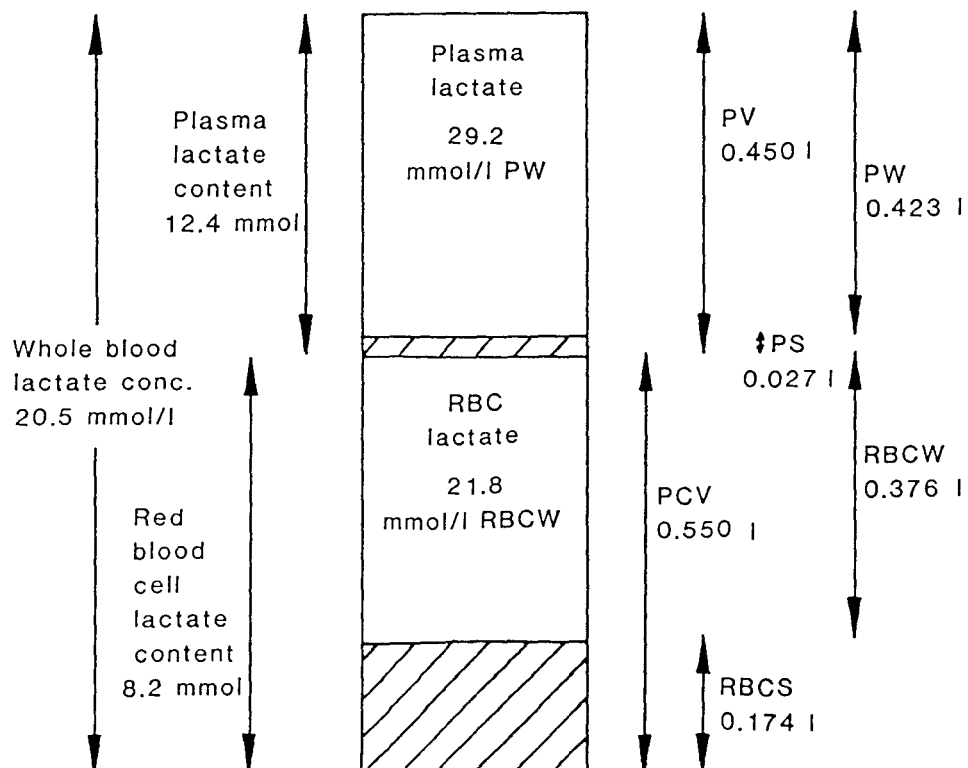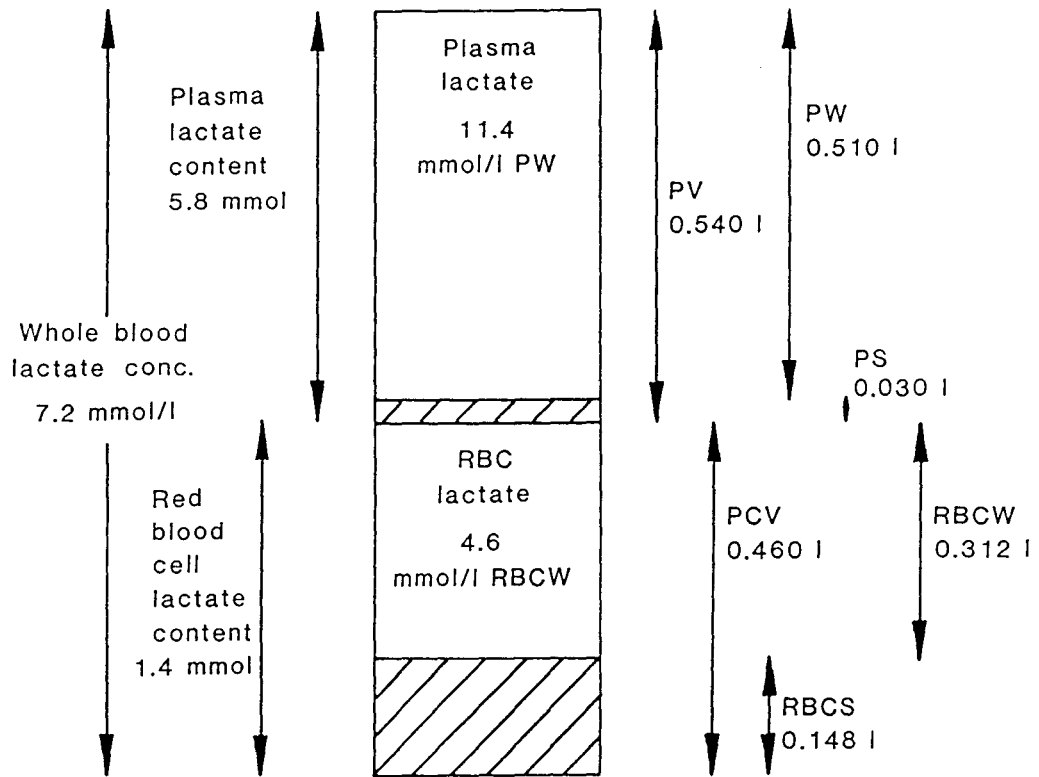PS 0.027 l

RBCW 0.376 l

PCV 0.550 l

RBCS 0.174 l

Figure 3.7. Lactate contents and concentrations in whole blood, RBC and plasma and volume of the different blood compartments at 70 min recovery following 2 min treadmill exercise at 12 m/s with 5° incline in horse SM. Abbreviations: PV - plasma volume; PW - plasma water; PS - plasma solids; PCV - packed cell volume; RBCW - red blood cell water; RBCS - red blood cell solids.

Plasma
lactate
content
5.8 mmol

Whole blood
lactate conc.
7.2 mmol/l

Red
blood
cell
lactate
content
1.4 mmol

Plasma
lactate

11.4
mmol/l PW

RBC
lactate

4.6
mmol/l RBCW

PV
0.540 l

PW
0.510 l

PS
0.030 l

PCV
0.460 l

RBCW
0.312 l

RBCS
0.148 l

3.8. Mean changes in plasma and RBC lactate content (where content refers the absolute mmol of lactate in the plasma or RBC space in 1 litre of blood) for the three horses are shown in Table 3.9. Mean whole blood lactate concentration was essentially unchanged during the period of the incubation, but mean plasma lactate concentration fell from 28.1±1.8 to 24.1±1.3 mmol/1 between time 0 and 10 min of incubation. Following this point in time there was only a slow downward drift. RBC and plasma lactate contents showed the same trends due to the fact that B-PCV remained constant.

Resting muscle blood content: Table 3.10 shows resting muscle blood contamination of nine biopsies of the middle gluteal estimated from muscle 2,3 DPG content. The mean resting blood content was estimated to be 68±35 ml/kg wet muscle.

DISCUSSION

Changes in PCV and TP with exercise and the effect on calculation of metabolite concentrations in whole blood and plasma

The present study demonstrated that although large changes occur with short term maximal exercise in the horse in the water content and density of whole blood and plasma, the errors introduced in the estimation of blood and plasma lactate by adopting values of 0.800 1/1 and 1.04 g/ml for B-Vw and B-D, respectively, and 0.935 1/1 for plasma P-Vw, are relatively small (approximately 2% for whole blood lactate and 1% for plasma lactate). For whole blood this is probably due to the changes in B-Vw and B-D being approximately equal and opposite, and therefore, cancelling each other out. The mean resting (Pre) values for B-Vw and B-D in this study were 0.831±0.016 1/1 and 1.0565±0.0055 g/ml, respectively, and it is proposed that adopting these values as opposed to the human values mentioned previously would be more appropriate and reduce the error. In any case, the error introduced by adopting either of these pairs of values is still likely to be within the analytical error. On the basis of these observations it would seem that there can be little justification for the extra complication of calculating individual values of B-Vw and B-D from the equations E(6) and E(7).

For plasma lactate, the use of a constant value of 0.935 1/1 for P-Vw did not result in any appreciable difference from the calculations based on P-Vw estimated from the regression with P-TP. Mean resting P-Vw in this study was 0.941±0.003 1/1 and is therefore very close to the value of 0.935

Table 3.9. Changes in whole blood and plasma lactate concentrations and RBC and plasma lactate content following addition of lactate in vitro.

		Incubation time (min)						
		0	5	10	15	20	25	30
Blood lactate concentration (mmol/l)	Mean	18.5	17.4	18.1	17.3	17.2	17.5	17.6
	sd	±1.3	±0.6	±0.8	±0.8	±0.8	±0.9	±0.8
	n = 3							
Plasma lactate concentration (mmol/l)	Mean	28.1	25.1	24.1	23.8	23.0	22.8	23.0
	sd	±1.8	±1.4	±1.3	±1.0	±1.0	±0.8	±0.7
	n = 3							
RBC lactate content (mmol)	Mean	1.7	3.4	4.0	4.2	4.6	4.7	4.6
	sd	±1.2	±1.2	±1.2	±1.3	±1.4	±1.4	±1.7
	n = 3							
Plasma lactate content (mmol)	Mean	16.4	14.7	14.1	13.9	13.5	13.4	14.0
	sd	±0.8	±0.6	±0.7	±0.7	±0.9	±0.8	±0.3
	n = 3							

Table 3.10. Resting muscle blood content estimated from the measurement of 2,3 DPG in paired muscle and blood samples.

Sample	Muscle 2,3 DPG mmol/kg d.m.	Blood 2,3 DPG mmol/l	kg dry blood /kg d.m.	1 blood /kg d.m. (a)	1 blood /kg w.m. (b)
1	0.87	1.75	0.0888	0.525	0.131
2	0.70	3.05	0.0410	0.243	0.060
3	0.28	3.12	0.0160	0.095	0.024
4	0.48	2.36	0.0363	0.215	0.054
5	1.20	3.65	0.0587	0.347	0.087
6	0.58	3.51	0.0295	0.175	0.044
7	0.34	3.13	0.0194	0.115	0.029
8	0.69	1.83	0.0673	0.398	0.100
9	0.64	2.12	0.0539	0.319	0.080
Mean	0.64	2.72	0.0457	0.270	0.068
sd	±0.28	±0.72	±0.0237	±0.140	±0.035

Footnote: (a) 1 blood/kg d.m. = kg dry blood per kg d.m. / (1 - B-Vw).B-Vw taken as resting mean value obtained in the present study of 0.831 1/1.

(b) calculated assuming a muscle water content of 75%.

w.m. = wet muscle.

1/1 used for P-Vw to date. Again, the similarity of the uncorrected and corrected plasma lactate concentrations would not appear to indicate the necessity of a plasma total protein estimation on every sample to be analysed for plasma lactate.

The present study defines the changes that occur in the density and water content of blood and plasma with short-term, high-intensity exercise in horses with normal hydration. Horses that are dehydrated, either through diet or as a result of prolonged sweating during exercise may be expected to show different relationships between PCV and B-D, and B-Vw, to those described in Figure 3.4. This may be particularly relevant to horses competing in long-distance endurance events.

In the comparison of P-Vw with P-TP, a slightly different relationship was found to that reported by Carlson and Harrold (1977) and shown in Figure 3.3 as a dashed line. The latter relationship was derived from the analysis of samples from 53 horses and although the slopes of the two lines are very similar, there is a marked difference in the intercepts. In the study of Carlson and Harrold, the estimated P-Vw at a protein concentration of 0 g/l was 0.985 1/1, whilst in the present study, the corresponding estimate was 0.997 1/1. This implies volumes for non-protein solids of 15 and 3 ml/l, respectively. The difference between the two estimates is unlikely to have arisen as a result of a failure to adequately dry samples, although it should be noted that the method employed by Carlson and Harrold was more extreme than in the present study. Most probably it reflects differences in the methods of protein estimation.

Distribution of lactate between plasma and red blood cell compartments

The distribution of lactate between red blood cell and plasma compartments has been investigated in man (Johnson et al, 1945; Huckabee, 1956; Buono and Yeager, 1986), sheep (Naylor et al, 1984), guinea pigs (Daniel et al, 1964) and in a variety of species, including cats and rabbits, by Devadatta (1935).

In the present study, a large gradient in lactate concentration was observed between plasma water (PW) and red blood cell water (RBCW). However, in terms of content, the RBC compartment of the blood is quantitatively almost as important as the plasma compartment in the transport of lactate in the blood as a whole. At any point in time, however, it is the lactate in the plasma which determines the gradient between the blood and the tissues, or more correctly, the plasma lactate concentration in terms of plasma water and the tissue content in terms of

intracellular water.

Following exercise, lactate is removed from the plasma by tissues such as heart, muscle and liver. In contrast to man, where there is no increase in B-PCV, in the horse the disappearance of lactate during recovery will also take place due to the removal of red blood cells containing lactate from the circulation when these are re-sequestered by the spleen. From data obtained post-mortem by Stadden, Weaver and Lunn (1984), the spleen of a 500 kg horse is estimated to have a blood content at rest of approximately 10 litres. In 9 horses, in which the spleen had been removed following either euthanasia or under anaesthesia (Catling, 1978), the mean splenic haematocrit (B-PCV) was around 60% greater than the circulating venous B-PCV. Assuming complete emptying of the spleen with exercise, during the recovery period, the uptake of 10 litres of blood with a final splenic B-PCV of 0.688 1/1 (based on splenic haematocrit being approximately 60% greater than a resting B-PCV of 0.43 1/1) represents the removal of around 7 litres of red cells from the circulation.

In the present study, by 70 minutes recovery B-PCV in all 4 horses was close to pre-exercise values. During this period the mean red blood cell lactate decrease amounted to approximately 11 mmol/1. The cells being taken up by the spleen at the start of the recovery period would obviously have had a higher lactate content than those being taken up at the end of the recovery period, but using an average value of 5.5 mmol/1 and assuming a linear fall in B-PCV and lactate during this time, the removal of red cells from the circulation by the spleen could account for the disappearance of around 40 mmol of lactate.

Mean peak whole blood lactate was 21.2 mmol/1 and with a blood volume in the region of 60 litres (Catling, 1978; Stadden, Weaver and Lunn, 1984), total lactate in the circulation would be of the order of 1300 mmol. On the basis of these estimates the uptake of red blood cells containing lactate by the spleen during the recovery period could only account for somewhere around 3-5% of the total lactate disappearance.

Lactate measurements: Whole blood or plasma ?

The results of the in vitro study in the present chapter indicate that at 37°C redistribution of lactate between plasma and red cells when added to whole blood at a final concentration of approximately 18 mmol/1 appeared to reach an equilibrium in around 5-10 minutes. This is in close agreement with studies on human blood with lactate added to final concentrations of between 10 and 19 mmol/1 whole blood (Johnson et al, 1945).

These observations suggest that in certain situations lactate in blood samples may have equilibrated between cells and plasma. Whilst this is irrelevant for whole blood lactate determinations there may be implications for treatment of samples for plasma lactate. Blood samples in which the lactate may not have reached an equilibrium distribution between cells and plasma are likely to be those taken during or in the early stages of recovery following moderate to high-intensity, short term exercise. This is in turn due to the large and relatively rapid efflux of lactate from muscle both during exercise and in the early stages of recovery.

In this situation, one of two procedures could be adopted. Ideally, the sample should be immediately separated in a refrigerated centrifuge at 0°C, as diffusion and/or transport of lactate into red cells has been shown to slow down dramatically with reduced blood temperature (Johnson et al, 1945). Alternatively, all samples should be allowed to stand to reach equilibrium before the plasma is separated.

As to whether whole blood or plasma lactate is analysed will depend to a certain extent on the conditions under which samples are being collected and the aims of the study. The collection of whole blood into PCA is well suited to field situations or when dealing with large numbers of samples. Determination of plasma lactate may be more appropriate in certain cases, such as when gradients between blood and tissues are being investigated, as it is the plasma lactate which is being exchanged with the perfused tissues.

Correction of muscle samples for blood contamination

Contamination of muscle biopsies by varying amounts of blood is a major problem in samples taken following exercise, and particularly so in the horse. To some extent this can be overcome by careful dissection of samples after freeze drying, but even then contamination can account for up to 40% of the sample weight. To allow for such variations in blood contamination, the contents of muscle located metabolites measured in a sequence of biopsies can be referred to a constant value of total creatine (TCr). This is usually either the resting or highest value in a series and acts as an internal reference (Harris et al, 1976). This will additionally correct for errors arising from the variable inclusion in the muscle samples of other non-muscle-cell elements, such as connective and fat tissues and variations in muscle glycogen content. Other reference bases include NAD (Sabina et al, 1984a) and DNA. Metabolites which occur in significant amounts in both muscle and blood, such as lactate and glucose, cannot be TCr adjusted until the amount present in the contaminating blood

has been calculated and eliminated. In order to do this an estimate of the resting muscle blood content must be obtained.

Dahlberg (1983) indicates a muscle blood content at rest of 10 ml/kg wet muscle for man. Based on a 75% muscle water content, the estimated blood content per kg dry muscle would be 40 ml which after drying would yield approximately 7g of dried blood per kg of dry muscle. This is probably an underestimate for the horse, as on visual examination alone, muscle biopsies from the horse are more bloody than those from human muscle. The analysis of 2,3 DPG in muscle and blood in the present study indicated a mean resting muscle blood content of 68 ml/kg wet muscle. However, this is probably an overestimate, as this method assumes the same B-PCV in central venous blood and the blood perfusing the muscles. Whilst this may be the case during exercise, it is unlikely that this is the situation at rest due to the low blood flow and its distribution.

A higher B-PCV in the blood perfusing the muscle bed would lead to an overestimation of muscle blood contamination, due to underestimation of the muscle blood 2,3 DPG content. For example, if the 2,3 DPG content of blood perfusing the muscle was 25% higher than that of central venous blood, this would result in a similar degree of overestimation in muscle blood contamination. Stadden, Weaver and Lunn (1984) estimated the resting muscle blood content in horses post-mortem to be 30-40 ml/kg wet muscle. Thus, an assumed resting muscle blood content of around 15 ml/kg wet muscle would seem reasonable and this would yield approximately 10g of dried blood per kg of dry muscle.

Although creatine is found in blood, its concentration is so low compared to muscle (Wu et al, 1983) that its contribution to the apparent TCr content in the biopsy can be ignored, even in the most heavily contaminated samples. Thus, the true TCr content of bloodless muscle (TCr~) is given by:

E(10) $TCr\text{~} = TCr / (1 - 0.01)$

assuming a resting muscle dry blood content of 0.01 kg/kg d.m. and where both TCr~ and TCr are in mmol/kg d.m.. TCr can now be used as a reference base to calculate within a series of muscle biopsies the volume of blood contaminating the sample. For this, values of B-D and B-Vw, corresponding to the time at which the biopsy was taken, are required. As noted previously, as B-Vw and B-D are calculated from the regression with B-PCV, an assumption has been made that central B-PCV (the B-PCV measured in blood collected from the jugular vein) corresponds to the peripheral B-PCV (the

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B-PCV in the blood actually perfusing the muscle bed).

E(11) Blood contamination = [(1 - TCr/TCr~)] / (B-D - B-Vw)
       (1/kg d.m.)

From a knowledge of the blood concentration (B-C, mmol/l) of the metabolite being assessed, the amount of that particular metabolite in the biopsy sample can be calculated. If this is then subtracted from the measured biopsy content (M-C, mmol/kg d.m.) and the result multiplied by TCr~/TCr, the true muscle content (TMC) expressed as mmol/kg blood free dry muscle is obtained.

E(12) TMC = [M-C - (B-C.   (1-TCr/TCr~)/(B-D - B-Vw))].TCr~/TCr

For metabolites such as ATP where the blood concentration (B-C) is very much lower than the muscle content, E(12) reduces to:

E(13)   TMC = M-C.TCr~/TCr

Tables 3.11 to 3.14 list the muscle contents of TCr, ATP and lactate for the four horses presented in the studies in Chapters 6 and 7 before exercise and during 70 minutes recovery. In Table 3.11 (horse JW) the 30 minute sample shows a marked drop in TCr content which is also reflected in the ATP content, indicating heavy blood contamination in this sample. To compensate for this contamination and underestimation of true muscle tissue present in the sample, values in (B) have been adjusted to TCr~ (in this case, corresponding to the 'Pre' sample divided by 0.99, see E(10)). This is justified by the small within-muscle variance in TCr (mean weighted within-horse standard deviation = 5.7 mmol/kg d.m., coefficient of variation = 5.1% - based on 3 resting biopsies from each of 5 horses). This greatly improves the trend in muscle ATP within this series but not the trend in lactate. In the latter case, the trend in lactate has clearly been made worse by TCr correction.

In (C), the estimated blood contents of the individual samples are shown. These contents are for biopsy samples which have already been dissected free of blood as much as possible, but in the case of the 30 minute sample, there is still a blood content equivalent to 1.5 litres for every kg of dry muscle solids. The true muscle contents of lactate are shown in (D) and were calculated using the corresponding blood lactate concentration for each time point. As ATP in blood is present in very low concentrations relative to muscle, no further correction to muscle ATP

Table 3.11 Muscle contents of TCr, ATP and lactate before and after 2 minutes treadmill exercise at 12 m/s on 5° incline and during 70 minutes (S) recovery in horse JW.

	Pre	0	10	30	70
(A) Raw data (mmol/kg d.m.)					
TCr	102.05	91.08	93.35	59.85	103.05
ATP	20.92	9.48	11.72	7.41	18.55
Lactate	9.41	128.17	83.09	63.26	13.58
(B) Adjusted to TCR~ (103.08 mmol/kg d.m.)					
ATP	21.13	10.73	12.94	12.76	18.56
Lactate	9.51	145.06	91.75	108.95	13.58
(C) Blood contamination (1/kg d.m.)	0.040	0.372	0.308	1.487	0.000
(D) True Muscle Content (mmol/kg d.m.)					
ATP	21.13	10.73	12.94	12.76	18.56
Lactate	9.48	138.28	85.72	59.04	13.58

Footnote: (B) Adjusted to TCr~ of 102.05/0.990 = 103.08 mmol/kg d.m.

(C) Calculated using E(6) and E(7) to estimate B-D and B-Vw from the changes in B-PCV (Table 3.4)

(D) Blood lactate concentrations are taken from row B), Table 3.2.

Table 3.12 Muscle contents of TCr, ATP and lactate before and after 2 minutes treadmill exercise at 12 m/s and during 70 minutes (S) recovery in horse SL.

	Pre	0	10	30	70
(A) Raw data					
(mmol/kg d.m.)					
TCr	106.02	100.49	80.84	90.17	72.55
ATP	29.29	22.98	19.81	24.34	19.32
Lactate	19.53	65.05	57.48	22.39	17.30
(B) Adjusted to TCr~					
(107.09 mmol/kg d.m.)					
ATP	29.59	24.49	26.24	28.91	28.52
Lactate	19.72	69.32	76.14	26.59	25.54
(C) Blood contamination					
(l/kg d.m.)	0.050	0.213	0.908	0.687	1.581
(D) True Muscle Content					
(mmol/kg d.m.)					
ATP	29.59	24.49	26.24	28.91	28.52
Lactate	16.69	66.81	59.26	19.08	20.03

Footnote: (B) Adjusted to TCr~ of 106.02/0.990 = 107.09 mmol/kg d.m.

(C) Calculated using E(6) and E(7) to estimate B-D and B-Vw from the changes in B-PCV (Table 3.4)

(D) Blood lactate concentrations are taken from row B), Table 3.2.

Table 3.13 Muscle contents of TCr, ATP and lactate before and after 2 minutes treadmill exercise at 12 m/s and during 70 minutes (S) recovery in horse SM.

	Pre	0	10	30	70
(A) Raw data (mmol/kg d.m.)					
TCr	101.56	84.45	74.60	74.95	95.56
ATP	23.59	13.73	11.56	15.53	21.29
Lactate	7.16	110.69	100.25	59.67	16.69
(B) Adjusted to TCr~ (102.59 mmol/kg d.m.)					
ATP	23.83	16.68	15.90	21.26	22.76
Lactate	7.23	134.47	137.86	81.68	17.84
(C) Blood contamination (1/kg d.m.)	0.044	0.563	0.886	1.005	0.273
(D) True Muscle Content (mmol/kg d.m.)					
ATP	23.83	16.68	15.90	21.26	22.76
Lactate	7.20	121.92	107.16	53.43	15.73

Footnote: (B) Adjusted to TCr~ of 101.56/0.990 = 102.59 mmol/kg d.m.

(C) Calculated using E(6) and E(7) to estimate B-D and B-Vw from changes in B-PCV (Table 3.4)

(D) Blood lactate concentrations are taken from row B), Table 3.2.

Table 3.14 Muscle contents of TCr, ATP and lactate before and after 2 minutes treadmill exercise at 12 m/s and during 70 minutes (S) recovery in horse HR.

	Pre	0	10	30	70
(A) Raw data					
(mmol/kg d.m.)					
TCr	114.07	104.07	106.84	110.12	100.33
ATP	24.86	15.26	18.23	21.76	22.04
Lactate	10.32	124.81	89.48	35.12	14.94
(B) Adjusted to TCr~					
(115.22 mmol/kg d.m.)					
ATP	25.11	16.85	19.66	22.77	25.31
Lactate	10.42	137.82	96.50	36.75	17.16
(C) Blood contamination					
(l/kg d.m.)					
	0.045	0.342	0.276	0.180	0.577
(D) True Muscle Content					
(mmol/kg d.m.)					
ATP	25.11	16.85	19.66	22.77	25.31
Lactate	10.40	131.41	89.47	33.22	13.49

Footnote: (B) Adjusted to TCr~ of 114.07/0.990 = 115.22 mmol/kg d.m.

(C) Calculated using E(6) and E(7) to estimate B-D and B-Vw from the changes in B-PCV (Table 3.4)

(D) Blood lactate concentrations are taken from row B), Table 3.2.

contents has been made and these correspond to the corrected values in (B). In (D), the corrected muscle lactate contents clearly show a more orderly progression with recovery than in (B). There is a close similarity between the lactate values in (A) and (D), due to the lactate content of dry blood being close to that of dry muscle. However, whilst this applies to lactate, this will not always be the case, particularly with metabolites such as glucose and glycerol, which are present in much higher amounts in muscle compared to blood following exercise.

In the correction of muscle lactate contents the assumption was made that the concentration of lactate in venous blood collected from the jugular vein can be used to estimate the concentration in the blood perfusing the middle gluteal. Improvements in the trend for lactate when correction for blood contamination was applied were also seen in the 10 minute recovery samples for both SL (Table 3.12) and SM (Table 3.13).

In conclusion, where concentrations of metabolites in blood are low compared to those in muscle, it is acceptable to refer muscle contents to a standard TCr content (TCr~), for example in the case of ATP. Where the concentrations of the metabolite in the blood is high, muscle contents should be corrected using equation E(12), for example in the case of lactate. If this is not possible, i.e. in the absence of a corresponding blood concentration, then no correction should be applied, though muscle located metabolites such as ATP should still be corrected.

With respect to calculation of blood and plasma metabolite concentrations, the use of constant values of 0.831 1/1 (B-Vw) and 1.0565 g/ml (B-D) for blood and 0.941 1/1 (P-Vw) for plasma would seem to be adequate in most cases and the calculation of individual values of B-Vw and B-D and P-Vw from B-PCV and P-TP, respectively, appears to be unnecessary in view of the relatively small improvement attained.

CHAPTER 4

MUSCLE BUFFERING CAPACITY IN THE THOROUGHBRED HORSE

INTRODUCTION

Intense physical exercise in the horse requires a high rate of glycolysis in muscle to support an adequate rate of energy production, resulting in the accumulation of lactate and hydrogen ions (H^+). In man, muscle lactate contents approaching 140 mmol/kg dry muscle (d.m.) and a decrease in muscle pH to around 6.5 have been observed after intense dynamic exercise (Sahlin et al, 1976). Higher muscle lactate contents have been observed in the horse; individual values of 200 mmol/kg d.m. have been reported following repeated maximal gallops (Snow, Harris and Gash, 1985). These authors estimated that such an increase will result in a fall in muscle pH to a value approaching 6.2. Following single bouts of maximal exercise under conditions and distances comparable to racing, muscle lactate contents of approximately 150 mmol/kg d.m. and muscle pH values of 6.5 have been observed (Chapter 6).

An increase in muscle lactate content of 150 mmol/kg d.m. with exercise represents the production of 150,000 μmol H^+/kg d.m. However, the fall in muscle pH from 7.1 at rest to 6.5 following exercise will result in the free H^+ content increasing by only 0.71 μmol/kg d.m. Although a proportion of the H^+ produced will be lost from the muscle to the circulation, this example clearly illustrates the importance of muscle buffering to muscle and whole body acid-base homeostasis.

Proton buffering in mammalian muscle is accomplished primarily by physico-chemical buffering by proteins (Sahlin, 1978), dipeptides (Burton, 1978; Somero, 1981) and inorganic phosphate and metabolic (dynamic) buffering by the removal of protons when bicarbonate is lost from the muscle, by the rephosphorylation of ADP by phosphocreatine and through the oxidation of amino acids (Hultman and Sahlin, 1980).

Muscle buffering of H^+ is of greatest importance in activities that result in the rapid and/or large accumulation of lactic acid. Failure to adequately buffer proton production may contribute to the onset of muscle fatigue, for example through disruption of the events involved in excitation-contraction coupling (Donaldson, Hermansen and Bolles, 1978) and effects on the contractile mechanism itself, for example a reduction in the affinity of calcium for troponin c (Fabiato and Fabiato, 1978).

The use of an index to estimate muscle buffering capacity could therefore be useful in evaluating athletic potential and physiological responses to training. The present study was undertaken to investigate in greater depth the homogenate method for determination of muscle buffering capacity (βm_{TIT}). More specifically, to determine which of the whole muscle

in vivo buffering mechanisms contribute to the in vitro determination. Factors affecting in vitro βm measurement and the reproducibility of the method were also investigated.

MATERIALS AND METHODS

(1) Standard Method: Forty mg of wet muscle (w.m.) or 10 mg of freeze-dried muscle (d.m.) were homogenized at 4°C in 1 ml of a solution containing 145 mmol/l KCl, 10 mmol/l NaCl and 5 mmol/l iodoacetic acid (sodium salt), pH 7.0, for 1 minute with a Polytron homogenizer at speed 7. The iodoacetic acid was included to block glycolysis which would otherwise occur on homogenization. Homogenates were equilibrated to 37°C for 5 minutes and the initial pH (pHm_i) recorded. If the pH was less than 7.1, this was adjusted upwards with 50 mmol/l NaOH.

Homogenates were titrated against 20 μl aliquots of a standard 10 mmol/l solution of HCl added with a 25 μl Hamilton glass syringe. Homogenates were stirred continuously and maintained at 37°C throughout the titration (approximately 5 min). Muscle homogenate pH was measured with a MI-410 microelectrode (Microelectrode Inc) fitted to a Corning 150 pH/ion meter (Figure 4.1). Muscle buffering capacity (βm) is expressed as the μmol H^+ required to change the pH of 1 g of w.m. or d.m. from pH 7.1 to pH 6.5 (see results for explanation).

(2) Effect of muscle concentration on βm_{TIT} and pHm_i: Portions from a large piece of fresh muscle were homogenized at concentrations of 2.5, 5, 10, 20, 30, 40 and 70 mg/ml. Homogenates were then titrated according to the standard method (1). Three homogenates were prepared for each concentration.

(3) Effect of different lengths of incubation at 37°C following homogenization on βm_{TIT}: Three series of five homogenates were made from pooled freeze-dried muscle as described in (1) and were incubated for 5, 10, 15, 30 and 60 min at 37°C before titration to investigate whether incubation time had any effect on βm_{TIT}.

(4) Loss of homogenate on homogenization: Significant amounts of homogenate remaining attached to the shaft of the homogenizer will result in an underestimation of βm_{TIT}. This possibility was investigated as follows: three tubes containing 7 mg, 3 containing 9 mg and 3 containing 11 mg of pooled freeze-dried powder were weighed out. Homogenizing reagent (HMR)

Figure 4.1. Apparatus for determination of βm_{TIT}: A - Electrode; B - Waterbath; C - Homogenate; D - Stirring bar; E - Magnetic stirrer.
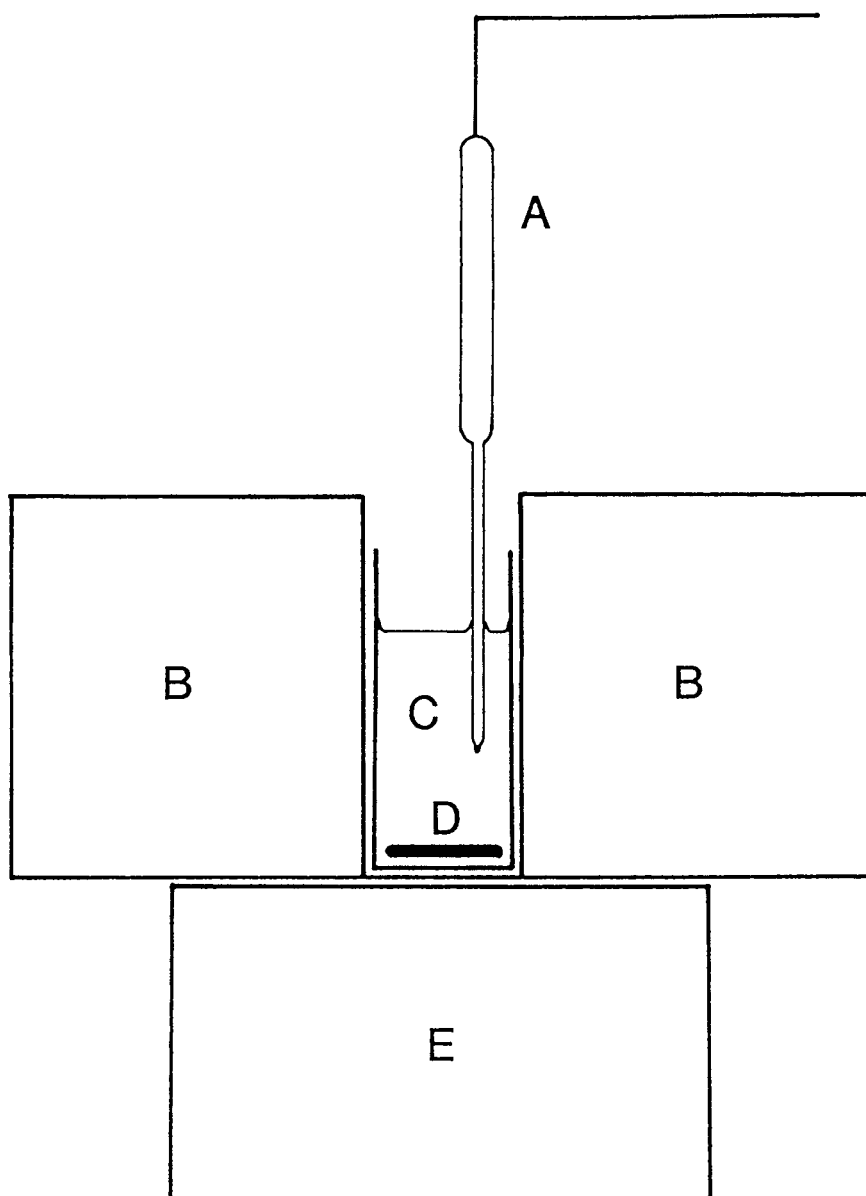
corresponding to 700 μl, 900 μl and 1100 μl was added to the appropriate tubes. Tubes containing muscle and HMR were weighed pre and post homogenization to calculate weight loss.

(5) Difference in βm_{TIT} between w.m. and d.m.: Two blocks of middle gluteal muscle from different regions (approximately 2-3 g each) were removed from a horse that had been euthanized 20 minutes previously. These were immediately frozen in liquid nitrogen and pulverized separately under nitrogen, until two relatively homogeneous pools of fragments were obtained. The pools were then divided in two and one half freeze-dried and powdered and the other kept under nitrogen. Five homogenates were made from each freeze-dried pool and five from each frozen pool. βm_{TIT} was measured as described in (1).

(6) Effect of added HCO_3^- on βm_{TIT}: Two pools of frozen and freeze-dried muscle were prepared as described in section (5). βm_{TIT} was determined as described in section (1) on 3 portions of w.m., on 3 portions of a d.m. pool and on 3 portions of the d.m. with HCO_3^- added in the proportion of 10 μl of 60 mmol $NaHCO_3$ per ml HMR.

(7) Muscle ATP, ADP, AMP and PCr content before and after homogenization: To attempt to establish whether or not dynamic creatine phosphokinase (CPK/PCr) buffering contributed to the βm_{TIT} measurements, ATP, ADP, AMP and PCr contents were analysed in muscle before and after homogenization. Two series of 4 tubes containing approximately 10 mg aliquots of d.m. powder were weighed out. HMR was added to one series of tubes and the samples homogenized as described in section (1). After 5 minutes incubation at 37°C, the samples were rapidly frozen in liquid nitrogen and then freeze-dried. When freeze-dried, the 4 homogenized samples and the 4 portions of original powder were extracted in perchloric acid and analysed for ATP, ADP, AMP and PCr as described in Chapter 2.

(8) Reproducibility of technique within and between homogenates: Five homogenates of approximately 3 ml each (i.e. 30 mg d.m. powder in 3 ml HMR) were made from a pool of d.m. βm_{TIT} was determined as described in section (1) in duplicate using two 1 ml aliquots to determine within homogenate variation. To determine between homogenate variation, a block of fresh/frozen muscle was pulverized under liquid nitrogen to yield a homogeneous pool. Five homogenates were prepared from this pool and titrated as in (1).

(9) <u>Comparison between βm determined by titration (βm$_{TIT}$) and βm calculated from the relationship between pH and lactate content (βm$_{pHvLAC}$).</u>

(a) <u>βm$_{pHvLAC}$ in samples collected following exercise</u>: Six muscle biopsies of the middle gluteal were collected from each of six horses on six separate occasions following exercise of 2 minutes duration at 7, 8, 9, 10, 11 or 12 m/s on a high speed treadmill and at rest to obtain samples with different muscle pH and lactate contents. Samples were collected within 30-60 seconds of the end of the exercise period and immediately frozen in liquid nitrogen. Samples were analysed for lactate and muscle pH as described in Chapter 2. βm$_{TIT}$ was determined by titration on the 2 samples collected from each horse at rest as described in section (1).

(b) <u>βm$_{pHvLAC}$ in samples collected post-mortem</u>: Following euthanasia there is a gradual increase in muscle lactate content and an accompanying fall in muscle pH due to anaerobic glycolysis. When estimating βm from the relationship between pH and lactate in samples obtained following dynamic exercise, the possibility exists that an underestimation or overestimation of βm may arise due to unequal loss of H$^+$ and lactate to the circulation. In estimating βm from the relationship between pH and lactate in muscle post-mortem, overestimation or underestimation of βm due to unequal loss of H$^+$ and lactate is unlikely due to the absence of muscle blood flow.

Muscle biopsies of the middle gluteal were taken at 20 minute intervals from each of 3 Thoroughbred horses that had been euthanized for reasons not connected with the musculoskeletal system. The first sample was obtained approximately 20 minutes post-mortem and samples were collected over a period of 2-3 hours. Samples were immediately frozen in liquid nitrogen and then freeze-dried. Muscle pH and lactate were determined as described in Chapter 2. βm$_{pHvLAC}$ was calculated from a plot of pH against muscle lactate as the amount of lactate (assumed to be equivalent to H$^+$ content) required to change the pH of 1g of d.m. from pH 7.1 to 6.5.

(10) <u>The HCO$_3^-$ content of HMR and effect of homogenization on HCO$_3^-$ content of homogenates</u>: Due to the vigorous nature of the homogenization process, the possibility that an increase in HCO$_3^-$ content may occur with homogenization was investigated. The HCO$_3^-$ content of 3 different stock solutions of HMR at 4°C was measured in duplicate on a Radiometer ABL3 blood gas analyser. To investigate whether there was any increase in HCO$_3^-$ content due to the homogenization process, the HCO$_3^-$ content of two homogenates prepared from each of the stock HMR solutions was analysed.

(11) <u>Normal values</u>: <u>Thoroughbred horse</u>, <u>Greyhound dog and Man</u>: Muscle biopsies of the middle gluteal were collected from 14 1yo, 35 2yo, 16 3yo and 9 4+yo Thoroughbred horses. (For a more detailed description of these populations see Chapter 5). Greyhound muscle samples were obtained from the biceps femoris, semitendinosis and deltoid muscles of 3 animals that had been euthanized. Human muscle biopsies were obtained from the vastus lateralis of 7 human subjects. All samples were frozen in liquid nitrogen, freeze-dried and βm_{TIT} determined as described in section (1).

(12) <u>Effect on</u> βm_{TIT} <u>of addition of carnosine to homogenates.</u>

(a) 1 ml of a 100 mmol/l standard solution of carnosine-HCL in distilled-deionized water was titrated against 50 μl aliquots of 100 mmol/l HCl at 37°C to estimate the β of carnosine over the pH range 7.1 to 6.5. The β of the carnosine standard was determined by titration rather than by calculation, as the latter would introduce errors due to impurity of the carnosine or to a higher water content than stated and errors in the titration method.

(b) Three homogenates of 2.5ml each (10 mg/ml d.m.) were prepared from a large pool of muscle powder. Each homogenate was then divided into two 1 ml aliquots. βm_{TIT} in 3 of the homogenates was then determined by the standard method (1). 20 μl of 100 mmol/l carnosine standard was added to each of the 3 remaining 1 ml aliquots. βm_{TIT} was then determined by the standard method (1).

(13) <u>Muscle dipeptide content and</u> βm_{TIT} <u>in Thoroughbred horse, Greyhound dog and man</u>: Muscle biopsies were obtained at rest from the middle gluteal of 5 Thoroughbred horses, from the vastus lateralis of 5 human subjects and post-mortem from the biceps femoris (n=3) and semitendinosus (n=2) of 5 Greyhound dogs. Samples were immediately frozen in liquid nitrogen, freeze-dried and powdered. Each sample was divided into two seperate portions for determination of βm_{TIT} and carnosine + anserine content as described in (1) and Chapter 2, respectively.

RESULTS

(1) <u>Observations on the standard method:</u> Titration of muscle homogenates, prepared from either d.m. or w.m., against 10 mmol/l HCl consistently showed a non-linear response (Figure 4.2). In every case a linear drop from 7.1 to around 6.8 was observed, followed by a second linear fall from 6.8 downwards, but with a different slope. The point at which the slope changed varied from as high as 6.9 to as low as 6.7. Because of this non-linearity in response to H^+, estimates of βm_{TIT} will vary according to the initial and final pH selected for the calculation. For this reason the calculations of βm_{TIT} are based on μmol required to change the pH of 1g d.m. or w.m. from 7.1 to 6.5. A pH of 7.1 represents the pH of resting muscle, and 6.5 is a pH frequently observed following moderate to intense field exercise in the horse (Harris et al, 1989).

(2) <u>Effect of muscle concentration on βm_{TIT} and pHm$_i$:</u> The effect of different ratios of HMR:muscle on βm_{TIT} and pHm$_i$ in homogenates prepared from the same block of muscle is shown in Figure 4.3. βm_{TIT} was independent of concentration above 10 mg w.m./ml, though pHm$_i$ continued to vary with concentration up to 40 mg w.m./ml. Consequently 40 mg w.m./ml or 10 mg d.m./ml was selected as the minimum concentration at which both βm_{TIT} and pHm$_i$ respectively are independent of concentration effects.

(3) <u>Effect of different lengths of incubation at 37°C following homogenization on βm_{TIT}:</u> The effect of incubation time from 5 to 60 minutes at 37°C before titration on βm_{TIT} are shown in Table 4.1. No significant effect of incubation time was found (P>0.05).

(4) <u>Loss of homogenate on homogenization:</u> Percentage weight loss on homogenization for samples of 7, 9 and 11 mg of d.m. are shown in Table 4.2. Combining the percentages for the 3 weights gives an error in the calculation of less than 3% due to loss on homogenization.

(5) <u>Difference in βm_{TIT} between w.m. and d.m.:</u> Differences in βm_{TIT} between d.m. and w.m. are shown in Table 4.3. Mean βm_{TIT} in w.m. was 29.7% greater in block A and 23.8% greater in block B compared to βm_{TIT} in d.m.. In both cases the difference was statistically significant (P<0.001). Freeze-drying, therefore, results in a loss in βm_{TIT} of approximately 25%.

(6) <u>Effect of added HCO_3^- on βm_{TIT}:</u> The βm_{TIT} of w.m., d.m. and d.m. plus HCO_3^- were 35.2±1.1, 27.3±1.1 and 32.2±0.6 μmol H^+/g w.m. 7.1-6.5

Figure 4.2. Representative titration curve of homogenate prepared from horse middle gluteal muscle as described in section (1).

Figure 4.3. Effect of different ratios of muscle:Homogenizing reagent (HMR) on βm_{TIT} (——) and pHm_i (- - -). Values are presented as the mean and standard deviation of three determinations.
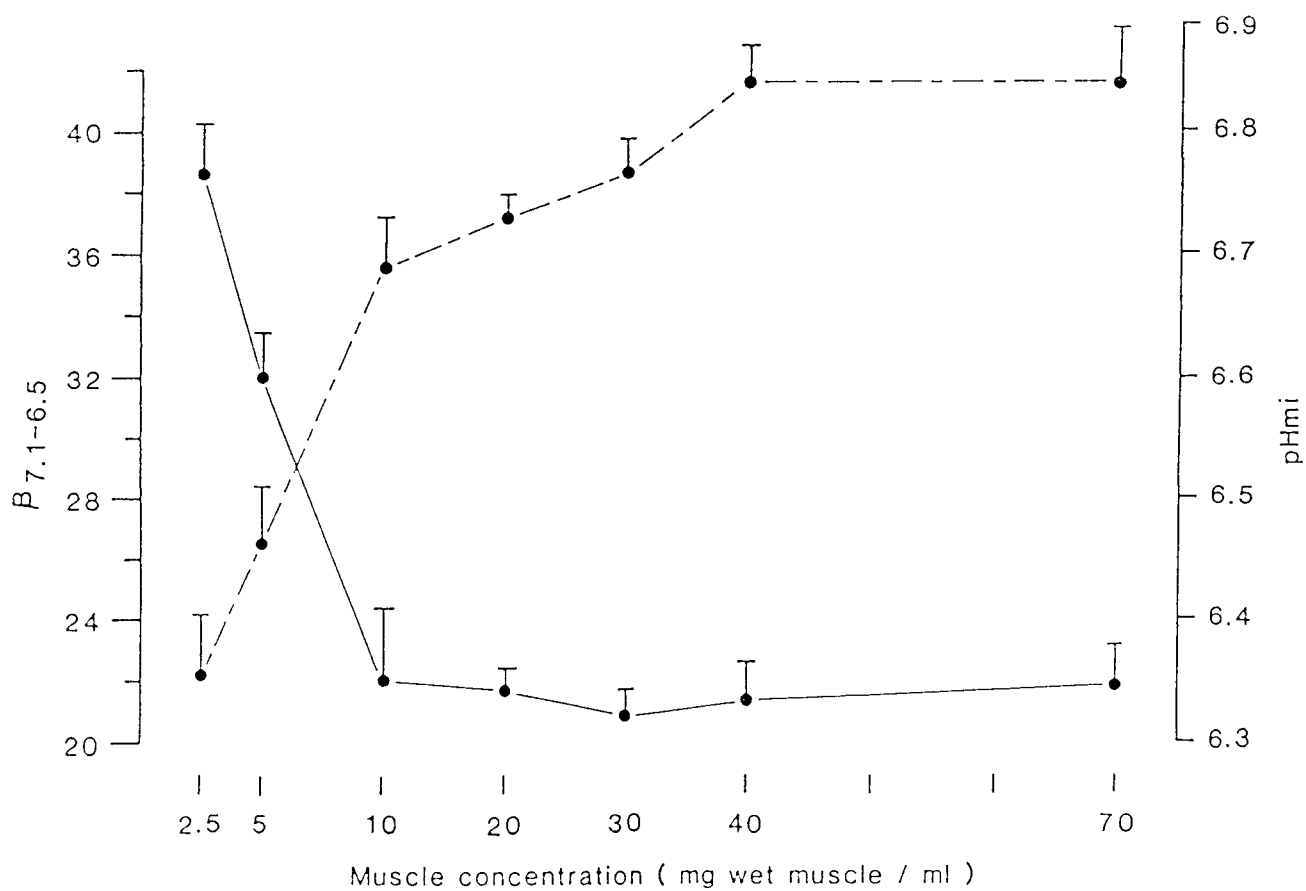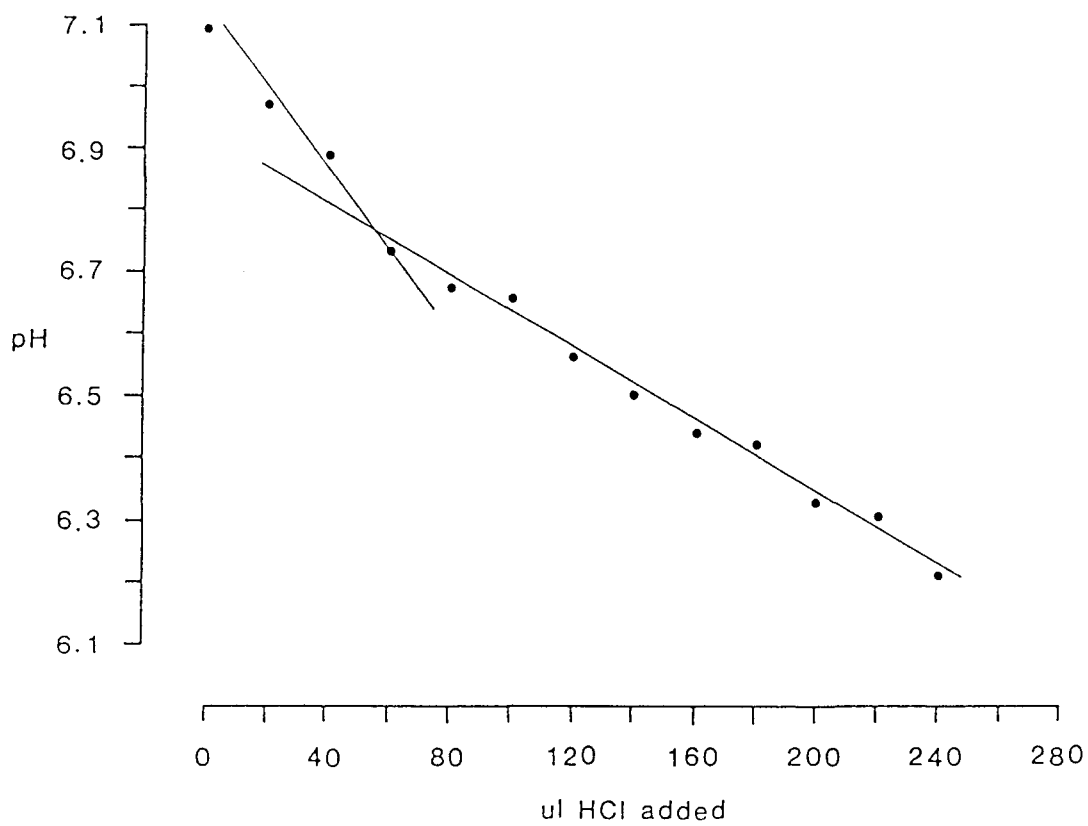
Table 4.1 Effect of incubation of homogenates on βm_{TIT} (μmol H$^+$/g d.m. pH 7.1- 6.5). Values are means of 3 determinations. Values for βm_{TIT} expressed in terms of wet muscle (w.m.) are in parentheses.

Incubation Time (min)	βm_{TIT}-d.m.	βm_{TIT}-w.m.
5	105.8	(26.5)
10	110.2	(27.5)
15	101.0	(25.3)
30	104.3	(26.0)
60	104.2	(26.0)

Table 4.2. Loss of homogenate due to homogenization technique. Values represent the mean±sd for 3 determinations.

Muscle + HMR	% Loss
7 mg in 700 μl	3.2±0.4
9 mg in 900 μl	2.3±0.2
11 mg in 1000 μl	3.0±0.3

Table 4.3. βm_{TIT} of d.m. versus w.m. (μmol H^+/g d.m. pH 7.1-6.5, 37°C).

	βm_{TIT}-d.m.		βm_{TIT}-w.m.	
	A	B	A	B
mean	65.5	98.9	85.0	122.4
sd	2.6	2.4	2.2	5.5
(n=5)				

A d.m. v w.m. % Difference +29.7%

B d.m. v w.m. % Difference +23.8%

A + B d.m. v w.m. % Difference +26.8% (mean)

Table 4.4. Muscle ATP, ADP, AMP and PCr contents in homogenized (H) and intact (I) muscle samples (mmol/kg d.m.) ND = not detected.

Sample	ATP		ADP		AMP		PCr	
	I	H	I	H	I	H	I	H
1	18.1	0.22	2.8	2.3	0.2	0.6	66.7	4.1
2	20.0	ND	2.9	2.0	0.2	1.4	72.6	4.2
3	18.3	ND	3.0	2.1	0.2	1.2	68.4	3.2
4	19.1	ND	2.9	2.1	0.2	1.2	69.8	2.7
mean	18.9	–	2.9	2.1	0.2	1.1	69.4	3.5
sd	0.9	–	0.1	0.1	0.0	0.3	2.5	0.7

respectively. Thus, the addition of HCO_3^- equivalent to 60 mmol HCO_3^-/kg d.m. (15 mmol HCO_3^-/kg w.m.) to the homogenate raised βm_{TIT} of the d.m. by 17.9%, bringing it close to that observed in w.m..

(7) <u>Muscle ATP, ADP, AMP and PCr content before and after homogenization:</u> Muscle ATP, ADP, AMP and PCr contents in homogenized and intact muscle samples are shown in Table 4.4. Homogenization resulted in the complete loss of ATP and PCr, with only small changes in ADP and AMP.

(8) <u>Reproducibility:</u> The coefficient of variation for βm_{TIT} in duplicate samples of the same homogenate was 2.7% (n = 5 pairs) and for total procedures (from division of muscle to titration) was 3.1% (βm_{TIT} = 10 determinations).

(9) <u>Comparison of βm determined by titration (βm_{TIT}) and βm calculated from the relationship between pH and lactate content (βm_{pHvLAC}).</u>

(a) βm_{pHvLAC} <u>in samples collected following exercise:</u> βm_{TIT} and βm_{pHvLAC} for the six horses are shown in Table 4.5. Mean βm was 115.0±12.0 and 166.8±51.3 μmol H+/g d.m. pH 7.1-6.5 for βm_{TIT} and βm_{pHvLAC}, respectively.

(b) βm_{pHvLAC} <u>in muscle samples collected post mortem:</u> βm_{TIT} and βm_{pHvLAC} for the 3 horses are shown in Table 4.6. Mean βm was 100.7±11.0 and 162.7±37.8 μmol/g d.m. pH 7.1-6.5 for βm_{TIT} and βm_{pHvLAC} respectively.

(10) <u>HCO_3^- content of HMR and effect of homogenizing on HCO_3^- content:</u> The mean HCO_3^- content of homogenates prepared from three stock HMR solutions was 0.70±0.01 mmol/l. This represents a mean increase in the HCO_3^- content due to homogenization of 0.07 μmol/ml HMR.

(11) <u>Normal Values: Thoroughbred horse, Greyhound dog and Man:</u> The mean, standard deviation and ranges for βm_{TIT} in muscle from Thoroughbred horse, Greyhound dog and man are shown in Table 4.7.

(12) <u>Effect on βm_{TIT} of addition of carnosine to homogenates</u>

(a) The titration curve of 1 ml of a 10 ml standard solution of carnosine in distilled-deionized water at 37°C is shown in Figure 4.4. The β of carnosine over the pH range 7.1-6.5 can be calculated as follows:

Table 4.5. Comparison of βm_{TIT} and βm_{pHvLAC} in samples collected following exercise.

	βm_{TIT} μmol H$^+$/g d.m. pH 7.1-6.5	βm_{pHvLAC} μmol H$^+$/g d.m. pH 7.1-6.5
H1	100.8	127.0
H2	131.5	125.0
H3	108.0	205.0
H4	120.6	246.0
H5	105.3	176.0
H6	123.5	122.0
mean	115.0	166.8
sd	12.0	51.3

Table 4.6. Comparison of βm_{TIT} and βm_{pHvLAC} in samples collected post mortem.

	βm_{TIT} μmol H$^+$/g d.m. pH 7.1-6.5	βm_{pHvLAC} μmol H$^+$/g d.m. pH 7.1-6.5
H1	88.0	192.0
H2	107.5	120.0
H3	106.7	176.0
mean	100.7	162.7
sd	11.0	37.8

Table 4.7. βm_{TIT} in the Thoroughbred horse, Greyhound dog and Man (μmol H^+/g d.m. pH 7.1-6.5).

Thoroughbred Horse

Gluteus medius

Age	Training Status	mean	sd	Range	n
1	UT	110.2	7.4	95.5-121.7	14
2	LT	115.3	8.2	100.8-132.2	28
	FT	106.1	7.4	91.7-114.2	7
3	FT	123.1	3.6	116.9-129.4	16
4+	LT-FT	117.8	13.4	102.2-149.8	9

Greyhound Dog

	Mean	sd	Range	n
Biceps femoris	118.4	2.2	115.9-120.0	3
Semitendinosis	119.0	6.0	114.2-125.8	3
Deltoid	103.4	5.3	98.4-109.0	3

Man

	Mean	sd	Range	n
Vastus lateralis	85.2	13.2	66.2-106.1	7

UT = Untrained
LT = Light/medium trained
FT = Fully trained

Figure 4.4. Titration curve of 1ml of a 100 mmol/l solution of Carnosine in distilled-deionized water at 37°C.
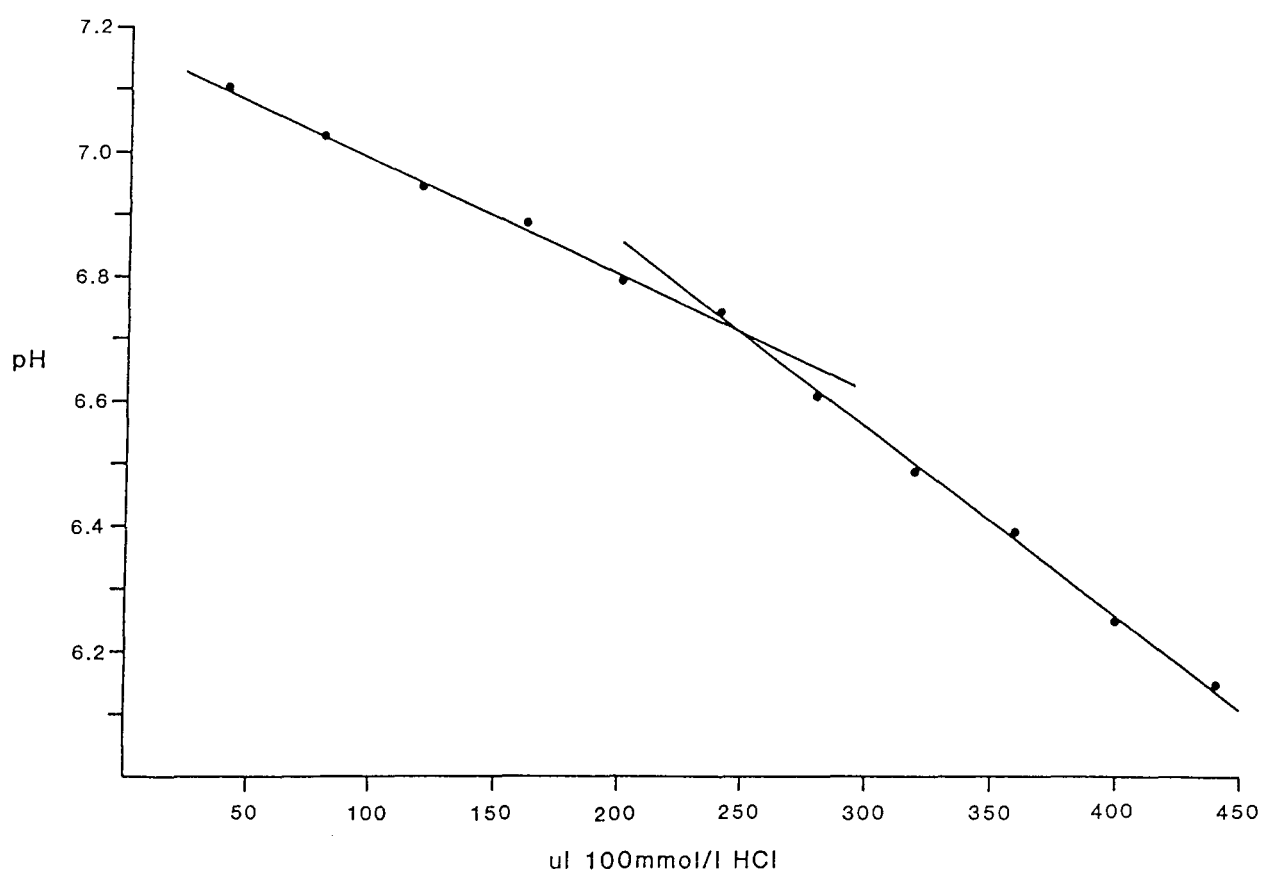
ul 100mmol/l HCl

Total H^+ required to change pH from 7.1 to 6.5:

280 μl 100 mmol/l HCl in a final volume of 1280 μl

= 28 μmol in 1280 μl

Carnosine standard = 1 ml of 100 mmol/l carnosine

= 100 μmol carnosine in 1280 μl

100 μmol carnosine will buffer 28 μmol H^+

or 1 mmol carnosine will buffer 280 μmol H^+ from pH 7.1
to 6.5.

β carnosine 7.1-6.5 = 280 mmol H^+/mol carnosine

or 1.237 mmol H^+/g carnosine

The pKa of carnosine has been estimated to be 6.83 at 37°C (Tanokura, Tasumi and Miyazawa, 1976). From the Hendersson-Hasslebach equation it is possible to calculate theoretically the buffering capacity of a 1 molar solution of carnosine as follows:

$$pH = pKa + \log_{10}\left(\frac{Salt}{Acid}\right)$$

$$pH = 6.83 + \log_{10}\left(\frac{Carnosine.NH_2}{Carnosine.COOH}\right)$$

$$pH\ 7.1 \quad \frac{Carnosine.NH_2}{Carnosine.COOH} = 1.86$$

$$pH\ 6.5 \quad \frac{Carnosine.NH_2}{Carnosine.COOH} = 0.47$$

$$pH\ 7.1\ 1M\ Carnosine = \frac{650\ mmol-SALT}{350\ mmol-ACID}$$

$$pH\ 6.5\ 1M\ Carnosine = \frac{320\ mmol-SALT}{680\ mmol-ACID}$$

The β of a 1M solution of carnosine from pH 7.1 to 6.5 is given by the increase in the acid form, i.e. 680-350

= 330 mmol $H^\pm$/mol carnosine.

The discrepancy between β from pH 7.1-6.5 measured by titration (280 mmol H^+/mol carnosine) and by calculation (330 mmol H^+/mol carnosine) most likely represents errors introduced at all stages from purity of carnosine standard to weighing and pipetting during titration.

(b) The addition of 20 μl of 100 mmol/l carnosine standard to homogenates of 10 mg d.m. in 1 ml HMR (final concentration of carnosine = 2 μmol) resulted in a mean increase in βm_{TIT} pH 7.1-6.5 from 108 to 155 μmol H^+/g d.m. (n = 3). Therefore, 2 μmol of carnosine buffered approximately 0.47 μmol H^+. This is close to the expected value of 0.56 μmol H^+ for 2 μmol of carnosine, calculated from the β pH 7.1-6.5 estimated by titration. (β pH 7.1-6.5 1 mol carnosine = 280 mmol H^+).

(13) <u>Muscle dipeptide content and</u> βm_{TIT} <u>in the Thoroughbred horse,</u> <u>Greyhound dog and man:</u> βm_{TIT} and carnosine and anserine contents of muscle samples from Thoroughbreds, Greyhounds and man are shown in Table 4.8. There was no apparent difference between either βm_{TIT} or carnosine and anserine contents of the biceps femoris and semitendinosus of Greyhound dogs and therefore the results for the two muscles have been pooled. Carnosine was detected in varying amounts in muscle from all 3 species but anserine was only detected in Greyhound muscle. The contributions made by the carnosine and anserine contents of the muscle samples to βm_{TIT} estimated by titration are shown in Table 4.9. Analysis of variance indicated significant differences between βm_{TIT} (P<0.001) between species, but no difference after subtraction of βm due to carnosine + anserine (P>0.05).

DISCUSSION

The shape of the titration curve for muscle homogenates prepared as described in section (1) of the Materials and Methods section and illustrated in Figure 4.1 was a consistent finding, not only with horse muscle, but also with Greyhound and human muscle . This suggests that the change in slope is either due to the homogenization reagent or alternatively to constituents of muscle common to all three species. However, as to why an identifiable change in slope should occur is still unclear. This phenomenon has also been observed by Cheetham (1988) in titration of human muscle homogenates. Despite the non-linearity of the titration curve, the change in slope close to pH 6.8 and the method of calculation should not introduce any further error in the estimation of βm_{TIT}.

In the present study βm_{TIT} and pHm_i were both found to be independent of concentration above 40 mg/ml w.m. Cheetham (1988) found in rat muscle that although pHm_i was unaffected by concentration at 40 mg/ml w.m. and above, βm_{TIT} varied with concentration between 20 and 100 mg/ml w.m. This is in contrast to the results from the present study and may be partly due to a much lower βm_{TIT} in rat muscle compared to the horse (Bate Smith, 1938; Cheetham, 1988).

Values for βm_{TIT} in the horse have been reported previously by Fox et al (1987) and McCutcheon et al (1987) and are summarized in Table 4.10 along with the methodology employed. In spite of differences in the age and breed of the horses sampled and some differences in methodology, the values

Table 4.8. Buffering capacity (βm_{TIT}) and carnosine and anserine content of muscle from Thoroughbred horse (middle gluteal) , Greyhound dog (biceps femoris + semitendinosus) and man (vastus lateralis). ND - not detected.

	βm_{TIT} $\mu mol\ H^+/g\ d.m.$ pH 7.1-6.5	Carnosine mmol/kg d.m.	Anserine mmol/kg d.m.
Thoroughbred	117.8±19.7	90.5±12.1	ND
Greyhound	113.6±4.6	41.6±21.8	59.5±19.2
Man	77.2±11.4	9.5±2.5	ND

Table 4.9. Comparison of the combined contribution of carnosine and anserine to βm_{TIT} in the Thoroughbred horse, Greyhound dog and man.

	βm_{TIT} $\mu mol\ H^+/g\ d.m.$ pH 7.1-6.5	βm^{C+A} $\mu mol\ H^+/g\ d.m.$ pH 7.1-6.5	$\beta m_{TIT} - \beta m^{C+A}$ $\mu mol\ H^+/g\ d.m.$ pH 7.1-6.5
Thoroughbred	117.8±19.7	30.0±4.0	87.6±19.5
Greyhound	113.6±4.6	32.3±9.6	81.3±12.7
Man	77.2±11.4	3.2±0.8	74.0±11.1
ANOVA	P<0.001		P>0.05

βm^{C+A} calculated buffering capacity of carnosine and anserine content of muscle between pH 7.1 and 6.5 based on contents from Table 4.8.

Table 4.10. In vitro muscle βm_{TIT} measurements in biopsy samples from equine middle gluteal muscle.

Author	Breed	Age	Training status	βm & units	βm range	Measurement conditions	βm recalculated to µmol H$^+$/g d.m. pH 7.0-6.0
Fox et al (1987)	SB	2-3	UT	223±14 µmol/g d.m. pH 7.0-6.0	197-238	HMR: 145 mmol/l KCl 10 mmol/l NaCl 5 mmol/l IAA Muscle: freeze-dried Conc: 10mg/ml Temp: Not given	223±214
McCutcheon et al (1987)	TB	5-8	UT	63±3 µmol/g v.m. pH 7.0-6.0	Not given	HMR: 145 mmol/l KCl 10 mmol/l NaCl 0.3 mmol/l IAA 0.3 mmol/l DNFB	252±12
			7 wks training	57±4 µmol/g v.m. pH 7.0-6.0		Muscle: fresh-frozen Conc: 200mg/ml Temp: 25°C	228±16
Present study	TB	1	UT	110.2±7.4	95.5-121.7	HMR: 145 mmol/l KCl 10 mmol/l NaCl	183.7±12.3
	TB	2	LT	115.3±8.2	100.8-132.2	5 mmol/l IAA Muscle: freeze-dried	192.2±13.7
	TB	2	FT	106.1±7.1	91.7-114.2	Conc: 10mg/ml Temp: 37°C	176.8±12.3
	TB	3	FT	123.1±3.6	116.9-129.4		205.2±6.0
	TB	4+	LT-FT	117.8±13.4 µmol/g d.m. pH 7.1-6.5	102.2-149.8		196.3±22.3

Breed: SB - Standardbred   Training status: UT - Untrained βm units: d.m. - dry muscle
         TB - Thoroughbred   LT - Light/Medium trained   v.m. - wet muscle
                                                    Ft - Fully trained

Measurement conditions: HMR - Homogenizing reagent
                        IAA - Iodoacetic acid
                        DNFB - 2,4 dinitrofluorobenzene

for βm_{TIT} are surprisingly close. The recalculated data of McCutcheon et al indicated a βm_{TIT} approximately 26% higher in w.m. (based on the mean of the data of McCutcheon et al and the mean of the data from the present study) at a temperature of 25°C and is similar to the difference observed for w.m. and d.m. in the present study. Thus, the values for βm_{TIT} determined by the method presented here relate to other observations of βm_{TIT} in the Thoroughbred and Standardbred horse. The similarity between values of βm_{TIT} observed in the present study and values found in other breeds or with other methods may indicate that βm_{TIT} in the horse shows very little inter-horse and inter-breed variation or alternatively, this may be an indication of the insensitivity of the homogenate-titration method.

Analysis of muscle samples from Thoroughbreds, Greyhounds and man for dipeptide content showed that whilst carnosine was present in muscle from all three species, anserine was only detected in Greyhound muscle. This is in agreement with previous studies in dogs (see Crush, 1970). The carnosine contents of Thoroughbred and human muscle are similar to previous reports of Davey (1960) and Parkhouse et al (1985), respectively. Furthermore, it seems evident that the higher muscle buffering capacities (determined by titration) in both Thoroughbreds and Greyhounds compared with man is due mainly to the higher levels of histidine dipeptides found in these species (carnosine in the Thoroughbred and carnosine and anserine in the Greyhound).

The muscle buffering system can be divided into static and dynamic buffering processes. Static buffering consists of buffering by proteins, dipeptides, amino acids such as histidine and inorganic phosphate. Dynamic buffering consists essentially of buffering by bicarbonate and the CPK/PCr mediated phosphorylation of ADP. It is therefore necessary to elucidate which components of the muscle buffering system are contributing to the βm_{TIT} measured in vitro by the methodology described.

The finding that 95% of PCr and virtually all the ATP are lost during homogenization would suggest an absence of any significant contribution from CPK/PCr. As for bicarbonate, this appears to be lost on freeze-drying (Harris et al, 1989) and will not, therefore, contribute to the measurement of βm_{TIT} in d.m. in vitro. Addition of HCO_3^- equivalent to a muscle content at rest of 15 mmol/kg w.m. (60 mmol HCO_3^- per ml HMR, estimated as the amount of HCO_3^- associated with 10 mg of d.m. solids in resting muscle: Sahlin et al, 1978) increased βm_{TIT} in d.m. by 18%. This is close to the difference observed between d.m. and w.m. of 25%. Ammonia lost on freeze-drying is unlikely to explain this discrepancy, as resting muscle levels

are relatively insignificant (0.1-0.3 mmol/kg w.m., Essén-Gustavsson and Valberg, 1987). The difference may indicate a higher resting muscle bicarbonate content in the horse or perhaps a slight underestimation of the resting muscle HCO_3^- content by Sahlin et al. There is little justification to suspect a difference in resting muscle HCO_3^- content between man and horse. An estimate of muscle HCO_3^- of around 22 mmol/kg w.m. would be expected to raise the βm_{TIT} of d.m. by approximately 26%, based on the observation that addition of HCO_3^- equivalent to a content of 15 mmol/kg d.m. gave an increase in βm_{TIT} of approximately 18%.

βm calculated from the relationship between muscle pH and lactate (βm_{pHvLAC}) following exercise was approximately the same in 2 horses and significantly higher in the other 4 when compared to βm estimated by titration (βm_{TIT}). βm_{pHvLAC} may be termed an internal titration as the 'acid' is being 'added' from within the muscle, in an intact state, due to the formation of lactic acid. The higher βm_{pHvLAC} in 4 out of the 6 horses can probably be best explained on the basis of contributions from dynamic buffering by the CPK/PCr buffer system, HCO_3^- buffering and unequal loss of H^+ and lactate$^-$ from the muscle during the period of the exercise as a higher rate of efflux of H^+ relative to lactate would result in an overestimation βm_{pHvLAC}. A faster efflux of H^+ relative to lactate has been demonstrated in stimulated rat diaphragm and frog sartorius muscle (Benadé and Heisler, 1978; Boutilier, Emilio and Shelton, 1986) although Seo (1984), also using frog sartorius muscle, found that H^+ efflux never exceeded lactate efflux. Therefore, the relative rates of H^+ and lactate$^-$ efflux during exercise are not clearly defined and the study by Seo (1984) further indicates that the relationship may change during exercise. The possibility must therefore be considered that estimates of βm from the relationship between pH and lactate may be affected by any differential loss of H^+ and lactate$^-$.

Hultman and Sahlin (1980) calculated that a decrease of PCr of approximately 60 mmol/kg d.m., and at a final pH of 6.6, would correspond to an uptake of 11.6 mmol H^+/l intracellular water. Assuming 3.3 l intracellular water associated with 1 kg d.m., this gives an estimate of CPK/PCr buffering from rest to post exercise of approximately 40 μmol H^+/g d.m. pH 7.1-6.6. If the mean value for βm_{TIT} of 115 μmol H^+/g d.m. pH 7.1-6.5 (Table 4.5) is increased by 25% to compensate for the absence of dynamic HCO_3^- buffering and then further increased by 40 μmol H^+/g d.m. pH 7.1-6.5 to allow for the contribution from dynamic CPK/PCr buffering, this gives an estimate of approximately 184 μmol H^+/g d.m.. If horses H2 and H6 are ignored (these being the two in which no significant difference was

found between βm_{TIT} and βm_{pHvLAC}), the mean βm_{pHvLAC} is 188.5 μmol H^+/g d.m., very close to the corrected value of βm_{TIT} in d.m..

The methodology employed for the determination of βm_{TIT} in d.m. estimates the contribution to total βm made by muscle proteins, dipeptides, amino acids and inorganic phosphate. It is unlikely that it includes any contribution from the dynamic buffering systems, CPK/PCr and HCO_3^-. Furthermore, no estimate of buffering due to removal or loss of H^+ from the muscle can be made in the estimate of βm_{pHvLAC} from samples taken post-exercise. In the experiments where βm was estimated in d.m. by titration and from the relationship between pH and lactate in muscle post-mortem, the confounding problem of H^+ loss is removed. Mean βm_{pHvLAC} in post mortem muscle samples was found to be approximately 63% higher than βm_{TIT} in the same muscle. This is very close to the mean difference of 65% between βm_{TIT} in resting samples and mean βm_{pHvLAC} in samples from the same horses following exercise (115.0 and 188.5 μmol H^+/g d.m. pH 7.1-6.5, respectively).

The finding that there is very little difference between βm_{pHvLAC} estimated essentially with and without blood flow (following exercise and at post-mortem, respectively) may indicate that if there is any loss of H^+ and lactate$^-$ ions from the muscle in the exercise condition, (i.e. with blood flow) that this loss is equal for both ions. The review by Hultman and Sahlin (1980) concluded that lactate and H^+ ions pass out of the muscle cell at approximately the same rate under most conditions. The only exception appears to be during the early phase of recovery (Sahlin et al, 1976) when there is an excess efflux of H^+ in relation to lactate.

Therefore, although overestimation of βm from the relationship between pH and lactate following exercise can be explained on the basis of an unequal efflux of H^+ and lactate$^-$ (i.e. H^+ efflux > lactate$^-$ efflux), perhaps in those horses where the muscle is particularly well vascularized, it is harder to explain an underestimation of βm_{pHvLAC} as this would only be possible in a situation where the H^+ ion concentration in the biopsy was overestimated, that is lactate was lost or removed from the muscle cell more rapidly compared to H^+.

On the basis of these observations and considerations the technique described for the measurement of βm_{TIT} on dry muscle essentially provides an estimate of the static buffering system of protein, dipeptides and phosphates and will allow comparison within species under different conditions and between species.

CHAPTER 5

MUSCLE CHARACTERISTICS OF 1, 2 AND 3 YEAR OLD THOROUGHBRED
RACEHORSES IN TRAINING.

INTRODUCTION

   A large number of studies have been undertaken to examine the
variation in muscle composition in a variety of breeds of horses and to
investigate the effects of age and training upon muscle characteristics.
Snow and Guy (1981) examined the muscle fibre composition of the middle
gluteal in Standardbred, Thoroughbred and Quarter horses and subsequently
(Snow, 1983) the changes with age. Other studies which have focused on the
effects of training on muscle composition include: Hodgson et al (1986);
Snow and Guy (1979); Lindholm et al, (1983); Essén-Gustavsson et al (1983).
However, possibly due to the small sample sizes involved, none of these
authors have investigated the relationship between muscle composition and
racing performance, although Snow and Guy (1980) demonstrated a
relationship between past optimal racing distance and fibre composition.

   The present study was undertaken with three aims. Firstly, to describe
the changes in muscle composition with age and training in Thoroughbred
horses in racing stables, especially with respect to muscle buffering
capacity and carnosine content. Secondly, to investigate the variability in
muscle characteristics within this population and thirdly, to determine
whether this variability is in any way related to racing performance.

MATERIALS AND METHODS

Animals: Muscle samples were collected, as described in Chapter 2, from
Thoroughbred racehorses from three different flat racing stables. The age,
sex, training status and date of sample collection are shown in Table 5.1.
The horses were sampled in April and October/November as this corresponds
to the start and finish of the flat racing season in Britain.

   The horses in group A1 represent animals that have had no training and
would have only been in the stable for a short period of time. Ten of these
horses were resampled 6 months later (group A2), following mainly low
intensity, mainly aerobic training, consisting of walking and trotting and
low speed cantering. Faster canters were introduced in this group towards
February and March.

   Group B1 corresponds to group A2 in that the horses are the same age
and at approximately the same stage of training. Twelve of the horses in
group B1 were resampled 6-7 months later, at the end of their first racing
season. Of these, 7 had been trained and raced, whilst 5 had undergone
little or no training.

Table 5.1. Age, sex, training status and date of sample collection of horses in survey.

Stable	Group	No.Horses sampled	Age	Sex	Training status	Date of sampling
A	A1	14	1	7c,1g,6f	UT	OCT 86
	A2	10	2	4c,6f	LT	APR 87
B	B1	20	2	10c,10f	LT	APR 85
	B2	7	2	5c,2f	FT	OCT/NOV 85
	B3	5	2	1c,4f	UT	OCT/NOV 85
C	C	16	3	12c,4f	FT	NOV 83

Footnote: Sex: c = colt (male)

                g = gelding (castrate)

                f = filly (female)

Training status: UT = Untrained

                     LT = Light-Medium trained

                     FT = Fully trained

Footnote

The horses to be sampled in each stable were selected by the trainer. Only horses that were due to race within a short period or those considered to be of unsuitable temperament were purposely avoided. Therefore the horses biopsied represent a random sample of the population.

Sixteen horses were sampled in the third Stable (C). These samples were collected in 1983 and, therefore, fibre area and LDH isoenzyme determinations were not possible.

Although the specific details of the training programmes for the different stables were not available, between April and October the horses can be considered to have been 'conventionally' trained. The weekly programme would be based on a daily regime very similar to that shown below.

Monday: 1 hr walking, 10 min trot, 1-2 slow canters.

Tuesday: 1 hr walking, 10 min trot, 1 medium canter, 1 fast canter.

Wednesday: 1 hr walking, 10 min trot, 1 medium canter, 1 gallop.

Thursday: 1 hr walking, 10 min trot, 1-2 slow canters.

Friday: 1 hr walking, 10 min trot, 1 medium canter, 1 fast canter.

Saturday: 1 hr walking, 10 min trot, 1 medium canter, 1 gallop.

Sunday: In box.

In general, canters and gallops would be between 4 and 8 furlongs.

Analysis: The treatment, storage and analysis of the muscle samples was as described in Chapter 2. Muscle histochemical sections were stained for mATPase and SDH. Fibre frequency and mean fibre area were determined for type I, IIA and IIB fibres. Muscle samples were analysed for total adenine nucleotide (TAN = ATP + ADP + AMP), TCr (PCr + Cr), glycogen, buffering capacity, carnosine, OGDH, PFK, CPK and LDH activity and LDH isoenzyme composition.

Performance: The performance rating given (where available) is Timeform (Timeform, Halifax, UK). This is a rating in pounds and is allocated to a horse according to its performance on the racetrack. The higher the rating, the better the horse. Elite racehorses have Timeform ratings of 125 upwards. In practice, in a handicap race, where the weight carried by each horse is related to the horses ability, a horse with a Timeform rating of 110 racing against a horse with a rating or 100, would have to carry 10 pounds more weight.

Statistics: As the data in this survey was collected from horses in different stables, this precluded the use of ANOVA. Therefore, differences between stables and groups were determined using Students' 't' test for paired and unpaired data.

RESULTS

Muscle characteristics for the horses in groups A1, A2, B1, B2 and C are presented in Table 5.2 as mean and standard deviation and in Figures 5.1 to 5.14.

Within stable comparisons

(1) A1 v A2: The six month period of aerobic training resulted in significant increases in the percentage of type IIA fibres ($P<0.05$), percentage of high staining SDH fibres ($P<0.01$), type I fibre area ($P<0.01$), percentage relative area occupied by type IIA fibres ($P<0.05$), glycogen ($P<0.001$) and TCr:TAN ratio ($P<0.05$). Significant decreases occurred in percentage relative area occupied by type IIB fibres ($P<0.05$), % LDH-5 isoenzyme ($P<0.05$), ATP, TAN and TCr ($P<0.001$) and LDH activity ($P<0.01$).

(2) B1 v B2: The effects of age and training on horses in group B1 resulted in an increase in the percentage of high staining SDH fibres ($P<0.01$) and a small increase in the percentage relative area occupied by type I fibres ($P<0.05$). Increases in glycogen content ($P<0.001$), TCr ($P<0.001$), TCr:TAN ratio ($P<0.01$), PFK ($P<0.001$), CPK ($P<0.001$) and OGDH ($P<0.05$) were also observed. The only significant decrease was in muscle buffering capacity ($P<0.001$).

Between stable comparisons

(1) A2 v B1: Groups A2 and B1 represent horses of the same age in different stables. Horses in Stable A (group A2) had a significantly greater percentage of high staining SDH fibres ($P<0.05$), a higher glycogen and TCr content ($P<0.001$), and higher PFK ($P<0.001$) and CPK ($P<0.01$) activities. Horses in Stable B (group B1) had significantly higher levels of TAN ($P<0.001$) and a greater muscle buffering capacity ($P<0.01$) compared with horses in Stable A.

(2) B2/B3 v C: The horses in group B2/B3 were 2 year old horses at the end of their first racing season and the horses in group C were 3 year olds at the end of their second racing season. Thus, there was approximately one years difference between groups B2/B3 and group C. The only significant difference between the two groups was a higher muscle buffering capacity ($P<0.001$) and muscle OGDH activity ($P<0.05$) in the horses in group C compared with group B2/B3.

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Table 5.2. Muscle characteristics of Groups A1, A2, B1, B2 and C. Values are presented as mean ± standard deviation.

Group	A1	A2	B1	B2	C
%I	19.9±5.0	19.4±4.9	19.8±4.8	16.6±5.7	15.1±3.3
%IIA	37.4±5.4	44.1±8.3	44.0±6.1	44.2±7.9	43.2±8.2
%IIB	42.6±8.5	36.5±8.5	36.2±7.4	39.2±9.2	41.8±9.2
Area I	1734±338	2150±285	2034±387	2224±555	ND
Area IIA	2502±472	2593±321	2571±526	2886±829	ND
Area IIB	4279±884	3816±315	4048±728	3986±1399	ND
%AOI	11.5±4.0	14.1±3.7	16.1±5.3	11.7±4.3	ND
%AOIIA	30.5±6.7	39.0±9.9	36.6±7.5	40.2±10.1	ND
%AOIIB	58.1±9.8	46.9±10.0	47.3±8.5	48.2±12.8	ND
%H-SDH	75.2±7.5	86.7±12.4	77.8±8.4	84.2±8.6	ND
Glycogen	469±62	555±40	427±47	566±63	ND
TAN	27.5±1.8	22.4±1.0	26.3±2.5	27.8±2.2	26.7±2.1
TCr	130.7±7.1	116.9±6.9	99.5±10.7	113.1±6.4	118.8±9.0
βm	110.2±9.1	108.2±4.2	118.1±8.9	107.0±7.2	123.1±3.6
Carnosine	121.5±14.8	109.4±13.5	109.3±13.6	113.8±16.1	108.8±17.0
OGDH	1.9±0.9	2.4±1.2	1.9±1.1	2.8±1.6	3.9±1.3
PFK	52.2±7.3	51.3±3.1	37.0±7.7	48.9±7.6	52.3±5.3
CPK	2168±121	2274±223	2004±198	2266±195	2206±130
LDH	858±111	691±103	684±151	683±151	693±101
%LDH-5	75.5±9.1	66.0±10.2	68.8±6.0	64.8±9.3	ND

UNITS: Fibre area – μm^2

%AO – percentage relative area occupied (see Chapter 2)

Glycogen, TAN, TCr, carnosine – mmol/kg/d.m.

OGDH, PFK, CPK, LDH – $\mu mol/g$ d.m./min 25°C

βm – $\mu mol/g$ d.m. pH$_{7.1-6.5}$

ND – not determined

Figure 5.1 Type I fibre frequency in horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.
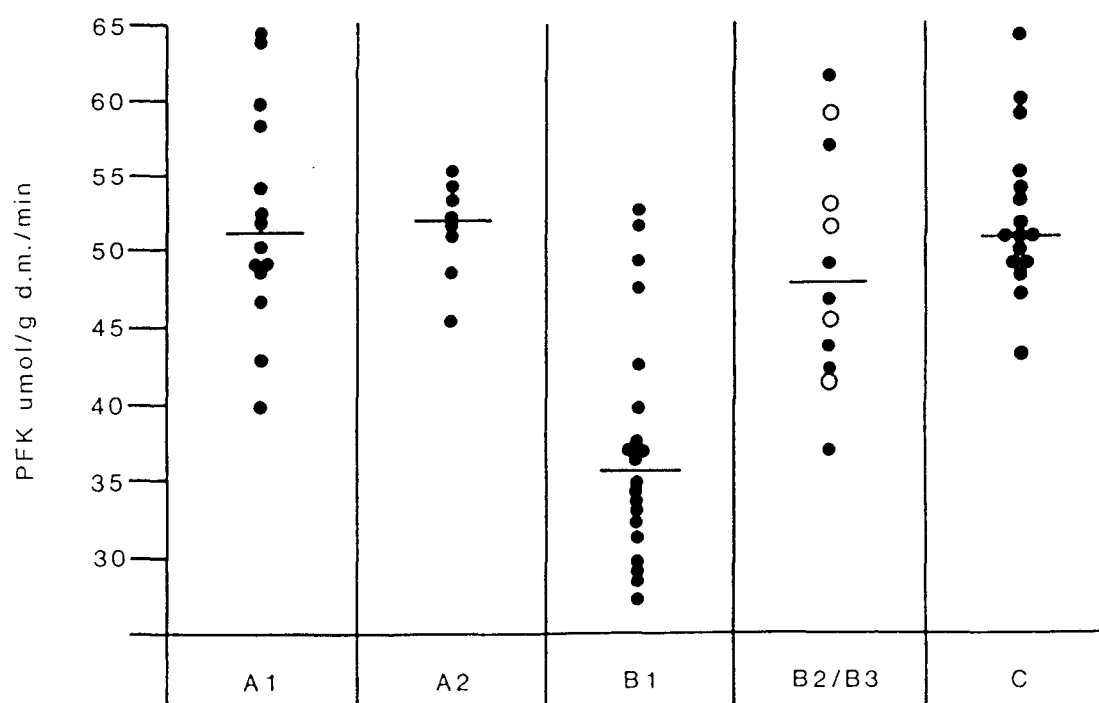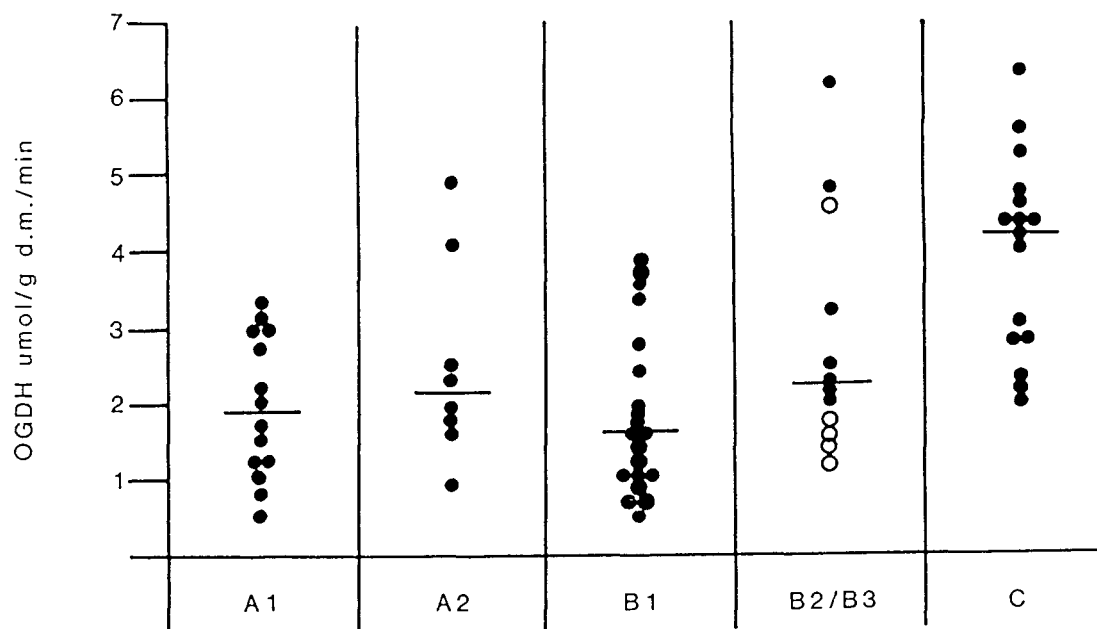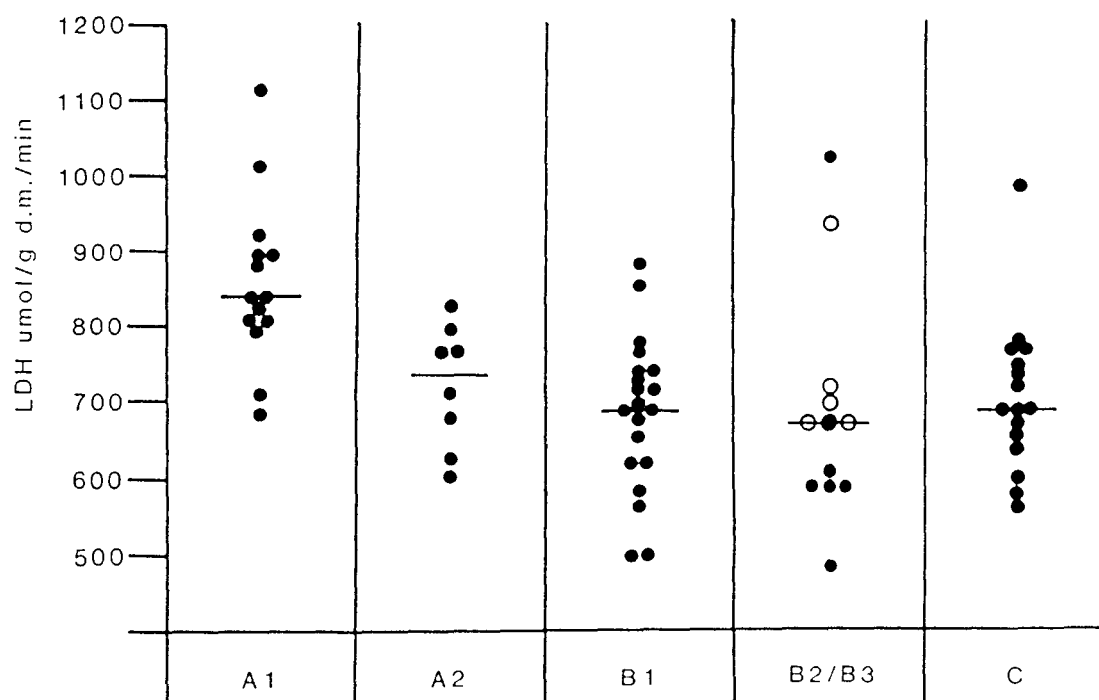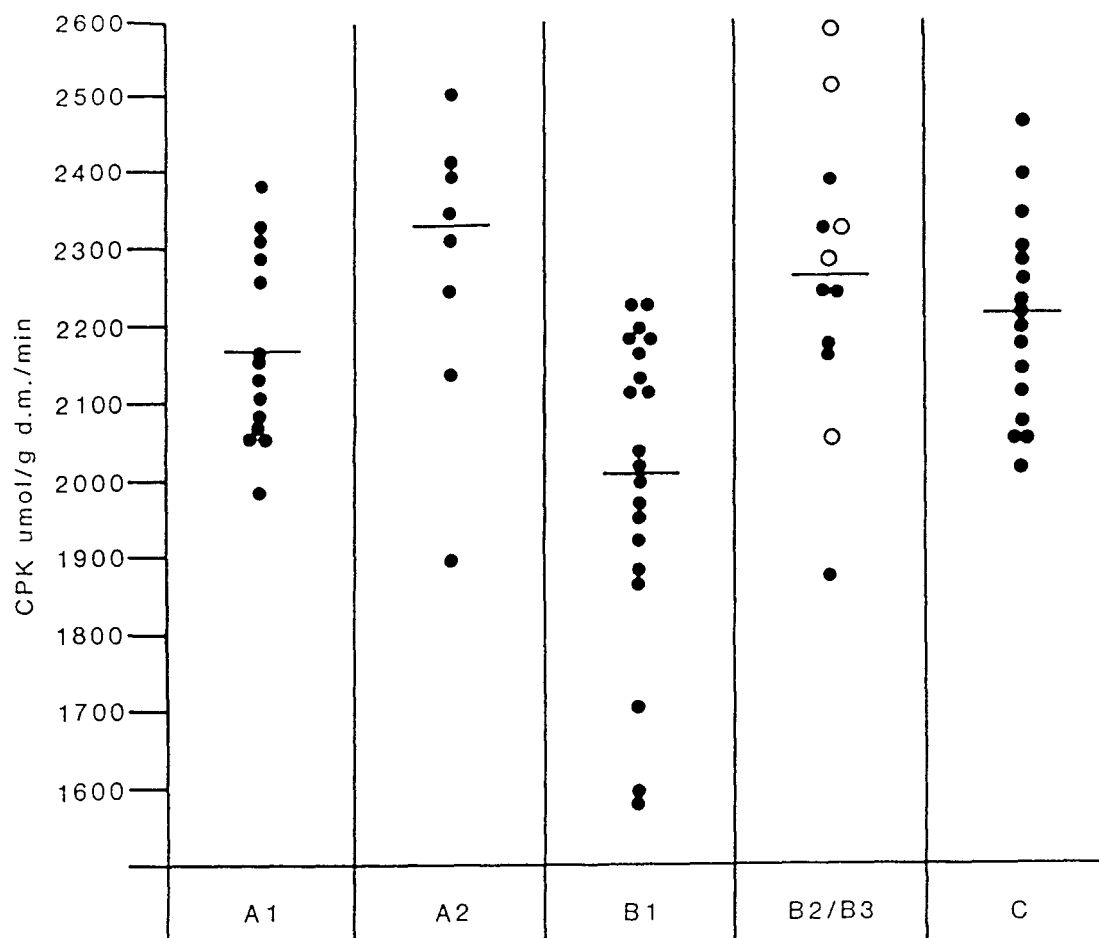
Figure 5.2 Relative percentage cross-sectional area occupied by type I fibres (%AOI) in horses in groups A1, A2, B1 and B2/B3. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.
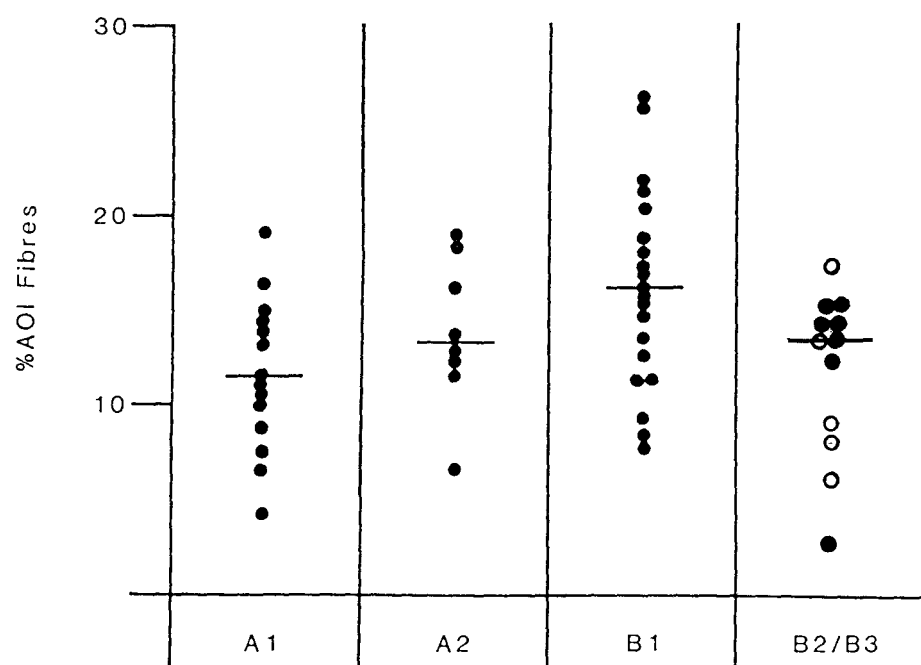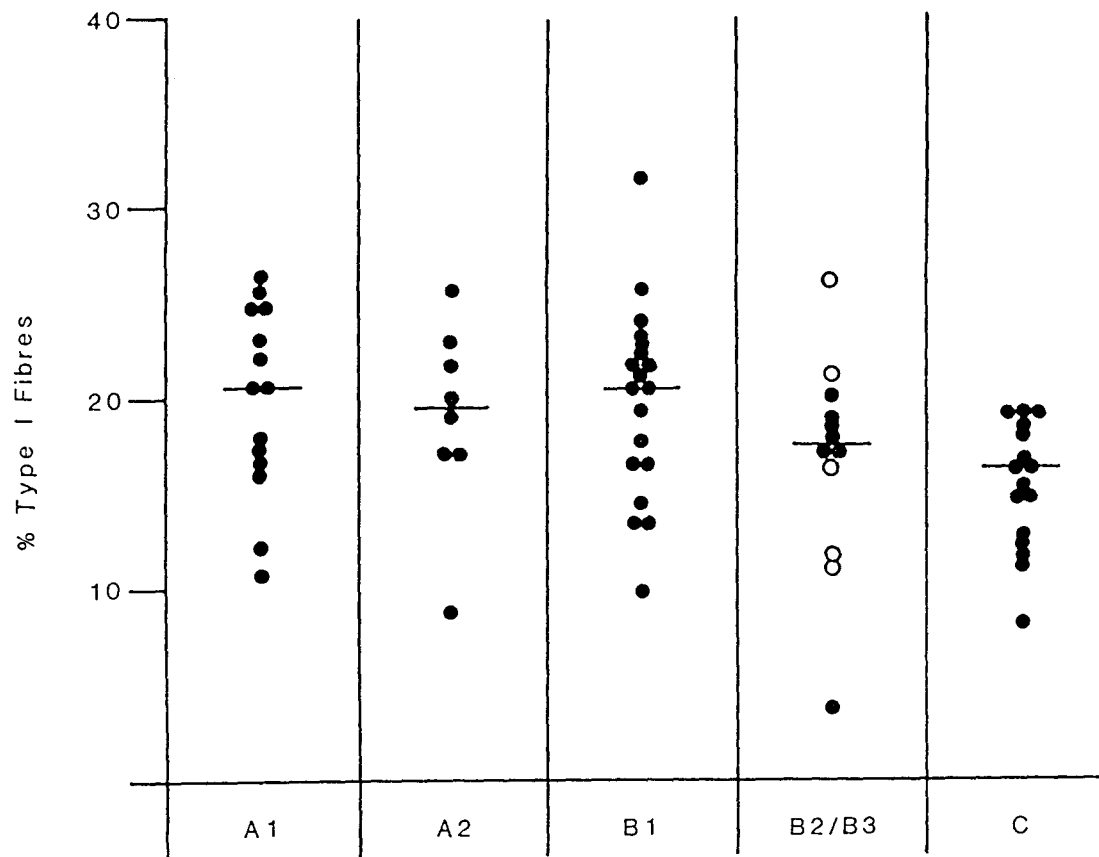
Figure 5.3 Percentage of high-staining SDH fibres (%H-SDH) in horses in groups A1, A2, B1 and B2/B3. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.

Figure 5.4 Relative percentage cross-sectional area occupied by type IIB fibres (%AOIIB) in horses in groups A1, A2, B1 and B2/B3. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.
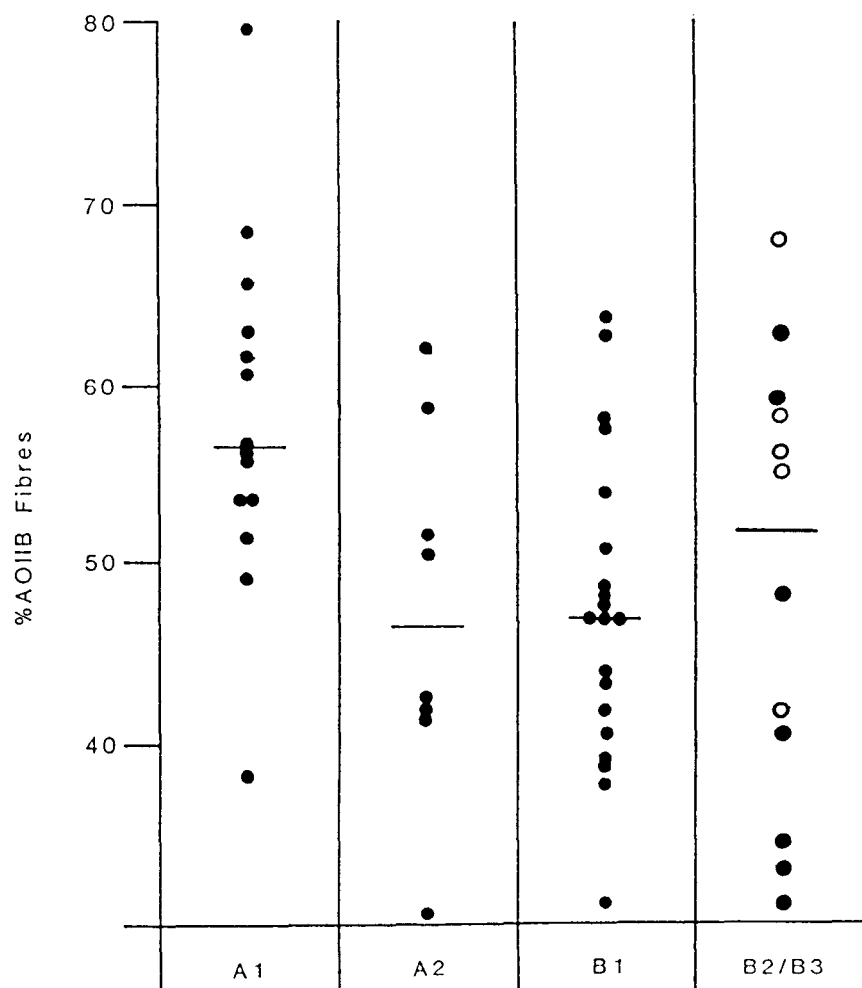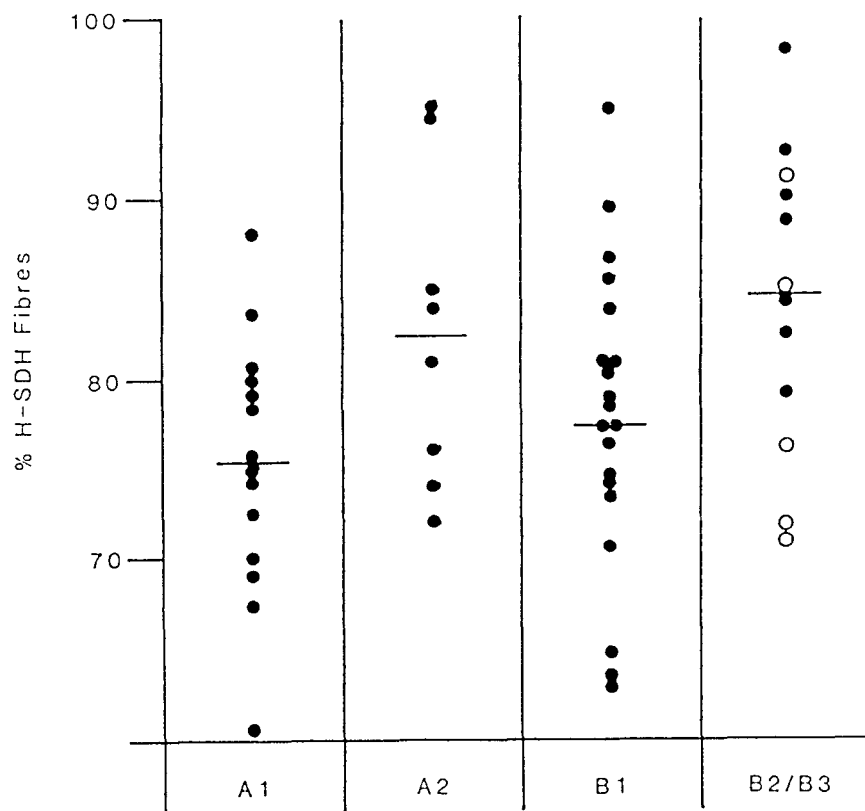
Figure 5.5 Muscle carnosine content of horses in groups A1, A2, B1 and B2/B3+C.The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.

Figure 5.6 Muscle buffering capacity (βm) of horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.
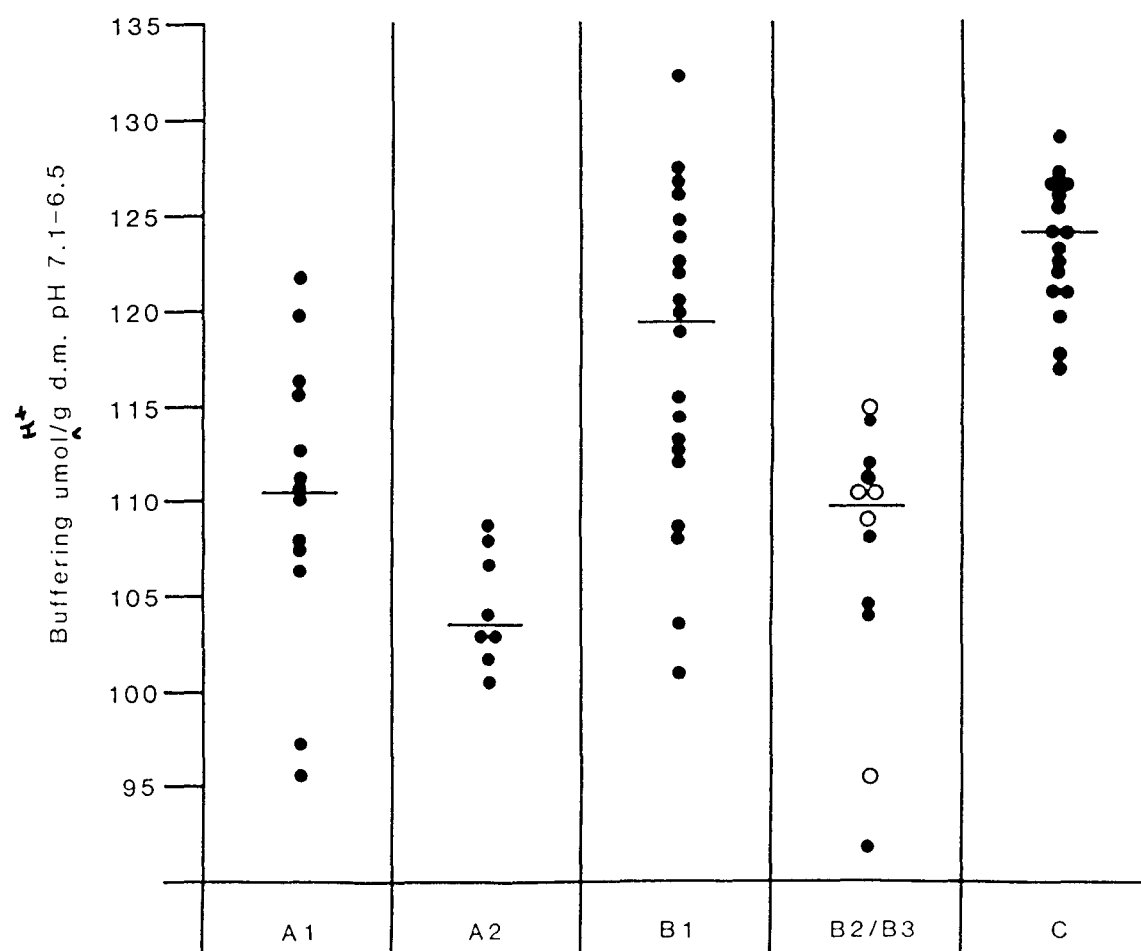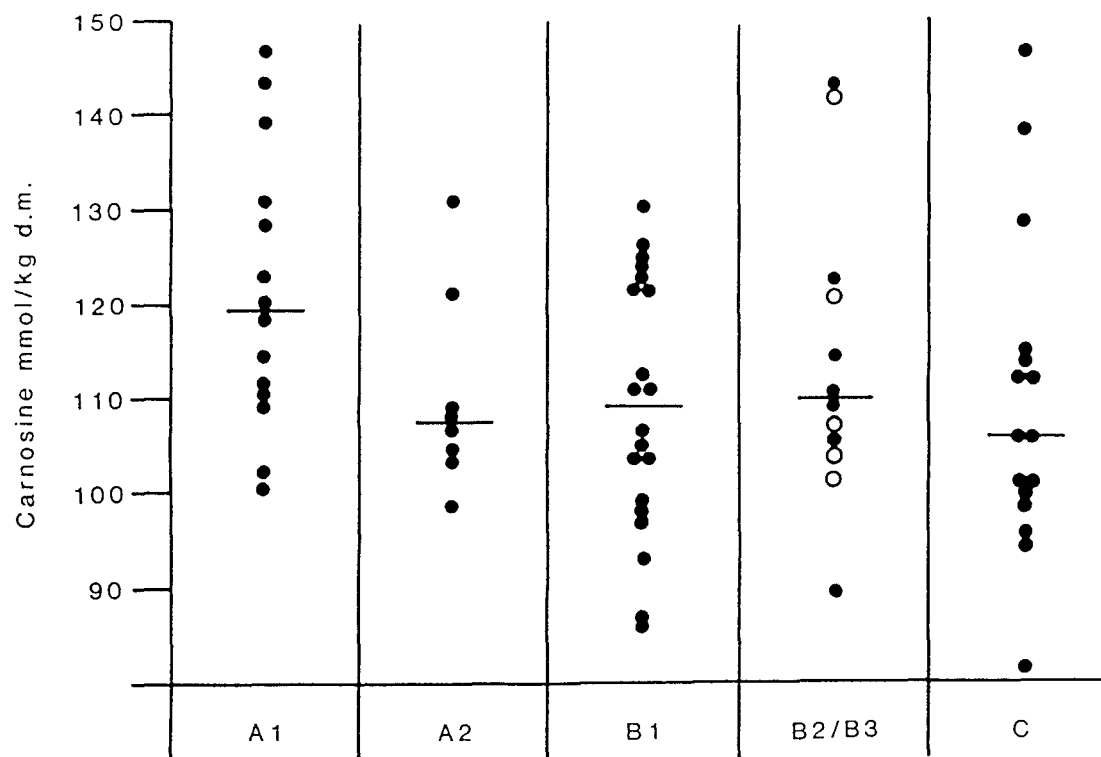
Figure 5.7 Muscle total creatine content (TCr) of horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.

Figure 5.8 Muscle total adenine nucleotide content (TAN) of horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.
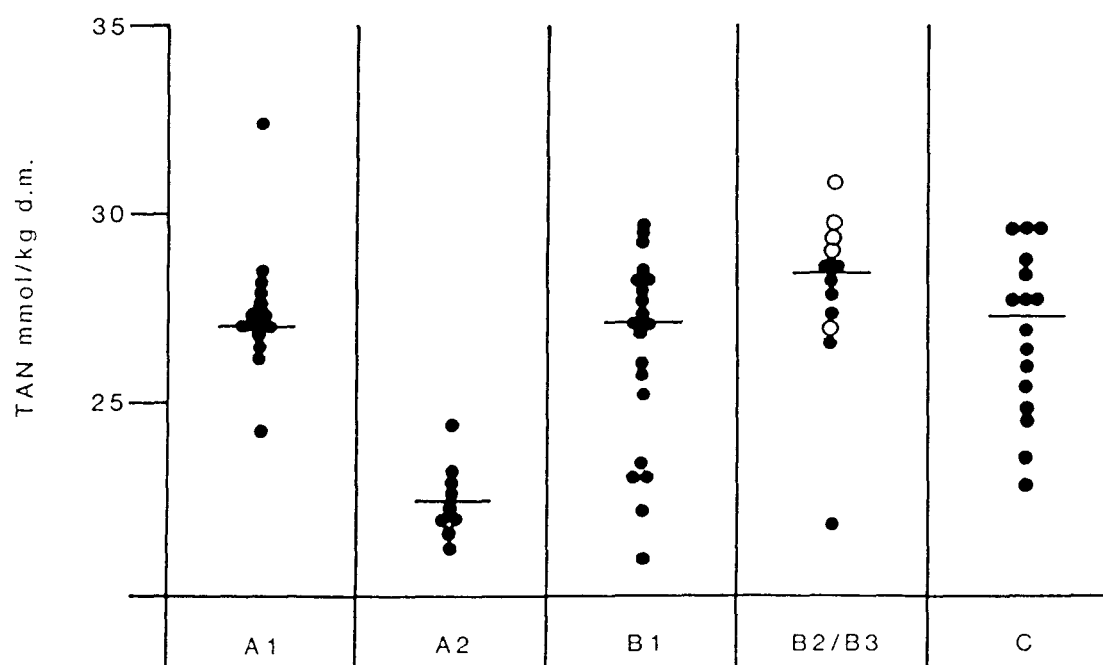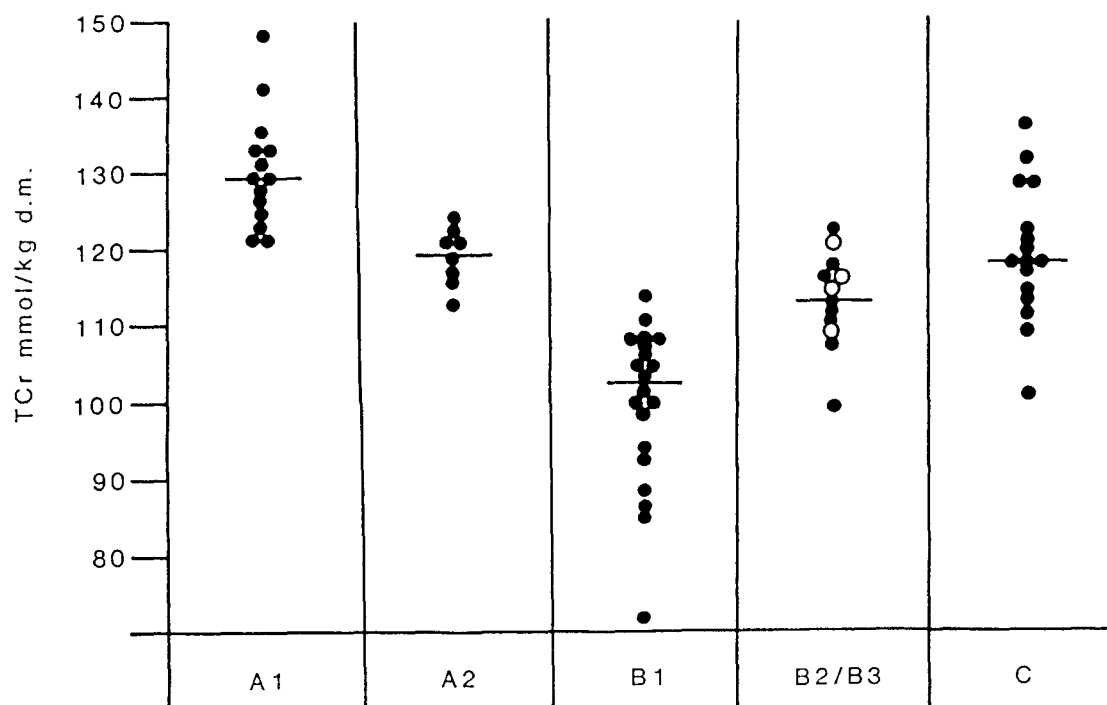
Figure 5.9 Muscle OGDH activity of horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.

Figure 5.10 Muscle PFK activity of horses in groups A1, A2, B1, B2/B3 and C. The median for each group is shown. In group B2/B3, open and closed circles represent untrained and trained horses, respectively.

Figure 5.11 Muscle CPK activity of horses in groups A1, A2, B1, B2/B3 and
C. The median for each group is shown. In group B2/B3, open and closed
circles represent untrained and trained horses, respectively.

Figure 5.12 Muscle LDH activity of horses in groups A1, A2, B1, B2/B3 and
C. The median for each group is shown. In group B2/B3, open and closed
circles represent untrained and trained horses, respectively.

Figure 5.13 Muscle %LDH-5 isoenzyme of horses in groups A1, A2, B1 and
B2/B3. The median for each group is shown. In group B2/B3, open and closed
circles represent untrained and trained horses, respectively.

Figure 5.14 Muscle glycogen content of horses in groups A1, A2, B1 and
B2/B3. The median for each group is shown. In group B2/B3, open and closed
circles represent untrained and trained horses, respectively.
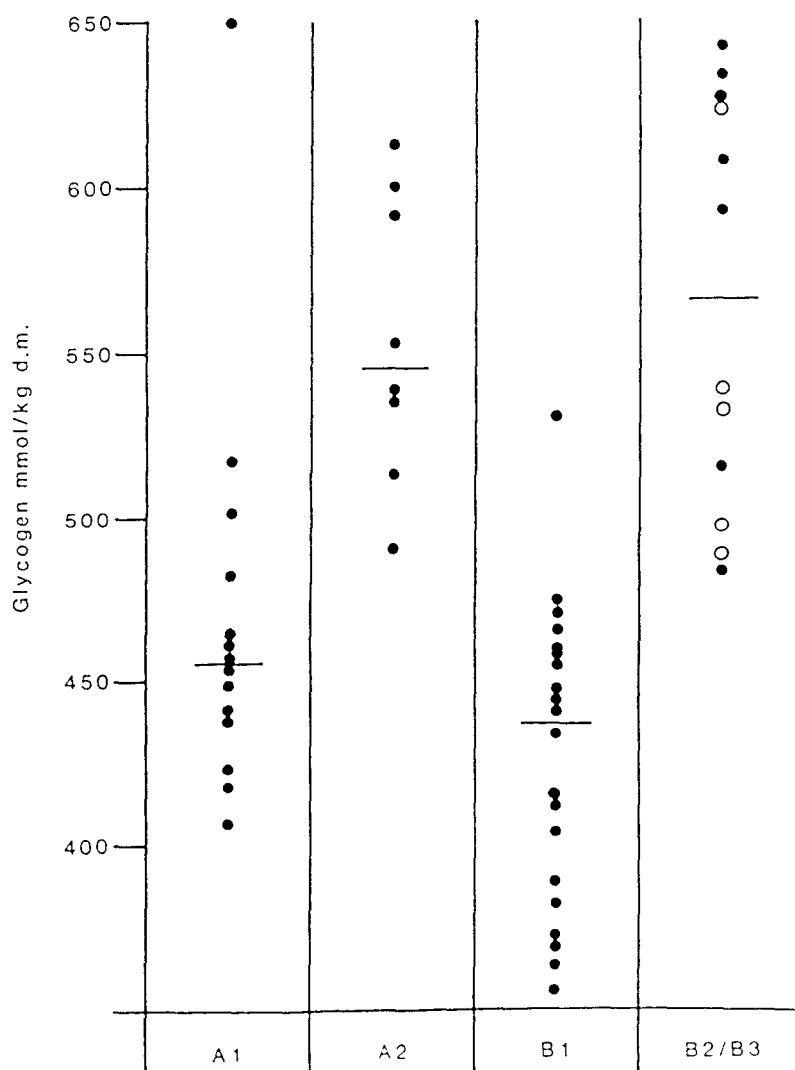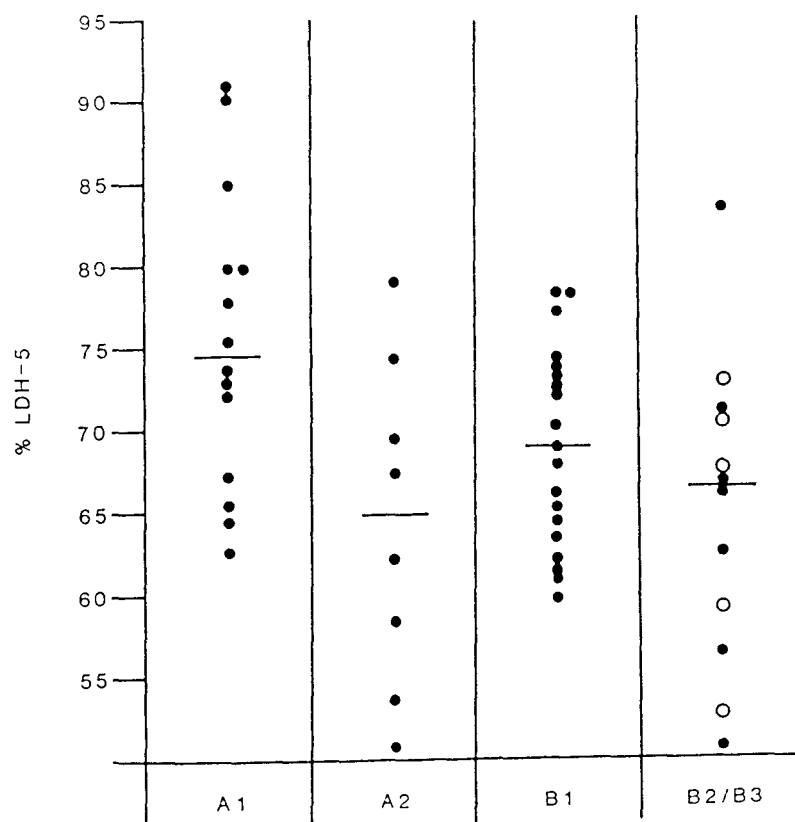
Correlation matrices for muscle parameters

For each group of horses (A1, A2, B1, B2/B3 and C) a correlation matrix was constructed by cross-correlating each muscle parameter. These are shown in Tables 5.3 to 5.8. In addition, where there was a sufficient number of horses with a Timeform rating, correlations were made between Timeform and each individual muscle parameter. A significant correlation between LDH and PFK activity was found in all five groups. Significant correlations with Timeform were found for PFK and LDH (+ve) in group B1, SDH (-ve) and %LDH-5 isoenzyme (+ve) in group A2/B1 combined, %AOIIB and PFK (+ve) in group B2 and %IIB fibre frequency (+ve) for group C as two year olds and %IIB (+ve) and %IIA (-ve) fibre frequency for group C as three year olds.

DISCUSSION

Changes in muscle composition with age and training.

The most apparent differences in the present material were found in the comparison of untrained one year old horses (group A1) and the same group of horses six months later (group A2). Over this period the horses exercise consisted of low intensity, aerobic exercise (trotting and slow cantering) used as a basis for the introduction of higher intensity exercise (i.e. faster canters and gallops) towards the end of this six month period in preparation for racing. The changes seen over this six month period suggest a decrease in muscle glycolytic capacity, as evidenced by decreases in the area occupied by type IIB fibres, percentage LDH-5 isoenzyme content and LDH activity. Correspondingly, this was accompanied by increases in the frequency of type IIA fibres and relative area occupied. The percentage of high staining SDH fibres also increased. There was also a significant decrease in the area occupied by IIB fibres and a trend towards decreased mean IIB fibre area, frequency and increased OGDH activity. This apparent decrease in muscle glycolytic capacity was accompanied by selective changes indicative of an increased muscle aerobic capacity.

Glycogen, the major fuel source during moderate to high-intensity exercise, was increased following training in both groups A1 and B2/B3. The mean increases were 18 and 33% for group A1 and B1, respectively. The greater change in glycogen in group B1 may be a result of the higher intensity training of these horses compared to those in group A1. However, a much greater increase in muscle glycogen of 37% was found by Hambleton et

Correlation matrix for group A1 (r values)

          %I

%IIB   -.798 %IIB

%AOI   +.927 -.823   %AOI

%AOIIB -.727 +.945 -.852 %AOIIB

%H-SDH +.571 -.748 +.738 -.784 %H-SDH

Gly   -.193 +.301 -.103 +.185 -.162   Gly

TAN   +.260 -.078 +.177 -.031 -.176 -.295   TAN

TCr   -.628 +.694 -.750 +.675 -.805 -.044 -.092 TCr

βm   +.293 -.047 +.279 -.092 +.161 -.096 -.194 +.007   βm

Car   -.707 +.537 -.628 +.471 -.311 -.003 +.003 +.315 -.073   Car

OGDH   +.533 -.573 +.542 -.553 +.244 -.197 +.461 -.416 +.156 +.034   OGDH

PFK   -.280 +.601 -.404 +.536 -.585 +.030 +.149 +.620 +.242 +.243 -.220   PFK

CPK   +.361 -.459 +.340 -.434 +.193 -.535 +.206 -.089 -.106 -.074 +.349 -.074   CPK

LDH   -.461 +.535 -.588 +.509 -.545 -.167 -.147 +.321 +.156 +.229 -.469 +.805 +.093   LDH

%LDH5 -.529 +.553 -.495 +.431 -.416 +.137 +.135 +.687 -.011 +.487 -.324 +.462 +.203 +.641 %LDH5

TF   -.019 +.986 +.069 +.049 -.052 -.033 +.322 -.275 -.194 +.158 +.098 -.262 +.681 -.245 +.396

N.B. n = 14 except for correlations with Timeform (n = 8)

Table 5.3. Correlation matrix for group A1 (Untrained 1 year olds).

```
                %I

%IIB      ***  %IIB
          -ve

%AOI      ***  ***  %AOI
          +ve  -ve

%AOIIB    **   **   ***  %AOIIB
          -ve  +ve  -ve

%H-SDH    *    **   **   ***  %H-SDH
          +ve  -ve  +ve  -ve

Gly                                     Gly

TAN                                     TAN

TCr       *    **   **        ***       TCr
          -ve  +ve  -ve       +ve

βm                                      βm

Car       **   *    *                             Car
          -ve  +ve  -ve

OGDH      **   *    *                                   OGDH
          +ve  -ve  +ve

PFK            *              *    ***                       PFK
               +ve           -ve  +ve

CPK                               *    **                         CPK
                                  -ve  -ve

LDH       *    *    *         *    **        ***                       LDH
          -ve  +ve  -ve       -ve  -ve       +ve

%LDH5     *    *                                                 * %LDH5
          -ve  +ve                                               +ve

TF
```

* - P<0.05   ** - P<0.01   *** - P<0.001

%H-SDH   % frequency of high SDH staining fibres
Gly   Glycogen
βm   Muscle buffering capacity
Car   Carnosine
%LDH5   % LDH5 isoenzyme
TF   Timeform

Correlation matrix for group A2 (r values)

```
          %I

%IIB    -.514  %IIB

%AOI    +.904  -.457  %AOI

%AOIIB  -.341  +.977  -.302  %AOIIB

%H-SDH  +.623  -.633  +.445  -.605  %H-SDH

Gly     -.489  -.221  -.659  -.321  -.064   Gly

TAN     -.127  +.276  -.143  +.333  -.363  +.152   TAN

TCr     -.658  +.125  -.730  -.020  -.213  +.429  +.149  TCr

βm      -.643  +.442  -.523  +.392  -.898  +.165  +.257  +.327   βm

Car     -.868  +.639  -.893  +.528  -.635  +.538  +.482  +.627  +.551   Car

OGDH    +.406  -.419  +.555  -.371  +.059  -.483  -.231  +.951  -.058  -.536   OGDH

PFK     -.393  +.400  -.319  +.285  +.208  -.055  -.074  +.147  -.266  +.286  -.521   PFK

CPK     +.684  -.509  +.744  -.439  +.652  -.608  -.237  -.366  -.495  -.834  +.448  +.049   CPK

LDH     -.499  +.403  -.526  +.323  -.065  +.419  +.284  +.002  +.041  +.550  -.935  +.701  -.341   LDH

%LDH5   -.611  +.035  -.693  -.106  -.168  +.697  -.459  +.302  +.281  +.414  -.438  +.116  -.604  +341   %LDH5
```

N.B. n = 8

Table 5.4. Correlation matrix for group A2 (Light-Medium trained 2 year olds).

	%I	%IIB	%AOI	%AOIIB	%H-SDH	Gly	TAN	TCr	βm	Car	OGDH	PFK	CPK	LDH	%LDH5
	%I														
%IIB		%IIB													
%AOI	** +ve		%AOI												
%AOIIB		*** +ve		%AOIIB											
%H-SDH					%H-SDH										
Gly						Gly									
TAN							TAN								
TCr			* -ve					TCr							
βm					** -ve				βm						
Car	** -ve		** -ve							Car					
OGDH											OGDH				
PFK												PFK			
CPK			* +ve						** -ve				CPK		
LDH											*** -ve	** +ve		LDH	
%LDH5															%LDH5

* – P<0.05   ** – P<0.01   *** – P<0.001

%H-SDH	% frequency of high SDH staining fibres
Gly	Glycogen
βm	Muscle buffering capacity
Car	Carnosine
%LDH5	% LDH5 isoenzyme

```
            %I

%IIB    -.559  %IIB

%AOI    +.269  -.063   %AOI

%AOIIB  -.292  +.687  -.488   %AOIIB

%H-SDH  +.414  -.182  +.442  -.175   %H-SDH

Gly     -.221  -.237  -.369  -.118  -.361   Gly

TAN     +.267  -.264  +.174  -.268  +.040  +.352    TAN

TCr     +.102  -.056  +.150  -.060  -.070  +.387  +.777  TCr

βm      +.345  -.377  +.309  -.375  +.167  +.170  +.478  +.359   βm

Car     -.346  +.328  +.109  -.014  -.062  +.160  +.407  +.636  +.119   Car

OGDH    -.363  -.116  -.246  -.131  -.125  +.262  +.039  -.035  -.026  +.109   OGDH

PFK     -.090  -.044  +.025  -.181  -.305  +.272  +.332  +.476  +.154  +.412  +.293   PFK

CPK     +.425  -.388  +.271  -.446  +.190  +.215  +.549  +.521  +.264  +.260  +.239  +.608   CPK

LDH     +.015  +.088  +.310  -.097  -.135  -.193  +.115  +.391  -.152  +.383  +.066  +.630  +.527   LDH

%LDH5   -.180  +.414  +.130  +.235  -.048  -.095  +.434  +.563  -.113  +.575  -.136  +.194  +.043  ±166   %LDH5

TF      -.223  +.322  +.155  +.242  -.458  -.394  -.322  -.092  +.033  -.091  -.095  +.569  -.096  +.640  -.095
```

N.B. n = 20 except for correlations with Timeform (n = 10)

Table 5.5. Correlation matrix for group B1 (Light-Medium trained 2 year olds).

	%I	%IIB	%AOI	%AOIIB	%H-SDH	Gly	TAN	TCr	βm	Car	OGDH	PFK	CPK	LDH	%LDH5	TF
%I	%I															
%IIB	* -ve	%IIB														
%AOI			%AOI													
%AOIIB		*** +ve	* -ve	%AOIIB												
%H-SDH			* +ve		%H-SDH											
Gly						Gly										
TAN							TAN									
TCr							*** +ve	TCr								
βm							* +ve		βm							
Car								** +ve		Car						
OGDH											OGDH					
PFK								* +ve				PFK				
CPK				* -ve			* -ve	** -ve					CPK			
LDH												** +ve	* +ve	LDH		
%LDH5								** +ve	** +ve					* +ve	%LDH5	
TF												* +ve		* +ve		

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

%H-SDH	% frequency of high SDH staining fibres
Gly	Glycogen
βm	Muscle buffering capacity
Car	Carnosine
%LDH5	% LDH5 isoenzyme
TF	Timeform

Correlation matrix for groups A2 and B1 combined (r values)

```
            %I

%IIB    -.471  %IIB

%AOI    +.026  +.179  %AOI

%AOIIB  -.104  +.737  -.309  %AOIIB

%H-SDH  +.467  -.682  -.153  -.437  %H-SDH

Gly     -.049  -.394  -.393  -.449  +.482   Gly

TAN     -.109  +.389  -.068  +.330  -.457  -.266    TAN

TCr     -.153  -.072  -.329  -.246  +.169  +.882  -.004  TCr

βm      +.530  +.317  -.001  +.427  -.648  -.512  +.306  -.411   βm

Car     -.642  +.623  +.038  +.189  -.513  +.073  +.613  +.316  -.048   Car

OGDH    -.539  +.098  -.397  +.009  +.094  +.453  -.159  +.510  -.331  +.296   OGDH

PFK     -.116  -.015  -.157  -.224  +.052  +.705  -.125  +.808  -.408  +.221  +.364   PFK

CPK     +.304  -.387  -.127  -.493  +.446  +.694  -.054  +.719  -.519  -.002  +.273  +.753   CPK

LDH     -.122  +.117  +.362  -.225  -.056  +.043  +.039  +.124  -.459  +.275  -.150  +.549  +.373   LDH

%LDH5   -.572  +.515  -.070  +.265  -.512  -.022  +.717  +.271  +.242  +.747  +.352  +.072  -.094  -.041  %LDH5

TE      -.304  +.278  +.167  +.174  -.522  -.379  -.060  -.257  +.232  +.019  -.141  +.198  -.240  +.548  +.093
```

N.B. n = 13

Table 5.6. Correlation matrix for groups A2 and B1 combined (i.e. all 2 year old horses before first racing season, horses with Timeform only)

	%I	%IIB	%AOI	%AOIIB	%H-SDH	Gly	TAN	TCr	βm	Car	OGDH	PFK	CPK	LDH	%LDH5
%IIB															
%AOI															
%AOIIB	** +ve														
%H-SDH		** -ve													
Gly															
TAN															
TCr						*** +ve									
βm					* -ve										
Car	* -ve	* +ve			* -ve		* +ve								
OGDH															
PFK						** +ve		*** +ve							
CPK						** +ve		** +ve				** +ve			
LDH													* +ve		
%LDH5	* -ve				* -ve		** +ve			** +ve					
TF					* -ve										* +ve

* - $P<0.05$ ** - $P<0.01$ *** - $P<0.001$

%H-SDH	% frequency of high SDH staining fibres
Gly	Glycogen
βm	Muscle buffering capacity
Car	Carnosine
%LDH5	% LDH5 isoenzyme
TF	Timeform

Correlation matrix for group B2 (r values)

	%I	%IIB	%AOI	%AOIIB	%H-SDH	Gly	TAN	TCr	θm	Car	OGDH	PFK	CPK	LDH	%LDH5
%IIB	-.571														
%AOI	+.580	-.401													
%AOIIB	-.590	+.704	-.654												
%H-SDH	+.496	-.638	+.072	-.368											
Gly	+.171	-.446	+.210	-.480	+.230										
TAN	+.114	+.326	-.195	+.312	-.425	+.180									
TCr	-.090	+.394	-.444	+.477	-.291	+.077	+.879								
θm	-.111	+.066	-.123	+.043	-.199	-.506	-.021	-.131							
Car	-.587	+.381	-.633	+.470	-.382	-.140	+.152	+.170	+.435						
OGDH	+.419	-.724	+.275	-.354	+.833	+.053	-.521	-.455	+.071	-.263					
PFK	-.394	+.460	-.628	+.713	-.338	-.277	+.472	+.575	-.044	+.253	-.431				
CPK	+.352	+.046	+.039	-.049	-.205	-.096	+.579	+.384	+.249	-.113	-.165	+.444			
LDH	-.578	+.734	-.737	+.859	-.568	-.454	+.477	+.527	+.346	+.721	-.560	+.728	+.182		
%LDH5	-.375	+.291	-.754	+.610	+.007	-.497	+.036	+.304	+.149	+.494	-.020	+.708	+.119	+.630	
TF	-.564	+.481	-.563	+.892	-.210	-.297	+.425	+.551	-.043	+.142	-.289	+.812	-.007	+.646	+.515

N.B. n = 12 except for correlations with Timeform (n = 7)

Table 5.7. Correlation matrix for group B2 (Fully trained two year olds).

	%I	%IIB	%AOI	%AOIIB	%H-SDH	Gly	TAN	TCr	βm	Car	OGDH	PFK	CPK	LDH	%LDH5
%I	%I														
%IIB	* -ve	%IIB													
%AOI	* +ve		%AOI												
%AOIIB	* -ve	** +ve	* -ve	%AOIIB											
%H-SDH		* -ve			%H-SDH										
Gly						Gly									
TAN							TAN								
TCr							*** +ve	TCr							
βm									βm						
Car	* -ve		* -ve							Car					
OGDH		** -ve			*** +ve						OGDH				
PFK			* -ve	** +ve				* +ve				PFK			
CPK								* +ve					CPK		
LDH	* -ve	** +ve	** -ve	*** +ve	* -ve					** +ve		** +ve		LDH	
%LDH5		** -ve	* +ve											* +ve	%LDH5
TF				** +ve								* +ve			

* - P<0.05   ** - P<0.01   *** - P<0.001

%H-SDH	% frequency of high SDH staining fibres
Gly	Glycogen
βm	Muscle buffering capacity
Car	Carnosine
%LDH5	% LDH5 isoenzyme
TF	Timeform

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Correlation matrix for group C (r values)

%I

%IIA   +.082   %IIA

%IIB   -.438   -.931   %IIB

TAN   -.081   -.364   +.363   TAN

TCr   -.074   -.338   +.077   +.799   TCr

βm   -.532   -.326   +.507   +.482   +.504   βm

Car   -.018   -.481   +.439   +.617   +.517   +.396   Car

OGDH   +.111   +.436   -.222   +.497   -.199   -.446   +.158   OGDH

PFK   +.176   +.296   -.251   +.578   +.567   -.206   +.769   -.234   PFK

CPK   +.486   -.448   -.292   +.165   -.248   -.107   +.635   +.260   +.673   CPK

LDH   +.053   -.335   +.303   +.561   +.701   +.350   +.812   -.391   +.687   +.557   LDH

TF (2YO) -.265 -.453 +.539 +.381 -.364 -.410 +.200 -.428 -.031 +-.302 -.379

TF (3YO) -.179 -.633 +.508 -.150 -.344 -.169 -.155 -.457 +.359 +.248 -.110

N.B. n = 16 except for correlations with Timeform: TF 2YO - n = 10

                                                          TF 3YO - n = 12

Table 5.8. Correlation matrix for group C (Fully trained 3 year olds).

	%I	%IIA	%IIB	TAN	TCr	βm	CAR	OGDH	PFK	CPK	LDH
%I	%I										
%IIA		%IIA									
%IIB		*** -ve	%IIB								
TAN				TAN							
TCr				*** +ve	TCr						
βm	* -ve		* +ve		* +ve	βm					
CAR				* +ve	* +ve		CAR				
OGDH								OGDH			
PFK				* +ve	* +ve		*** +ve		PFK		
CPK							** +ve		** +ve	CPK	
LDH				* +ve	** +ve		*** +ve		** +ve	* +ve	LDH
TF (2YO)			* +ve								
TF (3YO)		** -ve	* +ve								

* - P<0.05   ** - P<0.01   *** - P<0.001

βm	Muscle buffering capacity
Car	Carnosine
TF	Timeform for 2YO and 3YO racing seasons

al (1980) following endurance training. In agreement with the present study is an increase of 32% in muscle glycogen content following a training programme incorporating both cantering and high-intensity, repeated gallops (Guy and Snow, 1977).

The changes due to age and training in the horses in Stable B with respect to histochemical parameters were relatively small. There were however, significant increases in high-staining SDH fibres and OGDH activity. A slight anomaly is the apparent increase in PFK activity, but this may be exaggerated due to the initial low activity in group B1, as the activity for group B2 is not different from that in groups A1, A2 or C.

The differences between groups A2 and B1 may reflect the differences between stables in nutrition and or training programmes. It appears that the horses in Stable A (group A2) as two year olds, at the start of the season, may have been trained either more effectively or to a greater degree than the horses in Stable B (group B1). This is interpreted from the fact that in certain parameters, the horses in group B1 are more like the horses in group A1 than A2. For example, high staining SDH fibre frequency, glycogen, TAN and OGDH activity were very similar in these two groups. Furthermore, group A2 was similar in many ways to group B2. Thus, group A2 may have reached a relatively more advanced stage of training than group B1, although all horses were of comparable age.

The overall trends across all groups indicates a tendency to increased aerobic capacity with age and training, for example the increasing trend in mean OGDH activity. However, there appears to be little change in glycolytic capacity in two year olds and upwards. The muscle composition of one year old horses represents the untrained condition and is therefore almost entirely determined by genotype. It is perhaps not surprising to find that young horses have relatively high muscle glycolytic and low muscle aerobic capacities. This could be related to a survival strategy that involves being able to accelerate rapidly and outrun a predator over a relatively short distance, in order to convince the predator that pursuit is uneconomical. Thus, horses in the wild would tend to 'exercise' themselves, infrequently and over relatively short distances, activity which might not be expected to induce changes in muscle composition, especially in terms of the muscle aerobic capacity. A similar situation is seen in rats, with young rats having a higher glycolytic capacity compared with aerobic capacity and aerobic capacity increases with age, irrespective of training.[*] In the Thoroughbred horse, it is likely that ageing and current training methods result in an increase in muscle aerobic capacity at the expense of glycolytic capacity.

[*] Alnaqeeb and Goldspink, 1986.

In Britain, flat racing under the control of the Jockey Club takes place over distances of 5 - 21 furlongs (1000 - 4200 metres). A higher percentage of races for two year old horses are run over eight furlongs (one mile) or less compared to three year old races, i.e. on the whole, the younger horses in their first season tend to run over the shorter distances (see Figure 5.15). This may be because it has been recognized that two year olds are generally faster than three year olds over shorter race distances or alternatively that two year olds do not have the same ability to 'stay', that is they do not have a sufficiently high aerobic capacity to race successfully over longer distances. The latter may be a more likely explanation as the records for races between 5 and 8 furlongs are all held by horses of 4 years or over (see Introduction).

In groups B1 and B2, where sufficient numbers of horses were sampled on both occasions to allow correlations with Timeform, correlations were found between performance (i.e. Timeform) and PFK and LDH activity and relative area occupied by IIB fibres. When groups A2 and B1 were combined, a significant positive correlation was found with LDH and a negative correlation with the percentage of high-staining SDH fibres. Also, in group C, two year old racing performance was positively correlated to %IIB fibre frequency and negatively correlated to %IIA fibre frequency (estimated when the horses were 3 years old, i.e. retrospectively). In both two and three year old horses, the horses with the higher glycolytic or sprinting capacities performed the best. The trainer of Stable B favoured entering his two year old horses in longer races (8.1±2.2 furlongs) compared to the trainer of Stable A (6.0±0.9 furlongs) and the trainer of Stable C (6.9±0.8 furlongs). This might well be expected to be reflected in different approaches to training and may in part explain some of the differences observed between groups A2 and B1. Horses in group B1 were first sampled as two year olds before their first racing season and the correlation of PFK and the LDH with Timeform (P<0.05) indicates there may be scope for selection of horses based on a single muscle biopsy. However, there were no significant correlations between muscle parameters and subsequent racing performance in one year old horses (group A1) and insufficient numbers of horses in group A2 with Timeform ratings to make a correlation, therefore the predictive aspect should still be treated with caution, especially as it has been demonstrated that large variations can occur in the distribution of both fibre types (Bruce and Turek, 1985; Kline et al, 1987) and enzyme activities (McCutcheon et al, 1987) within the middle gluteal. However, it has also been demonstrated that careful standardization of the sampling site results in an acceptably small amount of variation (Wood et al, 1988b)

Figure 5.15. Mean racing distance of horses sampled. Although horses in Stable C were only sampled at 3 years of age, their previous seasons mean racing distance is also shown.

Distance, furlongs

12 —

11 —

10 —

9 —

8 —

7 —

6 —

5 —

2200

1800

1400

1000

metres

Stable A
2 y.o.
Season
41 races

Stable B
2 y.o.
Season
115 races

Stable C
2 y.o.
Season
31 races

Stable C
3 y.o.
Season
70 races
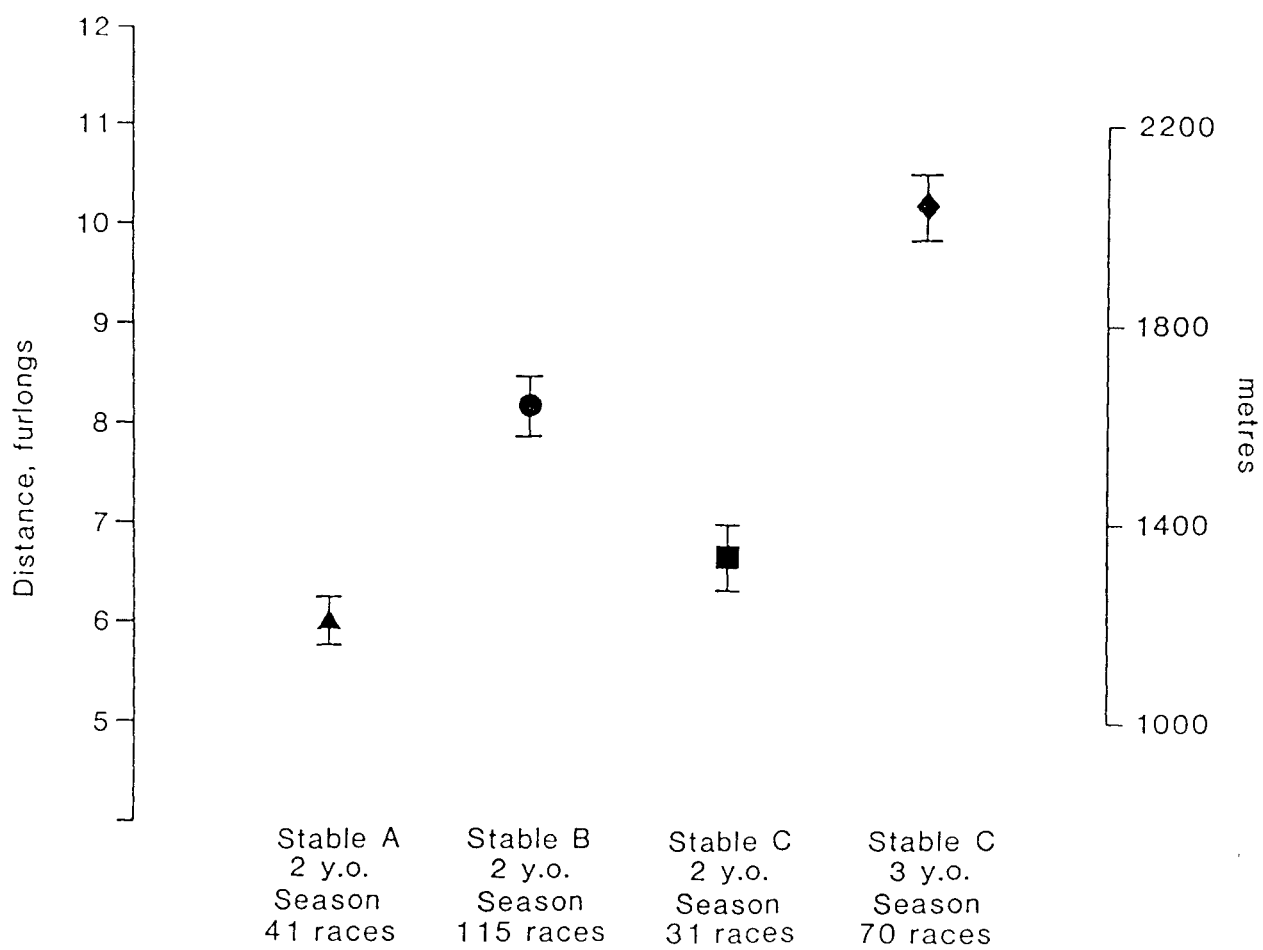
Evidence of a relationship between muscle fibre composition and racing performance has previously been documented. Barlow et al (1984) found that Thoroughbred yearlings with a percentage of type II fibres in the semitendinosus of greater than 90% subsequently won more races and had higher total earnings compared to those with less than 90% type II fibres. Recently, Wood et al (1988a) found that successfully raced Quarter Horses possessed a significantly lower percentage of type I and a higher percentage of type IIB fibres.

Evidence of an increased muscle aerobic capacity in Thoroughbreds has been found following both conventional racehorse training programs (Guy and Snow, 1977 ; Lindholm et al, 1983; Hodgson et al, 1986; McCutcheon et al, 1987) and high-intensity interval training programs (Wilson et al, 1987) as indicated by increases in the activities of oxidative enzymes such as citrate synthase (CS) and 3-hydroxy-acyl coenzyme A dehydrogenase (HAD) and increases in the percentage frequency of type IIA fibres at the expense of IIB fibres. Additionally, similar changes have also been observed in Standardbred horses following a programme of submaximal treadmill training (Hodgson et al, 1985) and also following a conventional race training programme (Essén-Gustavsson et al, 1983). Therefore, the indication of an increasing muscle aerobic capacity of Thoroughbred horses with age and training in the present study is in agreement with previous observations.

In relation to glycolytic capacity, lactate dehydrogenase (LDH) activity and % IIB fibre composition have been frequently used as markers. Muscle LDH activity has been shown to decrease with submaximal exercise training (Hodgson, 1985), to remain unchanged (Essén-Gustavsson et al, 1983; Hodgson et al, 1986) and to increase (Guy, 1978) following conventional type racehorse training. Guy (1978) also found a decrease in the percentage of the LDH-5 isoenzyme following training. The present study also indicated a decrease in %LDH-5 with age and training, however this was accompanied by a decrease in LDH between groups A1 and A2. Both %LDH5 and LDH activity were similar in all other age/training groups, the changes between groups A1 and A2 most likely occurring due to the high aerobic input to the training programme between sample collections. This is supported by the study of Hodgson et al, (1985).

Overall, the changes observed in muscle composition in the present study in relation to age and training tend to indicate an increased muscle aerobic capacity at the expense of the muscle glycolytic capacity. However, in view of the apparent relationship between muscle glycolytic capacity and performance in the present study, an increase in aerobic capacity with training may not be desirable, and possibly detrimental to performance.

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This suggests that current training practices may be less than optimal, especially in view of the fact that horses now race over shorter distances and training practices do not seem to have changed significantly to take this into account.

In accordance with previous calculations (see Chapter 4), the contribution of carnosine to total physicochemical buffering in horse muscle is estimated to be 37, 34, 31, 35 and 29% for groups A1, A2, B1, B2/B3 and C, respectively. This did not appear to be different due to age, sex or training. The positive correlations between IIB fibres and carnosine content indicates that the concentration of carnosine is probably higher in IIB fibres. This is consistent with its function as a major buffer to lactic acid production.

Throughout the present investigation, muscle biopsies were taken at a constant depth of 6cm. However, this may represent a different relative depth in horses of different size. As discussed previously, Bruce and Turek (1985) have observed pronounced heterogeneity within the middle gluteal of the horse and clearly this could have contributed to the overall variance in the data in the present study.

CHAPTER 6

INVESTIGATIONS INTO THE METABOLIC RESPONSE TO HIGH-INTENSITY
EXERCISE IN THE THOROUGHBRED HORSE: CHANGES WITH EXERCISE.

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INTRODUCTION

The studies presented in the present chapter were undertaken with two main aims. Firstly, to define the metabolic changes in muscle and blood associated with single bouts of high-intensity field and treadmill exercise and secondly, to investigate the metabolic stress of repeated maximal gallops.

The high percentage of type IIA and IIB muscle fibres and the high activities of both oxidative and glycolytic enzymes in horse muscle (see Chapter 1) endow the horse with a high capacity for both aerobic and anaerobic energy production. Maximal exercise in the horse results in very high rates of glycogen utilization. The accumulation of lactate and other intermediary metabolites in the horse (Snow and Mackenzie, 1977; Nimmo and Snow, 1983; Snow, Harris and Gash, 1985) is generally greater than that seen in humans following high-intensity exercise (e.g. Sahlin et al, 1976; Jacobs et al, 1982; Cheetham et al, 1988). In a study of intermittent maximal exercise (Snow, Harris and Gash, 1985) a 1-26% decrease in the muscle ATP content was observed in four Thoroughbred horses after a single maximal gallop of 620m and a 16-51% decrease after four consecutive 620m gallops with a five minute interval between gallops. The decline in muscle ATP with consecutive gallops was also accompanied by a reduction in running speed. In addition, little recovery in muscle lactate or ATP was found in a thirty minute recovery period following the fourth gallop (see Chapter 7).

The exercise model used in the study of Snow, Harris and Gash (1985) indicated the changes that occur with strenuous, intermittent, maximal exercise. Under these conditions the changes seen are likely to be more pronounced than after a single exercise bout.

The first study reported in this chapter was designed to specifically investigate the metabolic changes associated with single maximal gallops of 800 and 2000m and in particular, to establish the extent of nucleotide loss under these conditions. These distances encompass sprint to middle-distance flat races.

The second study reported involved 3 sessions, each consisting of two repeated maximal gallops of 900m and was undertaken to further investigate the findings of Snow, Harris and Gash (1985) and also to estimate the reproducibility of maximal exercise under field conditions.

The third study presented in this chapter consisted of four maximal gallops of 700m, with a 20 min recovery period between each gallop and is similar to the study of Snow, Harris and Gash (1985), with the exception

that these authors only allowed 5 min recovery between gallops. This study was undertaken in an attempt to explain the slow recovery observed in blood lactate during the 30 min recovery period following the fourth gallop (see Chapter 7) and to further investigate the metabolic changes associated with intermittent, high-intensity exercise.

In the fourth study, blood samples were collected from a number of Thoroughbred racehorses competing in flat-races of various distances at Newmarket racecourse. This was undertaken to determine if similar metabolic changes occur under true race conditions compared to in a group of experimental animals.

The relevance and reproducibility of treadmill exercise compared with field exercise is important in the validation of the treadmill for use in exercise studies. Thus, the metabolic changes associated with high-intensity treadmill exercise, their reproducibility and relevance to changes seen in the field were investigated. Preliminary treadmill studies indicated that 2 min exercise at 12 m/s (distance = 1440m) on a 5° incline was sufficient to induce signs associated with fatigue towards the end of the exercise period in the majority of experimental horses. Fatigue was defined as an increasing inability of the horse to maintain its position at the front of the treadmill. This exercise protocol was therefore selected to minimize the risk of injury and for its ability to induce fatigue in a relatively short time period.

MATERIALS AND METHODS

The results presented in this chapter were obtained in the following studies of field exercise:

(1) Single maximal gallops of 800 and 2000 metres.

(2) Repeated maximal gallops of 2 x 900 metres.

(3) Intermittent maximal exercise of 4 x 700 metres.

(4) Flat-racing at Newmarket racecourse.

The results for treadmill exercise were taken from the following study:

(5) Single gallops of 1440 metres at 12 m/s on a 5° incline.

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(1) SINGLE MAXIMAL GALLOPS OF 800 AND 2000M

Animals and exercise protocol: Seven trained Thoroughbred horses (3 mares, 4 geldings) were used in this study. The horses were walked and trotted approximately 2.5km from the stables to the track where the gallops took place. On arrival at the track the horses were given a short warm-up canter of approximately 200m and were then walked back to the start. The horses then galloped maximally over a distance of either 800m on an all-weather wood-chip track or 2000m on an all-weather sand track. Two horses (AL and JW) completed the 2000m gallop only; three horses (PE, MR and CL) completed the 800m gallop only; two horses (LW and RS) completed both the 800 and 2000m gallops. Two jockeys of approximately the same weight rode the horses in all gallops. On all occasions the horses were exercised in pairs and the jockeys instructed to ride the horses maximally from the start of the gallop. At least one week was allowed between gallops for horses LW and RS. For the 60 minute period following either gallop the horses were walked without a rider (i.e. led).

Sampling: Muscle biopsies were obtained from the middle gluteal as described in Chapter 2. Venous blood samples were collected by venepuncture. Pre-gallop muscle and blood samples were collected following the warm-up canter, except in the case of two horses (LW - 800m gallop, JW - 2000m gallop) which were known to be very excitable and difficult to sample and were therefore sampled prior to the warm-up canter. Horses were biopsied and blood sampled within 1-2 minutes of the completion of the gallop.

Analysis: Muscle ATP, ADP, AMP, phosphocreatine (PCr), creatine (Cr), hexosemonophosphate (HMP), glycerol, glycerol 3-phosphate (glycerol 3-P), pyruvate, lactate and alanine, plasma ammonia, uric acid and pH and blood glycerol, glucose and lactate were analysed as described in Chapter 2.

(2) REPEATED MAXIMAL GALLOPS OF 2 X 900M.

Animals and exercise protocol: Five trained Thoroughbred horses (4 geldings, 1 mare) were used in this study. One of the horses (RS) was used in the previous study of 800 and 2000m gallops. All gallops took place on the same track that was used for the 800m gallops in the previous study. On arrival at the track the horses were given a warm-up canter, were walked back to the start and then were galloped maximally over 900m. The horses were then walked and trotted back to the start (approximately 10 minutes) and then completed the second gallop, following which the horses were walked without a rider for 60 minutes. The exercise was repeated three

times for each horse, one week apart.

Sampling: Muscle biopsies were obtained and catheterization performed as described in Chapter 2. Muscle biopsies were collected before and immediately after the second gallop. Blood samples were collected following the warm-up canter (Pre) and at 2 minutes following the second gallop.

Analysis: ATP, lactate, PCr, Cr and glycogen in muscle, glucose in plasma and lactate in blood were analysed as described in Chapter 2.

(3) INTERMITTENT MAXIMAL EXERCISE OF 4 X 700M

Animals and exercise protocol: Four trained Thoroughbred geldings were used in this study. Two of the horses (KJ and SM) were used in the previous study of 2 x 900m. All gallops took place on the same track as the 800 and 2 x 900m gallops in the previously described studies. On arrival at the track all horses were given a warm-up canter of 200m, were walked back to the start and were then galloped maximally over 700m. The horses were then walked back to the start for the next gallop. Twenty minutes recovery was allowed between gallops and all horses were walked continuously during this period.

Sampling: The horses were catheterized as described in Chapter 2. Venous blood samples were collected after the warm-up canter (Pre) and at 2 minutes following each gallop.

Analysis: Blood lactate, plasma lactate, ammonia and uric acid and PCV were analysed as described in Chapter 2.

(4) SAMPLES FROM HORSES COMPETING IN FLAT RACES.

Venous blood samples were collected from sixteen Thoroughbred horses (2 to 3 years old) following racing over distances of 1200 to 2400 metres (6-12 furlongs) on the Newmarket July course. Samples were collected by venepuncture between 6 and 12 minutes post-racing.

(5) SINGLE TREADMILL GALLOPS OF 1440M

Animals and exercise protocol: Five trained Thoroughbred geldings accustomed to treadmill exercise were used in this study. All horses completed two experimental sessions (except horse KJ) in a period of seven weeks. At least one week was allowed between each session. Each experimental session consisted of 4 minutes walking (5° incline, 1.6m/s), 4 minutes trotting (5°, 3.2 m/s) and a 40 sec acceleration period to 12 m/s which was then maintained for 2 minutes (5° incline).

<u>Sampling</u>: Muscle biopsies were obtained and catheterization performed as described in Chapter 2. Muscle biopsies were collected at rest (pre) and following exercise (approximately 40-90 sec from the end of the gallop, due to the time for deceleration of the treadmill). Venous blood samples (20ml) were taken at rest (pre) and at the end of 2 minutes at 12 m/s.

<u>Analysis</u>: Muscle ATP, ADP, AMP, PCr, Cr, glycogen, HMP, glucose, glycerol 3-P, glycerol, pyruvate, lactate and alanine, blood lactate and glucose and plasma ammonia and pH were analysed as described in Chapter 2.

RESULTS

(1) <u>SINGLE MAXIMAL GALLOPS OF 800 AND 2000 METRES</u>

<u>Performance</u>: The mean gallop times for horses AL, LW, JW and RS over 2000m was 2 min 29 sec (range 2 min 20 sec to 2 min 38 sec; mean speed 13.4 m/s) and for horses LW, RS, PE, MR and CL over 800m was 56 sec (range 54 to 60 sec; mean speed 14.3 m/s). Individual gallop times for the 800 and 2000m gallops are shown in Tables 6.1 and 6.2, respectively.

<u>Muscle metabolite changes</u>: Muscle metabolite contents before (pre) and after exercise (post) are shown in Tables 6.3 (800m) and 6.4 (2000m). All horses showed a decrease in ATP with exercise that was significantly greater ($P<0.01$) after the 2000m gallop ($47\pm3\%$) compared with the 800m gallop ($30\pm7\%$). There were no significant changes in ADP or AMP. The initial content of PCr was approximately 60 mmol/kg d.m. and the mean percentage decrease was greater following the 2000m gallop ($70\pm14\%$) compared with the 800m gallop ($46\pm8\%$). Rates of glycogen utilization were 2.7 ± 0.9 mmol glucosyl units/kg d.m./sec over the 800m gallop and 1.1 ± 0.1 mmol glucosyl units/kg d.m./sec over the 2000m gallop. Mean glycogen decrease was 162 ± 57 and 177 ± 35 mmol glucosyl units/kg d.m. for the 800 and 2000m gallops respectively. In both cases, $45\pm7\%$ of the glycogen disappearance could be accounted for as accumulation of HMP, glycerol 3-P, lactate and alanine.

No significant difference was found when comparing the accumulation of HMP after the 800 and 2000m gallops ($P>0.05$). Contents were 29.1 ± 6.2 and 28.5 ± 6.1 mmol/kg d.m., respectively. Glycerol 3-P accumulation was significantly greater ($P<0.01$) after the 2000m gallop when compared with the 800m gallop. The increase in glycerol 3-P with both the 800 and 2000m gallops was accompanied by relatively small increases in free glycerol: approximately 2.5 mmol/kg d.m. in either case. Mean muscle lactate contents at the end of exercise were 123.5 ± 49.7 and 167.3 ± 20.7 mmol/kg d.m. for the

Table 6.1. Gallop times for horses completing the 800m gallop.

Horse	Gallop Time (sec)	Mean Speed (m/s)
LW	57.0	14.0
RS	60.0	13.3
PE	55.0	14.5
MR	54.5	14.7
CL	54.0	14.8

Table 6.2. Gallop times for the horses competing the 2000m gallop.

Horse	Gallop Time (sec)	Mean speed (m/s)
AL	149.0	13.4
LW	149.0	13.4
JW	140.0	14.3
RS	158.0	12.7

Table 6.3. Changes in muscle and blood metabolites, blood PCV and plasma AST and CPK activity following a single maximal gallop of 800 metres in 5 Thoroughbred horses.

	PRE	POST	PRE v POST
Muscle			
(mmol/kg d.m.)			
ATP	21.8±2.4	15.3±4.0	P<0.01
ADP	2.9±0.3	2.7±0.4	NS
AMP	0.20±0.05	0.23±0.12	NS
PCr	58.4±13.4	31.1±6.6	P<0.01
Glycogen	586±115	425±86	P<0.01
HMP	3.5±0.6	29.1±6.2	P<0.001
Glycerol 3-P	2.7±0.6	13.6±1.7	P<0.001
Glycerol	0.4±0.5	2.3±1.6	P<0.05
Pyruvate	0.2±0.2	0.4±0.3	NS
Lactate	13.0±5.4	123.5±49.7	P<0.01
Alanine	2.8±1.0	5.8±1.6	P<0.01
Blood			
Lactate (mmol/l)	0.4±0.3	17.8±3.9	P<0.001
Glucose (mmol/l)	4.7±0.6	5.2±0.9	NS
Glycerol (μmol/l)	116±26	192±86	NS
PCV (l/l)	0.495±0.010	0.604±0.031	P<0.001
Plasma			
pH	7.43±0.03	7.13±0.04	P<0.001
Ammonia (μmol/l)	27±22	216±39	P<0.001
Uric acid (μmol/l)	21.4±7.9	46.4±10.9	P<0.01
AST (IU/l)	134±19	132±17 *	NS
CPK (IU/l)	21±12	27±4 *	NS

* Samples for post-exercise AST and CPK were collected 60 minutes after the gallop.

Table 6.4. Changes in muscle and blood metabolites, blood PCV and plasma AST and CPK activity following a single maximal gallop of 2000 metres in 4 Thoroughbred horses.

	PRE	POST	PRE v POST
Muscle			
(mmol/kg d.m.)			
ATP	21.4±3.2	11.3±2.6	P<0.01
ADP	2.6±0.7	2.4±0.8	NS
AMP	0.37±0.19	0.21±0.13	NS
PCr	62.2±7.6	18.5±8.6	P<0.01
Glycogen	550±43	374±86	P<0.01
HMP	4.9±0.3	28.5±6.1	P<0.01
Glycerol 3-P	3.0±0.9	19.4±2.4	P<0.001
Glycerol	0.5±0.2	2.7±1.2	P<0.01
Pyruvate	0.4±0.5	0.4±0.4	NS
Lactate	15.1±6.3	167.3±20.7	P<0.01
Alanine	3.3±0.8	6.0±0.8	P<0.05
Blood			
Lactate (mmol/l)	1.7±0.8	26.5±1.6	P<0.001
Glucose (mmol/l)	5.1±0.7	6.7±1.0	P<0.01
Glycerol (μmol/l)	44±48	273±135	P<0.05
PCV (l/l)	0.518±0.065	0.605±0.047	P<0.001
Plasma			
pH	7.39±0.08	7.05±0.07	P<0.001
Ammonia (μmol/l)	37±10	257±93	P<0.001
Uric acid (μmol/l)	29.3±9.4	59.8±8.6	P<0.001
AST (IU/l)	114±9	123±5 *	NS
CPK (IU/l)	14±4	20±2 *	NS

* Samples for post-exercise AST and CPK were collected 60 minutes after the gallop.

800 and 2000m gallops, respectively. In each case, exercise resulted in a small, non-significant increase in pyruvate. Alanine, formed from the amination of pyruvate, increased approximately 50% with exercise. Neither muscle pyruvate or alanine were significantly different between the two gallops.

Blood changes: Changes in blood parameters following exercise over 800 and 2000m are shown in Tables 6.3 and 6.4, respectively. Blood lactate after exercise was 17.8±3.9 and 26.5±1.6 mmol/l for the 800 and 2000m gallops, respectively. In both cases levels were significantly elevated from pre-exercise concentrations (P<0.001). Lactate accumulation was significantly greater (P<0.01) following the 2000m gallop. The large increase in blood lactate with exercise was accompanied by a pronounced fall in plasma pH from 7.43±0.03 prior to exercise to 7.13±0.04 after the 800m gallop and from 7.39±0.08 to 7.05±0.07 after the 2000m gallop.

Large increases in plasma ammonia were found with exercise, although these were not significantly different between the two gallops. However, there was a trend towards higher levels following the 2000m gallop (257±93 μmol/l) compared to the 800m gallop (216±39 μmol/l). In contrast, uric acid showed a small but significant increase with exercise, which amounted to 25±12 (P<0.01) and 31±4 (P<0.001) μmol/l for the 800 and 2000m gallops, respectively. Blood glycerol only increased significantly following the 2000m gallop (P<0.05) from 44±48 (pre-exercise) to 273±135 μmol/l (post-exercise).

Blood glucose increased from 4.7±0.6 to 5.2±0.9 mmol/l (P>0.05) after the 800m gallop and from 5.1±0.7 to 6.7 ±1.0 mmol/l (P<0.01) after the 2000m gallop. Packed cell volume (PCV) at rest was 0.495±0.010 and 0.518±0.065 l/l before the 800 and 2000m gallops, respectively. Values increased to 0.604±0.031 (800m) and 0.605±0.047 l/l (2000m) following exercise. The increase with exercise was significant (P<0.001) in each case, although there was no difference between gallops. No significant changes in plasma AST or CPK activity were found at 24 hours following each gallop.

(2) REPEATED MAXIMAL GALLOPS OF 2 X 900 METRES

Performance: Mean time for the first 900m gallop was 63.2±3.2 sec (mean speed 14.2 m/s) and for the second 900m gallop 69.4±3.4 sec (mean speed 13.0 m/s). For each horse, the exercise time is based on the mean time for each of the three exercise sessions. Gallop times ranged from 60.4 - 77.0 seconds for G1 and from 63.8 - 79.0 seconds for G2. Individual gallop times

are shown in Table 6.5.

Muscle metabolite changes: Muscle metabolite changes with exercise are shown in Table 6.6. All horses showed a fall in muscle ATP content which amounted to a mean decrease of 43±10% of the original store (P<0.001). Muscle PCr content decreased to 28.5±5.7 mmol/kg d.m. from a resting content of 60.5±7.9 mmol/kg d.m. (P<0.001). Mean muscle glycogen utilization was 239±30 glucosyl units/kg d.m., which indicates a mean glycogen utilization rate of 1.8 mmol glucosyl units/kg d.m./sec.

Blood changes: Blood parameters before and after exercise are shown in Table 6.6. Exercise resulted in a significant increase in blood lactate from 1.1±0.2 mmol/l before exercise to 26.6±1.0 mmol/l following the second gallop (P<0.001). Plasma glucose increased from 5.4±0.4 to 9.0±1.0 mmol/l (P<0.01). No significant increase in plasma AST or CPK activity was found in samples collected 24 hours post-exercise.

Reproducibility of field exercise: The reproducibility of metabolite changes with exercise and gallop times over the three exercise sessions for each horse (calculated as the pooled within-horse standard deviation and expressed as the precision) are shown in Table 6.7.

(3) INTERMITTENT MAXIMAL EXERCISE OF 4 X 700M

Blood and plasma lactate, plasma ammonia and uric acid and PCV before (pre) and following each of 4 maximal gallops are shown in Table 6.8 and individually in Figures 6.1 to 6.5.

Performance: The individual gallop times for the 4 horses are shown in Figure 6.6. Total times for the 4 gallops were 199 sec - CP, 193 sec - KJ, 200 sec - SM and 197 sec - BR. Mean speed for the each of the four gallops G1 - G4 was 14.9, 14.9, 14.1 and 13.0 m/s, respectively. Horse CP completed the fastest G1, but in contrast, showed the slowest G4 and was one of the slowest in terms of total time for the four gallops. In contrast, BR who completed the slowest time for G1, completed the second fastest G4 and was second fastest in terms of overall time. On each subsequent gallop following G1, horses CP, KJ and SM were slower or could only equal their time for the preceeding gallop. In contrast, horse BR recorded faster times for both G2 and G3 compared to G1.

Changes in blood parameters: Each gallop resulted in a significant increase in blood lactate concentration, although the mean increment was reduced with each successive gallop: Pre - G1 = 14.6 mmol/l; G1 - G2 = 5.7 mmol/l; G2 - G3 = 4.2 mmol/l; G3 - G4 = 1.7 mmol/l. The patterns of plasma lactate

Table 6.5. Gallop times for horses completing 2 x 900m gallops.

Horse	Week 1		Week 2		Week 3	
	G1	G2	G1	G2	G1	G2
MN	61.0	65.0	62.0	66.1	59.7	63.8
HR	63.0	74.0	62.2	76.2	61.0	68.0
RS	77.0	79.0	67.3	69.5	68.0	69.6
KJ	60.4	67.0	62.3	70.0	64.0	70.6
SM	60.4	70.2	62.6	65.6	62.5	67.0

Table 6.6. Changes in muscle ATP, PCr, glycogen and lactate content and blood lactate concentration in 5 Thoroughbred horses following two maximal gallops of 900 metres. The exercise was repeated 3 times, each session being separated by one week. The results shown are based on a mean for each horse for the 3 sessions.

	PRE	POST	PRE v POST
Muscle (mmol/kg d.m.)			
ATP	22.3±0.4	12.7±1.3	P<0.001
PCr	60.5±7.9	28.5±5.7	P<0.001
Lactate	14.4±2.9	155.2±18.7	P<0.001
Glycogen	612±44	374±71	P<0.001
Blood			
Lactate (mmol/l)	1.1±0.2	26.6±1.0	P<0.001
Plasma			
Glucose (mmol/l)	5.4±0.4	9.0±1.0	P<0.01
AST (IU/l)	192±34	207±50 *	NS
CPK (IU/l)	29±15	45±17 *	NS

* Post-exercise samples for AST and CPK were collected 24 hours after the exercise.

Table 6.7. Reproducibility of gallop times and muscle and blood metabolite changes in 3 separate exercise sessions, each consisting of 2x900m gallops, calculated as pooled within-horse standard deviation and expressed as the precision.

Muscle	Precision (mmol/kg d.m.)	Blood	Precision (mmol/1)	Gallop Time	Precision (sec)
ATP	2.2	Lactate	1.6	G1	1.5
PCr	5.2			G2	3.4
Lactate	20.7	Plasma			
Glycogen	48	Glucose	0.5		

Table 6.8. Blood lactate, plasma lactate, ammonia and uric acid and PCV in 4 horses before and after each of 4 maximal gallops of 700 metres with 20 min recovery between gallops.

	Pre	G1	G2	G3	G4
Blood lactate (mmol/1)	2.0±0.8	16.6±1.8 ***	22.3±4.5 *	26.5±5.2 **	28.2±6.1 *
Plasma lactate (mmol/1)	4.0±1.4	27.5±3.3 ***	34.2±6.4 *	39.6±6.2 **	41.2±7.0 NS
Ammonia (μmol/1)	124±12	470±175 ***	789±383 *	907±418 *	956±526 NS
Uric acid (μmol/1)	37±21	42±27 NS	144±82 *	196±100 *	211±98 NS
PCV (1/1)	0.58±0.04	0.63±0.05 **	0.65±0.06 NS	0.64±0.06 NS	0.65±0.06 NS
Gallop time (sec)	–	47.0±2.5 –	47.0±0.8 NS	49.5±1.3 *	53.8±3.9 NS

Statistical comparisons were made using Students' t-test for paired data. Significance levels are indicated as follows: NS – non-significant; * – P<0.05; ** – P<0.01; *** – P<0.001. The significance level shown under each column refers to the comparison with the preceeding column.

Figure 6.1. Venous whole blood lactate concentration in four horses before (Pre) and after each of four maximal gallops of 700m in the field, each gallop separated by a 20 min recovery period.

Figure 6.2. Venous plasma lactate concentration in four horses before (Pre) and after each of four maximal gallops of 700m in the field, each gallop separated by a 20 min recovery period.
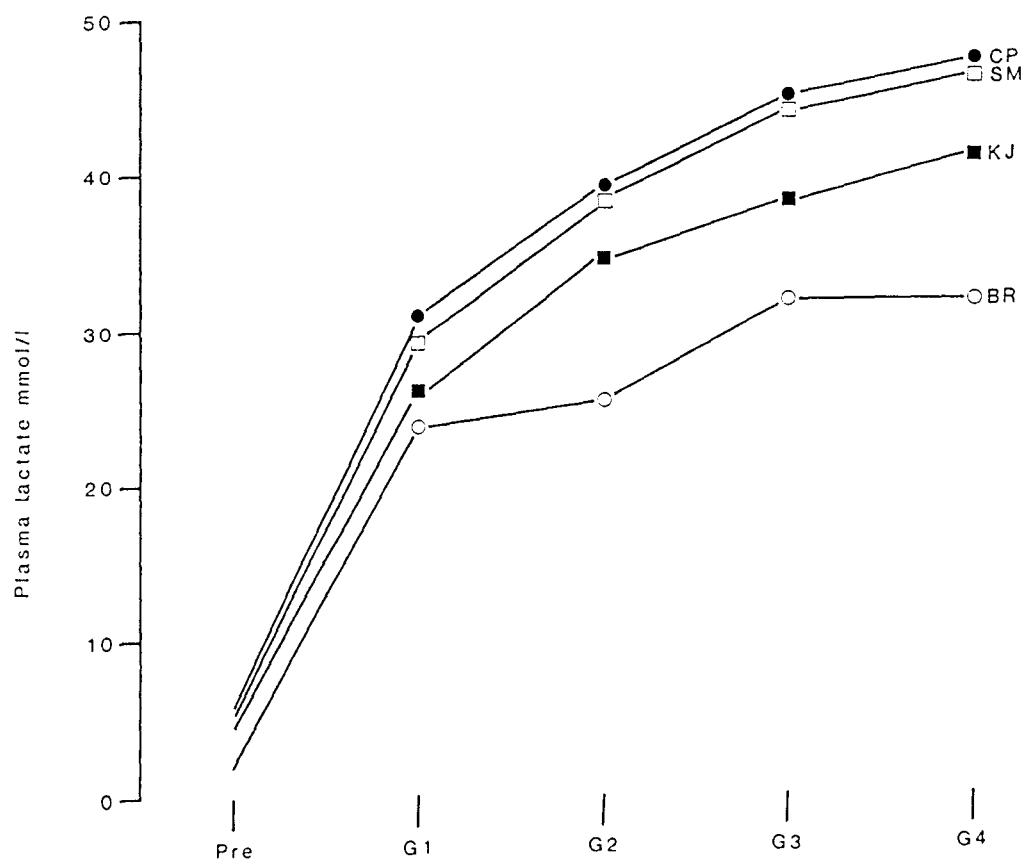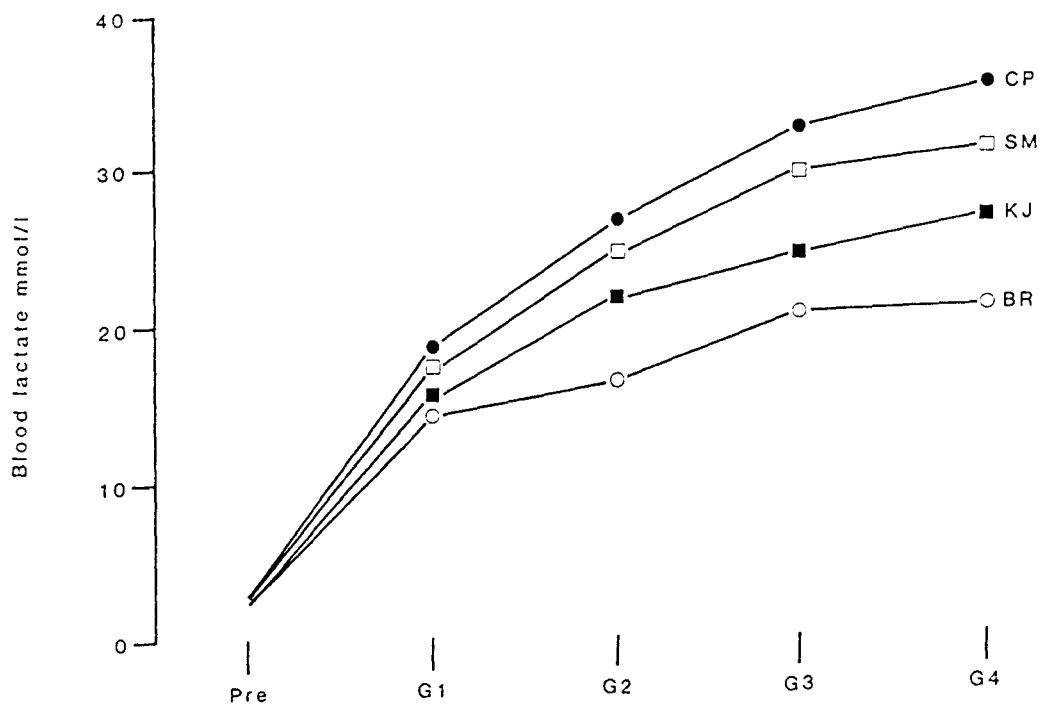
Figure 6.3. Venous plasma ammonia concentration in four horses before (Pre) and after each of four maximal gallops of 700m in the field, each gallop separated by a 20 min recovery period.

Figure 6.4. Venous plasma uric acid concentration in four horses before (Pre) and after each of four maximal gallops of 700m in the field, each gallop separated by a 20 min recovery period.
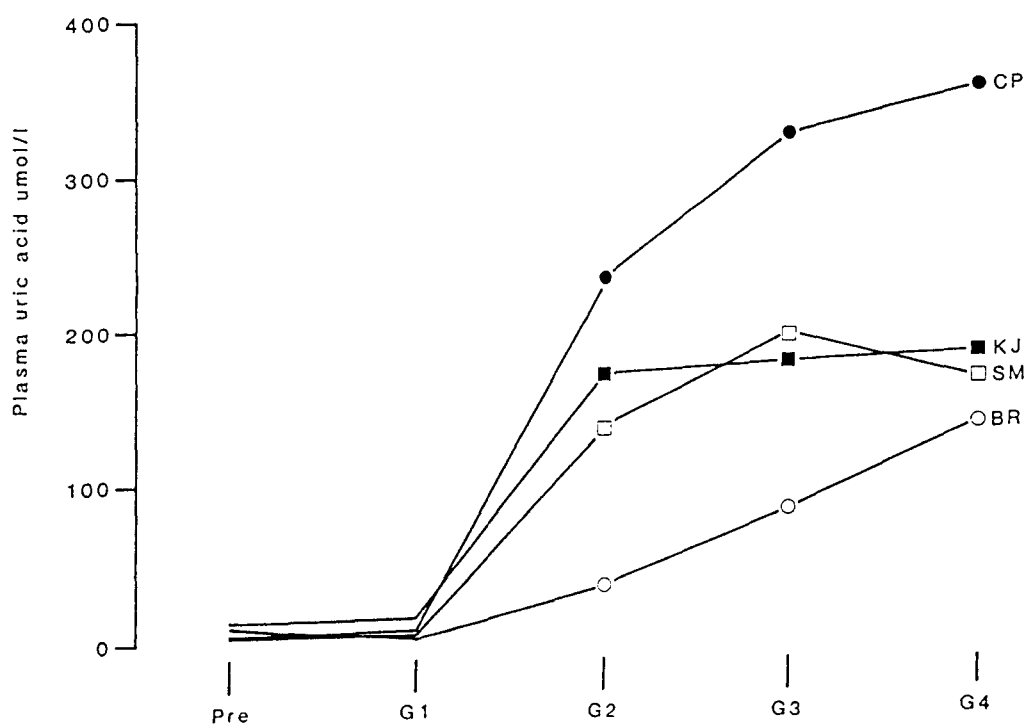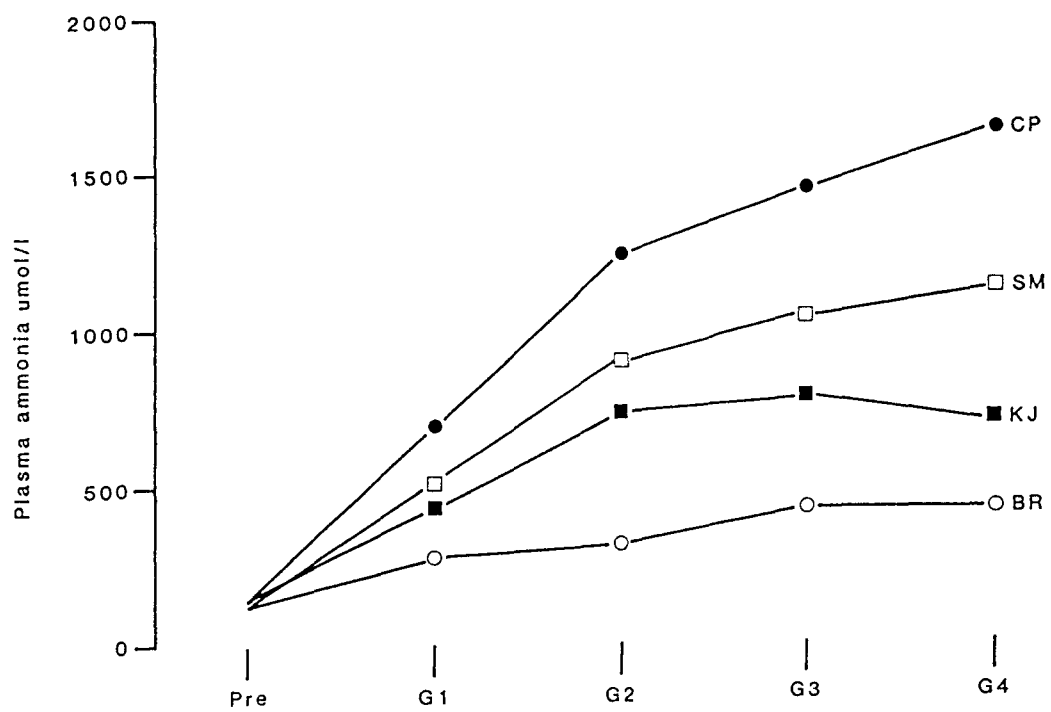
Figure 6.5. Venous blood packed cell volume (PCV) in four horses before (Pre) and after each of four maximal gallops of 700m in the field, each gallop separated by a 20 min recovery period.

Figure 6.6. Gallop times (sec) for each of 4 maximal gallops of 700m each, with a 20 min recovery period allowed between each gallop.
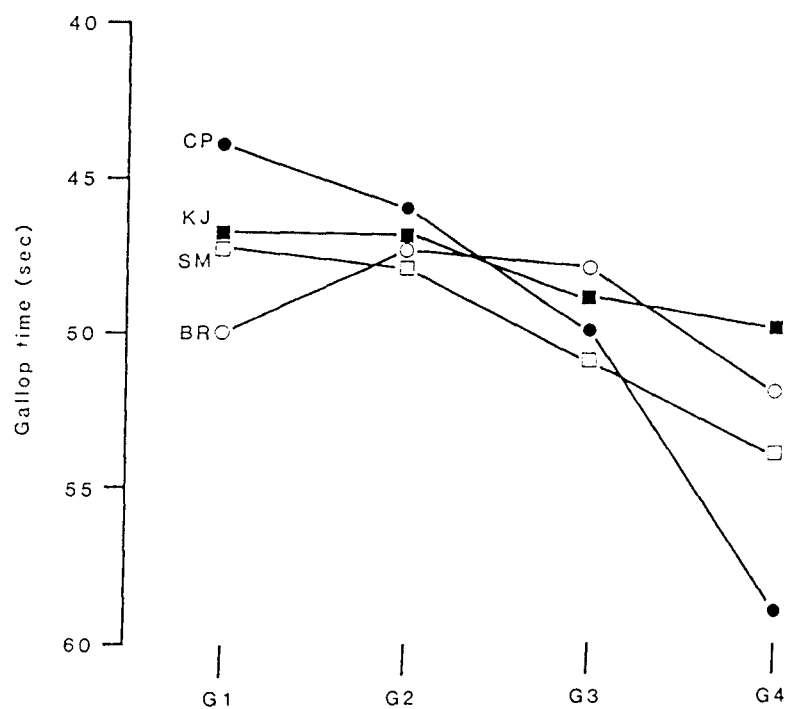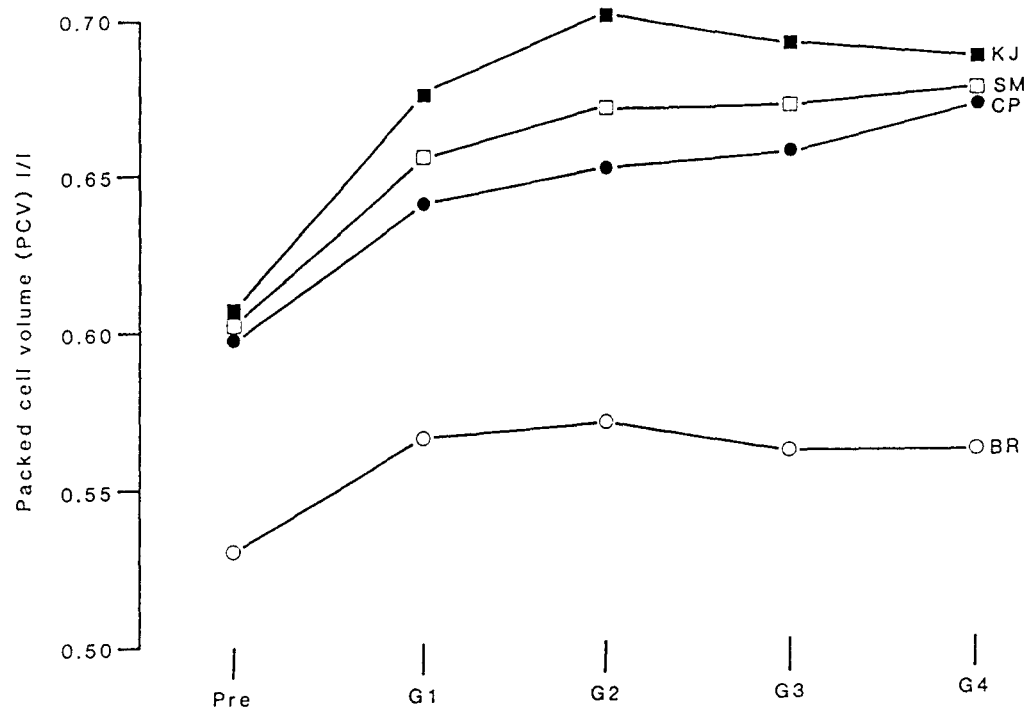
and ammonia increase followed a similar pattern to that for whole blood lactate. The first gallop did not result in any significant increase in plasma uric acid above the pre-exercise level, although significant increases (P<0.05) occurred between G1 and G2, and G2 and G3. No further increase in uric acid occurred following the fourth gallop. PCV increased significantly from the pre-exercise value (P<0.01) following the first gallop. There was no significant difference in PCV within horses between gallops. Following each gallop plasma ammonia and whole blood lactate were significantly correlated (r = 0.837, P<0.001, Figure 6.7.)

(4) BLOOD LACTATE FOLLOWING FLAT RACING OVER DISTANCES OF 1200-2400 METRES

Blood lactate, distance raced, placing in race and running time for 16 Thoroughbred horses competing in races at Newmarket are shown in Table 6.9. Considering the range of distances over which the horses ran, the range in lactate and mean running speed was relatively small (19.2 to 26.9 mmol/l and 15.6 to 16.4 metres/sec, respectively). No significant relationship was found between lactate and racing distance (r = -0.07, P>0.05) and there was no significant difference in blood lactate between horses finishing first and those that were unplaced (P>0.05).

(5) SINGLE TREADMILL GALLOPS OF 1440 METRES

Muscle metabolite changes: Muscle metabolite contents at rest and following a two minute gallop at 12 m/s on a 5° incline are shown in Table 6.10. The two minutes of high-intensity exercise resulted in a 30±10% fall in muscle ATP content, without any significant change in the ADP or AMP content. At the end of exercise PCr was 57±8% lower compared with the value recorded prior to exercise (P<0.01). Glycogen utilization during the two minutes of exercise was 96±48 mmol glucosyl units/kg d.m. Mean glycogen decrease following exercise amounted to 19±8% of the resting content and the mean glycogen utilization rate was 0.8±0.4 mmol glucosyl units/kg d.m./sec. The accumulation of glycerol 3-P, representing the reduction of dihydroxyacetone phosphate (DHAP) and the oxidation of one NADH to NAD, was approximately one seventh of the lactate accumulation. In contrast, the accumulation of glycerol with exercise was much smaller, amounting to a mean increase of only 2.3 mmol/kg d.m. The large increase in muscle lactate of approximately 100 mmol/kg d.m. was accompanied by a fall in pH from 7.21±0.03 at rest to 6.79±0.12 after exercise (P<0.01). There was only a trend towards increased muscle pyruvate content from 0.3±0.1 at rest to 0.5±0.2 mmol/kg d.m. following exercise.

Figure 6.7. Relationship between plasma ammonia and whole blood lactate immediately following each of four 700m maximal gallops. r = 0.837, ammonia = - 425 + 49.8.lactate, P<0.001.
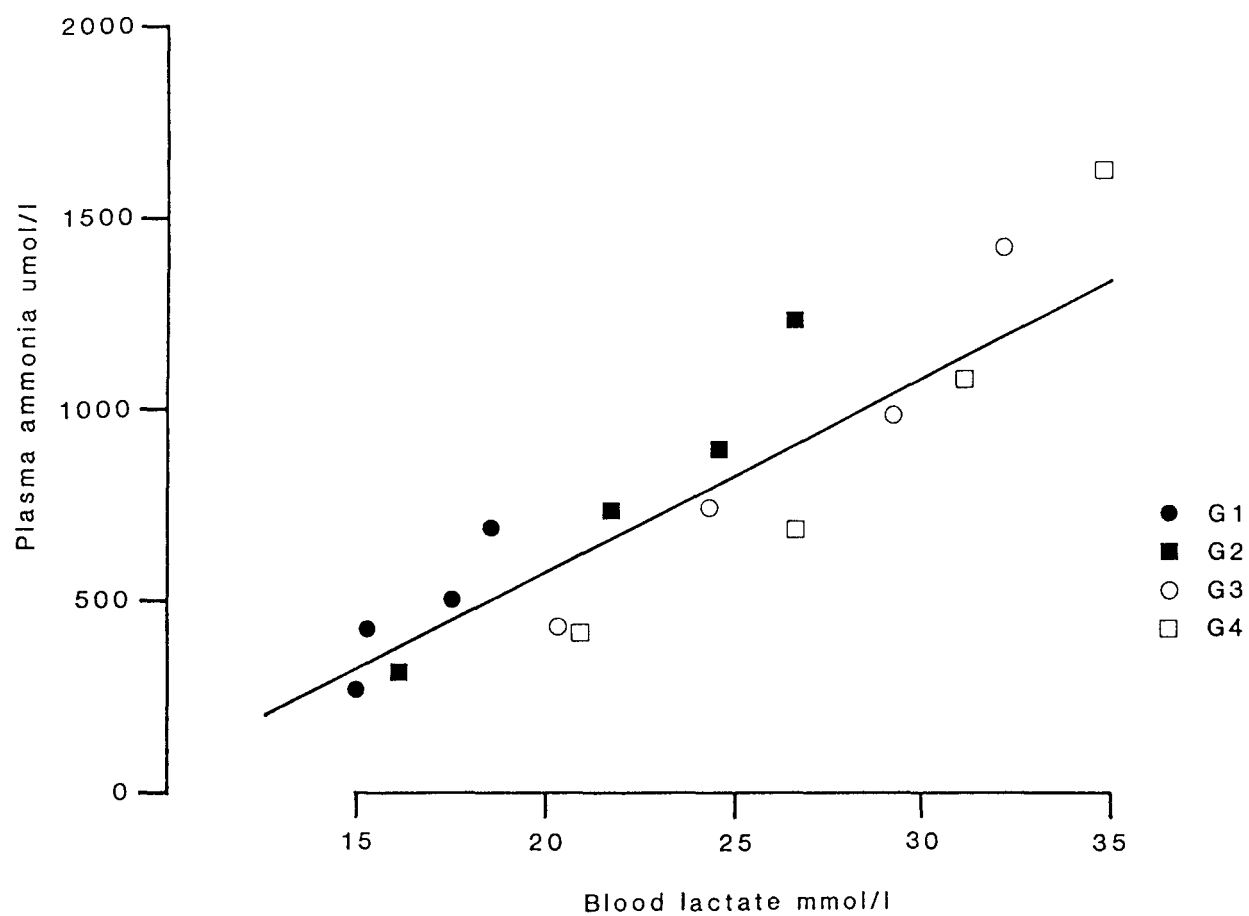
Table 6.9. Blood lactate concentrations (mmol/l) in Thoroughbred racehorses following racing over distances of 1200-2400 metres.

Horse no.	Distance (metres)	Position in race	Time (min:sec)	Mean speed (metres/sec)	Lactate (mmol/l)
1	1200	8	1:16	15.8	24.2
2	1200	1	1:15	16.0	21.4
3	1200	1	1:15	16.0	24.6
4	1200	1	1:14	16.2	24.4
5	1200	1	1:13	16.4	23.4
6	1400	1	1:29	15.7	22.9
7	1400	5	1:30	15.6	26.9
8	1600	1	1:41	15.8	22.7
9	1600	1	1:40	16.0	26.2
10	1600	9	1:43	15.5	25.1
11	2000	1	2:06	15.9	23.7
12	2000	1	2:05	16.0	24.7
13	2000	1	2:05	16.0	21.5
14	2000	4	2:07	15.7	26.7
15	2400	1	2:35	15.5	26.7
16	2400	1	2:34	15.6	19.2

Table 6.10. Muscle and blood metabolite contents and pH at rest (Pre) and following a two minute gallop at 12 m/s on a 5° incline (Post). Values are based on a mean for the two sessions for each horse.

	PRE	POST	PRE v POST
Muscle			
(mmol/kg d.m.)			
ATP	24.0±2.5	16.7±4.9	P<0.01
ADP	2.9±0.2	2.9±0.5	NS
AMP	0.16±0.04	0.17±0.06	NS
PCr	60.3±12.0	26.0±3.0	P<0.01
Glycogen	519±28	423±63	P<0.05
HMP	4.0±1.0	25.4±1.8	P<0.001
Glucose	3.0±1.4	11.0±3.1	P<0.01
Glycerol 3-P	2.9±0.6	16.9±4.4	P<0.01
Glycerol	0.3±0.2	2.6±0.5	P<0.01
Pyruvate	0.3±0.1	0.5±0.2	NS
Lactate	10.1±3.2	113.1±31.5	P<0.01
Alanine	2.9±0.8	6.7±1.2	P<0.05
pH	7.21±0.03	6.79±0.12	P<0.01
Blood			
Lactate (mmol/l)	0.6±0.1	15.5±3.7	P<0.01
Glucose (mmol/l)	3.7±0.4	3.1±0.3	P<0.01
Plasma			
pH	7.42±0.02	7.00±0.09	P<0.01
Lactate (mmol/l)	0.7±0.2	27.5±9.7	P<0.01
Ammonia (μmol/l)	33±4	188±109	NS
Heart rate (bpm)	55±22	190±8	P<0.001

Blood changes: The changes in blood lactate and pH were similar to those observed in muscle, i.e. the large increase in both whole blood and plasma lactate was paralleled by a drop in blood pH of the same order as that seen in muscle. Blood glucose showed a small but significant decrease from 3.7±0.4 mmol/l at rest to 3.1±0.3 mmol/l after exercise (P<0.01). There was only a small variation between horses in resting plasma ammonia content (33±4 μmol/l) but large variation in the response to exercise. The immediate post exercise plasma ammonia content of 188±109 μmol/l was not significantly different to the pre-exercise content (P>0.05), although all horses showed an increase with exercise.

Reproducibility of treadmill exercise: As each of the horses completed two separate exercise sessions, the reproducibility of muscle and blood metabolite changes was estimated. The greatest variation was seen in muscle glycogen (38%). In general, a much smaller variation was seen in blood parameters (10% - lactate; 9% - glucose; 5% - plasma pH). The reproducibility of the metabolite changes with exercise, calculated as the pooled within-horse standard deviation and expressed as precision, are shown in Table 6.11.

DISCUSSION

Muscle ATP decrease: In the two studies of field exercise and the study of treadmill exercise where muscle biopsies were taken, a consistent finding was a pronounced fall in the muscle ATP content with exercise. The decrease was of the same order as that described by Snow, Harris and Gash (1985) in their study of intermittent maximal exercise. Furthermore, the extent of the decrease was related to the distance over which the horses exercised (Figure 6.8).

In agreement with the present study is the evidence demonstrating that intense exercise in man can result in the depletion of muscle ATP (Sutton et al, 1980; Boobis, Williams and Wooton, 1983). However, pronounced muscle ATP decrease still seems to be a much more common feature of exercise in horses compared to humans. This may possibly be related to fibre composition as in general, the major locomotor muscles of the horse are composed of a higher percentage of IIA and IIB fibres compared to humans (see Chapter 1). Whilst TAN depletion and IMP formation have been shown to occur in both IIA and IIB fibres, this does not appear to occur so readily in muscle composed predominantly of type I fibres (Dudley and Terjung, 1985; Meyer, Dudley and Terjung, 1980). Thus, changes at the single fibre level will be masked by examining contents in a biopsy consisting of 200-

Table 6.11. Reproducibility of muscle and blood changes over two treadmill exercise sessions (2 minutes gallop at 12m/s, 5° incline) calculated as the pooled within-horse standard deviation and expressed as the precision.

Muscle	Precision	
ATP	1.3	mmol/kg d.m.
PCr	7.8	mmol/kg d.m.
Glycogen	32	mmol/kg d.m.
HMP	3.6	mmol/kg d.m.
Glucose	0.7	mmol/kg d.m.
Glycerol 3-P	1.5	mmol/kg d.m.
Glycerol	0.6	mmol/kg d.m.
Lactate	9.6	mmol/kg d.m.
Alanine	0.8	mmol/kg d.m.
pH	0.05	

Blood		
Lactate	1.6	mmol/l
Glucose	0.7	mmol/l
pH	0.02	

Plasma		
Ammonia	41	μmol/l

Heart rate	6.9	b.p.m.

Figure 6.8. Mean decrease in muscle ATP content in relation to distance galloped for field gallops of 800, 2000 and 2 x 900m and single treadmill gallops of 1440m.
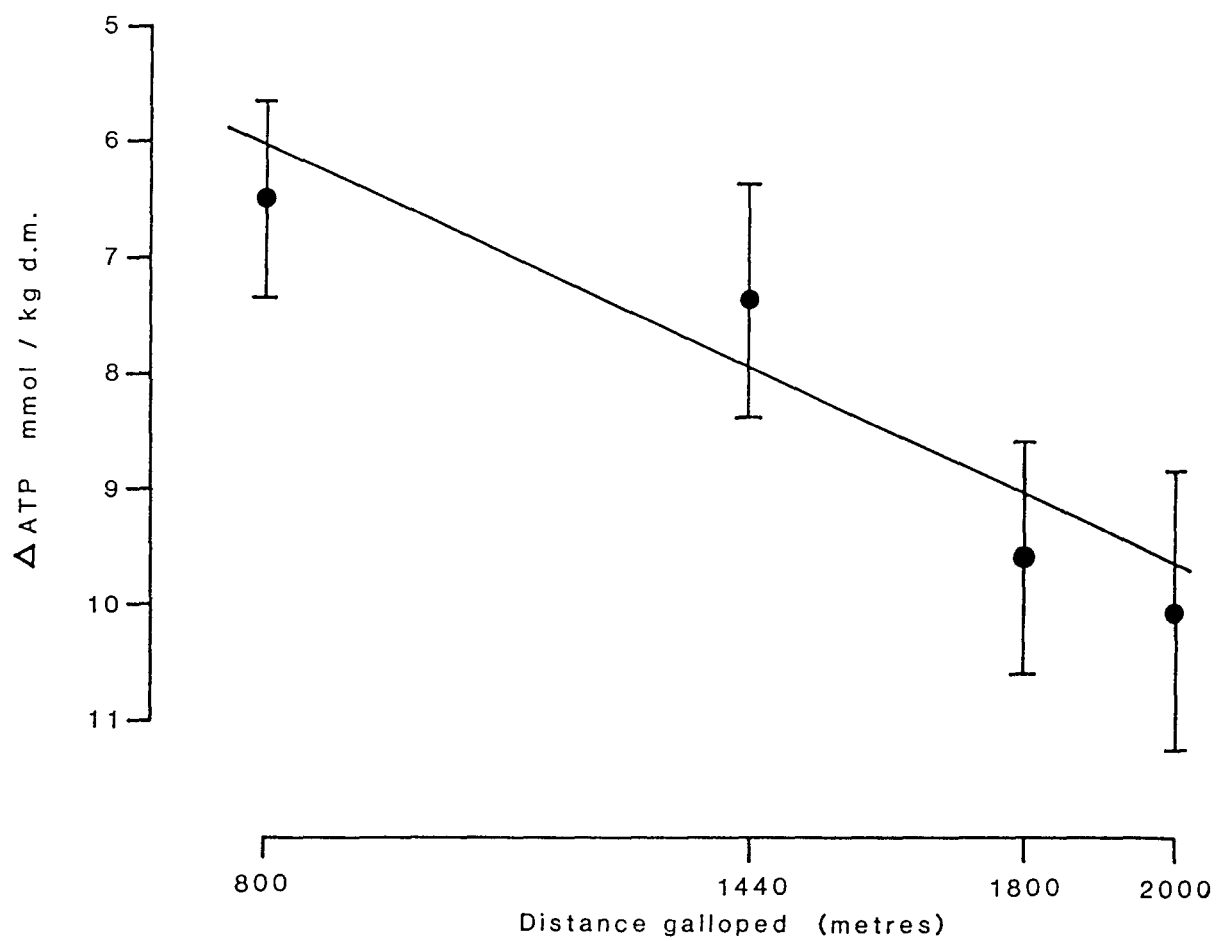
300 fibres of different types. Therefore, the smaller decrease in muscle ATP content in man following high-intensity exercise may simply be a reflection of a lower percentage of type II fibres.

Many human exercise studies have used 'normal' subjects as opposed to elite athletes so the ability to exercise at higher workloads for longer periods of time may be important in the development of the conditions in the muscle leading to reduction in muscle ATP. For example, trained elite human athletes have been shown to have a high capacity for lactate production during high-intensity exercise (Boobis, Williams and Wooton, 1982) comparable to that of the Thoroughbred horse. Like the horse, these subjects also exhibit a high degree of muscle ATP loss.

In the absence of any increase in muscle ADP or AMP, the fall in ATP content represents a loss of adenine nucleotide. The decrease in ATP is matched by a stoichiometric increase in muscle IMP content (Snow, Harris and Gash, 1985), formed from the deamination of AMP by AMP deaminase. This is followed by further degradation of IMP to inosine and uric acid, as indicated by the progressive rise in plasma uric acid concentration following exercise (see Chapter 7). The majority of the IMP formed, however, is retained in the muscle and is resynthesized back to AMP during recovery (see Figure 6.9).

The main function of the AMP deaminase reaction during maximal exercise appears to be in the removal of AMP, thereby maintaining the myokinase reaction in favour of ATP synthesis. Together, the myokinase and AMP deaminase reactions function to maintain a high ATP to ADP ratio and a low ADP content. Accumulation of ADP has been implicated in the development of force or tension fatigue (Dawson, Gadian and Wilkie, 1978), probably as a result of decreased myofibrillar ATPase activity.

From studies on rat muscle (Wheeler and Lowenstein, 1979) it has been suggested that ATP, ADP, AMP, protons and inorganic phosphate (Pi) are the major regulators of AMP deaminase. More recently it has been postulated that AMP deaminase may be regulated allosterically by di-adenosine tetraphosphate, a likely intermediate in the myokinase reaction (Fernandez et al, 1984). At rest the activity of the enzyme is very low due to lack of substrate, low ADP, H^+ and Pi and inhibition by ATP. During intense exercise, acidosis and increasing concentrations of AMP, ADP and H^+ favour an increase in activity, despite the increased concentrations of inorganic phosphate. AMP deaminase exhibits a pH optimum of approximately 6.2 (Setlow and Lowenstein, 1967). The muscle lactate contents attained in the single gallops of 800 and 2000m, would indicate muscle pH values of 6.62 and 6.43,

Figure 6.9. Biochemical pathways associated with muscle ATP utilization and AMP deamination/dephosphorylation.
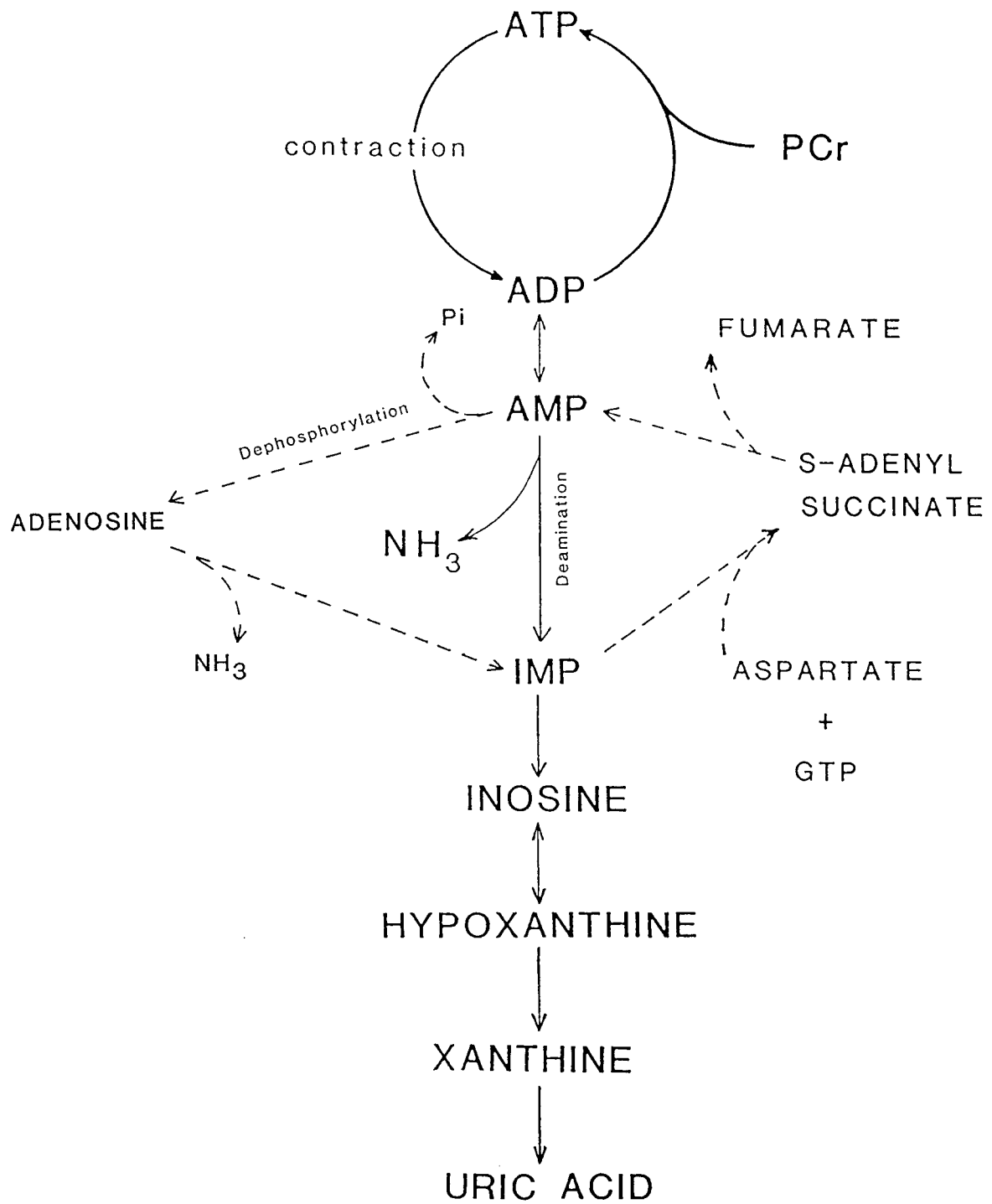
respectively (calculated from Harris et al, 1989).

High rates of energy demand under conditions of acute acidosis and a decreased pool of PCr to buffer ATP supply would both be expected to bring about an increase in AMP. Thus, deamination of AMP has been shown to occur in rat and human muscle only after depletion of the PCr store and accumulation of lactate of 40 and 60 mmol/kg dry muscle, respectively (Dudley and Terjung, 1985; Harris and Hultman, 1985).

Alternatively, AMP can be removed by dephosphorylation to adenosine and further breakdown to inosine. This route appears to be more important in slow-twitch compared to fast-twitch muscles (Bockman and McKenzie, 1983). However, when significant TAN depletion occurs, the AMP deaminase reaction resulting in IMP formation predominates in both man (Sabina et al, 1984) and the horse (Snow, Harris and Gash, 1985), as well as, presumably, in other species.

Deamination of AMP will also result in the formation of ammonia (Figure 6.9). A proportion of the ammonia produced will be removed due to uptake by glutamate in the formation of glutamine, and the remainder released into the bloodstream. Increases in blood ammonia of the same order as those presented here have also been reported to occur in humans during intense bicycle exercise (Babij, Matthews and Rennie, 1983; Mutch and Banister, 1983). Studies in humans have also demonstrated a parallel rise in blood ammonia and lactate with increasing work intensity (Babij, Matthews and Rennie, 1983; Buono, Clancy and Cook, 1984), implying that ammonia accumulation may be linked to the metabolic stress of the exercise. An increase in blood ammonia may only indicate an increased rate of purine nucleotide cycling and not necessarily purine catabolism. However, there is strong evidence against purine nucleotide cycling occurring during exercise. Meyer and Terjung (1979) demonstrated that in fast-twitch muscle deamination of AMP occurred during contraction and reamination of IMP during recovery. The similarity between ammonia increase and TAN decrease following both dynamic and isometric exercise (Katz et al, 1986; Katz, Sahlin and Henriksson, 1986) has also been interpreted as evidence against the operation of the purine nucleotide cycle during exercise (Sahlin and Katz, 1988). However, there is some evidence that the purine nucleotide cycle may operate during exercise of moderate intensity (70% VO_2 max; Broberg, 1989).

In the present study the significant correlation between muscle ATP decrease and plasma ammonia increase following single gallops of 800 and 2000m in the field and 1440m on a treadmill (Figure 6.10) seems to indicate

Figure 6.10. Muscle ATP decrease in relation to peak post-exercise plasma ammonia following single gallops of 800 and 2000m in the field and 1440m on a treadmill. + - Single 800m gallop, * - Single 2000m gallop, x - Single 1440m treadmill gallop. ATP = 2.06 + 0.0198.Ammonia. r = 0.718, df = 15, P<0.01.

Figure 6.11. Gallop time versus peak plasma ammonia concentration prior to gallop. For G1 the pre-exercise plasma ammonia concentration is used. • - Pre-exercise v G1 time, + - Peak post G1 v G2 time, * - Peak post G2 v G3 time, x - Peak post G3 v G4 time. Gallop time = 45.7 + 0.00573.Ammonia. r = 0.683, df = 14, P<0.01.

ATP decrease mmol/kg d.m.

Peak plasma Ammonia, umol/l

Gallop time sec

Plasma Ammonia, umol/l

140
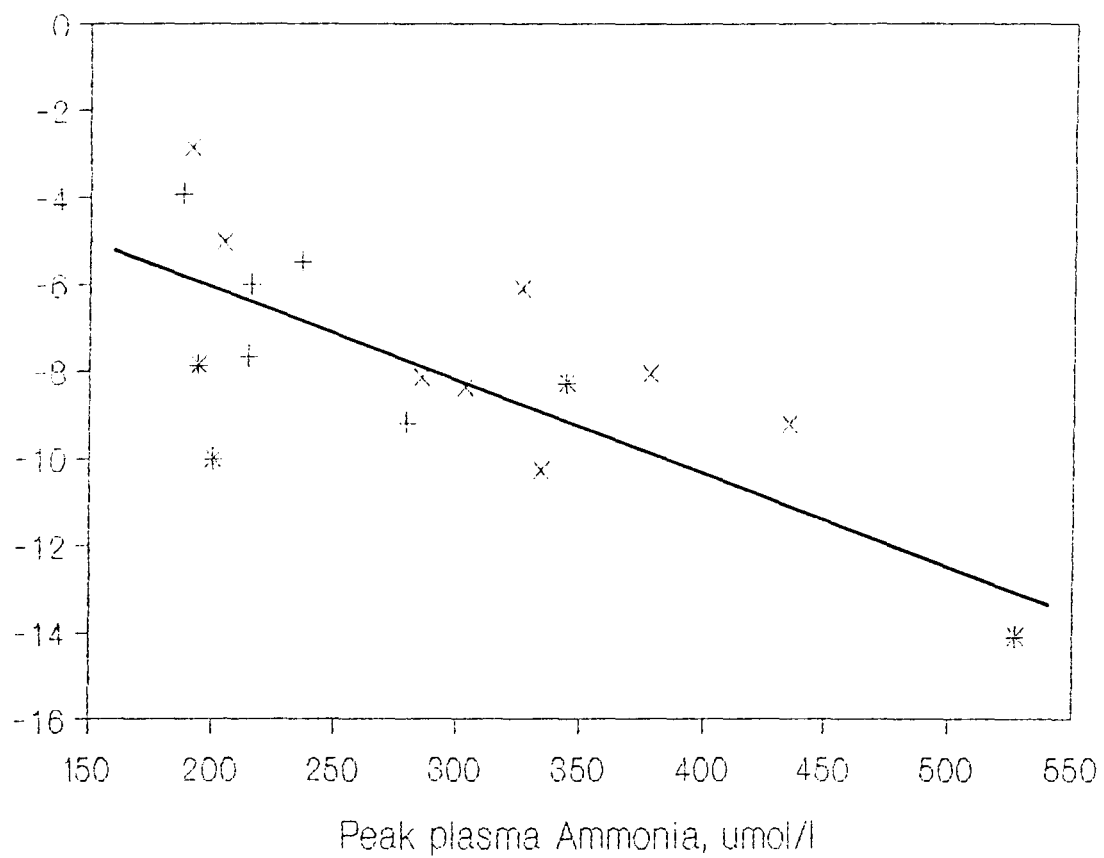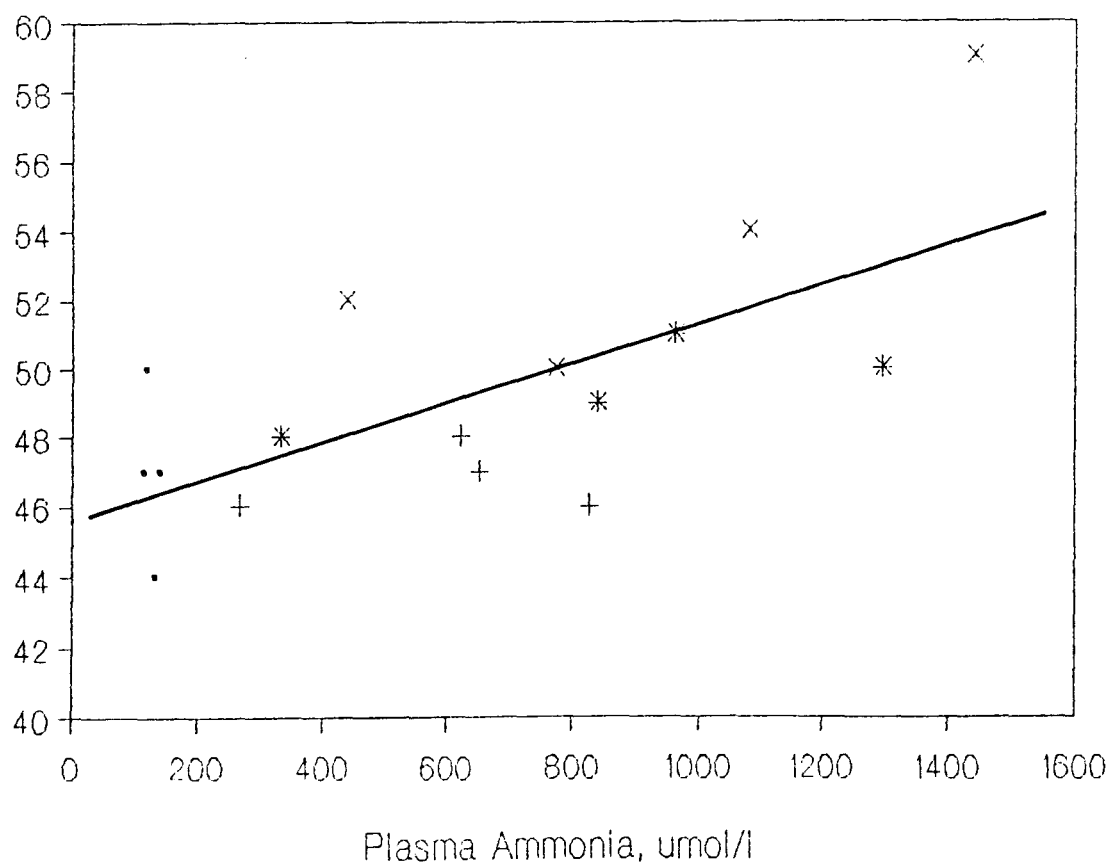
that at least in the horse, plasma ammonia is a good marker for muscle ATP status following high-intensity exercise.

In the study of intermittent high-intensity exercise (4 x 700m gallops), gallop time was positively correlated to the plasma ammonia content prior to the gallop (Figure 6.11). This implies that a high plasma ammonia and hence, a depressed muscle ATP content may reduce running speed (see later discussion).

Glycogen utilization: The mean rate of glycogen utilization was 2.7 ± 0.9 mmol glucosyl units/kg d.m./sec over the 800m gallop, which is much higher than previous rates reported for the Thoroughbred over both shorter and longer distances (Guy, 1978; Nimmo and Snow, 1983; Snow, Harris and Gash, 1985). The decrease in the mean rate of glycogen utilization from 2.7 for the 800m gallop to 1.8 and 1.1 mmol glucosyl units/kg d.m./sec for the 2x900m and 2000m gallops, respectively, can possibly be explained on the basis of increasing cardiovascular adjustment as the time of exercise increases. Also, despite the instruction to ride the horses maximally, the riders may have 'paced' the horses for the longer distances, resulting in the first 800m of the 2000m gallop being completed in a much slower time than in the single 800m. A slower gallop time would almost certainly result in a lower rate of glycogen utilization.

Despite the apparently high rates of utilization, the total glycogen utilized only amounted to 27%, 39% and 33% of the pre-exercise content for the 800, 2x900 and 2000m gallops, respectively. The similarity in the amounts of glycogen utilized resulted in a decrease in glycogen utilization rate with increasing distance (equivalent to exercise duration). The similar glycogen utilization despite different durations of exercise may be due to a similar decline in phosphorylase activity in all three gallops.

During exercise the release of Ca^{2+} and cAMP (due to binding of adrenaline to receptors in the muscle cell membrane) activate phosphorylase kinase which mediates the conversion of phosphorylase $\underline{b}$ (inactive) to $\underline{a}$ (active). The reconversion of phosphorylase $\underline{a}$ to $\underline{b}$ is brought about by phosphorylase phosphatase. Thus, the proportion of phosphorylase in the active ($\underline{a}$) form is a function of the activity of both phosphorylase kinase and phosphorylase phosphatase. Accumulation of H^+ during exercise may reduce the activity of phosphorylase, and therefore the glycogenolytic rate, in one of two ways. Firstly, a reduction in pH has been shown to reduce the activity of phosphorylase kinase (Krebs, Graves and Fischer, 1959; Krebs et al, 1964) which in vivo would result in a reduction in phosphorylase $\underline{a}$ ($\underline{b} \longrightarrow \underline{a}$ < $\underline{a} \longrightarrow \underline{b}$). Secondly, decreased pH has been shown

to reduce the proportion of Pi in the HPO_4^{2-} form, which is thought to be the true substrate for phosphorylase (Kasvinsky and Meyer, 1977; Chasiotis, 1983).

It has also been demonstrated that the pre-exercise glycogen content can affect the glycogenolytic rate during stimulation of isolated muscles (Richter and Galbo, 1986) and in man during high-intensity exercise (Greenhaff, 1988). This is despite the glycogen content of resting muscle being far in excess of the apparent K_m of phosphorylase $\underline{a}$ for glycogen of 1.5 mmol/kg d.m. (Brown and Cori, 1961; Chasiotis, 1983). How might this paradox come about? It could be hypothesised that as glycogen exists as a spherical conglomorate of glucosyl units, phosphorylase will initially phosphorylate the outermost molecules. Thus, the activity of phosphorylase $\underline{a}$ will depend to a certain extent on the availability or presentation of substrate (i.e. branches of glucosyl units). As the glycogen molecule becomes degraded this will result in a reduction in its surface area and therefore, a reduction in the amount of glucosyl units available or presented to phosphorylase. In the same way as the in vitro activity is affected by substrate concentration, the in vivo activity of phosphorylase may be affected by the spatial and structural presentation of glucosyl units, which may result in rates of glycogen breakdown below those predicted from purely kinetic parameters, such as the K_m.

Lactate: Blood lactate following single gallops in the field was related to distance galloped up to about 1500m, after which a 'plateau' in values was reached (Figure 6.12). Multiple gallops also fitted this line despite the disappearance of lactate from the blood during recovery periods. The same relationship has also been reported for plasma lactate following single gallops of 600-1800m (Bayly, Grant and Pearson, 1987).

It is also apparent that the treadmill exercise model did not result in the same lactate accumulation as would have been predicted for a maximal gallop of 1440m in the field. Whereas in the field, running speeds were in the range of 14-16 m/s, the speed on the treadmill was only 12 m/s. Also, in the field the horses accelerated rapidly, whereas on the treadmill the speed increased over a period of 40 seconds. Rapid acceleration might reasonably be expected to result in greater lactate production compared to a slower rate of acceleration which would allow more time for cardiovascular adjustment.

Furthermore, a 5° incline may not compensate for the slower speed, lack of wind resistance and the absence of the weight of a rider. However,

<u>Figure</u> <u>6.12.</u> Post-exercise blood lactate concentration in relation to distance galloped. Round symbols = single gallops, square symbols = multiple gallops.

R – Samples collected following racing

T – Treadmill exercise of 2 min 12m/s & 5° incline

F1 – Single field gallop of 700m

F2 – Single field gallop of 800m

F3 – Two field gallops of 700m

F4 – Two field gallops of 900m

F5 – Single field gallop of 2000m

F6 – Three field gallops of 700m

F7 – Four field gallops of 700m
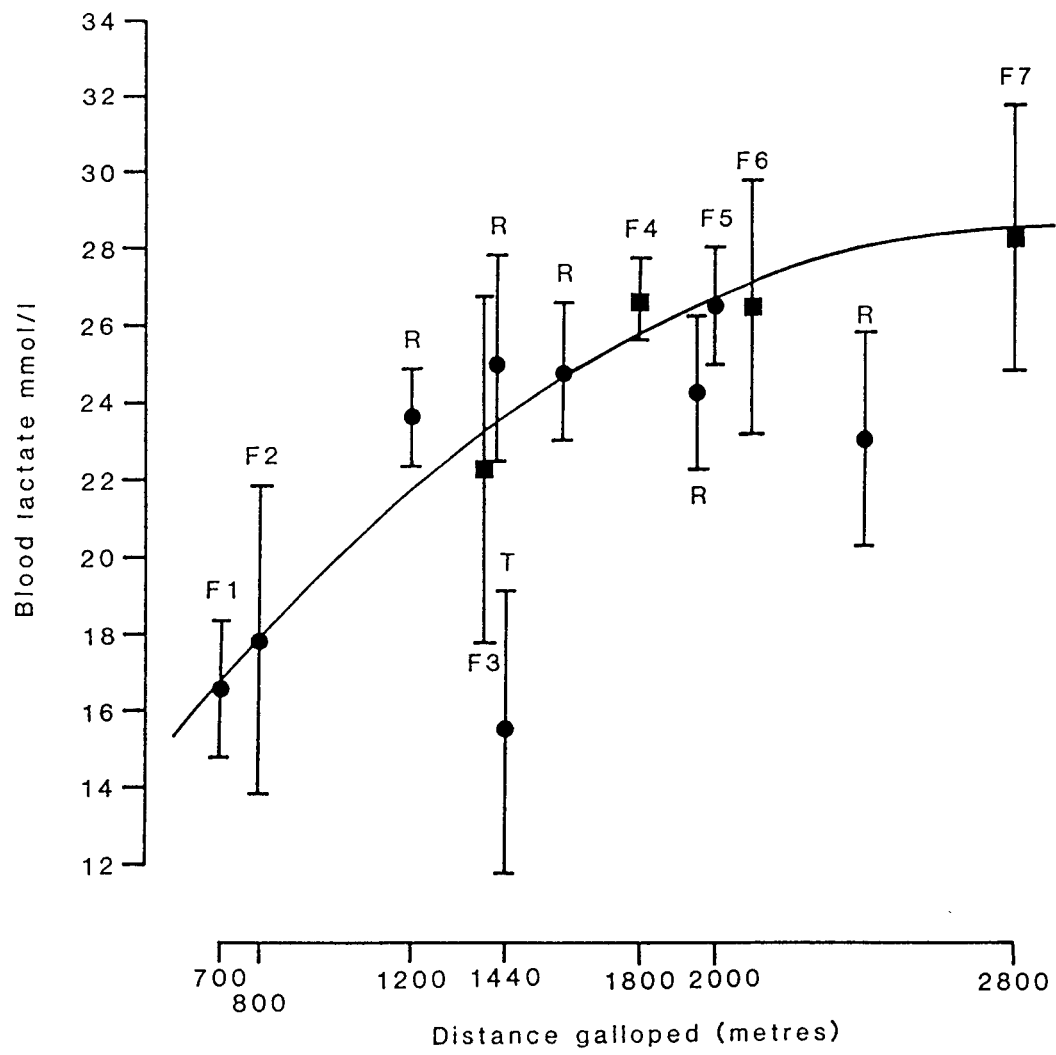
the horses that were used in these treadmill studies did appear to be experiencing fatigue at the end of the two minute exercise period, judged by their inability to maintain their position on the treadmill and, as already described, showed marked ATP depletion.

It may be that running horses on an incline results in fatigue of muscle groups not recruited to the same extent on the flat and therefore, fatigue occurs at a much earlier stage in the exercise or at a lower workload. It may therefore be preferable to use a combination of a higher speed and lower incline in order to achieve lactates of the order seen in maximal field exercise and to carry out studies on treadmills on which the acceleration can be controlled.

Reproducibility: Field versus treadmill exercise

Comparison of the precision of muscle and blood metabolite changes following high-intensity field and treadmill exercise (Tables 6.7 and 6.11, respectively), indicate that for most of the metabolites a higher degree of reproducibility was achieved with treadmill exercise, although the difference was not great. This may be explained on the basis of several considerations. A likely cause for the differences in reproducibility may be that the treadmill exercise took place under very closely controlled conditions. On each exercise session conditions such as temperature, humidity, wind resistance, running surface and speed would have been almost identical. In contrast, in the field variations in environmental conditions could easily introduce variation between sessions. Also, as shown in Table 6.7 there was some variation in running times from session to session. Differences in the manner in which the riders tackled the gallop may also have varied between sessions in spite of the same riders being used and the instruction to ride the horses maximally.

With respect to the collection of muscle biopsy samples, it was generally found to be easier to collect biopsies under laboratory conditions (i.e. on the treadmill) than in the field. This may have enabled samples to be collected with a greater consistency with regard to site. Furthermore, it was also easier to collect samples within a relatively narrow range of times following the treadmill exercise. In the field, horses were frequently more excited and more awkward to sample, resulting in a greater variation in actual sample time from the target time.

In spite of the number of ways in which the reproducibility of exercise in the field could be affected, the precision for most metabolites was surprisingly good and comparable to that for treadmill exercise. Even

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for the latter, the precision will be affected by such factors as subclinical conditions developing between sessions, such as slight lameness, sampling site variation and analytical error. Overall, these results suggest that both field and treadmill exercise can be carried out on separate occasions with an acceptable degree of precision for muscle and blood metabolite contents. However, it must be remembered that this applies to high-intensity exercise only and a lesser degree of precision may be found at lower exercise intensities due to psychological factors, in particular, the catecholamine response.

Relationship between ATP, Lactate, Ammonia and speed

Muscle ATP decrease has previously been shown to be positively related to both muscle ammonia and lactate concentrations in Standardbred horses following racing (Essén-Gustavsson and Valberg, 1987). In the present studies involving single and multiple gallops, both muscle lactate and plasma ammonia concentration were found to be positively correlated to muscle ATP decrease (Figures 6.13 & 6.10). Using plasma ammonia as a marker for muscle ATP depletion, in the study of 4 x 700m gallops, gallop time was found to be significantly correlated to the peak plasma ammonia content preceeding each gallop (Figure 6.14).

This would seem to confirm the finding in the similar study of intermittent maximal exercise by Snow, Harris and Gash (1985) that gallop time was inversely related to the muscle ATP content prior to the gallop (Figure 6.15). In this study, one horse showed a different pattern of ATP depletion and performance to the others. It completed the first gallop in the slowest time compared to the other 3 horses with no corresponding decrease in ATP. In subsequent gallops this horse only showed a small decrease in ATP content and achieved the fastest overall time (G1 + G2 + G3 + G4). In contrast the horse completing the fastest first gallop was joint slowest in the fourth gallop.

Large scale muscle ATP depletion and decreased performance has also been observed in human subjects (Hultman, Bergström and McLennan-Anderson, 1967). More recently, Hultman, Spriet and Söderlund (1987) have demonstrated that when muscle ATP content falls below approximately 20 mmol/kg d.m., there is a rapid decline in force production with further decreases in the ATP content. In the study of intermittent maximal exercise in the present chapter, horse BR showed a pattern similar to that seen by the horse S in the study of Snow, Harris and Gash (1985), namely, a slow first gallop, the fastest G3 and the second fastest overall time (Figure 6.14). Horse CP who completed the fastest G1 also completed the slowest G4

Figure 6.13. Muscle ATP decrease in relation to change in muscle lactate following single gallops of 800 and 2000m in the field and 1440m on a treadmill and multiple gallops of 2x900m in the field. ATP = 2.91 + 0.0446.Lactate. r = 0.625, df = 28, P<0.001. + - Single 800m gallop, * - Single 2000m gallop, x - 1440m Single treadmill gallop, · - 2x900m gallops.

ATP decrease
mmol/kg d.m.

Muscle lactate, mmol/kg d.m.
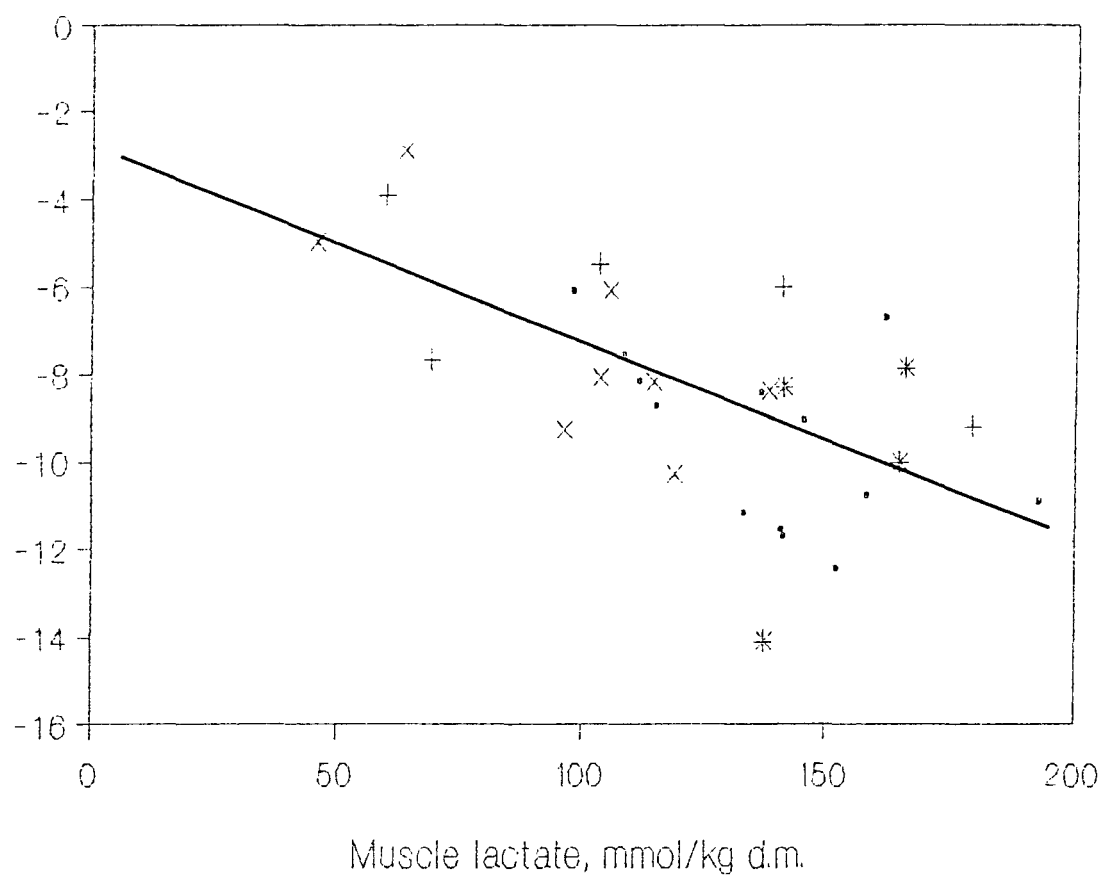
and was second slowest overall. These two horses also showed the least and greatest plasma ammonia accumulation, respectively, inferring differences in the extent of muscle ATP depletion.

In all studies in the present chapter involving single gallops no significant relationships were found between ATP decrease or ammonia increase and speed. Thus, whilst a reduced muscle ATP content from a previous bout of exercise would seem likely to reduce subsequent exercise performance, it is not clear as to whether the extent of ATP depletion is related to performance within a single bout of exercise undertaken following low intensity warm-up exercise.

The studies presented in the present chapter indicate the major metabolic changes that occur in muscle and blood with single gallops in the field. The changes occurring with high-intensity treadmill exercise and multiple gallops are also outlined. The major finding of this chapter is that ATP depletion occurs during single gallops as well as during more intense multiple gallops. The possibility that a low muscle ATP content may affect subsequent exercise performance has also been considered. However, the importance of ATP depletion on performance during a single, isolated bout of exercise remains unclear.

Figure 6.14. Comparison of running time over each of 4 gallops with venous plasma ammonia concentration. • = G1, + = G2, * = G3 and x = G4. The ammonia concentration refers to that measured before the start of the relevant gallop.

Figure 6.15. Comparison of running time over each of 4 gallops with ATP content of the middle gluteal. Closed circles, open circles, closed triangles and open triangles signify gallops G1, G2, G3 and G4, respectively. Muscle ATP content shown is average of that measured before start of relevant gallop [i.e., at start of exercise (G1) or at end of previous gallop (G2, G3 and G4) and at end of gallop]. Reproduced from Snow, Harris and Gash (1985).

Gallop time
sec

Plasma Ammonia, umol/l
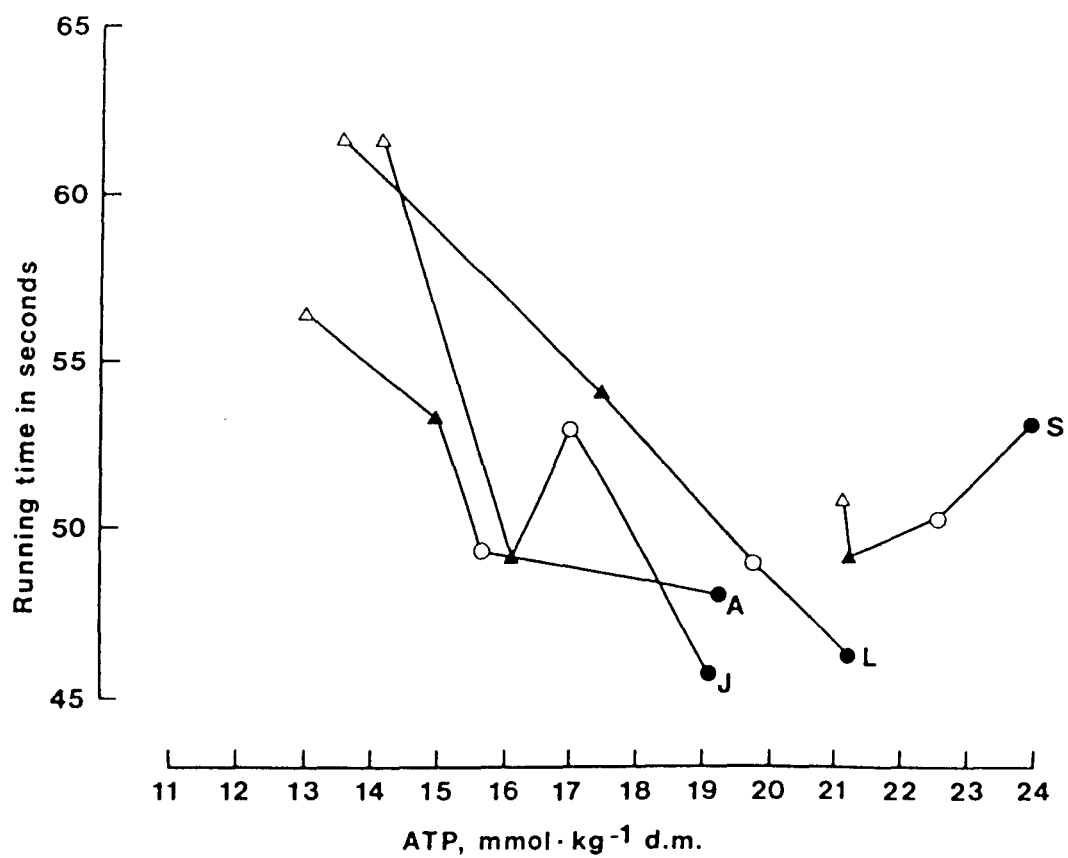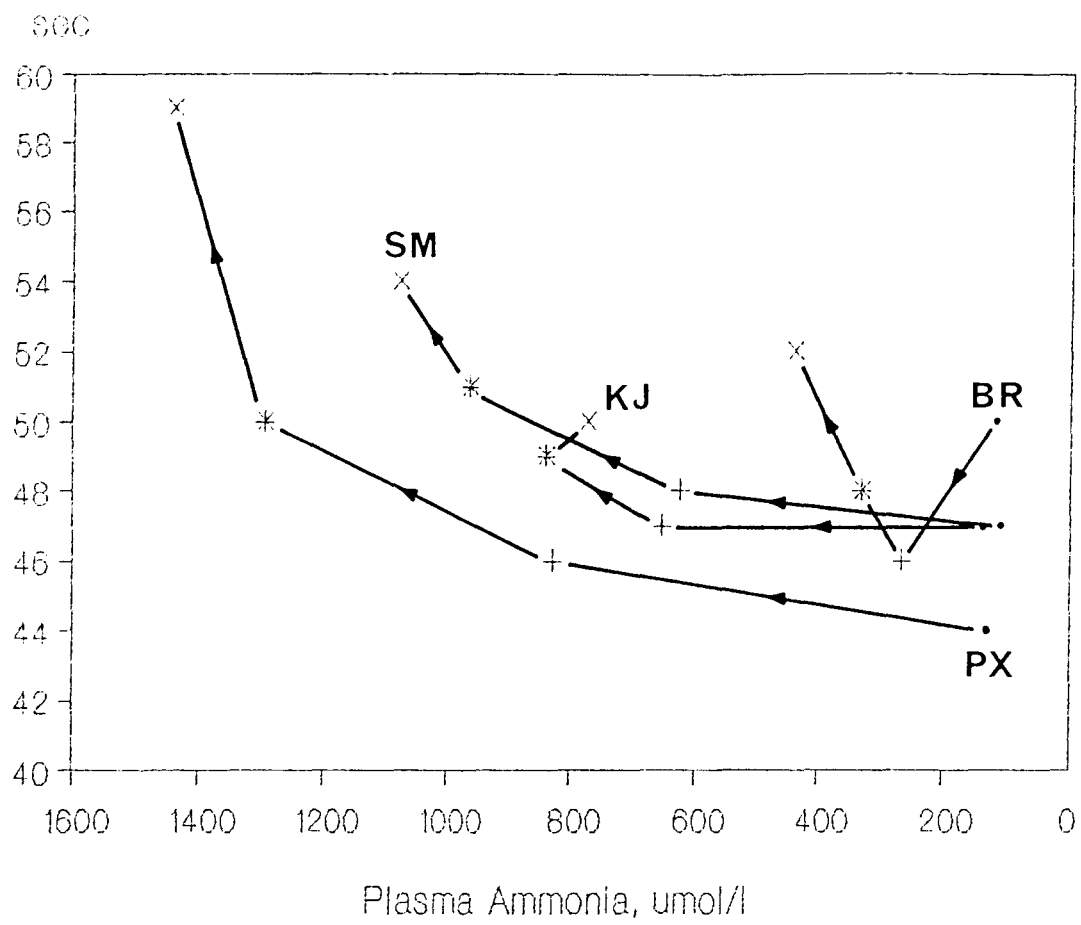
CHAPTER 7

FACTORS AFFECTING POST-EXERCISE RECOVERY IN MUSCLE AND BLOOD
METABOLITES FOLLOWING HIGH-INTENSITY EXERCISE.

INTRODUCTION

High-intensity exercise in the Thoroughbred horse results in large changes in muscle and blood metabolites (see Chapter 6). A study of intermittent maximal exercise in the horse by Snow, Harris and Gash (1985) demonstrated marked muscle and blood lactate accumulation and loss of muscle adenine nucleotide, with little recovery in 30 minutes following the last of four gallops.

There appears to be a limited amount of information available on post-exercise recovery in muscle and blood metabolites following high-intensity exercise in the horse and with respect to ATP recovery following deamination there is little information on any species.

The first study in the present chapter was undertaken to investigate recovery in muscle and blood metabolites, with particular emphasis on muscle ATP and lactate, following single bouts of high-intensity exercise in the field over distances comparable to flat racing. In the second study, a treadmill exercise model was used to investigate the effect of post-exercise activity on recovery in muscle and blood metabolites following high-intensity exercise. In the third and fourth studies, multiple-intermittent exercise models were used to examine the effect of different exercise protocols on post-exercise recovery. The fourth study utilized an exercise protocol consisting of four intermittent gallops, each of 700m and is similar to the study of Snow, Harris and Gash (1985), with the exception that in the present study 20 minutes recovery was allowed between gallops, compared to 5 minutes in the study of Snow et al.

An understanding of the metabolic recovery processes following high intensity exercise in the horse may also be of importance in the application of interval training programs.

MATERIALS AND METHODS

The results presented in this chapter are taken from the following studies of field and treadmill exercise:

(1) Single maximal field gallops of 800 or 2000 metres with walking recovery.

(2) Treadmill exercise consisting of 2 minutes galloping at 12 m/s on a 5 degree incline with either standing, walking or trotting and walking recovery.

(3) Intermittent maximal exercise, consisting of two field gallops of 900 metres with walking recovery.

(4) Intermittent maximal exercise consisting of four field gallops of 700 metres with walking recovery.

A more detailed description of the animals and exercise protocols is given in Chapter 6.

(1) SINGLE MAXIMAL GALLOPS OF 800 AND 2000 METRES

Following the completion of either an 800 or 2000m gallop, paired muscle and venous blood samples were collected at 0, 5, 10, 20, 40 and 60 minutes post-exercise. Except when being sampled, the horses were walked by their rider for the whole of the 60 minute recovery period. Muscle samples were treated as described in Chapter 2 and analysed for ATP, ADP, AMP, PCr, Cr, glycogen, HMP, glycerol 3-P, glycerol, pyruvate, lactate and alanine. Lactate, glycerol and glucose in whole blood and pH, ammonia and uric acid in plasma were analysed as described in Chapter 2.

(2) EFFECT OF DIFFERENT INTENSITIES OF POST-EXERCISE ACTIVITY ON RECOVERY FROM HIGH-INTENSITY TREADMILL EXERCISE

Following two minutes exercise at 12 m/s on a 5° incline, three different recovery protocols were used:

S   70 minutes standing recovery.
W   70 minutes walking at 1.6 m/s and 0° incline.
T   30 minutes trotting (3.2 m/s and 0° incline) and
        40 minutes walking (1.6 m/s and 0° incline).

Each horse completed three experimental sessions, at least one week being allowed between sessions and in a total period of seven weeks. The order of recovery sessions was allocated using a semi-randomized design in order to avoid the effect of any training response.

Horses were prepared for muscle biopsying and catheterized as described in Chapter 2. Venous blood samples were collected at the end of exercise (0' recovery) and at 2 minute intervals to 20 minutes recovery, at 5 minute intervals to 40 minutes recovery and at 10 minute intervals to 70 minutes recovery. Venous blood samples for the measurement of plasma pH were collected immediately following exercise (0' recovery) and at 10, 30 and 70 minutes recovery on S and T sessions only. Muscle biopsies were taken at the end of exercise and at 10, 30 and 70 minutes recovery on S and T sessions only.

ATP, ADP, AMP, PCr, Cr, glycogen, HMP, glucose, glycerol 3-P, lactate, alanine and pH in muscle, lactate and glucose in blood, pH and ammonia in plasma and heart rate were determined as described in Chapter 2.

(3) INTERMITTENT MAXIMAL EXERCISE OF 2 X 900 METRES.

Four trained Thoroughbred geldings each completed two gallops of 900 metres on two separate occasions, one week apart. Following the first 900m gallop, the horses were walked and trotted back to the start (10 minutes) and then completed the second gallop. The horses were catheterized as described in Chapter 2 and venous blood samples for whole blood lactate analysis were collected at 2, 4, 6, 8, 10, 12, 14, 16, 20, 25, 30, 35, 40, 50 and 60 minutes recovery following the second gallop. During the 60 minute recovery period the horses were walked by their rider, including when being sampled.

(4) INTERMITTENT MAXIMAL EXERCISE OF 4 X 700 METRES.

Each of the four horses completed four maximal gallops of 700 metres, separated by a 20 minute recovery period during which the horses were walked with their rider mounted. The horses were catheterized and venous blood samples were collected at 2, 10 and 20 minutes recovery following each of the first 3 gallops and at 2, 10, 20, 30, 40 and 50 minutes recovery following the fourth gallop, during which time the horses continued to be walked. Lactate and PCV in whole blood and lactate, uric acid and ammonia in plasma were analysed as described in Chapter 2.

RESULTS

(1) RECOVERY FOLLOWING SINGLE MAXIMAL FIELD GALLOPS OF 800 AND 2000 METRES

Muscle metabolites: Muscle metabolite contents during 60 minutes recovery following maximal gallops of 800 or 2000 metres are shown in Tables 7.1 and 7.2, respectively. Restoration of muscle ATP contents to pre-exercise levels was essentially complete by 60 minutes of recovery for both the 800 and 2000 metre gallops. In either case, there was a rapid increase in muscle ATP immediately post-exercise to 5 minutes recovery. This was followed by a much slower increase from 5 to 60 minutes. Recovery in muscle PCr contents showed a similar pattern to ATP. Following either the 800 or 2000 metre gallop, muscle PCr contents at 20 minutes recovery were no longer significantly lower than pre-exercise contents.

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Table 7.1. Muscle metabolite contents during 60 minutes walking recovery following a maximal gallop in the field of 800 metres.

				Recovery (min)			
	PRE	0	5	10	20	40	60
ATP	21.8±2.4	15.3±4.0 **	18.0±3.4 *	18.8±4.6	18.9±5.2	19.6±3.6	20.3±4.3
ADP	2.9±0.3	2.7±0.4	2.7±0.2	2.6±0.3	2.9±0.4	2.8±0.2	3.2±0.6
AMP	0.20±0.05	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.3±0.2	0.3±0.1
PCr	58.4±13.4	31.1±6.6 **	35.6±7.9 **	39.5±11.4 *	59.4±1.7	63.8±9.6	59.9±14.1
Gly	586±115	425±86 ***	ND	ND	469±69 **	ND	461±93 *
HMP	3.5±0.6	29.1±6.2 ***	23.3±10.5 **	18.2±8.8 *	12.9±6.4 *	6.8±1.8 **	5.8±2.1 *
G3P	2.7±0.6	13.6±1.7 ***	12.0±2.2 **	10.7±0.6 ***	8.4±2.0 **	4.7±2.4	2.9±0.8
Glyc	0.4±0.5	2.3±1.6 *	5.8±1.4 **	8.3±2.7 **	9.8±5.2 *	8.8±5.7 *	5.3±3.4 *
Pyr	0.2±0.2	0.4±0.3	0.4±0.3	0.6±0.5	0.7±0.7	0.5±0.5	0.5±0.4
Lac	13±5	124±50 **	85±39 **	75±38 **	51±22 *	29±11 *	17±6 *
Ala	2.8±1.0	5.8±1.6 **	5.2±0.9 **	5.0±1.1 **	5.2±1.3 **	3.9±0.8 **	3.1±1.3

Values are expressed in mmol/kg dry muscle as mean ± sd.

Significantly different from pre-exercise values: * P<0.05

                                                    ** P<0.01

                                                   *** P<0.001

Abbreviations: Gly   Glycogen

               HMP   Hexosemonophosphate

               G3P   Glycerol 3-P

               Glyc   Glycerol

               Pyr   Pyruvate

               Lac   Lactate

               Ala   Alanine

ND - not determined

Table 7.2. Muscle metabolite contents during 60 minutes walking recovery following a maximal gallop in the field of 2000 metres.

Recovery (min)

	PRE	0	5	10	20	40	60
ATP	21.4±3.2	11.3±2.6 **	13.2±1.6 **	14.4±4.5 *	14.4±3.7 **	17.7±4.2 *	18.9±4.1
ADP	2.6±0.7	2.4±0.8	2.2±0.6	2.1±0.3	2.3±0.5	2.2±0.6	2.4±0.9
AMP	0.37±0.19	0.2±0.1	0.4±0.3	0.3±0.2	0.3±0.3	0.5±0.3	0.6±0.3
PCr	62.2±7.6	18.5±8.6 **	40.1±12.5 **	45.4±12.9 *	53.0±16.9	68.2±15.5	69.2±14.8
Gly	550±43	374±74 **	ND	ND	385±129 *	ND	413±81 *
HMP	4.9±0.3	28.5±6.1 **	22.7±6.1 **	17.5±4.7 *	13.8±2.7 **	6.3±2.3	6.6±1.5
G3P	3.0±0.9	19.4±2.4 ***	11.7±0.9 ***	8.9±2.5 *	9.5±1.9 **	4.6±2.1	5.2±1.0 *
Glyc	0.5±0.2	2.7±1.2 *	8.9±2.7 **	9.8±2.3 **	11.7±2.7 **	9.7±4.4 *	7.3±2.1 **
Pyr	0.4±0.5	0.4±0.4	1.2±0.2	0.8±0.3	1.1±0.6	1.0±0.9	0.5±0.3
Lac	15±6	167±21 ***	147±11 ***	115±35 ***	94±56 *	44±32 *	28±21
Ala	3.3±0.8	6.0±0.8 *	6.0±0.5 *	6.0±0.2 **	6.2±0.6 *	4.2±0.1 **	3.9±1.3

Values are expressed in mmol/kg dry muscle as mean ± sd.

Significantly different from pre-exercise values: * $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Abbreviations: Gly   Glycogen

HMP   Hexosemonophosphate

G3P   Glycerol 3-P

Glyc   Glycerol

Pyr   Pyruvate

Lac   Lactate

Ala   Alanine

ND - not determined

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There was no evidence of any increase in muscle glycogen content during the recovery period following either the 800 or 2000 metre gallops. In both cases, muscle glycogen contents were significantly lower than pre-exercise contents at 0 ($P<0.01$), 20 ($P<0.05$) and 60 minutes recovery ($P<0.001$). Muscle HMP accumulation and disappearance during recovery was very similar for both the 800 and 2000 metre gallops and showed an approximately exponential decline, with mean half-times of 17.5 and 18.4 minutes, respectively. In both cases, contents approached pre-exercise values by 40 minutes recovery.

Glycerol 3-P peaked immediately post-exercise with either gallop and thereafter decreased gradually, approaching resting contents by 60 minutes recovery. In contrast, the muscle free glycerol content showed only a small increase with exercise, but a much greater increase during the first 20 minutes of the recovery period. The increase in glycerol was mirrored closely by the decrease in glycerol 3-P. Peak contents in either case were found at 20 or 40 minutes recovery and were still significantly elevated above pre-exercise contents at 60 minutes recovery ($P<0.05$).

Peak muscle lactate contents occurred immediately post-exercise in either case, and showed a mono-exponential decline with mean half-times of 22.9±4.2 and 18.9±6.6 min after the 800 and 2000 metre gallops, respectively. These were not statistically different ($P>0.05$). Pyruvate contents remained essentially unchanged during the recovery period, with no difference between gallops. Alanine, formed from the amination of pyruvate, increased significantly with exercise ($P<0.05$) and showed little change over the first 20 minutes of recovery. There was a decrease with both the 800 and 2000 metre gallops following 20 minutes recovery, with resting contents being approached by 60 minutes. The changes were not statistically different between either gallop.

Changes in blood indices: Changes in blood metabolites, pH and PCV following maximal gallops in the field of 800 and 2000 metres are shown in Tables 7.3 and 7.4, respectively. There was a large increase in plasma ammonia with exercise and although there was a trend towards lower concentrations during the recovery period, these were still elevated at 60 minutes. There was no significant difference in recovery between the 800 and 2000 metre gallops. In contrast, plasma uric acid showed only a small increase with exercise and a much larger increase during the recovery period, with peak concentrations occurring around 40 to 60 minutes. As for ammonia, there was no significant difference between gallops.

Table 7.3. Venous blood lactate, glycerol, glucose and PCV and plasma ammonia, uric acid and pH before (PRE) and during 60 min recovery following a maximal gallop in the field of 800 metres

		Recovery (min)					
	PRE	0	5	10	20	40	60
Lac mmol/l	0.4±0.3	17.8±3.9 ***	20.6±3.6 ***	20.5±4.3 ***	17.0±4.9 **	8.4±4.9 **	3.0±2.7
Glyc µmol/l	116±26	192±86	493±159 **	858±267 **	1185±332 ***	1259±677 **	916±709 *
NH₃ µmol/l	27±22	216±39 ***	210±31 ***	207±31 ***	215±24 ***	181±29 ***	152±23 ***
Urate µmol/l	21±8	46±11 *	65±5 ***	100±7 ***	141±32 **	175±62 **	149±71 *
Gluc mmol/l	4.7±0.6	5.2±0.9	6.1±0.6 *	6.0±0.7 *	5.7±0.7	5.7±0.8	5.6±0.8
pH	7.43±0.03	7.13±0.04 ***	7.11±0.05 ***	7.12±0.08 **	7.22±0.08 **	7.38±0.05	7.42±0.02
PCV l/l	0.495±0.01	0.60±0.03 **	0.59±0.02 **	0.58±0.02 **	0.53±0.04	0.44±0.01	0.43±0.01

Significantly different from pre-exercise: * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Abbreviations: Lac   Lactate
                NH₃   Ammonia
                Glyc   Glycerol
                Urate   Uric acid
                Gluc   Glucose
                PCV   Packed cell volume

Table 7.4. Venous blood lactate, glycerol, glucose and PCV and plasma ammonia, uric acid and pH before (PRE) and during 60 min recovery following a maximal gallop in the field of 2000 metres

Recovery (min)

	PRE	0	5	10	20	40	60
Lac mmol/l	1.7±0.8	26.5±1.6 ***	30.9±5.3 ***	30.5±2.6 ***	26.3±3.7 ***	15.4±4.6 **	7.1±4.5 *
Glyc μmol/l	44±48	272±135 *	579±255 *	1146±392 *	1421±485 *	1636±590 *	1267±689 *
NH₃ μmol/l	37±10	257±92 *	232±94 *	211±107 *	229±101 *	230±80 *	212±41 **
Urate μmol/l	29±9	60±9 *	90±26 *	116±27 **	162±34 **	190±46 **	193±53 **
Gluc mmol/l	5.1±0.7	6.7±1.0	7.7±1.5 *	7.2±1.5	6.8±1.4	6.4±1.0	7.0±1.3
pH	7.39±0.08	7.05±0.07 **	7.01±0.06 **	7.02±0.07 **	7.12±0.08 *	7.32±0.07 *	7.41±0.05
PCV l/l	0.518±0.07	0.61±0.05 *	0.59±0.04	0.58±0.06	0.54±0.07	0.47±0.07	0.45±0.07

Significantly different from pre-exercise: * P<0.05   ** P<0.01   *** P<0.001

Abbreviations: Lac   Lactate
                NH₃   Ammonia
                Glyc   Glycerol
                Urate   Uric acid
                Gluc   Glucose
                PCV   Packed cell volume

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In contrast to muscle lactate, which peaked immediately at the end of exercise, blood lactate following either gallop peaked after 5 to 10 minutes of recovery. Concentrations approached pre-exercise values by 60 min recovery. Half-times for blood lactate disappearance over the last 40 min of recovery, during which time the decrease was exponential, were 15.2±5.9 and 21.1±7.7 min for the 800 and 2000 metre gallops, respectively.

The large increases in lactate with exercise were accompanied by pronounced decreases in plasma pH, the lowest values being found at 5 min recovery. Following exercise, recovery in plasma pH showed a similar pattern to lactate disappearance, with an approximately exponential increase between 10 and 60 min recovery and values approaching pre-exercise pH by the end of the recovery period.

Blood glycerol showed similar kinetics to muscle contents, showing only a small increase with exercise, followed by a rise during the recovery period. However, in contrast to muscle, where peak contents were observed at 20 min recovery, in blood peak concentrations were not found until 40 min recovery. Peak blood glucose concentrations occurred 5 min post-exercise with either the 800 or 2000 metre gallop, with only a small decrease during the recovery period.

Between 0 and 40 minutes recovery following either gallop, PCV showed a near linear fall with time. No significant difference was found between gallops in the rate of recovery in PCV and by 40 minutes recovery values approached pre-exercise levels.

(2) EFFECT OF POST-EXERCISE ACTIVITY ON RECOVERY IN MUSCLE AND BLOOD METABOLITES

Muscle metabolite changes after exercise and during 70 minutes recovery with S and T are shown in Table 7.5. Blood metabolite changes are shown in Tables 7.6 and 7.7.

Muscle: Two minutes of high intensity exercise resulted in a mean decrease in muscle ATP content of 30±10% (P<0.01). During the first ten minutes of recovery there was an initial rapid increase in ATP. This was followed by a slower increase towards pre-exercise contents by 70 minutes of recovery. The mean increases in ATP during S and T recoveries were exponential with half-times of 24.5 and 29.7 minutes, respectively and were not significantly different. At the end of exercise PCr was 57±8% lower compared to the pre-exercise content (P<0.01). By 10 minutes of recovery this had increased to within 17-22% of the pre-exercise value. As for ATP, the increase in PCr was approximately exponential, with half-times of

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Table 7.5. Muscle metabolite contents (mmol/kg d.m.) at rest and the changes with exercise and during 70 minutes S or T recovery.

	PRE	EX	S Recovery (min) 10	30	70	T Recovery (min) 10	30	70
ATP	24.0 ±2.5	-7.3** ±2.4	-6.2* ±2.3	-3.5 ±3.3	-1.1 ±1.1	-4.3* ±2.3	-2.5 ±1.8	-0.5 ±1.5
ADP	2.9 ±0.2	-0.1 ±0.4	-0.1 ±0.3	-0.3 ±0.4	-0.2 ±0.2	-0.1 ±0.4	-0.1 ±0.5	-0.1 ±0.5
PCr	60.3 ±12.0	-34.3** ±11.0	-13.1 ±12.1	-4.1 ±5.2	-6.7 ±7.4	-10.2 ±8.9	-4.8 ±8.2	-0.3 ±3.2
Glycogen	519 ±28	-101 ±43	ND	-57* ±31	-50 ±59	ND	-91* ±46	-78* ±40
HMP	4.0 ±1.0	+21.4*** ±2.6	+9.8*§ ±5.9	+1.8 ±1.8	+0.9 ±2.0	+14.5** ±5.5	+3.7* ±1.8	+2.2 ±2.4
Glucose	3.0 ±1.4	+7.9** ±2.4	+14.4** ±3.9	+13.1 ±5.5	+4.8 ±5.4	+13.5** ±3.5	+12.4* ±4.9	+4.9 ±4.8
Glycerol 3-P	2.9 ±0.6	+14.0* ±5.0	+6.7* ±3.1	+3.2 ±2.2	+0.7 ±2.4	+7.6** ±1.9	+3.1* ±1.9	+0.3 ±0.7
Lactate	10.1 ±3.2	+103.0** ±34.5	+73.2** ±23.8	+28.8 ±21.5	+4.4§ ±3.2	+59.3** ±10.5	+21.2 ±16.5	+0.6 ±5.0
Alanine	2.9 ±0.9	+3.8* ±1.4	+3.6* ±1.4	+3.4 ±2.4	+1.2§ ±0.8	+3.3** ±0.6	+1.2 ±1.1	-0.2 ±1.1
pH	7.21 ±0.03	-0.42** ±0.13	-0.28** ±0.06	-0.14* ±0.06	-0.05* ±0.02	-0.22* ±0.08	-0.08* ±0.12	-0.00 ±0.07

ND - Not determined

Significantly different from pre-exercise: * P<0.05 ** P<0.01 *** P<0.001
Significant difference between S and T : § P<0.05

Table 7.6 Venous blood lactate concentration (mmol/l) at rest, immediately following exercise and the changes during 70 minutes S, W or T recovery.

Minutes of recovery

	PRE	0	10	30	70
S	0.5	+15.6**	+20.3**§	+16.1**§§§	+5.2*
	±0.1	±3.9	±4.9	±5.2	±2.6
W	–	–	+21.9**	+13.7**	+2.1
			±5.8	±6.4	±1.7
T	–	–	+18.7**	+7.5*	+0.9*
			±5.1	±4.1	±0.8

Significantly different from pre-exercise: * P<0.05 ** P<0.01 *** P<0.001
Significant difference between S and T: § P<0.05 §§§ P<0.001
No significant difference between S and W or W and T.

Table 7.7. Venous blood glucose concentration (mmol/l) and PCV (1/1) and plasma pH and ammonia (μmol/l) before exercise, and the changes with exercise and during 70 minutes S or T recovery.

			S Recovery			T Recovery		
	PRE	EX	10	30	70	10	30	70
Ammonia	33	+156	+203**	+243*	+217*	+215**	+255*	+142
	±4	±112	±49	±100	±138	±48	±121	±116
Glucose	3.6	-0.7*	+0.3	+0.8	+1.1	+0.6	+1.0*	+1.4
	±0.6	±0.4	±0.8	±1.2	±0.9	±0.8	±0.5	±1.2
pH	7.42	-0.41**	-0.35**§§	-0.20*§§	-0.24§§	-0.27	-0.00	-0.04**
	±0.02	±0.12	±0.09	±0.10	±0.03	±0.10	±0.05	±0.01
PCV	0.43	+0.21	+0.18	+0.09	+0.01	+0.16	+0.07	-0.01
	±0.05	±0.05	±0.05	±0.02	±0.01	±0.05	±0.03	±0.02

Significantly different from pre-exercise: * P<0.05 ** P<0.01
Significant difference between S and T : § P<0.05 §§ P<0.01

approximately 12 minutes in either case.

Mean glycogen depletion at the end of exercise amounted to 19% of the mean pre-exercise content. Only limited resynthesis of this was observed over 70 minutes with either S or T recovery. HMP declined rapidly with both S and T, with a mean half-time in either case of approximately 11 minutes. However, the muscle content at 10 minutes recovery was significantly lower with S compared to T (P<0.05). The muscle free glucose content increased approximately threefold with exercise, with a further doubling during the first ten minutes of recovery with both S and T. Following this, little change occurred in muscle glucose with either S or T recovery between 10 and 30 minutes recovery and contents were still elevated at 70 minutes recovery .

Peak muscle lactate contents were found immediately post-exercise, following which the disappearance was essentially mono-exponential with either recovery mode. Half-times for muscle lactate disappearance (Table 7.8) were significantly lower with T compared to S (P<0.02). With both S and T, muscle lactate contents approached pre-exercise values by 70 minutes recovery. Although an obvious difference was observed between S and T with respect to lactate, no corresponding trend was observed with muscle pH (P>0.05).

Blood: Changes in blood lactate with exercise and recovery are shown in Figure 7.1. There was a trend towards shorter times to peak blood lactate with T (7.0±1.2 min) compared to W (8.0±2.3 min) compared to S (10.0±3.3 min). Mean half-times for blood lactate disappearance (Table 7.9) were: S - 26.8±5.2 min; W - 16.9±4.3 min; T - 12.2±3.9 min (S < W < T, P<0.01). Rate constants for blood lactate disappearance (calculated from plots of the natural logarithm of lactate concentration versus time) were linearly related to post-exercise activity, expressed in terms of treadmill speed (Figure 7.2). The greatest response in blood lactate disappearance to increased post-exercise activity was shown by horse SL, whilst the other four horses showed a similar response. Interestingly, horse SL also showed the lowest lactate accumulation compared to the other 4 horses.

Venous plasma pH decreased from 7.417±0.021 at rest to 7.000±0.092 immediately post-exercise (P<0.01). During T recovery plasma pH was significantly higher at 10, 30 and 70 minutes recovery (P < 0.01) compared to S. Peak plasma ammonia concentrations occurred between 10 and 30 minutes recovery with both S and T. A variety of responses was seen between horses in terms of the pattern of ammonia increase, although only one horse showed a marked difference between S and T. Only in horse SL did plasma ammonia

Table 7.8. Half-times (min) for post-exercise muscle lactate disappearance with S and T recovery.

Horse	S	T
SM	22.7	11.6
HR	26.4	21.1
SL	39.6	19.0
JW	21.5	9.0
Mean	27.6	15.2
±	8.3	5.8

Table 7.9. Half-times (min) for post-exercise blood lactate disappearance with S, W and T recovery.

Horse	S	W	T
SM	28.2	18.7	15.8
HR	24.4	18.3	12.9
SL	21.3	10.4	6.6
JW	33.3	19.7	13.5
KJ	25.9	17.2	--
Mean	26.8	16.8	12.2
±	5.2	4.3	3.9

Footnote: Data from KJ has been excluded from statistical comparisons as this horse completed S and W sessions only.

Figure 7.1. Venous blood lactate contents in 4 horses at the end immediately following 2 min treadmill exercise at 12m/s on 5° incline (0 min recovery) and during 70 minutes recovery with Stand, Walk and Trot recovery.

Figure 7.2. Exponential rate constants for blood lactate disappearance during recovery in relation to post-exercise activity, expressed as treadmill speed, in 5 horses.
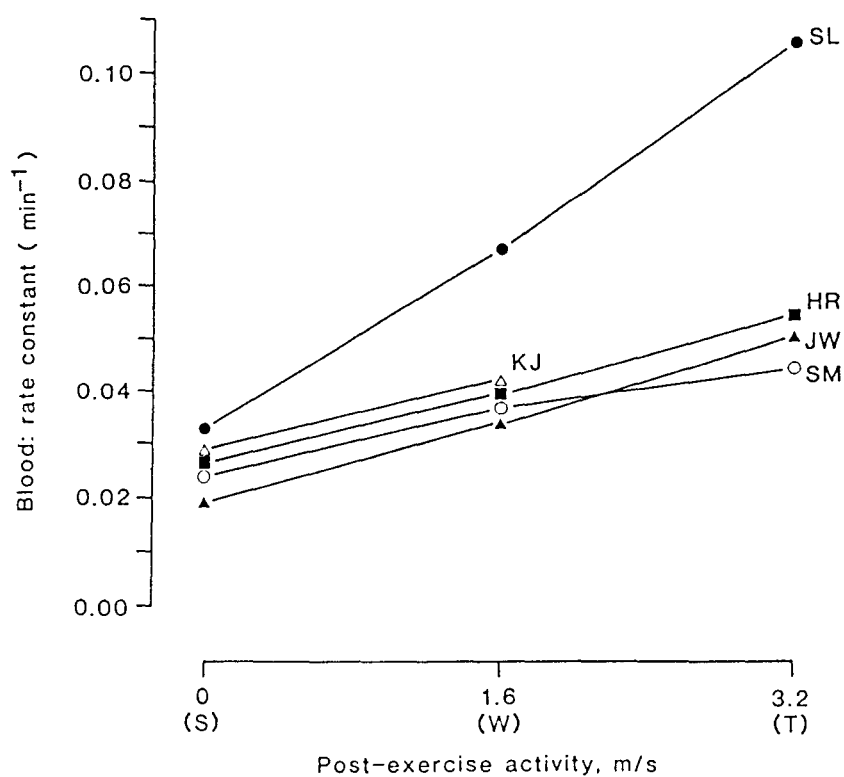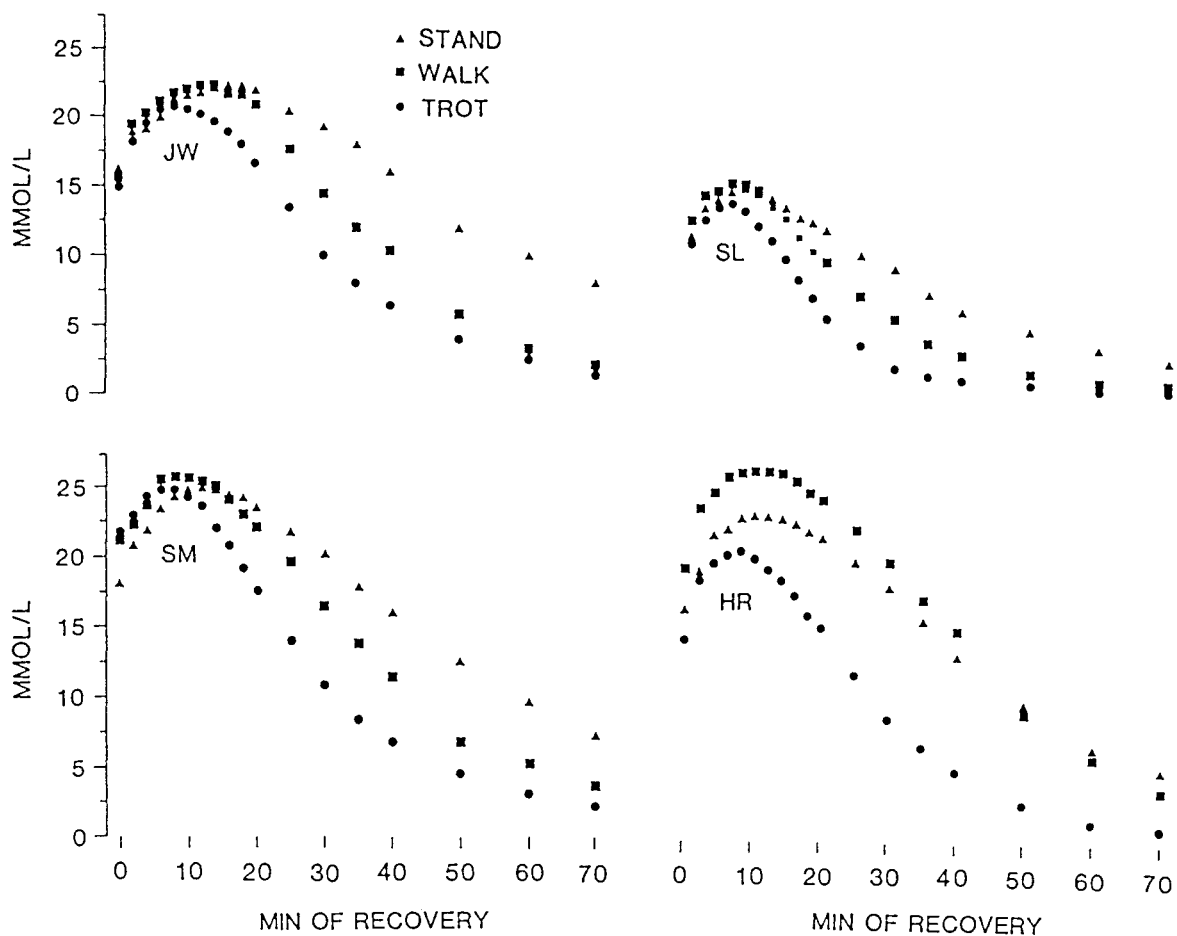
approach resting levels by the end of the 70 minute recovery period. This horse also showed the least increase with exercise. A small but significant increase in blood glucose occurred with exercise (P<0.05), followed by a further increase during the recovery period (Table 7.7). There was no significant difference between S and T.

Mean muscle to plasma lactate gradients, calculated from the ratio of intracellular lactate concentration in muscle (mmol/l intracellular water, assuming a water content of 3 litres per 4kg of wet muscle) to the lactate concentration in plasma water (mmol/l plasma water) at rest, after exercise and during recovery are shown in Table 7.10. No significant difference was found between S and T, however the muscle to plasma gradient was significantly different from unity at 10 and 30 minutes recovery with S, whereas no significant differences from unity were observed with T throughout the whole of the recovery period.

Heart rate decreased rapidly from the end of the two minute exercise period with both S and T (Figure 7.3). However, with T heart rate between 5 and 30 minutes recovery was significantly higher than with S (P<0.05 and P<0.01).

(3) RECOVERY FOLLOWING INTERMITTENT MAXIMAL EXERCISE OF 2 X 900M

Blood lactate recovery curves for each of the four horses on the two separate exercise sessions are shown in Figures 7.4 to 7.7. Following exercise, there was a small increase in blood lactate concentration over the initial phase of recovery. Only by 20 minutes of recovery did blood lactate begin to show a definite downward trend in all horses. Mean half-times calculated over the last 30 minutes of recovery (Table 7.11) were 22.7±4.6 and 19.9±1.9 minutes for the first and second exercise sessions, respectively, and were not significantly different.

(4) RECOVERY DURING AND AFTER INTERMITTENT MAXIMAL EXERCISE OF 4 X 700 M

Changes in venous blood lactate and PCV and plasma ammonia and uric acid during intermittent exercise of 4 x 700 metres are shown in Figures 7.8 to 7.11, respectively.

The greatest increase in blood lactate occurred following the first gallop, with progressively smaller increases following successive gallops. There was also a trend for the post-gallop increase and the decrease during the 20 minute recovery period between gallops to become reduced. This is shown quite clearly in Figure 7.8. There was a significant decrease in the rate of lactate removal (mmol/l/hr) following each gallop, calculated from

Table 7.10. Muscle to plasma lactate gradients at rest, following 2 min exercise and at 10, 30 and 70 min recovery with S and T recovery.

Recovery time (min)	S	T
Pre-exercise		4.73±2.01**
0		1.45±0.36*
10	0.82±0.07**	0.77±0.30
30	0.52±0.12**	1.25±1.26
70	0.90±0.86	1.40±0.66

Values differing significantly from unity - * P<0.05, ** P<0.01

Table 7.11. Half-times (min) for blood lactate disappearance following two gallops of 900 metres.

HORSE	WEEK1	WEEK2
KJ	16.9	18.2
SM	22.1	22.6
RS	23.6	19.8
MN	28.0	19.1
Mean	22.7	19.9
± sd	4.6	1.9

Table 7.12. Rates of blood lactate disappearance (mmol/l/hr) following each of four successive maximal gallops of 700 metres each.

	G1 - G2	G2 - G3	G3 - G4	G4 - 20'R	G4 - 50'R
PX	23.5	13.7	12.0	9.6	13.5
KJ	22.1	17.2	15.6	6.6	20.2
SM	21.1	14.9	8.7	5.4	12.5
BR	22.1	15.4	12.0	10.2	19.9
Mean	22.2	15.3	12.1	8.0	16.5
±sd	±1.0	±1.5	±2.8	±2.3	±4.1

Rates of lactate disappearance are calculated from the fall from the peak post-gallop lactate to the 20 min recovery lactate, with the exception of the last column where the rate is based on the decrease from the immediate post-G4 lactate to the 50 min recovery sample. Rates are not based on calculations from semi-log plots as the disappearance following each gallop was not exponential.

Figure 7.3. Heart rate (b.p.m.) during exercise and during T (filled circles) and S (open circles) recovery. S v T - * P<0.05, ** P<0.01.
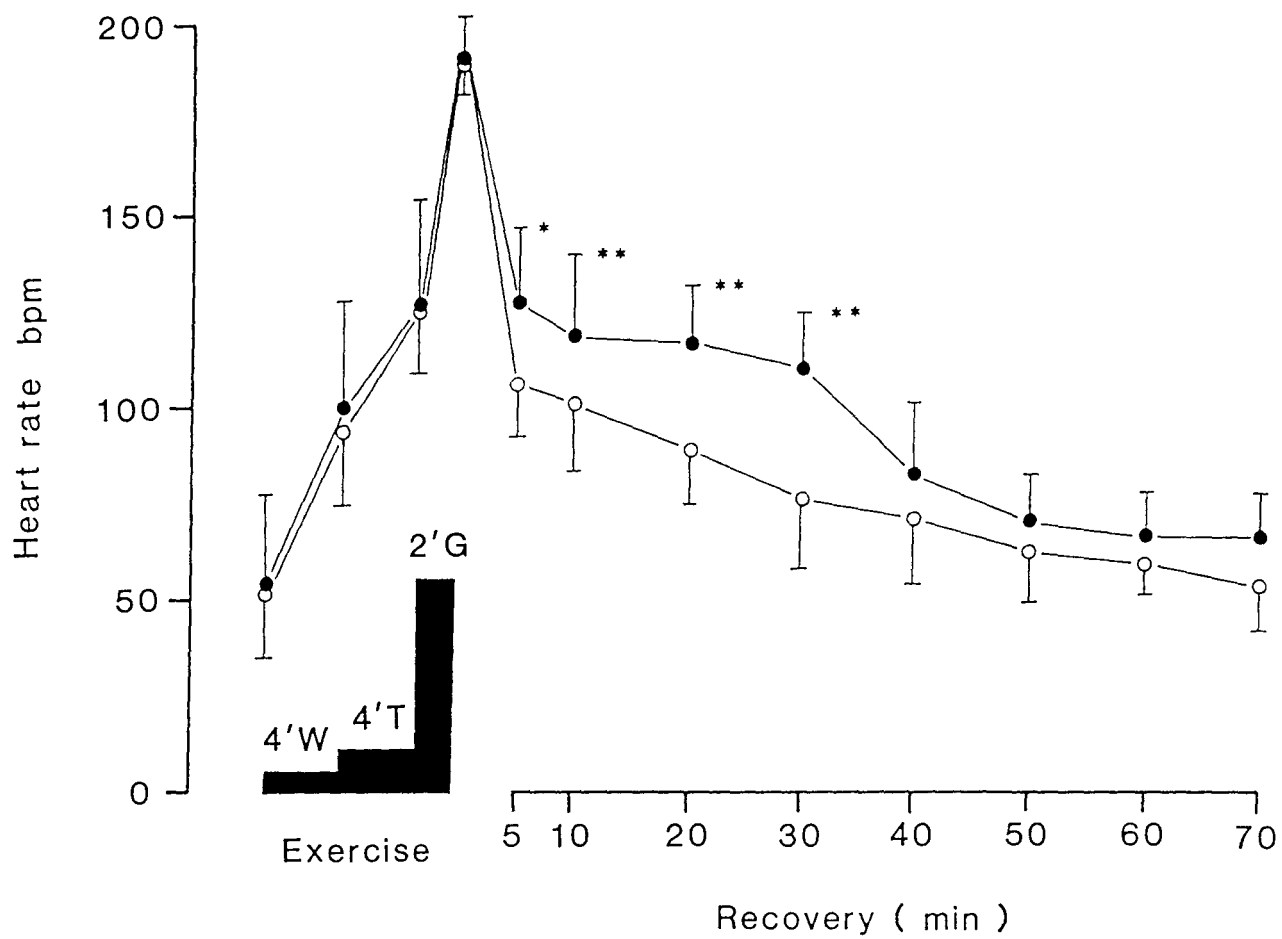
Figure 7.4. Venous blood lactate concentration (mmol/l) in horse MN during 60 minutes recovery following two gallops of 900m each.

Figure 7.5. Venous blood lactate concentration (mmol/l) in horse KJ during 60 minutes recovery following two gallops of 900m each.

MN

mmol/l

MIN OF RECOVERY

—*— WEEK 1   —□— WEEK 2

KJ

mmol/l

MIN OF RECOVERY

—*— WEEK 1   —□— WEEK 2
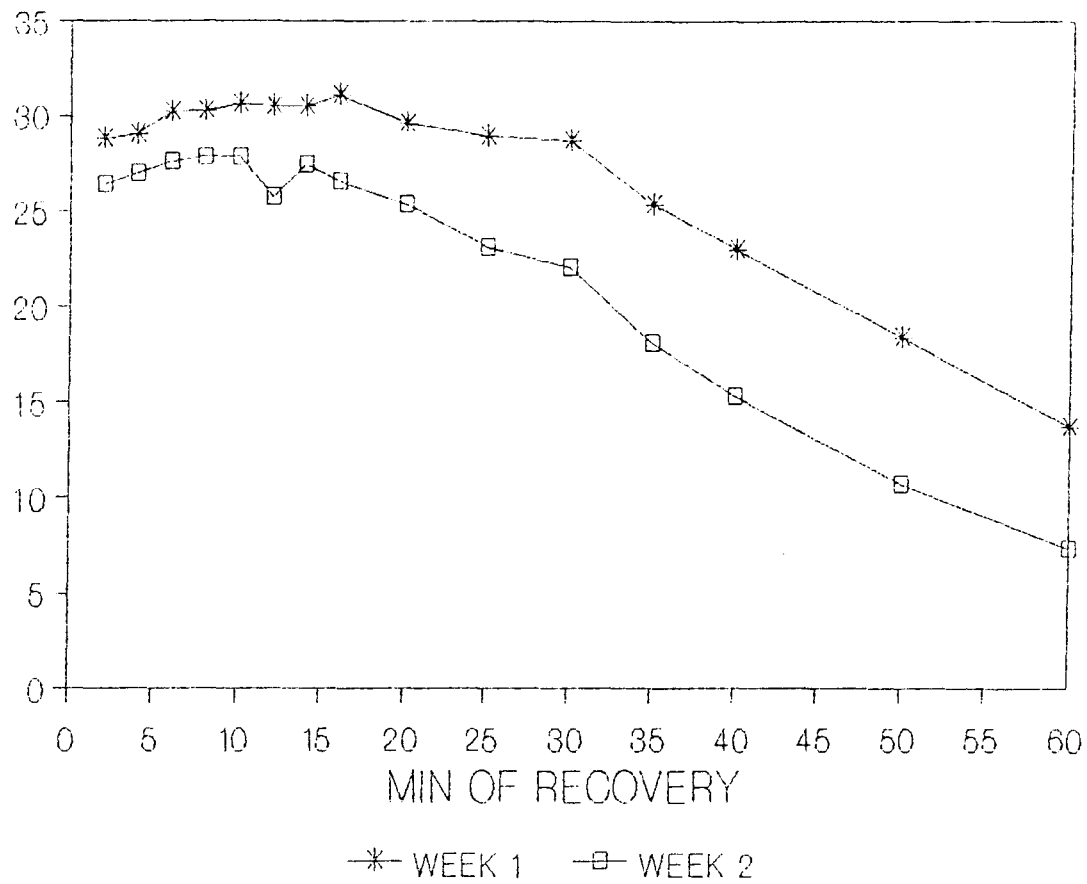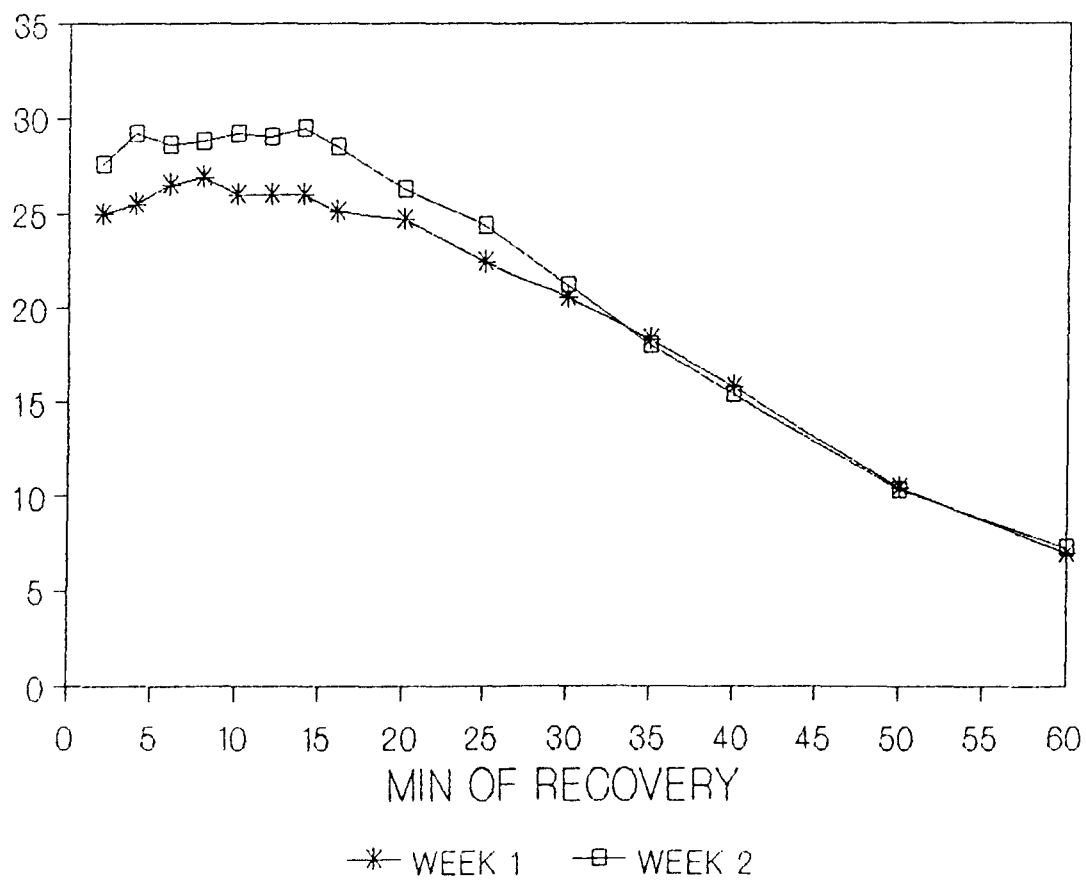
Figure 7.6. Venous blood lactate concentration (mmol/l) in horse SM during 60 minutes recovery following two gallops of 900m each.

Figure 7.7. Venous blood lactate concentration (mmol/l) in horse RS during 60 minutes recovery following two gallops of 900m each.

SM

mmol/l

WEEK 1   WEEK 2

MIN OF RECOVERY

RS

mmol/l

WEEK 1   WEEK 2

MIN OF RECOVERY

170
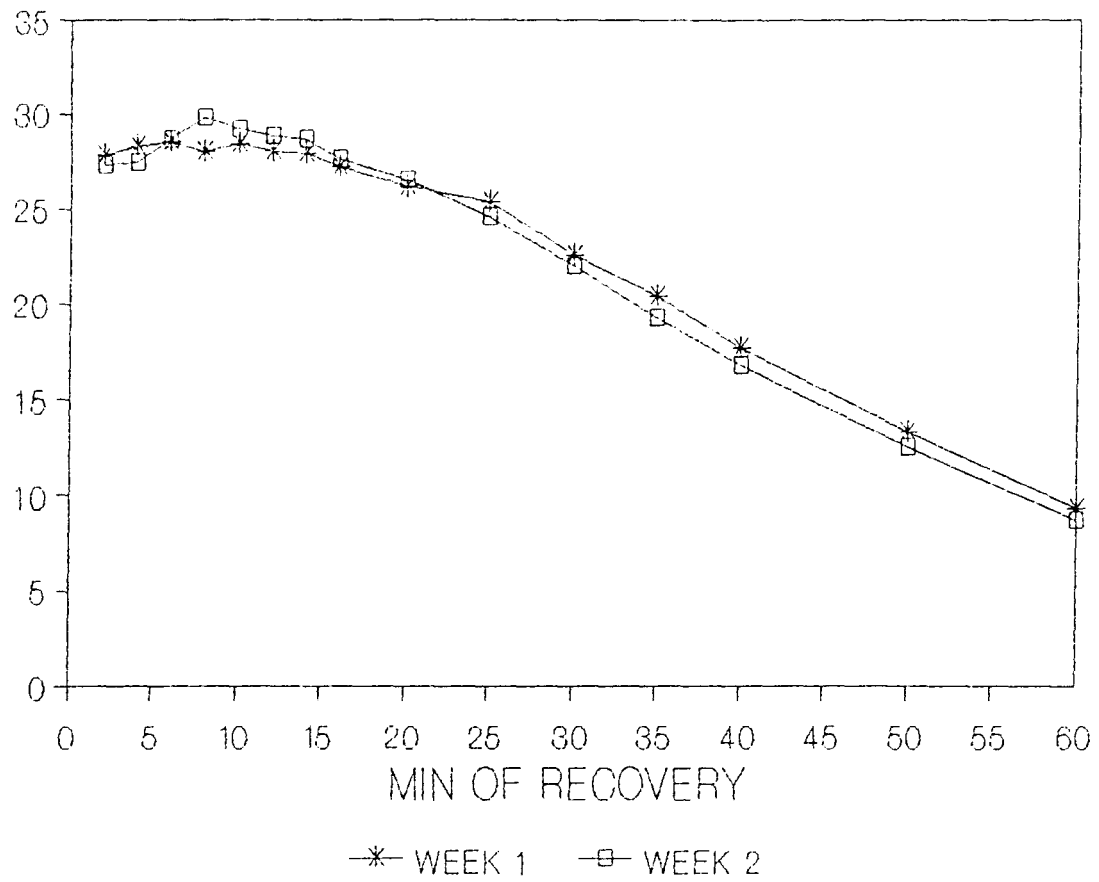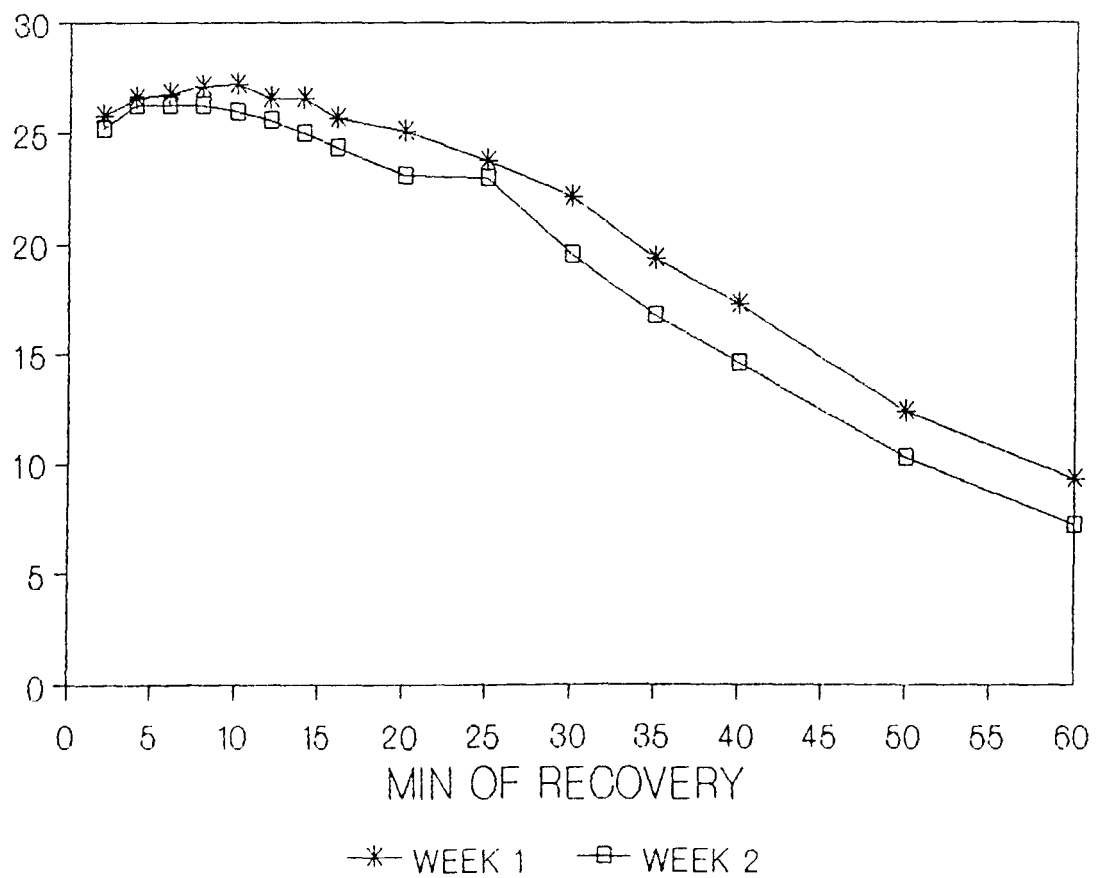
Figure 7.8. Venous whole blood lactate concentration (mmol/l) before and during 20 min recovery from each of four maximal gallops of 700m and during 50 min recovery following the 4th gallop.

Figure 7.9. Venous blood packed cell volume (PCV, 1/1) before and during 20 min recovery from each of four maximal gallops of 700m and during 50 min recovery following the 4th gallop.
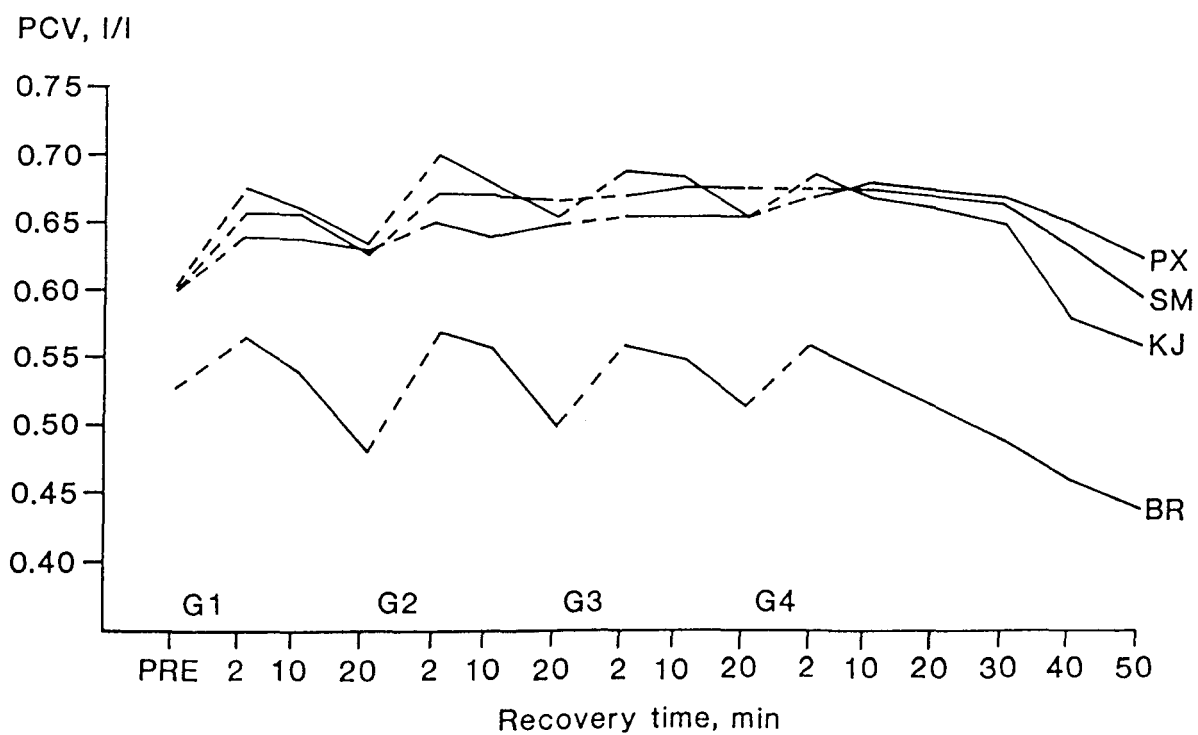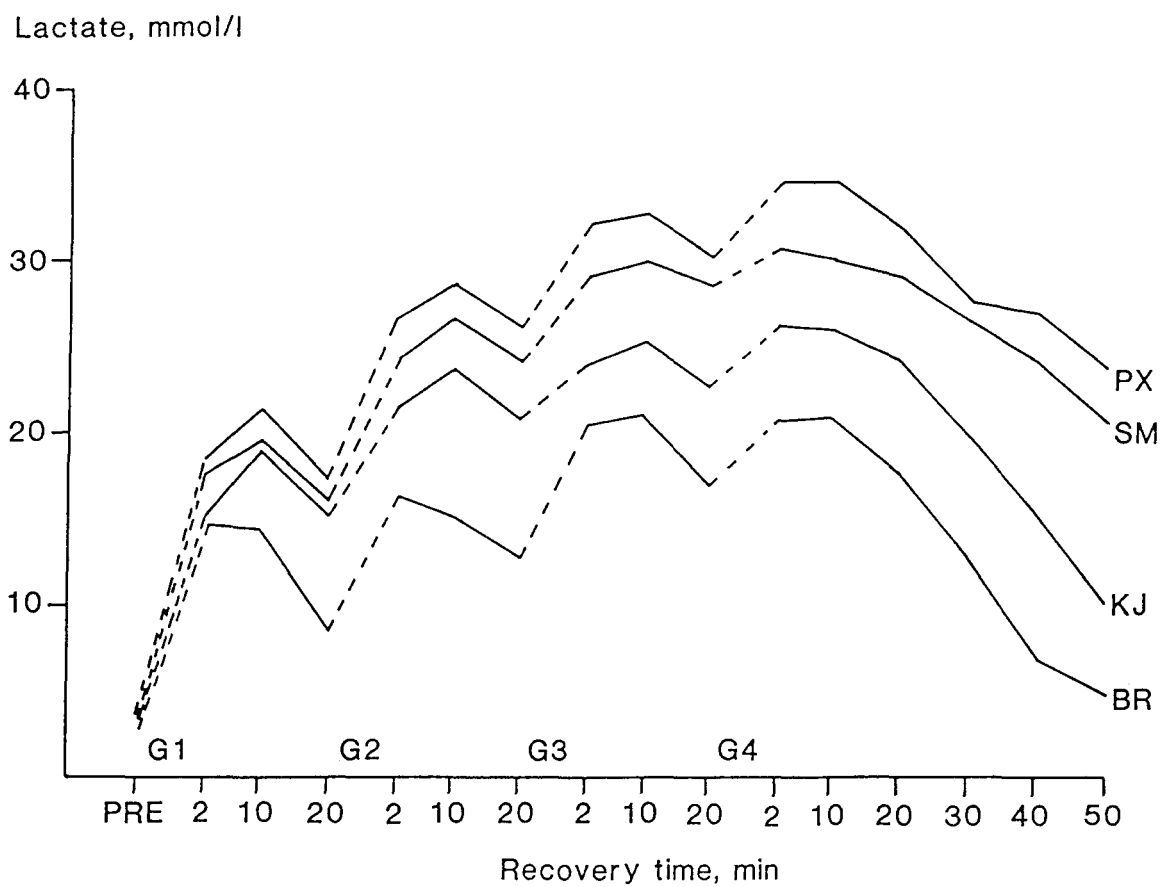
Figure 7.10. Venous plasma ammonia concentration (μmol/l) before and during 20 min recovery from each of four maximal gallops of 700m and during 50 min recovery following the 4th gallop.

Figure 7.11. Venous plasma uric acid concentration (μmol/l) before and during 20 min recovery from each of four maximal gallops of 700m and during 50 min recovery following the 4th gallop.

NH$_3$, umol/l

Recovery time, min

Uric acid, umol/l

Recovery time, min
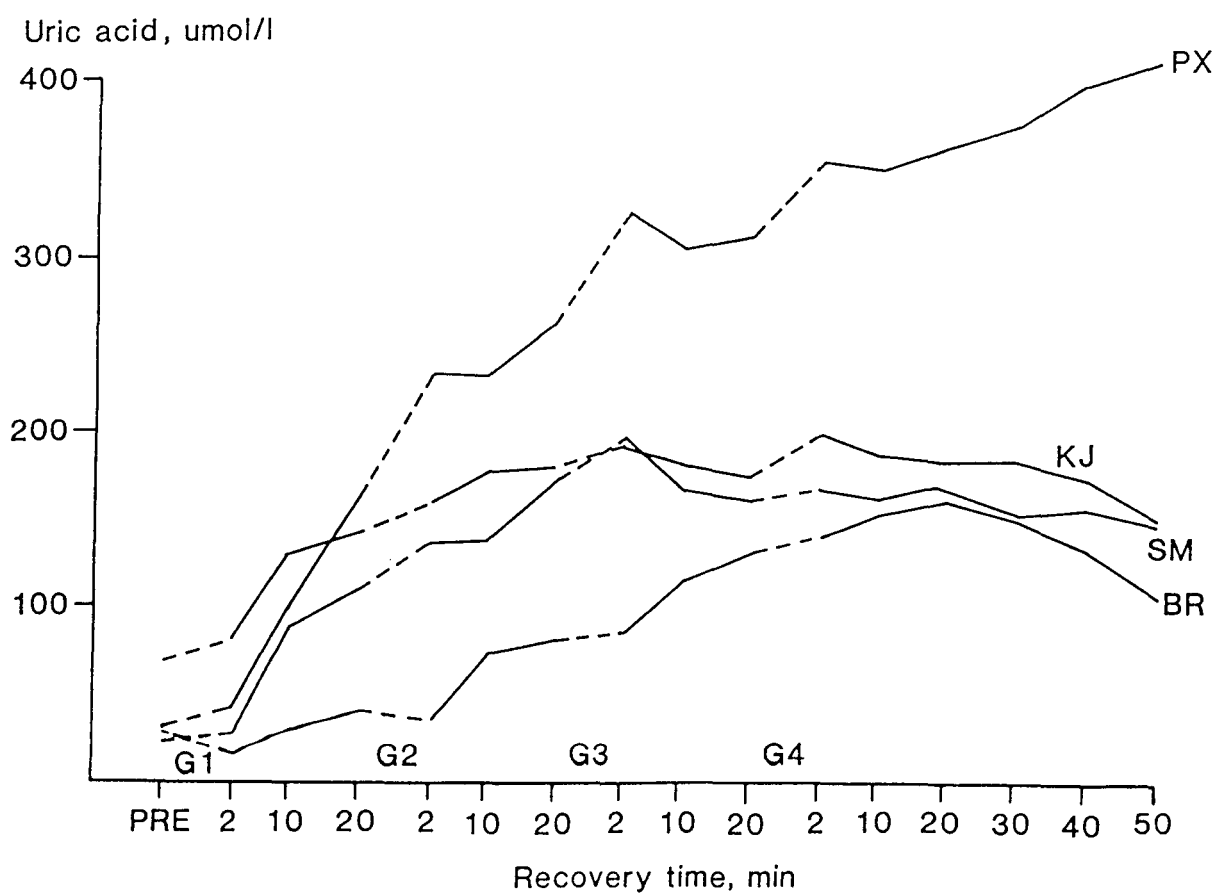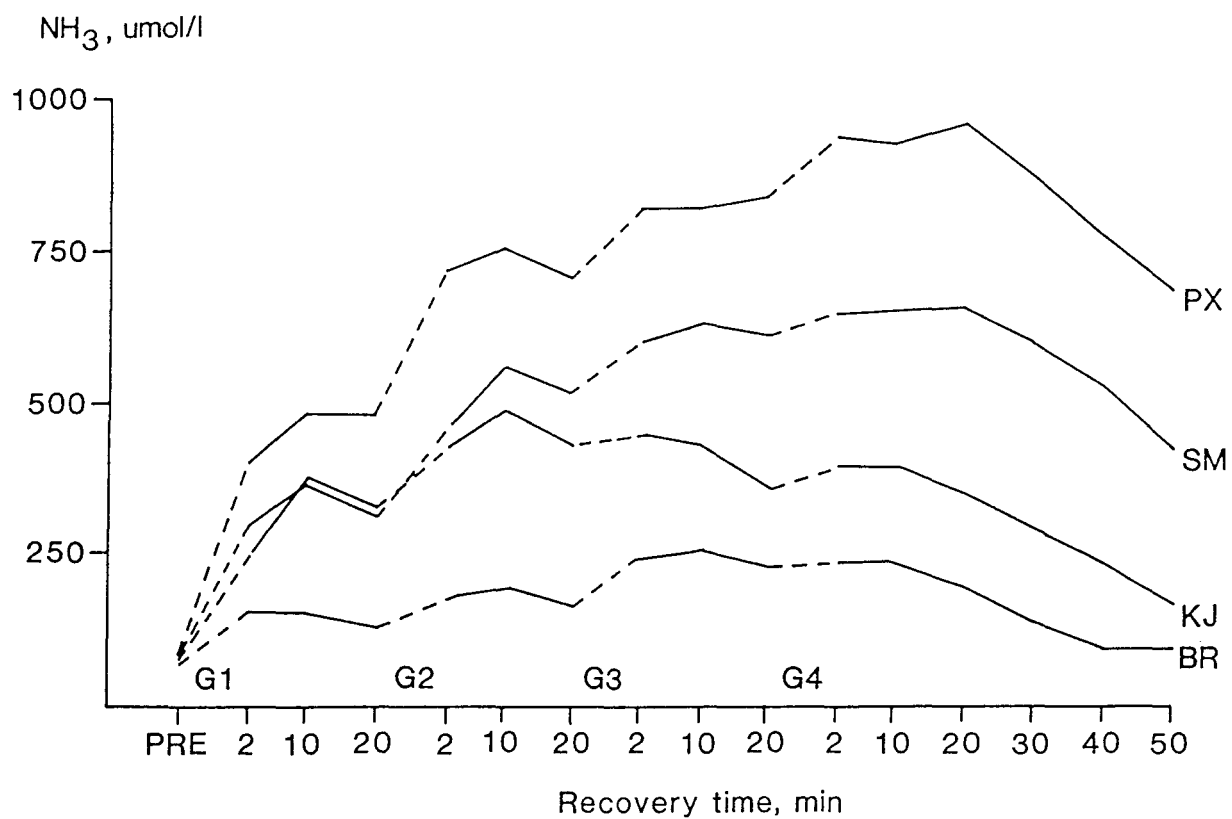
the reduction in lactate from the peak to the 20 minute recovery sample, as shown in Table 7.12. Half-times for lactate disappearance, calculated from the linear portion of a semi-log plot of lactate against time following the fourth gallop, were BR - 16.2 min, KJ - 24.1 min, SM - 63.0 min and PX - 74.9 min. The rate of decrease was positively correlated to peak blood lactate concentration following G4 (r = 0.96, P<0.01).

Two of the four horses showed a decrease in PCV during the 20 minute recovery period following each gallop (BR and KJ). Horse PX showed a small decrease in PCV following the first gallop, but showed no change with subsequent exercise or recovery periods, until around 30 minutes recovery following G4. In contrast, horse SM showed virtually no change in PCV with exercise and recovery periods from G1 to 30 minutes recovery following G4.

Plasma ammonia recovery showed a similar pattern to lactate with the decrease during the 20 minute recovery period between gallops becoming less with successive gallops (Figure 7.10). As for lactate, half-times for ammonia disappearance were positively correlated to peak concentration (r = 0.97, P<0.01). Half-times for ammonia and lactate disappearance were also very similar and were positively correlated (r = 0.88, P<0.05).

As shown in Figure 7.11, in most instances plasma uric acid showed an increase with each gallop and a further increase during the following 20 minute recovery period. With the exception of horse PX, peak values had been attained close to the end of the fourth gallop. In the case of the former, the peak was not seen until 50 minutes recovery following the fourth gallop. Uric acid disappearance (excluding horse PX) was relatively slow: SM - 27 μmol/l/hr; BR - 45 μmol/l/hr; KJ - 69 μmol/l/hr. By three hours following the fourth gallop, plasma uric acid was approaching pre values in these three horses, although in horse PX, plasma uric acid was still significantly elevated.

DISCUSSION

(1) 800 AND 2000 METRE GALLOPS

Post-exercise ATP and PCr resynthesis: Depletion of the muscle ATP store immediately following exercise was 6.5±2.0 and 10.1±2.8 mmol/kg d.m. for the 800 and 2000m gallops, respectively. Restoration of this began immediately after the end of exercise and by 60 min of recovery the deficit was no longer significant - 1.5±3.5 and 1.4±3.1 mmol/kg d.m., respectively. This is in contrast to the study of Snow, Harris and Gash (1985) using

intermittent maximal exercise, where virtually no resynthesis of muscle ATP was observed during 30 minutes of recovery. However, virtually no recovery occurred in most of the other metabolites either. The difference in recovery noted between the present study and the study of intermittent exercise may be due to the horses being stood following exercise in the latter and walked in the present study. Where deamination of AMP to IMP occurs during exercise, reamination via the purine nucleotide cycle may be limited by factors such as the size of the aspartate store (see Figure 7.12).

Restoration of the muscle PCr store occurred at the same rate following both the 800 and 2000m gallops. Studies in humans (Harris et al, 1976) have shown that following exercise resulting in immediate post-exercise muscle PCr contents of less than 10 mmol/kg d.m., resynthesis of PCr may be resolved into a fast (half time: 20-21s) and a slow (half time > 3 min.) exponential phase. With the delay in biopsying in the present study it is unlikely that the fast phase would have been seen, therefore, the rates of PCr resynthesis observed most likely correspond to the slow phase seen in humans.

Lactate and glycogen: The half-times for muscle lactate disappearance (800m - 22.9 min; 2000m - 18.9 min) compare with values of 9.4 min after 6-11 min exhaustive bicycle exercise and 2.5 min after intense isometric contraction in human quadriceps femoris (Harris, Sahlin and Hultman, 1981). Immediately after exercise muscle to blood lactate gradients were 1.66 ± 0.41 (800m) and 1.55 ± 0.16 (2000m), but had fallen to near unity or below within 5-10 min of recovery. This is similar to observations of Sahlin et al (1976) in humans, but contrary to previous findings following maximal exercise in the horse (Snow, Harris and Gash, 1985). The continuing rise in blood lactate from the end of exercise to 5-10 minutes into recovery suggests that during and immediately after exercise lactate efflux from muscle is in excess of lactate removal from blood. After 10 minutes recovery, and with the lactate gradient close to unity, efflux of lactate from muscle would follow lactate removal from blood. This is supported by the similarity in half-times for muscle and blood lactate disappearance.

Muscle lactate disappearance could also occur without export of lactate from the muscle via glyconeogenesis and oxidation and in this case the lactate will not appear in the blood. The combined data from both gallops indicates some evidence of an increase in muscle glycogen by the end of 60 minutes recovery, but the trend was not statistically significant $(P>0.05)$. The initial resynthesis of glycogen would be at the expense of

HMP which declined during the same period by an amount equivalent to around 50% of the mean increase in muscle glycogen. This would leave a maximum increase of around 25 mmol glucosyl units/kg d.m. explainable on the basis of glyconeogenesis from muscle lactate or approximately 23 and 18% of the muscle lactate disappearance for the 800 and 2000m gallops, respectively. Other studies have not succeeded in defining the fate of lactate during recovery from high-intensity exercise. For example, Hermansen and Vaage (1977) presented evidence suggesting that around 90% of lactate formed during high-intensity exercise was resynthesized back to glycogen during the recovery period, whilst Brooks and Gaesser (1980) indicated that the principal fate of lactate is oxidation. More recently, Astrand et al (1986) have demonstrated that following high-intensity exercise in man, oxidation and muscle glyconeogenesis can account for the disappearance of 40 and 43% of lactate produced, respectively.

The relatively low energy demands of the muscle during recovery make it unlikely that intramuscular pyruvate oxidation could account for a significant proportion of muscle lactate disappearance in the horse. From these considerations it would seem likely that the majority of muscle lactate disappearance is due to export of lactate from the working muscles (see later Discussion).

The changes in blood lactate as shown in Figure 7.13 are similar to those described in humans (Asmussen, 1950; Davies, Knobbs and Musgrove, 1970; Freund and Zouloumian, 1981). As in the present study peak blood lactate concentrations after heavy exercise occurred during the early part of recovery and overall the changes can be described using a biexponential equation (Freund and Gendry, 1978; Freund and Zouloumian, 1981). This form of equation was found to closely describe the time course of lactate recovery after both the 800 and 2000m gallops (Figure 7.13), such that

$$La_{(t)} = A_1 - A_1 e^{-\tau_1 t} + A_2 - A_2 e^{-\tau_2 t} + La_{(0)}$$

800m: $\tau_1 = 0.13$; $A_1 = 20$; $\tau_2 = 0.039$; $A_2 = -37$; $La_{(0)} = 17.8$

2000m: $\tau_1 = 0.13$; $A_1 = 28$; $\tau_2 = 0.039$; $A_2 = -50$; $La_{(0)} = 26.5$

where $La_{(t)}$ is the blood lactate concentration at time t in mmol/l and $La_{(0)}$ is the measured venous blood lactate concentration at the end of exercise. To satisfy the data from the two gallops only the amplitudes, A_1 and A_2, had to be varied, the velocity constants, τ_1 and τ_2, could be left unchanged. Compared with values of Freund and Zouloumian (1981)

Figure 7.12. Pathway for muscle AMP resynthesis from IMP following high-intensity exercise.

Figure 7.13. Venous blood lactate concentration (mmol/l) before (Pre) and after a single maximal gallop of 800m (- - -) or 2000m (-----) and during 60 min walking recovery. The curves have been computed from the following equation and data sets:

$$La_{(t)} = A_1 - A_1 e^{-\tau_1 t} + A_2 - A_2 e^{-\tau_2 t} + La_{(0)}$$

800m: $\tau_1 = 0.13$; $A_1 = 20$; $\tau_2 = 0.039$; $A_2 = -37$; $La_{(0)} = 17.8$

2000m: $\tau_1 = 0.13$; $A_1 = 28$; $\tau_2 = 0.039$; $A_2 = -50$; $La_{(0)} = 26.5$
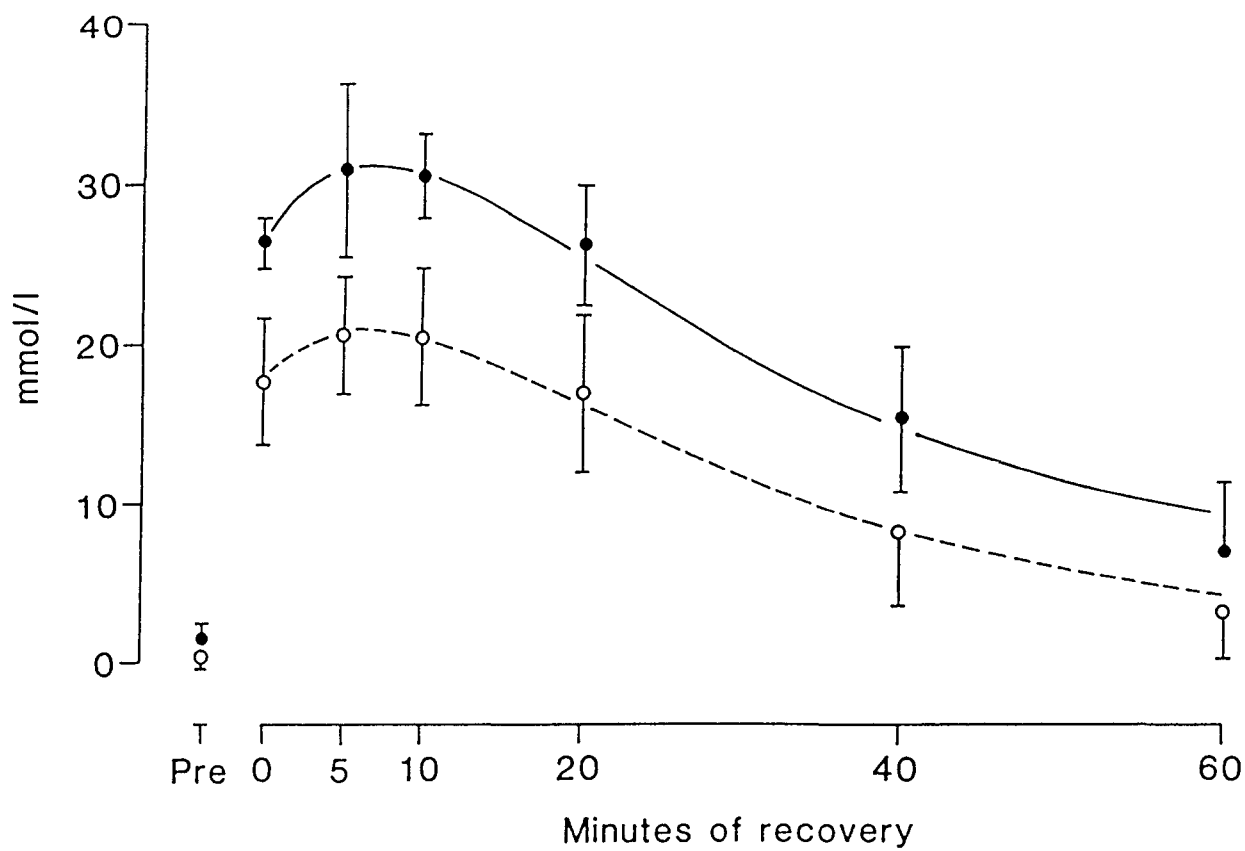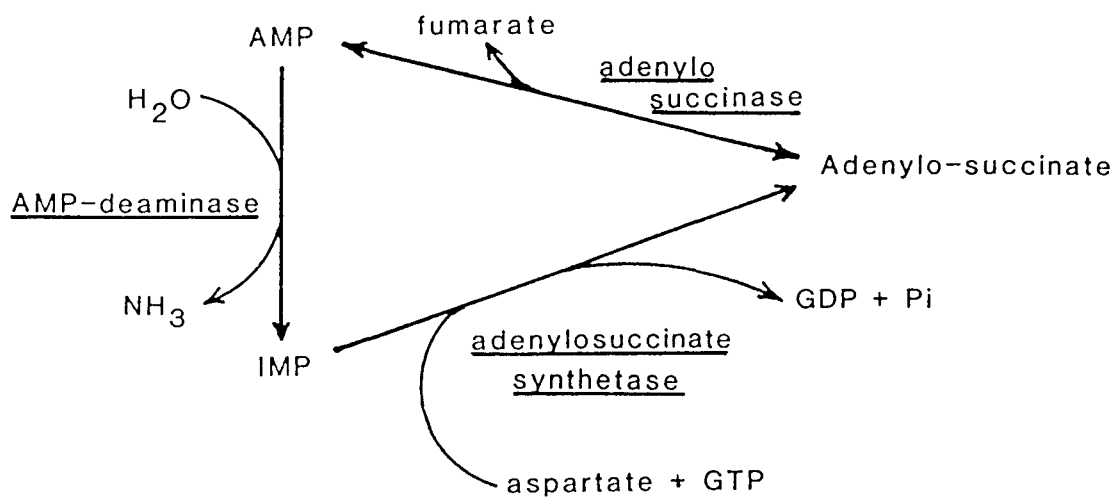
obtained in humans during recovery while in the seated or supine position, both τ_1 and τ_2 were considerably lower, consistent with a longer time to peak concentration and slower rate of disappearance (see earlier discussion of half-times).

It may have been anticipated that in an elite athlete such as the horse, the rate of blood lactate disappearance would have been much faster than in man, but apparently this is not the case. Although total lactate production is obviously very much greater in the horse, this does not appear to have been matched by any equivalent increase in the blood and muscle removal capacities. It could be inferred that whilst a high glycolytic capacity would confer a definite advantage to the horse in terms of its survival strategy, the benefit of a high rate of lactate removal is much less.

(2) ACTIVE AND PASSIVE RECOVERY

In the field studies in which distances of 800 and 2000m were covered at mean running speeds of 14.3 and 13.4 m/s respectively, mean peak blood lactates of 20.6±3.6 and 30.9±5.3 mmol/l were recorded. Half-times for blood lactate disappearance during recovery, where the horses were walked for 60 min, were 15.2±5.9 and 21.7±7.7 min, respectively. These times compare well with a value of 16.9±4.3 recorded in this study with W recovery and with a mean peak post-exercise blood lactate concentration of 21.3±4.7 mmol/l. Compared to man, half-times for blood and muscle lactate disappearance tend to be longer in the horse. Half-times for lactate disappearance from the vastus lateralis of 2.5 and 9.5 min have been observed following intense isometric contraction (Harris et al, 1981) and 6-11 min exhaustive bicycle exercise (Sahlin et al, 1976), respectively. Immediate post-exercise muscle lactate contents in either case were of the order of 100 mmol/kg d.m.

The range in half-times observed in the human studies can probably be best explained on the basis that in the isometric exercise model, only one major muscle group was used and the blood perfusing the muscle during recovery would have had a lactate concentration not very different from that at rest. Therefore, a large gradient would exist between muscle and blood. With respect to the dynamic exercise study, the perfusing blood would have had a much higher lactate concentration, simply due to the involvement of more muscle groups and loss of lactate from the muscles to the blood during the exercise period. Thus, the muscle to blood gradient would have been less following dynamic exercise, resulting in a slower rate

of efflux.

Half-times for blood lactate disappearance in trained subjects following intermittent exercise calculated from the data of Dodd et al (1984) were 15.0 min with passive recovery and 9.8 min with active recovery when subjects continued to exercise at 35% VO_2max. Furthermore, these authors also observed that a combination of exercise performed at 35 and 65% VO_2max during the recovery period produced no further increase in the rate of blood lactate disappearance. As might be expected, exercise at 65% VO_2max produced a slower rate of blood lactate disappearance than that observed during passive recovery, presumably due to continued lactate production. Gisolfi, Robinson and Turrell (1966) also reported a faster rate of blood lactate disappearance in subjects who continued to exercise at between 39 and 52% of their VO_2max following 2-5 min of treadmill exercise, when compared to controls undertaking a passive recovery.

The effect of activity during recovery in man is therefore similar to that seen in the present study, with the mean half-time for T being about half that for S recovery (12.2±3.9 compared to 26.8±5.2 min).

The process of lactate release from muscle is still not fully understood. In the study of Dodd et al (1984), the failure to obtain an early peak in blood lactate during active recovery was seen as evidence that lactate is not freely diffusible from the muscle. This appeared to agree with the studies of Jorfeldt et al (1978) who reported that the translocation rate of lactate from muscle to blood increased linearly with intracellular concentrations up to 4 mmol lactate/kg wet muscle (about 16 mmol/kg d.m.) after which the rate of efflux was constant. This suggests that lactate transport from the muscle is wholly carrier mediated, and that the carrier becomes saturated, even at relatively low concentrations of lactate. If this were true, then differences in blood flow through the muscle bed would be unlikely to affect the rate of muscle lactate efflux, implying that the difference in muscle lactate disappearance rates between S and T recovery resulted from differences in rates of local utilization, namely glycogen resynthesis and oxidation.

Although there was some evidence of glycogen resynthesis during recovery, this was not statistically significant and did not appear to differ between S and T. Also, most of the glycogen increase could be accounted for by the disappearance of HMP. Between 0 and 30 min recovery, the difference between S and T recoveries in muscle lactate disappearance amounted to approximately 30 mmol/kg d.m., sufficient for the aerobic synthesis of 495 mmol ATP/kg and support of an ATP turnover rate of 0.3

mmol/kg d.m./sec. Although the increased metabolic cost to the middle gluteal of trotting compared to standing is not known, by comparison to human rates (Harris, 1981) this is a reasonable figure, if not somewhat conservative. In other words, local utilization of lactate to meet the increased metabolic cost of trotting above standing could explain the increased rate of lactate disappearance.

Alternatively, if lactate was considered to be freely diffusible across the muscle membrane then its release would depend to a certain extent on the muscle to plasma lactate gradient. This is supported by the earlier mentioned studies of muscle lactate disappearance in man following isometric and dynamic exercise, and by the exponential nature of the decrease in muscle lactate in both man and horse.

Even considering that some of the lactate may be utilized within the muscle in which it was produced, the fate of the majority appears to be efflux into and redistribution by the blood. Examination of the muscle to plasma lactate gradients showed a fall from 1.45 ± 0.36 following exercise to near unity after 10 min recovery with both S and T recoveries, implying an equilibrium in terms of concentration. However, with S recovery the gradient showed a further fall to 0.53 ± 0.12 at 30 min which was significantly ($P<0.01$) lower than unity. This had risen to 0.90 ± 0.86 by 70 min recovery. With T the recovery gradients actually showed a small increase at 30 and 70 min, however, neither were significantly different from unity, although there was clearly an upward trend. The changes in gradients are complex and difficult to interpret, though a consistent trend was seen with both recovery modes.

Recovery in gradients may be linked to recovery in muscle pH. It has been shown that in frog sartorius muscle (Seo, 1984) permeability of the muscle membranes to lactate is decreased when extracellular pH is reduced. In T recovery, plasma pH increased more rapidly compared to S which on this basis would favour an increase in muscle lactate efflux and this may in part explain the shorter half-times for muscle lactate disappearance with T. Although, the faster recovery in plasma pH with T was not matched by a faster recovery in muscle pH, this does not preclude an effect of extracellular pH on lactate efflux.

Muscle pH determined in samples taken immediately after exercise was linearly correlated to muscle lactate content (Figure 7.14) and with a slope similar to that found previously in the horse (Harris et al, 1989). Surprisingly, the same relationship was also found for samples collected during the 70 min recovery period (Figure 7.15) indicating a continued

Figure 7.14. Relationship between muscle pH and muscle lactate content (mmol/kg d.m.) in muscle samples taken after 2 min treadmill exercise at 12m/s on 5° incline. T session - ● , S session - ○ . Mean and standard deviation of resting pH and lactate content is shown but not included in correlation and regression. r = -0.93, pH = 7.202 - 0.003626.(Lactate), P<0.001.

Figure 7.15. Relationship between muscle pH and muscle lactate (mmol/kg d.m.) in muscle samples taken during recovery following 2 min treadmill exercise at 12m/s on 5° incline. ■ - 10 min T, □ - 10 min S, ● - 30 min T, ○ - 30 min S, ▲ - 70 min T, △ - 70 min S. Dashed line is from Figure 7.14. Mean and standard deviation of resting pH and lactate content is shown but not included in correlation and regression. r = -0.92, pH = 7.218 - 0.003326.(Lactate), P<0.001.
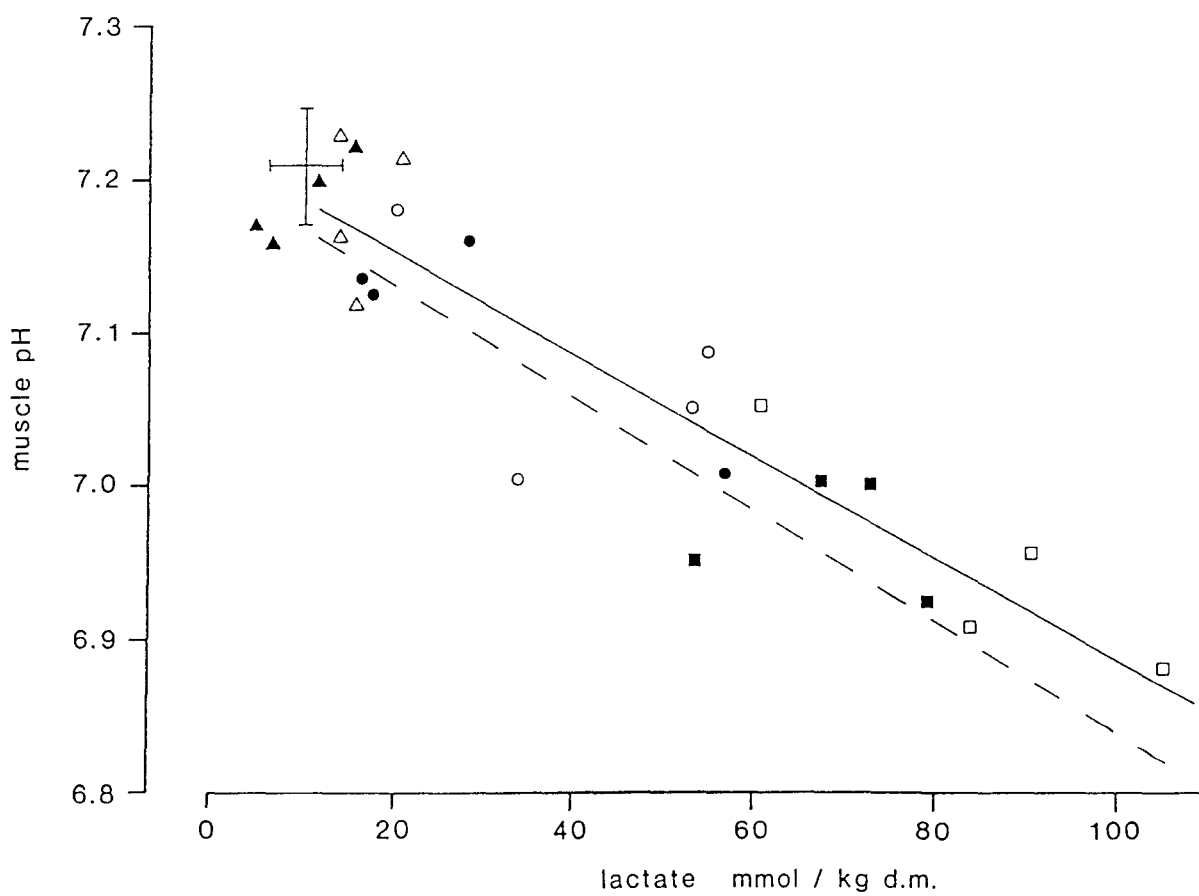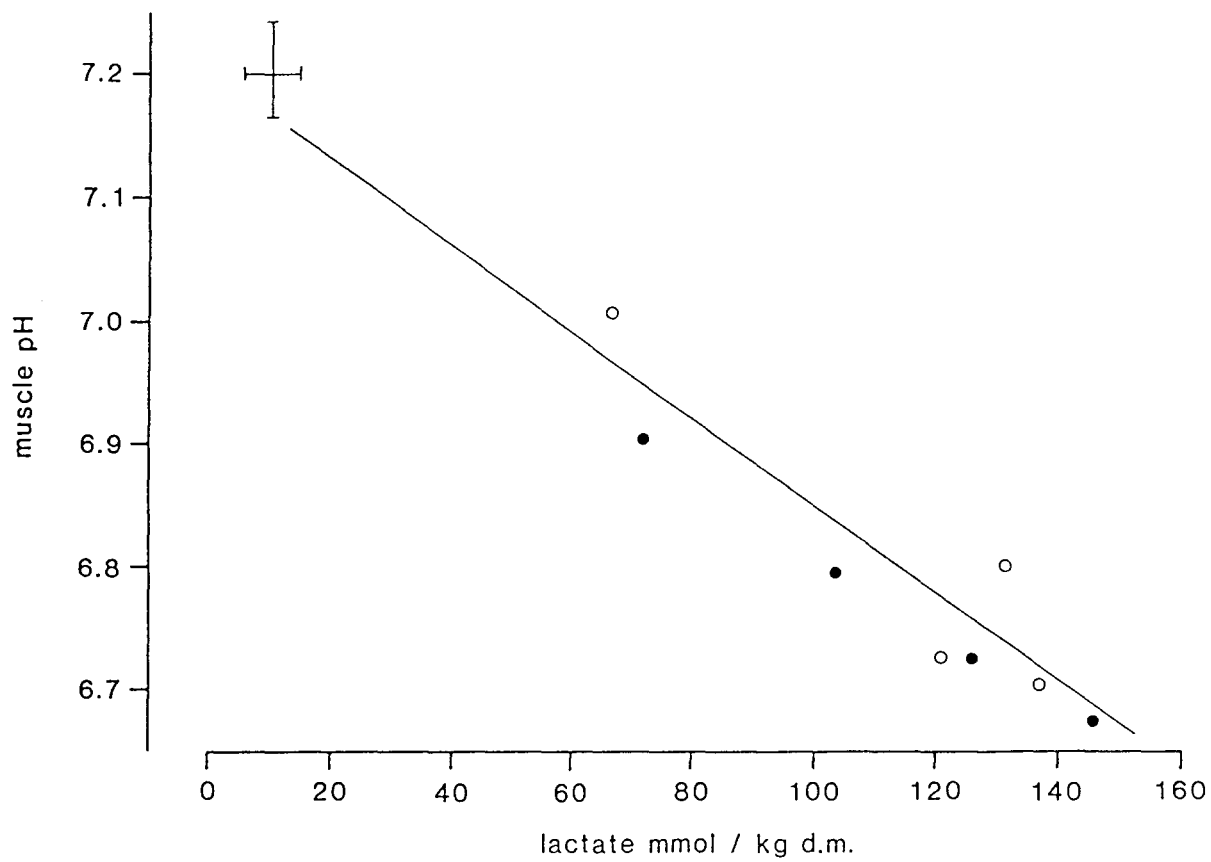
Figure 7.16. Rate constants for muscle lactate disappearance (min) in relation to rate constants for blood lactate disappearance (min) in four horses for S and T recoveries.
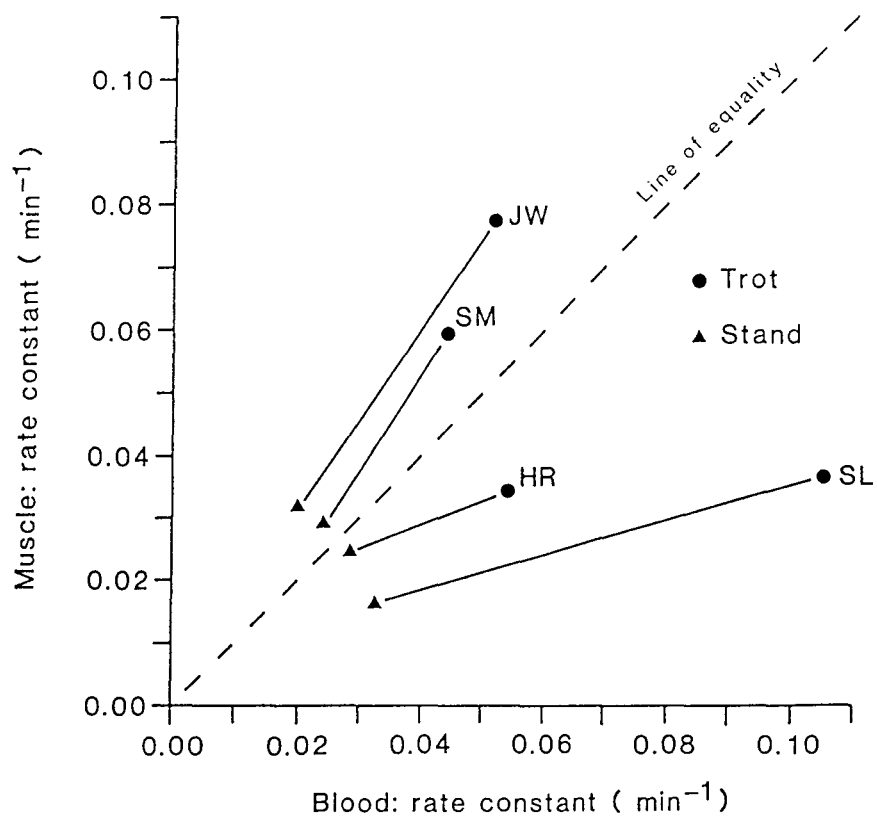
stoichiometry between H+ release and lactate release. Previous studies in man (Sahlin et al, 1976) have indicated that there is a trend towards an increased slope between muscle pH and lactate content during recovery, suggesting an increase in the rate of H+ release relative to that of lactate. Despite the linearity of the relationship between muscle pH and muscle lactate content, the faster disappearance of lactate from muscle with T was not accompanied by any identifiable increase in the rate of muscle pH recovery. This may be a reflection of the uncertainty of the relationships expressed in Figures 7.14 and 7.15.

The ability to rapidly remove or eliminate metabolites during exercise from both muscle and blood could be considered to be a distinct physiological advantage to an athlete, particularly so in the case of lactic acid. As may be seen from Figure 7.16, rate constants for blood lactate disappearance varied between the four horses, as did their response to increased activity. Horse SL consistently showed the highest rate constants and was easily the most capable of the horses used in this study. This is consistent with studies in man showing that the rate of blood lactate disappearance is increased following physical conditioning (Evans and Cureton, 1983). Paradoxically, SL showed the lowest rate of muscle lactate disappearance with S and was still one of the lowest when trotting (Figure 7.16). In general, muscle lactate disappearance rates with S recovery were inversely related to blood disappearance rates, as were also the proportionate changes in either with increased activity. Put simply, where the capacity of the muscle to release lactate was high, then the fall in the concentration in blood was slower. This is exemplified in the case of JW. In the case of SL, a slower rate of lactate release from muscle with both S and T would seem to be the explanation for a faster rate of disappearance from blood. Also the magnitude of the lactate increase with exercise in this horse was much less when compared to the other horses, therefore a relationship may exist between the magnitude of lactate production and rate of removal. Other factors which could account for the variation between individuals in muscle and blood lactate disappearance rates are muscle perfusion (Tesch and Wright, 1983), fibre composition (Bonen et al, 1979) and level of fitness (Evans and Cureton, 1983).

Muscle metabolic profiles for the five horses are shown in Table 7.13. With such a small number of animals and a relatively narrow range of responses, there were no clear indications as to the importance of muscle composition in determining rates of muscle and blood lactate disappearance. The highest correlation coefficients were found for the relationship between HAD activity and half-times for blood (W) and muscle (S) lactate

Table 7.13. Muscle characterization of the five horses used in the present study. Values are presented as mean of left and right side.

Horse	PFK	CPK	LDH	HAD	%I	%IIA	%IIB
SM	29.3	1861	340	9.4	4.3	46.2	49.5
HR	36.7	1700	607	8.3	5.8	39.9	54.3
SL	42.2	1803	511	12.7	19.3	62.7	18.0
JW	38.0	1633	458	9.5	11.9	62.7	25.4
KJ	41.7	1700	635	8.3	6.8	24.2	69.1

PFK, CPK, LDH & HAD activities are expressed as μmol/g d.m./min at 25°C.

disappearance, r=-0.856 in both cases, which was just below the correlation coefficient indicating P<0.05 (r=0.878).

Metabolite levels measured with the biopsy technique do not represent the immediate post-exercise content as it takes approximately 1-2 min from the end of the exercise period to the freezing of the muscle sample. Instead, these metabolite contents represent an equilibration of the factors in the creatine kinase equilibrium. Therefore, the resynthesis seen in PCr is secondary to the restoration of the resting creatine kinase equilibrium, principally the H^+ concentration. As discussed previously, there was no evidence of a faster rate of muscle pH recovery with T compared to S and the absence of any significant difference in PCr recovery would seem to support this observation.

In the absence of any evidence for a direct effect of pH on ATP recovery, ATP resynthesis is most likely limited by the rate of AMP regeneration from IMP. The rates of ATP resynthesis in the present study (42% in 30 min) are much higher than those in the study of intermittent maximal exercise of Snow, Harris and Gash (1985), when less than 5% was restored in 40 min following the fourth gallop and depletion amounting to 40% of the original store. Quite possibly the availability of aspartate or guanosine triphosphate (GTP) for synthesis of s-adenylosuccinate could be the limiting factor in the resynthesis of AMP, and therefore ATP, when there is significant loss of adenine nucleotide.

The large increase in muscle free glucose content is most likely a result of increased uptake from the blood. However, free glucose would be prevented from entering the glycolytic pathway due to inhibition of hexokinase at this time by the high levels of glucose 6-P (Crane and Sols, 1953; Colowick, 1973). There was little change in the free glucose content up to 30 min of recovery, but between 30 and 70 min contents decreased significantly. This coincided with the return of HMP to resting contents. Although it is likely that most of the increase in muscle free glucose is due to uptake from the blood, a proportion may arise within the muscle itself from the breakdown of glycogen, via the debranching enzyme complex. This has been shown to account for approximately 8-10% of the increase in intracellular free glucose during dynamic exercise in man (Field, 1960) and there is little reason to suspect a difference in the horse.

(3) 2 X 900 METRE GALLOPS

In contrast to the study of single maximal gallops of 800 and 2000m where peak blood lactate occurred between 5 and 10 minutes recovery, there

was a trend following two gallops of 900m to peak blood lactates occurring later into the recovery period. Furthermore, none of the horses began to show a significant decline in blood lactate until around 20 minutes recovery. Despite differences in the shape of the lactate curves during the initial phase of recovery, the distance covered of 1800m resulted in a mean peak post-exercise lactate concentration of around 27 mmol/l, which compares with 30.9 mmol/l for the single 2000m gallop. However, a mean half-time calculated over the last 30-40 minutes of recovery of 21.3 minutes (combining week one and week two) is not dissimilar to that observed for the single gallop of 2000m (18-19 minutes). The 'plateauing' of blood lactate following repeated bouts of maximal exercise seen here is similar, although less pronounced, to that observed by Snow, Harris and Gash (1985). This could indicate that the efflux of muscle lactate is limited by the blood concentration. With the repeated exercise model used here, when the horse begins its second bout of exercise it would already have a high blood lactate concentration. Thus, following the second gallop, the muscle to blood gradient would be nearer to unity, resulting in a slower rate of lactate efflux. The prolonged plateau could also indicate a slower rate of blood lactate removal.

(4) 4 X 700 METRE GALLOPS

As stated in the introduction, one of the aims of this study was to investigate the very slow rate of recovery in blood lactate observed previously in a study of intermittent maximal exercise of 4 x 600m gallops (Snow, Harris and Gash, 1985). This may have been due the large amounts of lactate produced leading to a saturation of the blood lactate carrying capacity (i.e. a fixed limit exists as to the amount of lactate that can be contained in the blood), a delayed or reduced rate of muscle lactate efflux or a slow rate of lactate removal from the blood. Alternatively, the slow rate of lactate disappearance could have been due to the horses being stood during the recovery period, as standing results in slower rates of lactate disappearance compared to walking (section 2).

The rates of blood lactate disappearance in the present study were much slower than previously observed in the studies of single and double gallops and were similar to those observed following similar exercise when horses were stood (Snow, Harris and Gash, 1985). Thus, in the present study walking recovery did not appear to result in faster rates of lactate disappearance than found in the study of Snow, Harris and Gash (1985). Therefore, the slow rates of blood lactate disappearance in both studies would seem to be due to the large amounts of lactate produced and a

saturation of the lactate removal processes (see also General Discussion this chapter).

GENERAL DISCUSSION

Lactate disappearance following high-intensity exercise.

The disappearance of lactate from muscle and blood was studied under different exercise protocols. Lactate disappearance from muscle and blood was inversely related to lactate accumulation, that is the higher the lactate production, the slower the removal. Several routes exist for the disappearance of lactate produced during high-intensity exercise. Intramuscular glyconeogensis has been estimated to account for the disappearance of the majority of lactate produced during high-intensity exercise (Hermanssen and Vaage, 1977). Contrary to this view, Brooks and Gaesser (1980) proposed that most of the lactate produced is removed by oxidation and that only a limited amount of glyconeogensis from lactate occurred within skeletal muscle. They also suggested that the majority of glycogen repletion in skeletal muscle occurred following gluconeogenesis in the liver and kidney. Some of the opposition to the view that intramuscular glycogen repletion can occur directly from lactate arises from Krebs and Woodford (1965) who failed to demonstrate the presence of the enzymes necessary for the conversion of pyruvate to phosphoenolpyruvate. However, more recently it has been demonstrated that in man during one hour of recovery following exhaustive high-intensity exercise, 40% of the lactate disappearance could be accounted for by oxidation and a further 43% by intramuscular glyconeogensis (Astrand et al, 1986). These authors also presented evidence for the existence of those enzymes necessary for muscle glyconeogensis, namely, malate dehydrogenase, phosphoenolpyruvate carboxykinase and fructose diphosphatase. They calculated that only around 10% of the lactate production was used for gluconeogenesis and suggested that the fall in liver pH during high-intensity exercise would limit lactate uptake and gluconeogenesis during recovery.

In the present studies it is clear that lactate disappearance following high-intensity exercise is affected by the exercise protocol (single or multiple gallops) the amount of lactate produced and the activity during the recovery period, although the fate of lactate following high-intensity exercise in the horse is still unresolved.

Implications for the training of horses

The study of intermittent maximal exercise in the horse by Snow, Harris and Gash (1985) demonstrated a considerable lactate accumulation in both muscle and blood and a loss in muscle adenine nucleotide content ranging from 38 to 51% of the resting content, with little recovery in the 30 minutes following the fourth gallop. The first study in the present chapter demonstrated mean muscle ATP depletion of 30 and 47% following single gallops of 800 and 2000m, respectively. However, in contrast to the study of Snow, Harris and Gash (1985), a significant degree of recovery in the muscle ATP content had occurred by 20-40 minutes recovery.

Intermittent exercise or interval training is widely employed in the training of human athletes and to a lesser extent in the training of horses. It is apparent that the use of an intermittent exercise model results in more extreme and prolonged metabolic changes within muscle and blood compared to single high-intensity exercise bouts. The exposure of muscles to these biochemical conditions may be as important in the initiation of training effects as the neural input to the muscle fibres (Viru, 1984). As demonstrated in the second study, exposure to the biochemical changes following high-intensity exercise can also be increased by undertaking a passive as opposed to an active recovery.

However, with intermittent exercise protocols, insufficient time allowed for recovery periods and an excessive number of intervals resulting in severe and prolonged acidosis, muscle hypoxia and low ATP contents may decrease the viability of individual muscle fibres (Foster et al, 1986). Complete depletion of ATP in individual fibres has been implicated in the etiology of exertional rhabdomyolysis. In this condition stiffness in locomotor muscles, particularly in the gluteus group, may be due to a small percentage of the fibres being in a hypercontracted state due to complete depletion of the ATP store (see Foster et al, 1986).

In summary, the studies in the present chapter indicate that recovery in muscle ATP and muscle and blood lactate will be affected by the nature of the preceeding exercise and the level of post-exercise activity. The metabolic characteristics of the muscles are also likely to be important in determining both the extent of metabolite changes with exercise and possibly rates of metabolite recovery.

CHAPTER 8

METABOLIC RESPONSE TO INCREASING EXERCISE INTENSITY
IN THE THOROUGHBRED HORSE

INTRODUCTION

The studies carried out in Chapter 6 examined the metabolic response to maximal exercise in the horse under both field and treadmill conditions. A feature of high-intensity exercise in the horse was shown to be a dramatic loss of muscle adenine nucleotide even after single gallops. This had been previously demonstrated to occur in the horse only under more extreme exercise conditions (Snow, Harris and Gash, 1985). Changes on this scale have been observed less frequently in human athletes (Boobis, Williams and Wooton, 1982). The present study was undertaken to examine the metabolic response to increasing work intensity in the horse, with particular emphasis on the muscle ATP decrease.

MATERIALS AND METHODS

Animals: Six trained Thoroughbred horses (five geldings, one filly) were used in this study. The ages, sex and weights of the horses are shown in Table 8.1.

Exercise protocol: Each horse performed seven experimental treadmill sessions, each separated by at least three days. Each experimental session consisted of a warm-up period during which the horses were walked for 15 min at 1.6 m/s, followed by a canter for 2 min at 6 m/s and a further 5 min walk at 1.6 m/s. After the warm-up the horses performed a 2 min test exercise at a speed of 6, 7, 8, 9, 10, 11, or 12 m/s. Only one speed was used for each experimental session and the order of sessions was randomized. The treadmill was set at 5° incline at all times during the warm-up and exercise periods and at 0° during the recovery.

In addition, each horse performed a "pre" and a "post" session in which only the warm-up exercise was completed. These were undertaken 1-2 weeks prior to and following the first and last experimental sessions, respectively. Environmental conditions were maintained between 18-20° centigrade and 60-70% relative humidity.

Sampling

Muscle: Muscle biopsies of the middle gluteal were collected as described in Chapter 2. A single muscle sample for metabolite analysis was collected in each experimental session following the 2 min test exercise period. In the "pre" and "post" sessions, muscle samples were collected at rest prior to exercise (PRE) and at the end of the warm-up exercise period (prior to the test exercise (ST)) for metabolite analysis. In addition, muscle

Table 8.1. Age (years), weight (kg) and sex (g=gelding, f=filly) of the six horses used in the present study.

Horse	Age	Sex	Weight (Kg)		
			Pre	Post	Mean
FR	5	g	422	408	415
HR	6	g	480	494	487
JW	13	g	519	514	517
LB	4	f	416	420	418
MR	4	g	414	416	415
TB	5	g	442	440	441

samples for histochemistry, enzyme, carnosine and βm analysis were collected at rest in the "pre" and "post" sessions.

Blood: Venous blood samples were collected via an indwelling catheter as described in Chapter 2.

Biochemical analysis

Muscle: ATP, ADP, AMP, PCr, Cr, glucose, glucose 1-P, glucose 6-P, fructose 6-P, glycerol 3-P, glycerol, pyruvate and lactate in the rest, pre and post-exercise samples and carnosine content, muscle buffering capacity (βm), OGDH, PFK, CPK and LDH activity, LDH isoenzyme pattern and fibre typing and area measurement in the pre and post rest samples were analysed as described in Chapter 2.

Blood: Lactate, base excess, bicarbonate and PCV in whole blood and lactate, ammonia, xanthine, hypoxanthine, uric acid, glucose, free-fatty acids, glycerol and pH in plasma were analysed as described in Chapter 2.

Heart rate and stride frequency were also determined as described in Chapter 2.

RESULTS

General

Six m/s was the minimum speed chosen for the test-exercise period and for the warm-up, as this was the lowest speed at which all of the horses would canter. Transition from canter to gallop would almost certainly have occurred in all horses by 11 m/s, although it is impossible to define precisely at what speed this would have taken place. Twelve m/s was chosen as the highest test speed as previous studies had indicated that significant muscle ATP loss occurred in most horses after 2 min exercise at this speed (Chapter 6).

Muscle metabolite changes

There was no significant difference in any of the metabolite contents between the rest (PRE) and immediate pre-exercise (ST) samples.

Mean muscle ATP content showed little or no change up to a speed of 9 m/s (Table 8.2). At 10 m/s all horses showed a fall in ATP content compared to the pre-exercise (PRE) content, the mean change being -2.74±1.72 mmol/kg d.m. A further decrease of 2.33±2.32 mmol/kg d.m. occurred between

10 and 11 m/s, followed by a much greater fall of 4.80±2.4 mmol/kg d.m. between 11 and 12 m/s. At 12 m/s the fall in ATP content corresponded to 41.4±6.7% of the ST content. Examination of the individual data (Table 8.3, Figure 8.1) showed the same pattern throughout. Essentially, the resting content was maintained over several speed increments, following which there was a marked decline in the ATP content. This threshold was first reached in JW at 10 m/s, in HR at 11 m/s and in MR, FR and TB at 12 m/s. A decrease also occurred in the sixth horse, LB, at 12 m/s, but the content remaining at the end of the two minute exercise was much higher compared to the other 5 horses.

Loss of muscle ATP was not accompanied by any corresponding increase in either ADP or AMP (Table 8.2). If anything, ADP showed a small decrease at 12 m/s (11 m/s for JW), the mean change being -0.46±0.15 mmol/kg d.m. (P<0.01).

Mean changes in PCr and Cr observed at the time of muscle biopsying are shown in Table 8.2. and individual changes in PCr in Figure 8.2. PCr showed a linear decline in relation to increasing speed. Muscle total creatine content (TCr) was not affected by exercise.

There was a varied response in muscle lactate accumulation between horses with increasing work intensity (Table 8.4). All horses demonstrated a point above which a marked increase in lactate occurred. For example, in the case of MR this occurred between 7 and 8 m/s. In LB the critical point in lactate accumulation was probably reached between 9 and 10 m/s, although a higher value, not consistent with the obvious trend was observed at 8 m/s (see also Discussion). A similar pattern of response to that in LB was observed in horse FR, with the critical point occurring between 10 and 11 m/s. Between 10 and 12 m/s lactate accumulation was exponential and all horses showed contents in excess of 100 mmol/kg d.m. at 12 m/s (11 m/s JW). The change in muscle ATP content was significantly correlated to muscle lactate accumulation (Figure 8.3).

As noted previously (see Chapter 6) exercise resulted in only small increases in muscle pyruvate content.

Muscle free glucose, glucose 1-P, glucose 6-P and fructose 6-P all showed an approximately linear increase with each speed increment up to 11 m/s. Examination of the mean contents indicated some evidence of a plateau being reached at 11 m/s with a much smaller increment between 11 and 12 m/s for all four metabolites. This was more evident when the individual data

Table 8.2. Muscle metabolite contents at rest (PRE), immediately before (ST) and after 2 minutes exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline. All values are mmol/kg d.m. and are presented as mean±sd. n=6 for each speed, except 12m/s (n=5).

					Speed, m/s				
	PRE	ST	6	7	8	9	10	11	12
ATP	22.8 ±1.3	23.1 ±1.6	22.4 ±1.8	22.2 ±1.7	21.8 ±1.7	21.6 ±2.0	20.4 ±2.1	18.0 ±2.9	13.8 ±1.9
ADP	2.5 ±0.3	2.7 ±0.1	2.7 ±0.4	2.6 ±0.3	2.6 ±0.3	2.9 ±0.3	2.6 ±0.3	2.8 ±0.4	2.3 ±0.1
AMP	0.09 ±0.05	0.15 ±0.17	0.11 ±0.03	0.09 ±0.02	0.14 ±0.11	0.13 ±0.06	0.08 ±0.06	0.09 ±0.03	0.10 ±0.07
PCr	64.7 ±4.5	64.6 ±3.8	58.1 ±6.1	58.5 ±7.6	45.0 ±5.2	36.1 ±5.2	35.0 ±11.2	26.5 ±5.8	24.9 ±9.6
Cr	51.6 ±3.9	52.9 ±5.2	58.9 ±6.8	60.1 ±7.7	71.5 ±8.8	81.2 ±4.0	81.3 ±10.1	89.9 ±3.9	93.3 ±13.1
Glucose	2.8 ±0.6	3.0 +1.2	5.1 ±2.5	5.5 ±1.6	7.2 ±2.7	9.3 ±2.0	8.6 ±2.0	11.2 ±2.1	13.4 ±0.7
G 1-P	0.23 ±0.06	0.19 ±0.08	0.32 ±0.09	0.28 ±0.10	0.46 ±0.10	0.60 ±0.23	0.83 ±0.32	0.93 ±0.28	1.09 ±0.33
G 6-P	3.8 ±1.3	3.9 ±0.9	6.4 ±2.4	6.9 ±2.2	10.4 ±2.7	12.3 ±3.8	17.3 ±5.4	20.1 ±3.2	19.4 ±5.3
F 6-P	0.7 ±0.3	0.7 ±0.2	1.1 ±0.5	1.2 ±0.5	1.6 ±0.8	2.3 ±0.8	3.6 ±1.4	4.4 ±0.8	4.5 ±1.3
Glyc 3-P	2.9 ±0.4	2.7 ±0.7	3.9 ±1.6	4.2 ±1.9	7.5 ±3.4	10.3 ±2.6	11.8 ±4.0	14.6 ±4.5	16.9 ±3.2
Glycerol	0.2 ±0.1	0.7 ±0.6	0.8 ±0.4	0.8 ±0.8	1.3 ±0.7	1.5 ±0.4	1.7 ±0.5	2.4 ±0.6	2.5 ±0.5
Pyruvate	0.4 ±0.1	0.4 ±0.1	0.5 ±0.2	0.3 ±0.2	0.5 ±0.2	0.6 ±0.3	0.8 ±0.4	0.7 ±0.4	1.0 ±0.5
Lactate	9.7 ±1.3	11.2 ±2.0	19.1 ±7.5	23.9 ±11.1	43.7 ±13.9	57.3 ±13.1	76.5 ±31.0	97.0 ±28.7	132.9 ±19.5

Values linked by lines are not significantly different (P>0.05)

Abbreviations: G 1-P   Glucose 1-phosphate

G 6-P   Glucose 6-phosphate

F 6-P   Fructose 6-phosphate

Glyc 3-P   Glycerol 3-phosphate

Table 8.3. Individual changes in muscle ATP content following 2 min exercise at 6, 7, 8, 9, 10, 11 or 12 m/s and 5° incline. (Comparison is with the PRE (resting) sample).

Speed, m/s

	6	7	8	9	10	11	12
FR	-1.80	-1.60	+0.50	-3.95	-0.46	-1.90	-8.90
HR	-0.60	+1.31	-1.36	+1.41	-2.08	-9.03	-10.57
JW	0.00	-0.30	+0.50	-0.10	-4.70	-5.90	-
LB	-0.60	+0.40	-2.10	-2.10	-2.00	-4.00	-7.40
MR	-1.22	-2.66	-5.10	-1.40	-4.90	-5.47	-10.36
TB	+0.10	-2.30	0.00	-2.60	-2.33	-4.14	-11.30

Table 8.4. Individual muscle lactate contents (mmol/kg d.m.) at rest (PRE), immediately before (ST) and following 2 min exercise at 6, 7, 8, 9, 10, 11 or 12 m/s. Values are presented as mean ± sd.

Speed, m/s

	PRE	ST	6	7	8	9	10	11	12
FR	8.57	8.60	14.48	17.16	25.14	64.84	38.99	62.44	120.95
HR	9.19	12.35	30.70	44.19	51.04	57.52	130.99	142.87	148.96
JW	9.96	11.80	17.68	33.07	50.77	78.40	102.18	123.05	-
LB	8.16	14.29	10.05	16.94	57.95	35.24	52.79	69.04	102.86
MR	9.78	8.71	14.17	12.77	53.62	50.41	69.23	84.34	134.18
TB	12.27	11.40	27.53	19.17	23.65	57.55	65.06	100.36	157.46

Figure 8.1. Individual muscle ATP content (mmol/kg d.m.) in each of six horses at rest (PRE), immediately before (ST) and following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline.

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Figure 8.2. Individual muscle Phosphocreatine (PCr) content (mmol/kg d.m.) in each of six horses at rest (PRE), immediately before (ST) and following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline.

ATP
mmol/kg d.m.

25 ·

20 ·

15 ·

10 ·

PRE   ST   6 m/s 7 m/s 8 m/s 9 m/s 10 m/s 11 m/s 12 m/s

—•—FR   —+—HR   —*—JW   —□—LB   —×—MR   —◇—TB

PCr
mmol/kg d.m.

80 ·

60 ·

40 ·

20 ·

0 ·

PRE   ST   6 m/s 7 m/s 8 m/s 9 m/s 10 m/s 11 m/s 12 m/s

—•—FR   —+—HR   —*—JW   —□—LB   —×—MR   —◇—TB
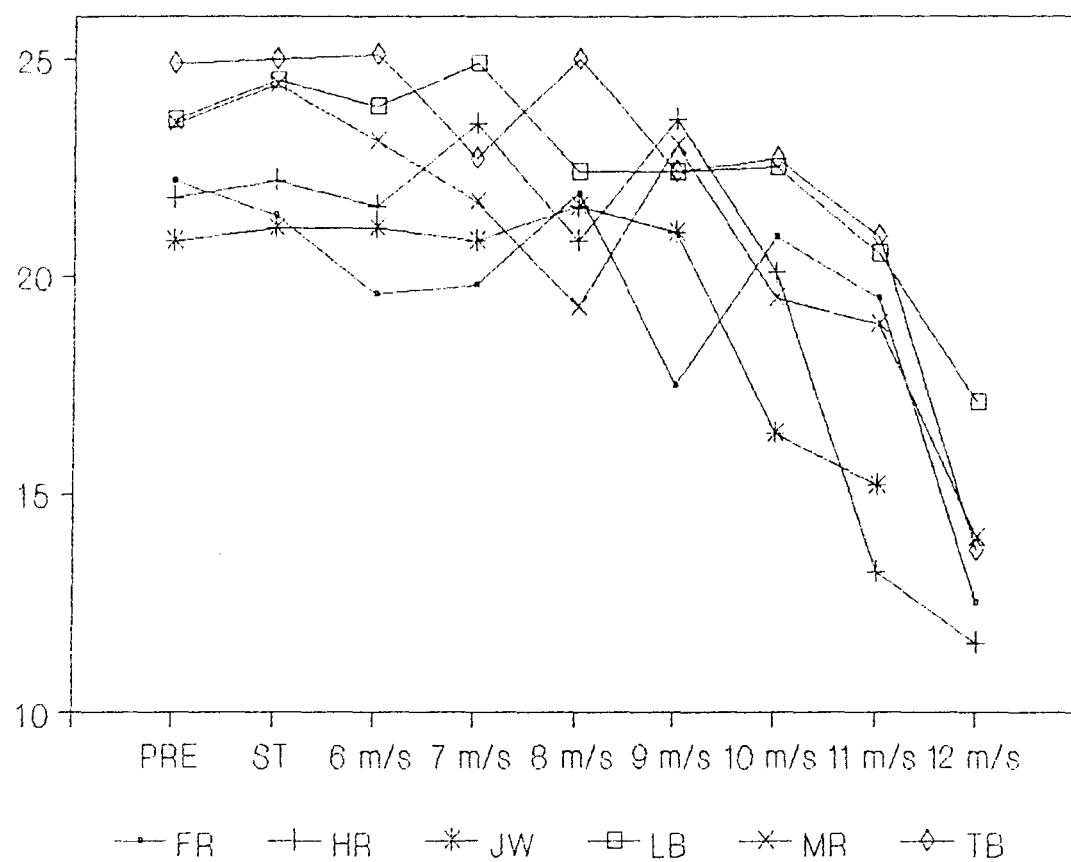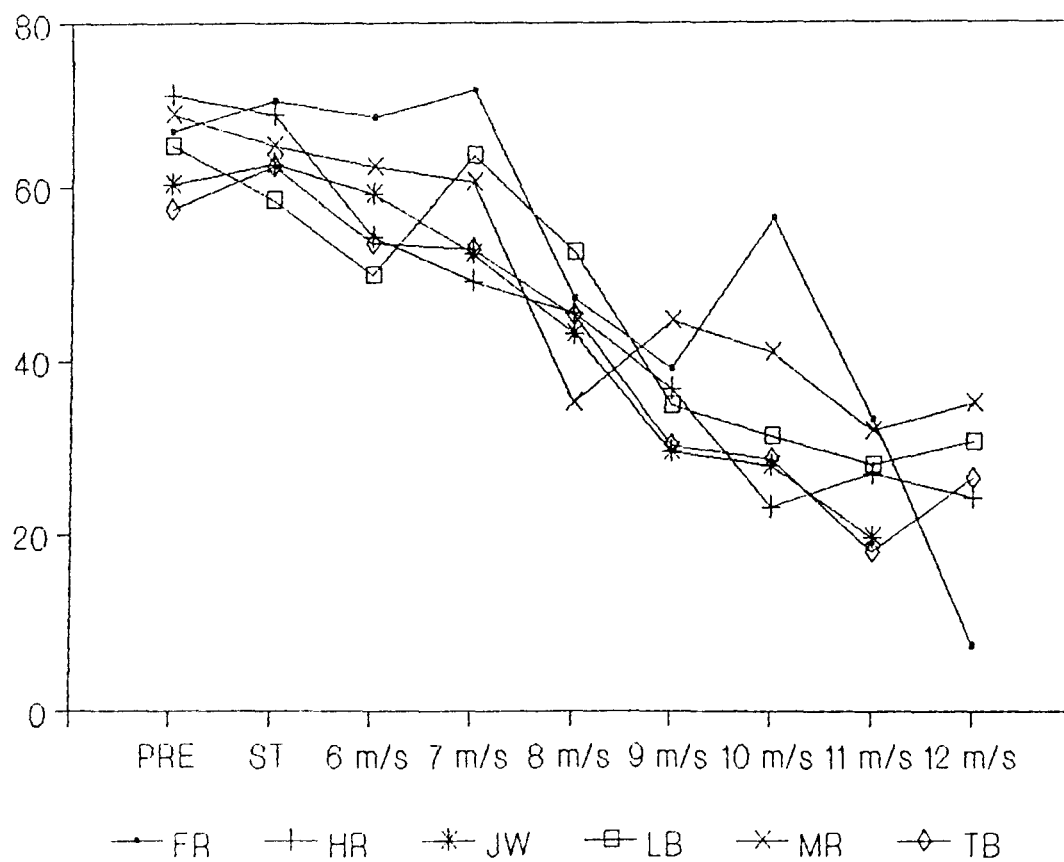
Figure 8.3. Change in muscle ATP content (mmol/kg d.m.) in relation to muscle lactate content (mmol/kg d.m.). The regression line is calculated on ATP and lactate contents obtained at 9, 10, 11 and 12 m/s only (see text). Delta ATP = 3.04 - 0.0881.Lactate, r = -0.899, P<0.001. * = 6, 7 & 8 m/s. + = 9, 10, 11 & 12 m/s.

Delta ATP
mmol/kg d.m.

Lactate, mmol/kg d.m.

196
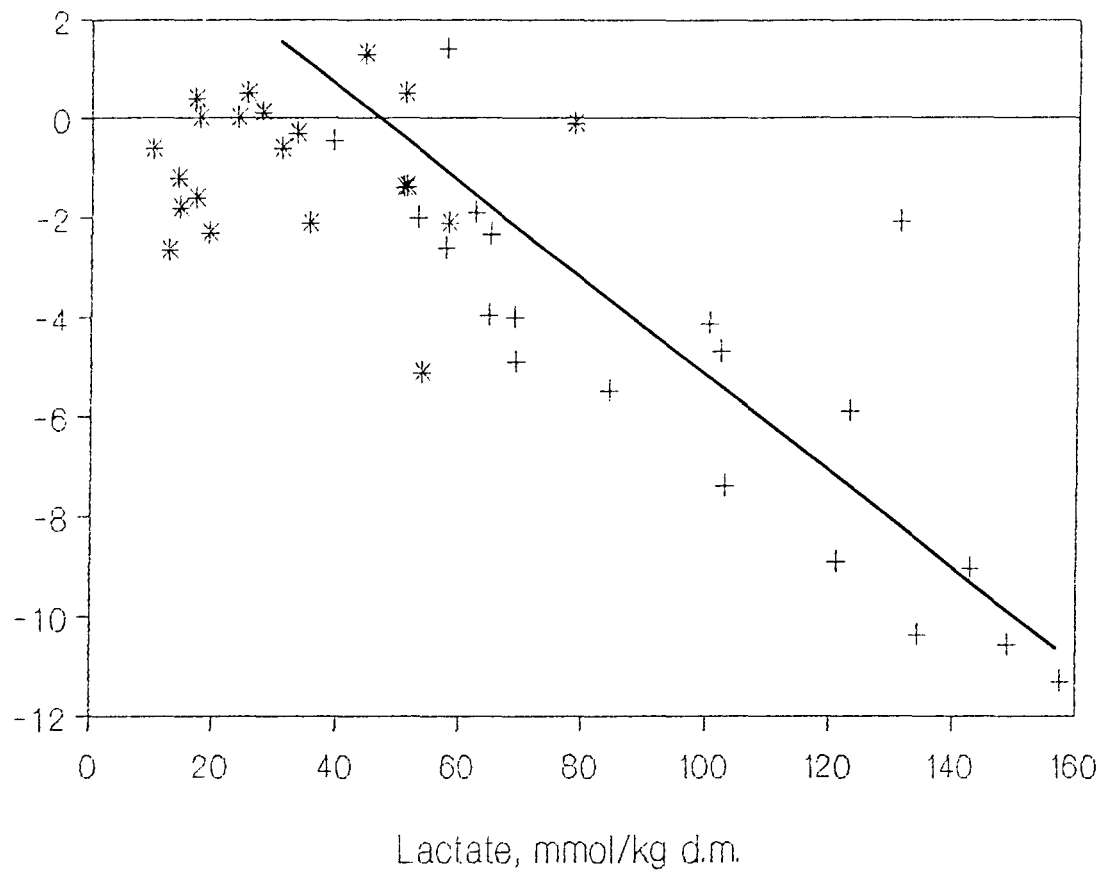
were examined. Of the hexosemonophosphates, the greatest accumulation seen was for glucose 6-P. Following exercise at 12 m/s, the highest content observed was 27.6 mmol/kg d.m. in horse HR. Calculated over all seven speeds, the mean ratio of glucose 6-P:fructose 6-P:glucose 1-P accumulation was 21:4:1.

Glycerol 3-P showed an approximately linear increase with speed. In relation to the lactate accumulation, the lactate:glycerol 3-P ratio showed a trend towards an increase with increasing speed, that is the relationship was not linear. Maximal glycerol accumulation averaged 1.8 mmol/kg d.m. following the 12 m/s test. The highest individual value noted was 3.4 mmol/kg d.m. in horse FR at 12 m/s.

Blood metabolite changes

At the lower speeds the blood lactate concentration peaked immediately after exercise, however at speeds resulting in an immediate post-exercise concentration of 10-12 mmol/l or greater, the peak occurred around 5 to 10 minutes into recovery (Figure 8.4). In most cases this occurred above 10 m/s. The times of occurrence of peak lactate values are shown in Table 8.5. Based on either the peak or 5 min recovery concentration, blood lactate accumulation showed an approximately exponential increase with speed (Figure 8.5). A ln-ln plot of mean peak concentration versus speed, as suggested by Beaver, Wasserman and Whipp (1985), indicated a marked change in slope at approximately 7.2 m/s (Figure 8.6).

Half-times for blood lactate disappearance (calculated from the linear portion of a plot of ln concentration versus time) following exercise at each speed are shown in Table 8.6. Exponential rate constants (change in ln concentration over time) were significantly correlated to peak blood lactate concentration (Table 8.7). However, in spite of significance being obtained following linear regression, on visual examination the relationship between rate constant and peak blood lactate concentration was clearly exponential (Figure 8.7).

At speeds of up to 10m/s, peak ammonia concentrations occurred between 0 and 5 minutes recovery (Figure 8.8). Above this speed there was a trend towards greater delay to peak concentration, the mode reaching 10 min recovery at 12m/s.(Table 8.8). Based on either the peak or 5 min recovery concentration, plasma ammonia also showed a curvilinear response to increasing speed (Figure 8.9), a ln-ln plot indicating a change in slope at approximately 8.6 m/s (Figure 8.10), or about 1.4 m/s higher than for lactate.

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Table 8.5. Time at which peak whole blood lactate concentrations were observed in six horses (except 12 m/s, n=5) following exercise for 2 minutes at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline.

	Speed, m/s						
Recovery (min)	6	7	8	9	10	11	12
0	6	6	5	5	2	0	0
5	0	0	1	1	3	5	3
10	0	0	0	0	1	1	2

Table 8.6. Half-times (min) for blood lactate dissapearance following 2 minutes exercise at 6, 7, 8, 9, 10, 11 or 12 m/s.

	Speed, m/s						
	6	7	8	9	10	11	12
FR	4.2	5.2	6.7	5.8	17.1	11.0	24.2
HR	5.9	7.0	8.7	9.8	11.4	21.9	25.1
JW	3.2	8.7	10.7	14.8	23.1	26.9	-
LB	2.6	4.1	10.3	10.9	12.2	14.2	20.0
MR	4.1	4.4	10.0	16.8	17.9	21.9	37.1
TB	5.6	5.4	7.9	9.9	11.1	17.3	33.0

Table 8.7. Correlation between exponential rate constant (min) and peak post-exercise blood lactate concentration (mmol/l) following exercise at 6, 7, 8, 9, 10, 11 or 12 m/s.

	r	slope	
FR	-0.854	-0.00717	P<0.02
HR	-0.971	-0.00369	P<0.001
JW	-0.776	-0.00730	P>0.05
LB	-0.781	-0.00907	P<0.05
MR	-0.886	-0.00764	P<0.01
TB	-0.947	-0.00494	P<0.001

Table 8.8. Time at which peak plasma ammonia concentrations were observed in six horses (except 12 m/s, n=5) following exercise for 2 minutes at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline.

				Speed, m/s			
Recovery (min)	6	7	8	9	10	11	12
0	4	3	0	1	0	0	1
5	1	3	6	5	4	2	0
10	1	0	0	0	0	2	2
15	0	0	0	0	1	1	1
35	0	0	0	0	0	1	0
40	0	0	0	0	0	0	1

Table 8.9. Peak plasma hypoxanthine, xanthine and uric acid concentrations (umol/l) in six horses (except 12m/s, n=5) following 2 min exercise at 9, 10, 11 or 12m/s and 5° incline. Values are presented as mean ± sd.

		speed, m/s		
	9	10	11	12
Hypoxanthine	0.18	0.60	3.05	10.30
	±0.14	±0.25	±3.28	±8.15
Xanthine	0.15	36.17	49.40	68.50
	±0.08	±14.09	±26.75	±52.10
Uric acid	31	47	103	145
	±23	±24	±68	±48

Figure 8.4. Mean time courses for whole blood lactate (mmol/l) for six horses at rest (PRE) immediately before (ST) and during 40 minutes walking recovery (1.6 m/s and 0° incline) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. For the sake of clarity, mean changes only are shown. A complete listing of values with standard deviations are given in Table 8.10 overleaf.

Figure 8.5. Mean five minute recovery and peak whole blood lactate concentration (mmol/l) in relation to speed following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline.

Blood lactate
mmol/l

PRE   ST   0'R   5'R   10'R   15'R   20'R   25'R   30'R   35'R   40'R

—■— 6 M/S   —+— 7 M/S   —*— 8 M/S   —□— 9 M/S
—×— 10 M/S   —◇— 11 M/S   —△— 12 M/S

Blood lactate
mmol/l

PRE   ST   6 M/S   7 M/S   8 M/S   9 M/S   10 M/S   11 M/S   12 M/S
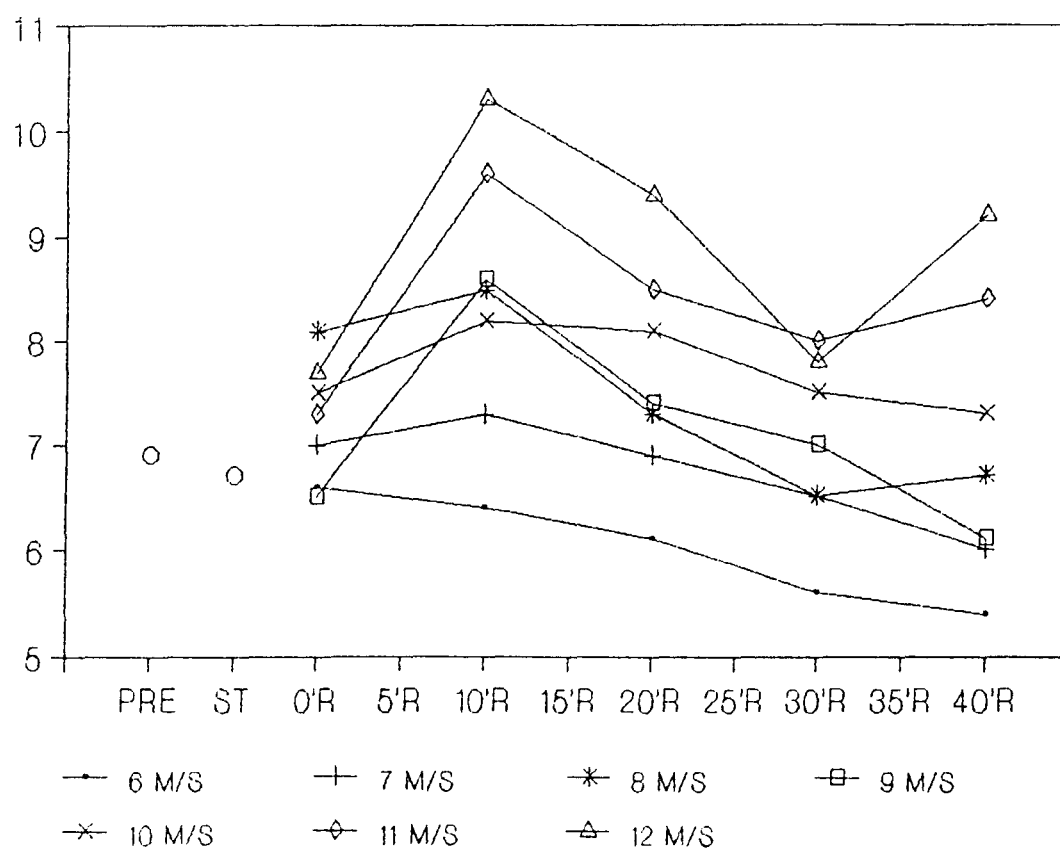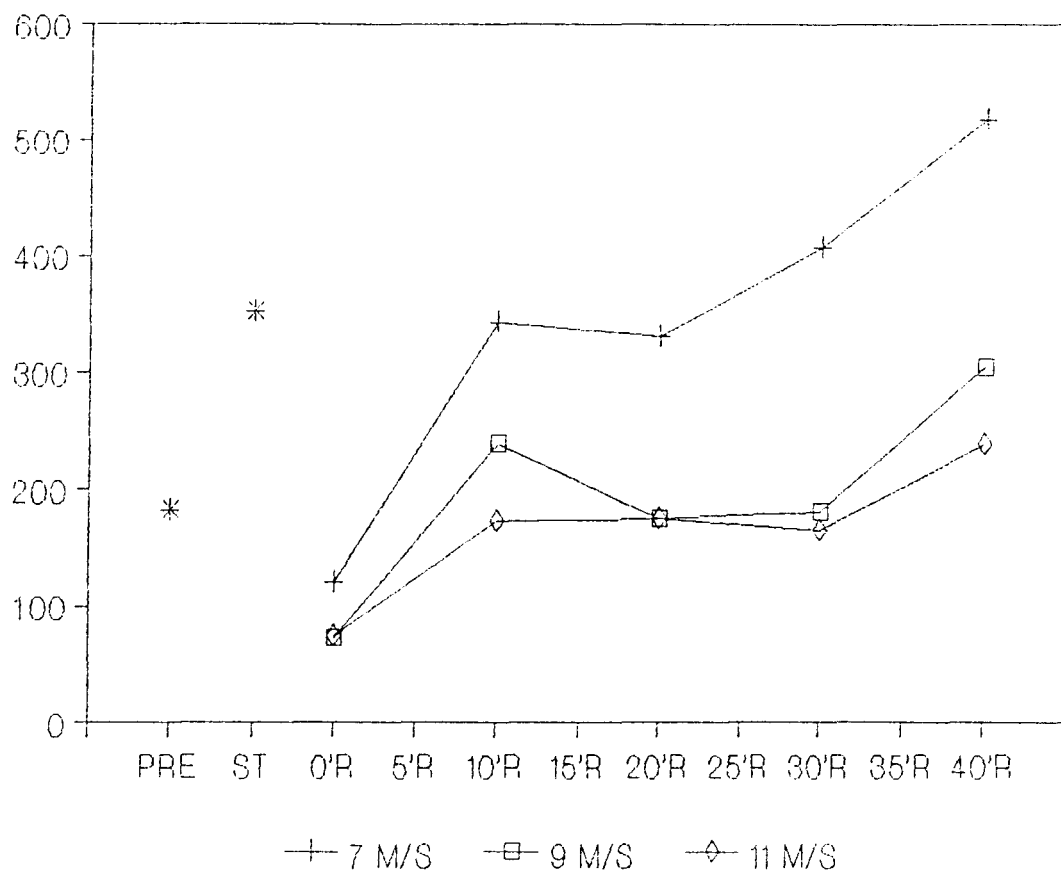
—□— 5'R   —+— PEAK

200
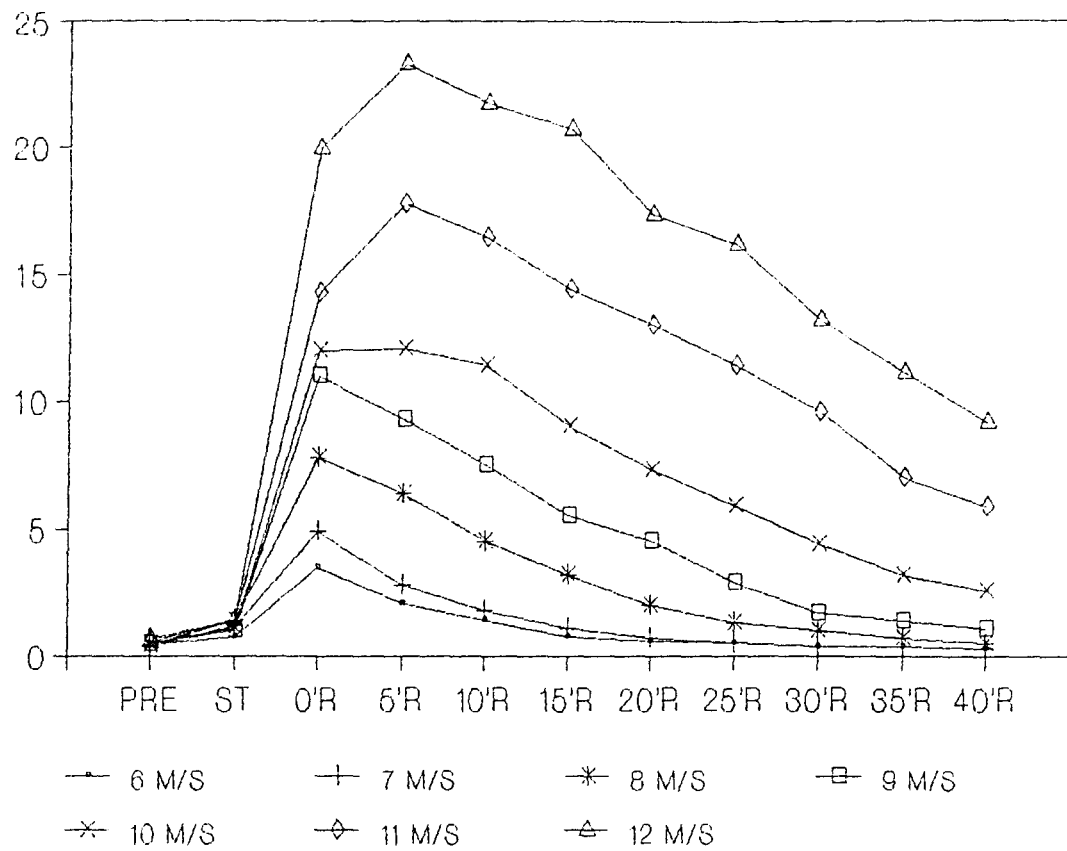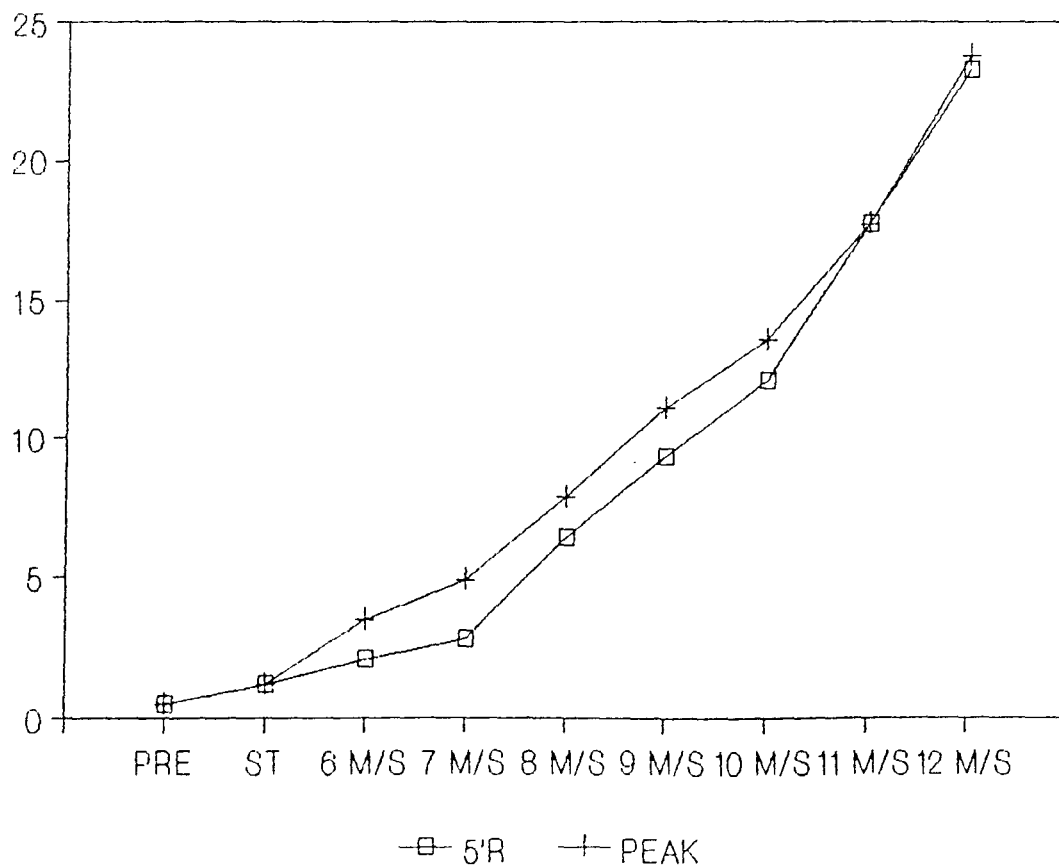
Table 8.10. Mean blood lactate concentration (mmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline.

Min of Recovery

M/s	PRE	ST	0	5	10	15	20	25	30	35	40
6	0.4 ±0.1	0.8 ±0.4	3.5 ±1.2	2.1 ±1.5	1.3 ±1.1	0.8 ±0.5	0.6 ±0.4	0.5 ±0.2	0.4 ±0.1	0.4 ±0.2	0.3 ±0.1
7	0.4 ±0.1	1.2 ±1.0	4.9 ±1.9	2.8 ±1.8	1.8 ±1.3	1.1 ±0.7	0.7 ±0.5	0.5 ±0.3	0.4 ±0.2	0.4 ±0.2	0.3 ±0.1
8	0.4 ±0.2	1.4 ±0.9	7.8 ±2.6	6.4 ±2.8	4.5 ±2.6	3.2 ±2.0	2.0 ±1.5	1.3 ±1.1	1.0 ±0.7	0.7 ±0.4	0.5 ±0.3
9	0.5 ±0.1	1.0 ±0.5	11.0 ±1.9	9.3 ±1.9	7.5 ±2.8	5.5 ±2.5	4.5 ±2.5	2.9 ±1.8	1.7 ±0.9	1.4 ±0.9	1.1 ±0.8
10	0.5 ±0.2	1.1 ±0.6	12.0 ±2.1	12.1 ±3.5	11.3 ±5.1	9.0 ±5.2	7.3 ±4.7	5.9 ±4.2	4.4 ±3.9	3.2 ±3.1	2.6 ±2.5
11	0.7 ±0.4	1.4 ±1.1	14.3 ±4.3	17.8 ±5.7	16.5 ±5.3	14.4 ±5.7	13.0 ±6.3	11.4 ±5.5	9.6 ±4.9	7.0 ±4.8	5.9 ±3.9
12	0.6 ±0.2	1.5 ±0.4	20.0 ±4.4	23.3 ±2.8	21.8 ±4.3	20.7 ±4.2	17.4 ±3.5	16.2 ±2.1	13.2 ±4.1	11.1 ±3.4	9.2 ±2.6

Figure 8.6. Mean ln peak whole blood lactate in relation to ln speed. Intercept corresponds to a speed of 7.2 m/s. Ln speed scale is based on the change in speed such that 6 m/s = 1, 7 m/s = 2 ...... 12 m/s = 7.

Figure 8.7. Rate constants for blood lactate dissappearance (min) in relation to peak blood lactate concentration (mmol/l) in each of six horses following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s. ● = FR; + = HR; ✳ = JW; □ = LB; X = MR; ◇ = TB.
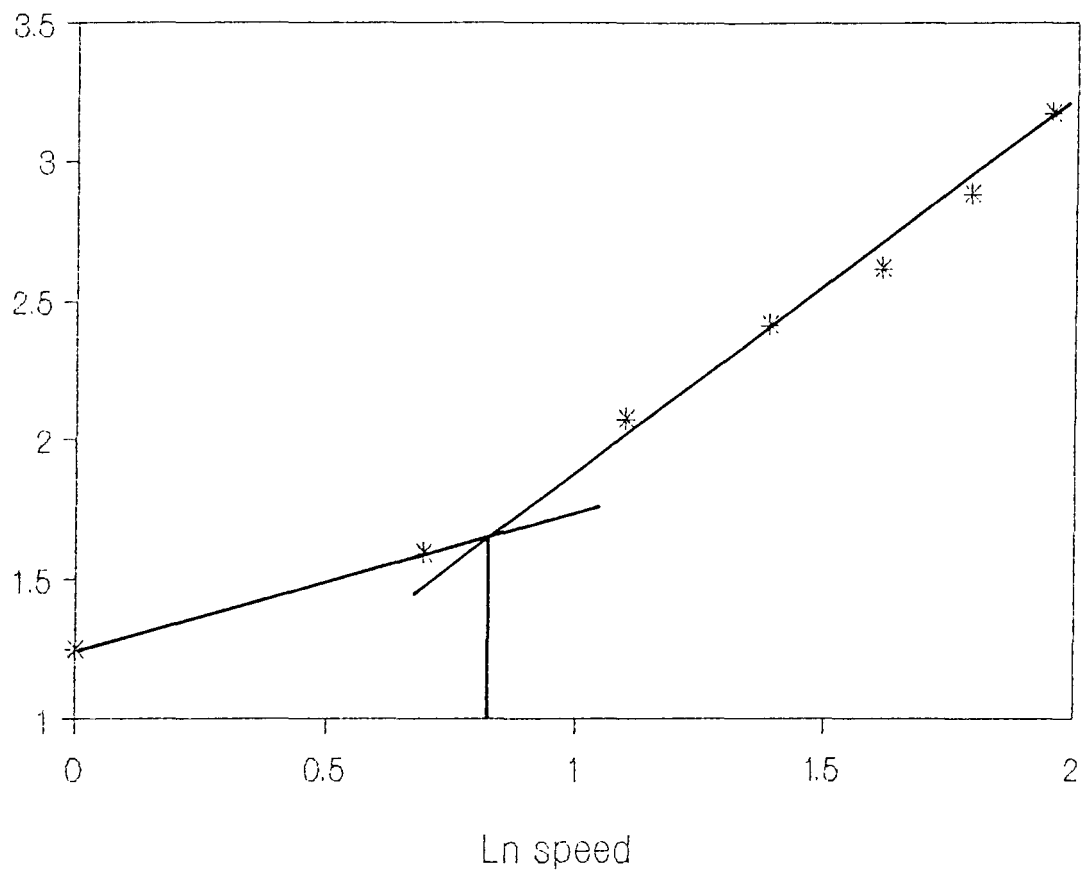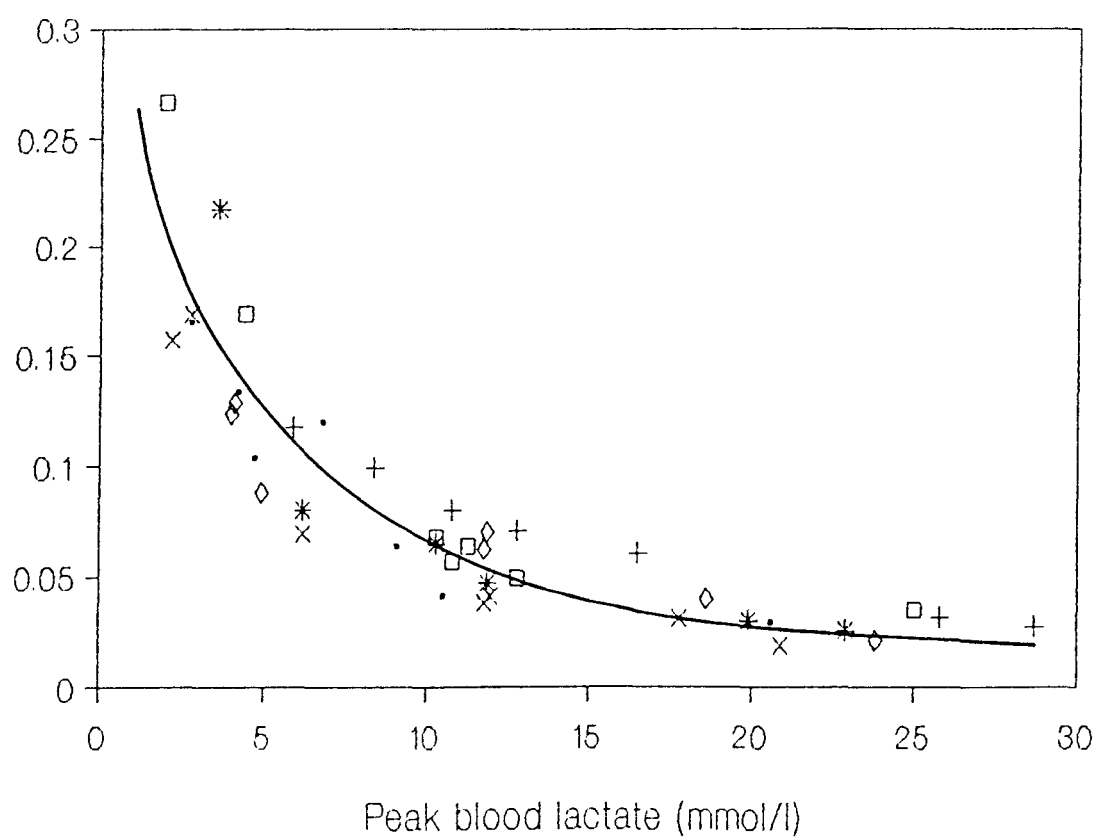
Figure 8.8. Mean time courses for plasma ammonia (μmol/l) for six horses at rest (PRE), immediately before (ST) and during 40 minutes walking recovery (1.6 m/s and 0° incline) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. For the sake of clarity, mean changes only are shown. A complete listing of values with standard deviations are given in Table 8.11 overleaf.

Figure 8.9. Mean five minute recovery and peak plasma ammonia concentration (μmol/l) in relation to speed following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline.

Plasma ammonia
umol/l

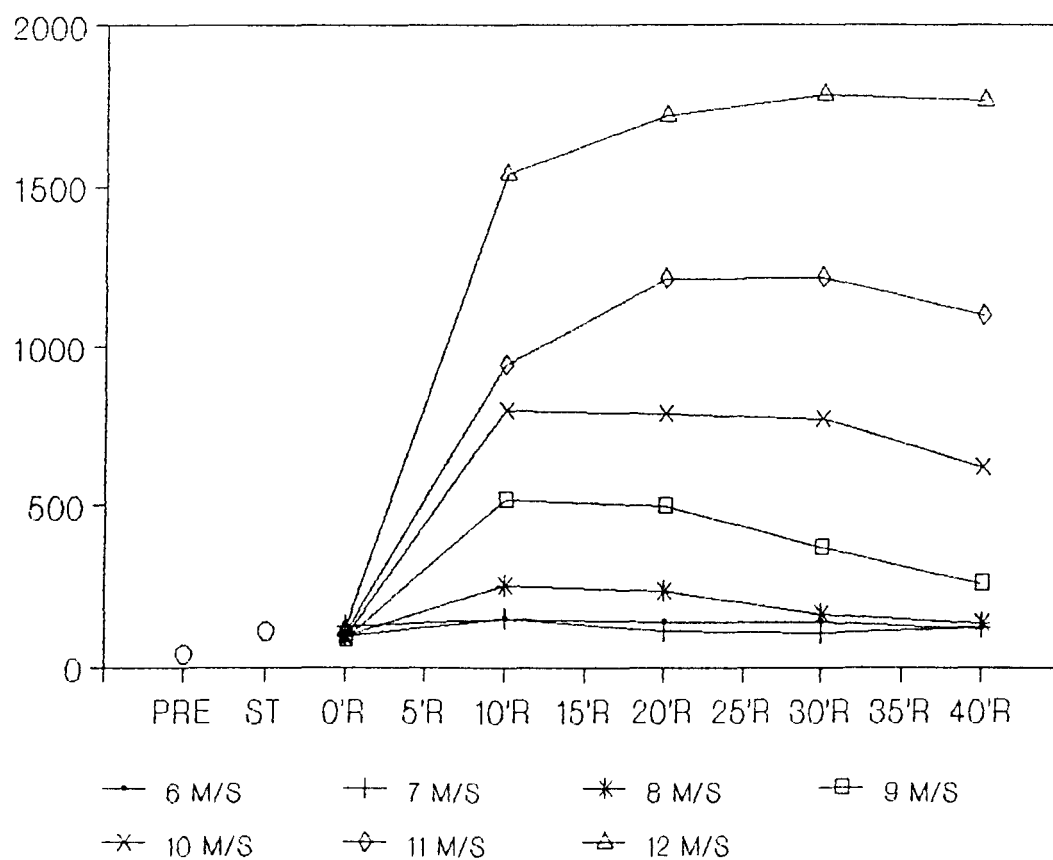|			
6 M/S	7 M/S	8 M/S	9 M/S
10 M/S	11 M/S	12 M/S	

Plasma ammonia
umol/l

5'R   PEAK
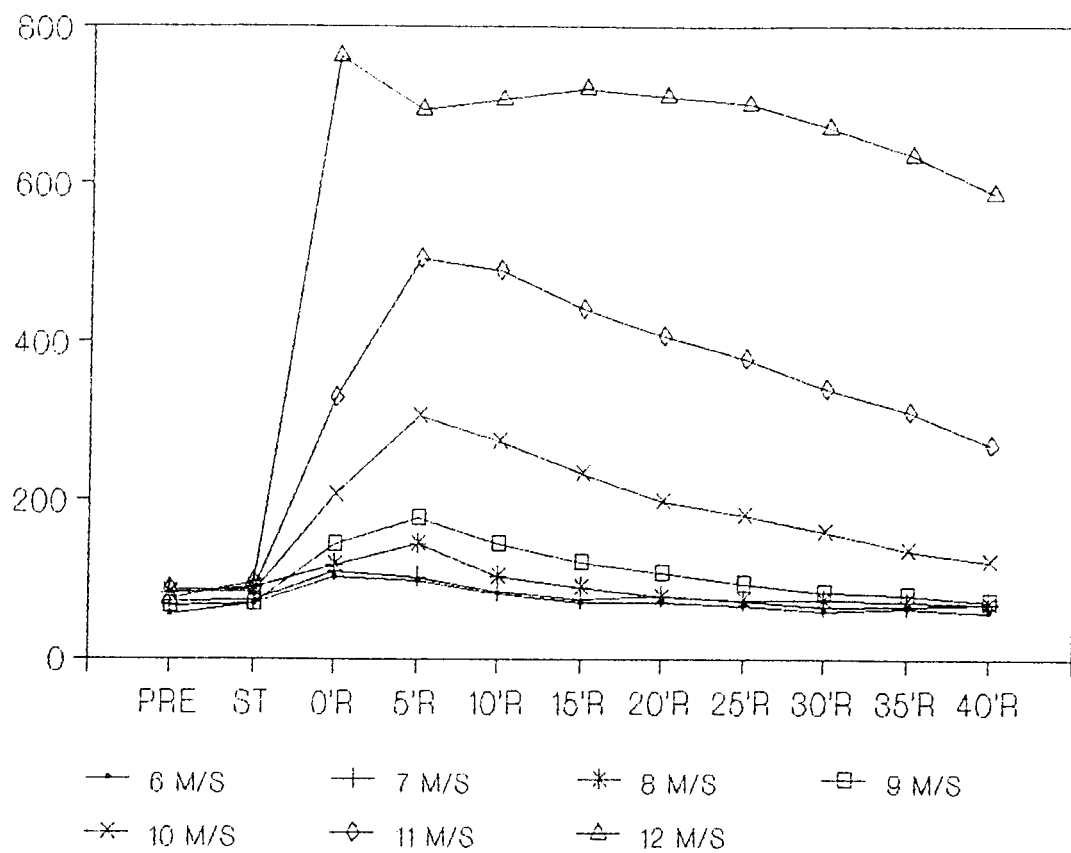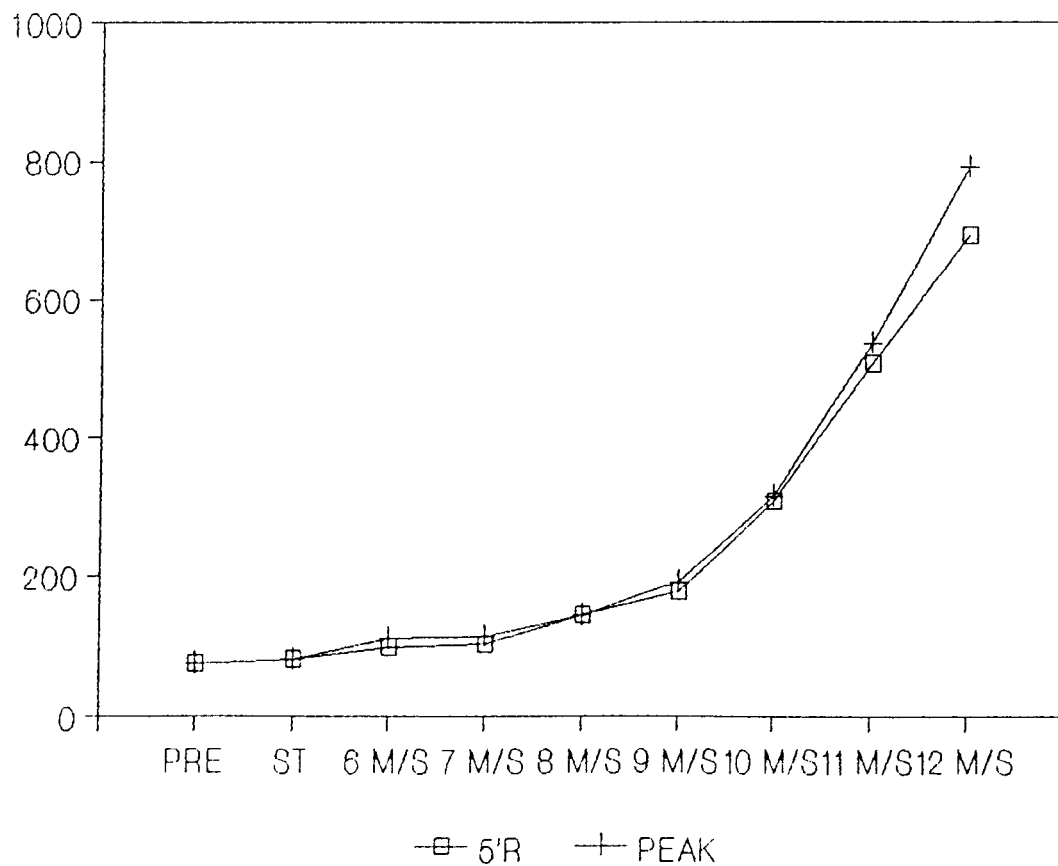
Table 8.11. Mean plasma ammonia concentration (μmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. (12 m/s n=5).

Min of recovery

M/S	PRE	ST	0	5	10	15	20	25	30	35	40
6	56 ±24	70 ±22	103 ±32	98 ±40	82 ±19	71 ±18	71 ±24	68 ±19	60 ±19	64 ±19	58 ±14
7	71 ±15	74 ±11	110 ±14	102 ±16	84 ±11	75 ±9	79 ±24	72 ±20	65 ±16	66 ±19	70 ±13
8	82 ±15	90 ±27	118 ±30	145 ±32	103 ±31	90 ±21	77 ±25	73 ±19	74 ±22	72 ±20	70 ±17
9	67 ±21	69 ±22	144 ±29	178 ±66	145 ±65	122 ±57	108 ±36	94 ±42	83 ±30	80 ±23	72 ±17
10	85 ±15	85 ±11	207 ±47	307 ±76	274 ±96	234 ±104	198 ±100	180 ±91	159 ±89	136 ±70	123 ±57
11	87 ±28	87 ±43	331 ±105	506 ±178	490 ±212	442 ±205	407 ±207	379 ±196	342 ±176	311 ±169	270 ±138
12	75 ±30	97 ±52	762 ±341	693 ±307	705 ±337	720 ±322	711 ±333	700 ±367	671 ±366	635 ±348	587 ±283

Figure 8.10. Mean ln peak plasma ammonia in relation to ln speed. Intercept corresponds to a speed of 8.6 m/s. Ln speed scale is based on the change in speed such that 6 m/s = 1, 7 m/s = 2 .... 12 m/s = 7.

Figure 8.11. Mean time courses for plasma uric acid (μmol/l) for six horses immediately before (ST) and during 40 minutes walking recovery (1.6 m/s and 0° incline) following 2 min treadmill exercise at 9, 10, 11 or 12 m/s on 5° incline. For the sake of clarity, mean changes only are shown. A complete listing of values with standard deviations are given in Table 8.12 overleaf.

Ln peak ammonia vs Ln speed

Plasma uric acid umol/l

Legend: 9 M/S, 10 M/S, 11 M/S, 12 M/S

X-axis: PRE, ST, 0'R, 5'R, 10'R, 15'R, 20'R, 25'R, 30'R, 35'R, 40'R
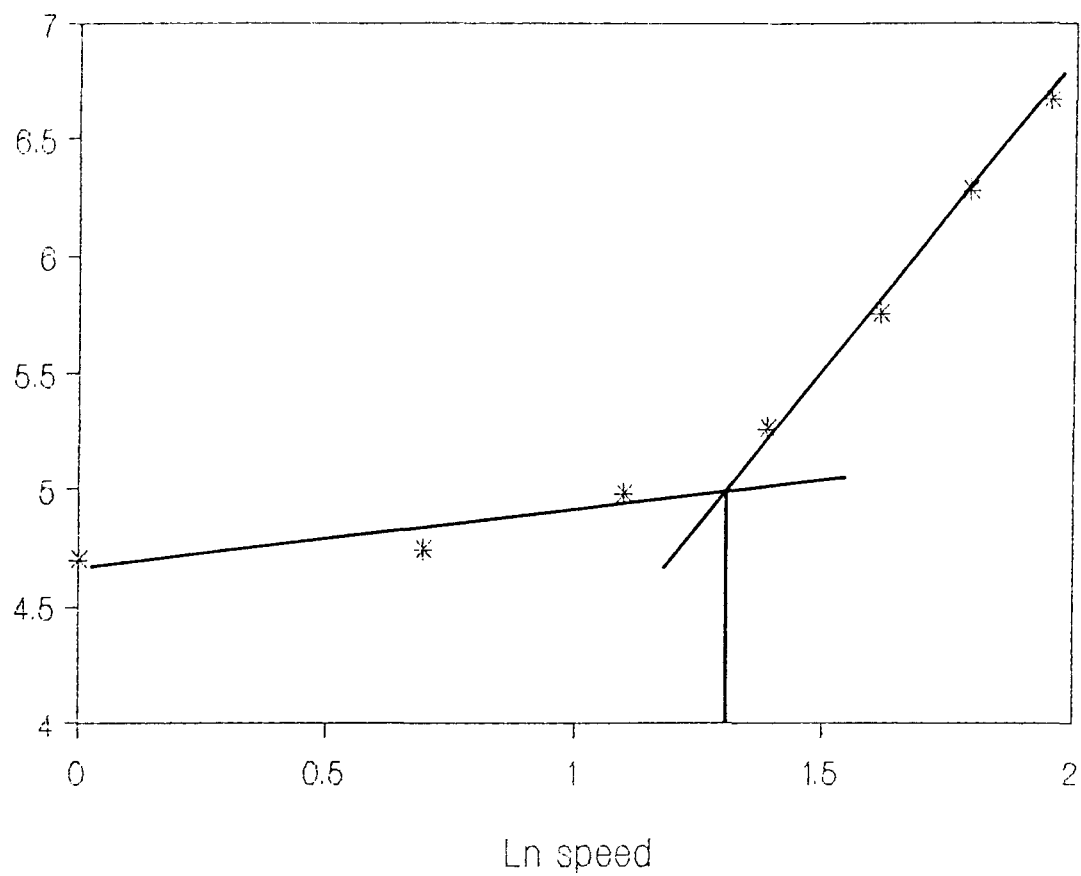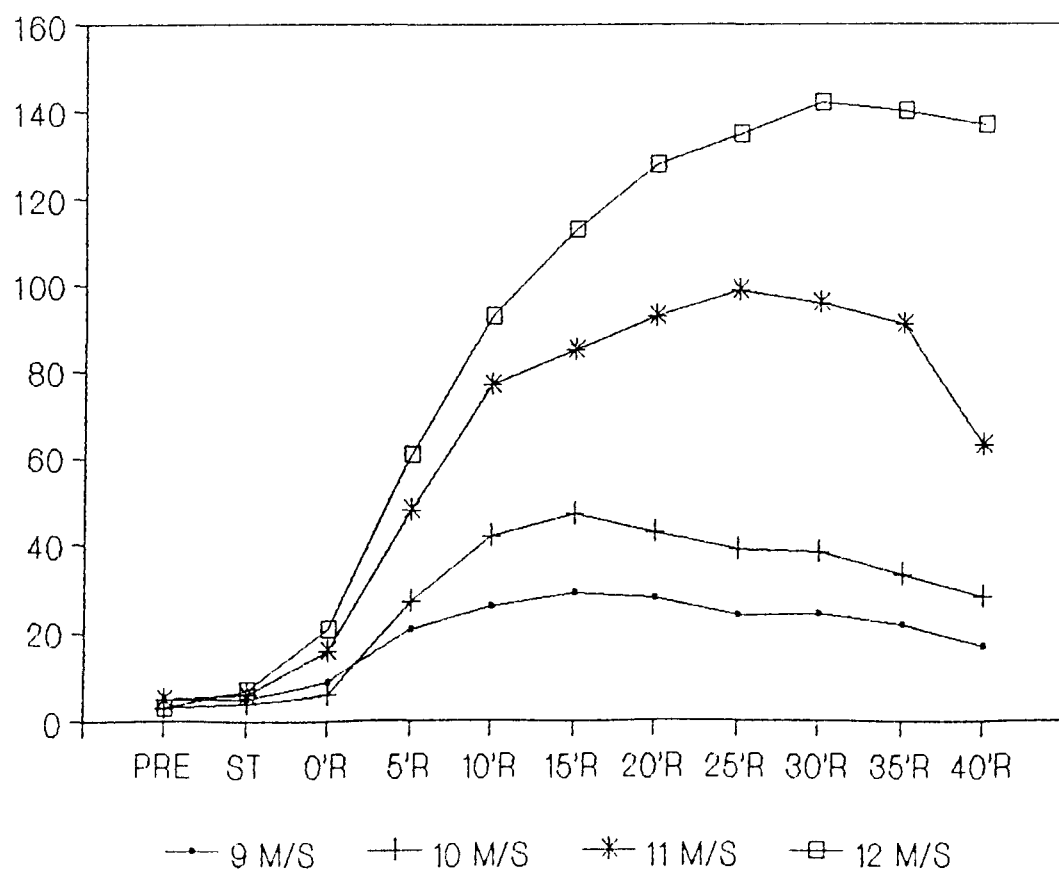
Table 8.12. Mean plasma uric acid concentration (μmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 9, 10, 11 or 12 m/s on 5° incline. (12 m/s n=5).

Min of Recovery

M/S	PRE	ST	0	5	10	15	20	25	30	35	40
9	5 ±5	5 ±4	9 ±6	21 ±8	26 ±12	29 ±17	28 ±19	24 ±22	24 ±23	22 ±23	17 ±24
10	3 ±4	4 ±3	6 ±4	28 ±10	43 ±20	47 ±22	43 ±22	40 ±20	38 ±22	33 ±16	28 ±17
11	5 ±7	6 ±4	16 ±14	48 ±23	77 ±41	85 ±44	93 ±52	99 ±62	96 ±64	91 ±68	63 ±47
12	3 ±3	7 ±2	21 ±5	61 ±7	93 ±12	113 ±20	128 ±28	135 ±36	142 ±43	140 ±46	137 ±49

Marked accumulation of plasma hypoxanthine and uric acid occurred following exercise at 11 and 12 m/s, whilst plasma xanthine showed an increase also at 10 m/s (Table 8.9). Peaks for all three metabolites occurred much later into recovery compared to ammonia, averaging 40 minutes plus for uric acid at 12 m/s (Figure 8.11, Table 8.13).

Significant correlations were found between ammonia and hypoxanthine (Figure 8.12) and ammonia and uric acid (Figure 8.13). The relationship between uric acid and hypoxanthine was less clear (Figure 8.14). No significant correlations were found between ammonia, hypoxanthine or uric acid and xanthine. In addition, it was observed that the horses showing the smallest increase in ammonia, hypoxanthine and uric acid with exercise at 11 and 12 m/s, showed the greatest increases in xanthine (up to 120 μmol/l).

Plasma glucose concentration was unaffected by the warm-up exercise and only small increases were found immediately post-exercise. Peak concentrations occurred at approximately 10 minutes recovery (Figure 8.15). Thereafter, there was a slow decline towards pre-exercise concentrations by 40 minutes recovery following exercise between 6 and 8 m/s. After exercise at 9 m/s and above, plasma glucose was still elevated at the end of the recovery period. Mean peak plasma glucose showed an approximately linear relationship with speed.

Plasma FFA showed an increase from 183±114 μmol/l at rest to 352±175 μmol/l at the end of the warm-up period. Exercise at 7, 9 and 11 m/s resulted in immediate post-exercise concentrations of 121±53, 73±21 and 75±30 μmol/l, respectively. The plasma FFA concentration increased during the recovery period, with peak values in most cases occurring at 40 min recovery (Figure 8.16). Little or no change was found in plasma glycerol immediately following exercise at each of the speeds from 6 to 12m/s (Figure 8.17). However, following exercise at 9m/s and above, substantial increases in plasma glycerol occurred during the first 10 min of recovery. The time to peak concentration increased with increasing speed. Exercise at 6 and 7m/s resulted in virtually no change in plasma glycerol over the 40 min recovery period, and only a small increase was seen following 8m/s.

Venous blood base excess, bicarbonate content and plasma pH at rest and the changes with exercise are shown in Table 8.14. Plasma pH was slightly elevated (mean increase 0.053) compared to the resting sample. Mean immediate post-exercise pH was linearly related to speed. Significant correlations were found between immediate post-exercise blood lactate and pH (r=-0.91, P>0.001) and lactate and base excess (r=-0.77, P>0.001).

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Table 8.13. Time at which peak concentrations (μmol/l) of plasma hypoxanthine, xanthine and uric acid were observed in 6 horses (except 12m/s, n=5) following 2 min exercise at 10, 11 or 12 m/s on 5° incline.

speed, m/s

	Recovery (min)	9	10	11	12
Hypoxanthine	5		5	1	
	10				1
	15		1	2	2
	20				1
	25			1	1
	30			1	
	35			1	
	40				
Xanthine	5		1	1	
	10		2	2	1
	15		2	2	
	20			1	1
	25				
	30		1		3
	35				
	40				
Uric acid	5		1		
	10	4	2	1	
	15	1	2	1	
	20				1
	25			2	
	30	1	1	1	1
	35			1	
	40				3

N.B. Peak times are not presented for hypoxanthine and xanthine at 9 m/s as all values were very low and showed little change during recovery.

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Table 8.14. Venous blood base excess (BE, mmol/l), bicarbonate (HCO₃⁻, mmol/l), packed cell volume (PCV, l/l) and plasma pH and mean stride frequency (SF, strides/min) and heart rate (HR, b.p.m.) at rest (PRE), immediately prior to exercise (ST) and during or at the end of 2 minutes at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline. n=6 except 12m/s (n=5).

			Speed, m/s						
	PRE	ST	6	7	8	9	10	11	12
pH	7.409 ±0.008	7.462 ±0.008	7.401 ±0.037	7.383 ±0.020	7.279 ±0.042	7.238 ±0.044	7.180 ±0.075	7.100 ±0.060	6.992 ±0.053
BE	5.7 ±1.0	5.7 ±1.1	2.9 ±2.1	2.2 ±2.0	-3.3 ±3.9	-3.0 ±4.5	-1.7 ±7.5	-10.0 ±3.8	-13.6 ±3.6
HCO₃⁻	30.7 ±1.1	29.2 ±1.4	27.5 ±1.7	27.1 ±1.9	23.7 ±3.7	24.1 ±3.2	23.0 ±3.4	21.1 ±3.8	19.8 ±3.8
PCV	0.42 ±0.01	0.53 ±0.01	0.57 ±0.02	0.59 ±0.03	0.62 ±0.03	0.62 ±0.03	0.63 ±0.04	0.64 ±0.03	0.64 ±0.03
SF (a)	–	–	110.2 ±3.8	114.2 ±1.9	117.2 ±1.6	120.4 ±3.5	122.6 ±3.4	126.7 ±4.7	130.8 ±2.6
HR (b)	46 ±11	99 ±9	175 ±8	182 ±8	199 ±8	205 ±4	207 ±4	209 ±3	214 ±5

Values linked by lines are not significantly different from PRE (P>0.05)

(a) Stride frequency calculated as mean of six 20 second periods.

(b) Exercise heart rates taken as the mean of heart rates at the end of two minutes exercise.

Figure 8.12. Peak plasma hypoxanthine (μmol/l) in relation to peak plasma ammonia (μmol/l) following 2 min treadmill exercise at 10, 11 or 12 m/s on 5° incline. Peak hypoxanthine = -3.26 + 0.0123.peak ammonia, r = 0.748, P<0.001.

Figure 8.13. Peak plasma uric acid (μmol/l) in relation to peak plasma ammonia (μmol/l) following 2 min treadmill exercise at 9, 10, 11 or 12 m/s on 5° incline. Peak uric acid = 8.8 + 0.154.peak ammonia, r = 0.771, P<0.001.

Peak plasma hypoxanthine, umol/l

Peak plasma ammonia, umol/l

Peak plasma uric acid, umol/l
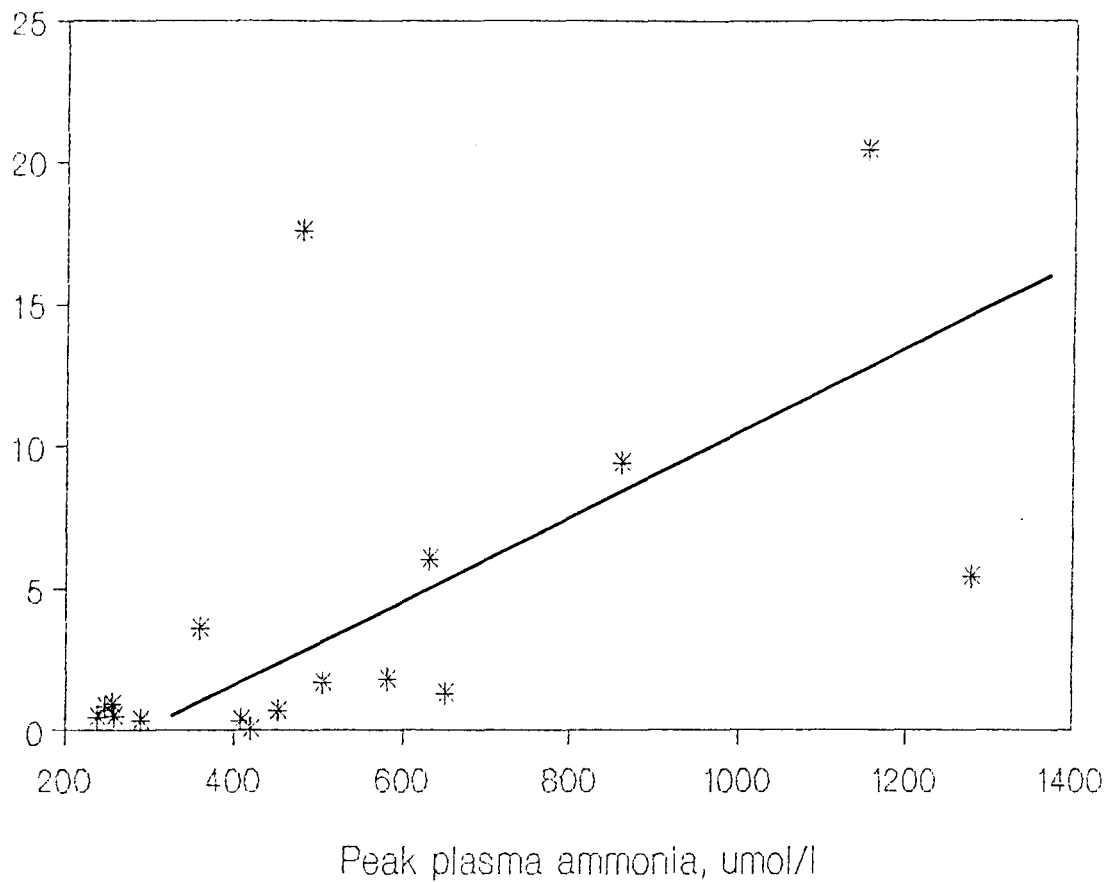
Peak plasma ammonia, umol/l
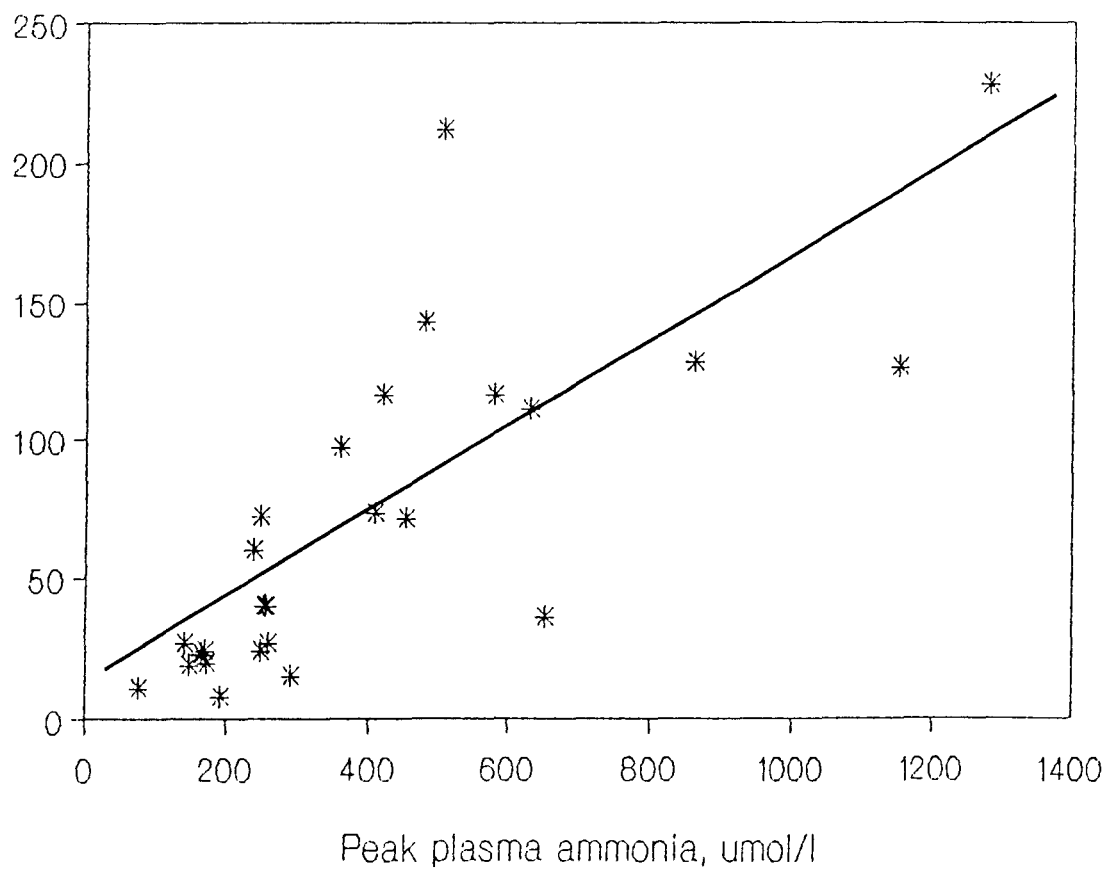
Figure 8.14. Peak plasma hypoxanthine (μmol/l) in relation to peak plasma uric acid (μmol/l) following 2 min treadmill exercise at 10, 11 or 12 m/s on 5° incline. r = 0.414, P>0.05.

Figure 8.15. Mean time courses for plasma glucose (mmol/l) for six horses immediately before (ST) and during 40 minutes walking recovery (1.6 m/s and 0° incline) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. For the sake of clarity, mean changes only are shown. A complete listing of values with standard deviations are given in Table 8.15 overleaf.

Peak plasma
hypoxanthine, umol/l

Peak plasma uric acid, umol/l

Plasma glucose
mmol/l

PRE   ST   0'R   5'R   10'R 15'R 20'R 25'R 30'R 35'R 40'R

─•─ 6 M/S   ─┼─ 7 M/S   ─∗─ 8 M/S   ─▢─ 9 M/S

─✕─ 10 M/S   ─◇─ 11 M/S   ─△─ 12 M/S
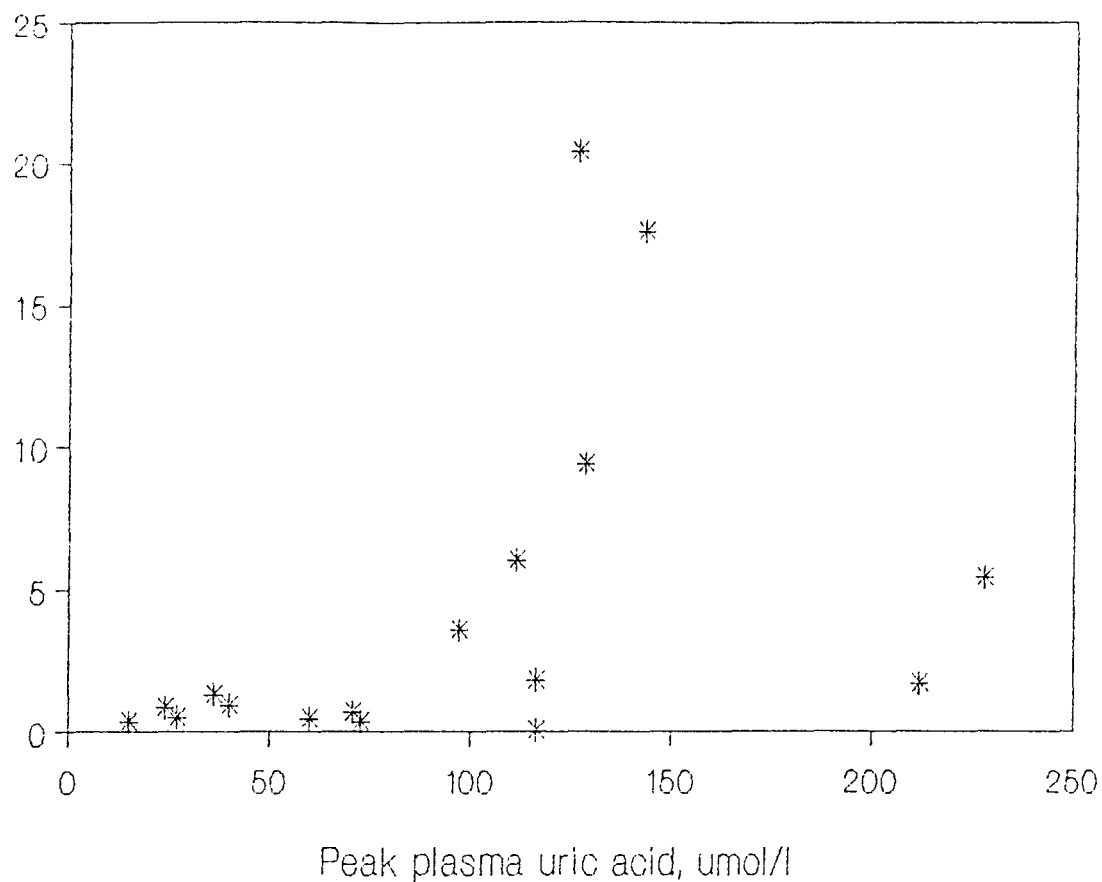
Table 8.15. Mean plasma glucose concentration (mmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. (12 m/s n=5).

| M/S | PRE | ST | Min of Recovery |||||
			0	10	20	30	40
6	6.4 ±0.8	6.6 ±1.3	6.6 ±1.3	6.4 ±1.1	6.1 ±1.4	5.6 ±1.1	5.4 ±0.8
7	6.9 ±0.9	6.3 ±0.5	7.0 ±0.6	7.3 ±0.6	6.9 ±0.9	6.5 ±0.4	6.0 ±0.3
8	6.9 ±0.7	7.8 ±1.4	8.1 ±2.2	8.5 ±1.1	7.3 ±1.3	6.5 ±0.8	6.7 ±1.5
9	7.2 ±0.5	6.3 ±0.6	6.5 ±0.4	8.6 ±2.4	7.4 ±0.5	7.0 ±0.8	6.1 ±0.9
10	7.2 ±1.3	6.6 ±0.8	7.5 ±1.2	8.2 ±1.5	8.1 ±1.3	7.5 ±1.6	7.3 ±2.0
11	7.1 ±0.8	7.1 ±1.2	7.3 ±1.3	9.6 ±1.2	8.5 ±0.8	8.0 ±1.3	8.4 ±1.4
12	6.5 ±0.7	6.2 ±0.6	7.7 ±1.1	10.3 ±1.7	9.4 ±1.4	7.8 ±0.4	9.2 ±1.7

Figure 8.16. Mean time courses for plasma free fatty acids (FFA, μmol/l)
for six horses at rest (PRE), immediately before (ST) and during 40 minutes
walking recovery (1.6 m/s and 0° incline) following 2 min treadmill
exercise at 7, 9, or 11 m/s on 5° incline. For the sake of clarity, mean
changes only are shown. A complete listing of values with standard
deviations are given in Table 8.16 overleaf.

Figure 8.17. Mean time courses for plasma glycerol (μmol/l) for six horses
at rest (PRE), immediately before (ST) and during 40 minutes walking
recovery (1.6 m/s and 0° incline) following 2 min treadmill exercise at 6,
7, 8, 9, 10, 11 or 12 m/s on 5° incline. For the sake of clarity, mean
changes only are shown. A complete listing of values with standard
deviations are given in Table 8.17 overleaf.

Plasma FFA
umol/l

PRE ST 0'R 5'R 10'R 15'R 20'R 25'R 30'R 35'R 40'R

—+— 7 M/S   —☐— 9 M/S   —◇— 11 M/S

Plasma glycerol
umol/l

PRE ST 0'R 5'R 10'R 15'R 20'R 25'R 30'R 35'R 40'R

—•— 6 M/S   —+— 7 M/S   —*— 8 M/S   —☐— 9 M/S
—✕— 10 M/S   —◇— 11 M/S   —△— 12 M/S

Table 8.16. Mean plasma FFA concentration (μmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 7, 9, or 11 m/s on 5° incline.

| M/S | PRE | ST | Min of Recovery | | | | |
			0	10	20	30	40
7	184 ±137	348 ±196	121 ±53	343 ±144	331 ±146	407 ±266	518 ±335
9	129 ±124	306 ±207	73 ±21	239 ±116	175 ±78	181 ±102	304 ±210
11	266 ±152	379 ±209	75 ±30	173 ±98	175 ±98	165 ±64	239 ±106

Table 8.17. Mean plasma glycerol concentration (μmol/l) in six horses at rest (PRE), following warm-up (ST) and during 40 min recovery following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on 5° incline. (12 m/s n=5).

				Min of Recovery			
M/S	PRE	ST	0	10	20	30	40
6	42 ±45	115 ±41	97 ±38	149 ±98	140 ±98	140 ±75	119 ±34
7	52 ±40	177 ±108	126 ±82	153 ±96	113 ±62	107 ±46	124 ±51
8	27 ±29	109 ±39	96 ±38	252 ±133	233 ±109	163 ±76	136 ±63
9	40 ±29	98 ±51	87 ±20	517 ±237	496 ±332	370 ±290	257 ±183
10	26 ±15	67 ±26	89 ±15	798 ±350	788 ±433	770 ±506	620 ±488
11	62 ±66	105 ±38	104 ±35	943 ±400	1215 ±636	1220 ±707	1100 ±742
12	35 ±18	97 ±22	116 ±25	1541 ±222	1716 ±349	1782 ±432	1766 ±516

Physiological parameters: PCV, stride frequency and heart rate

The warm-up period resulted in a significant increase in PCV in all horses from a mean of 0.42 at rest to 0.53 1/1. Maximal PCV appeared to have been reached in all horses by 8 m/s (Table 8.14). Heart rate was also elevated by the warm-up exercise, with a mean increase of 53 b.p.m. Maximum heart rate appeared to be reached around 9 m/s, though all horses showed some upward drift from 9 to 12 m/s (Table 8.14). Mean stride frequency, calculated over the two minute exercise period, was linearly related to speed (Table 8.14, r=0.997, P<0.001).

Comparison of blood changes with muscle changes

Plasma ammonia and uric acid both showed a significant correlation to the change in muscle ATP content (Figures 8.18 and 8.19). Peak plasma hypoxanthine appeared to show an exponential increase with muscle ATP decrease (Figure 8.20). This was made clearer by a plot of ln peak hypoxanthine against muscle ATP decrease (Figure 8.21). In contrast, there was no evidence of any correlation between peak plasma xanthine and muscle ATP change.

Muscle histochemical, enzymic and metabolic characterization

Enzymic, histochemical and metabolic parameters of muscle samples obtained prior to ("pre") and following ("post") the complete test period for each of the six horses are shown in Tables 8.18, 8.19 and 8.20, respectively. Comparison of results using Students' t test for paired data indicated no significant difference between "pre" and "post" samples for any of the parameters analysed, with the exception of IIB fibre frequency (%IIB, P<0.05).

The relationship between the muscle metabolic response to exercise and muscle composition was also examined. Significant correlations between muscle lactate accumulation and histochemical and enzymic parameters were found at different exercise intensities (Table 8.21).

Significant positive correlations were found between muscle lactate accumulation and relative area occupied by IIB fibres (6, 7 and 11 m/s, P<0.05), LDH activity (7 and 11 m/s, P<0.01 and P<0.05, respectively) and %LDH-5 isoenzyme (7, 10 and 11m/s, P<0.05). Significant negative correlations were found between muscle lactate accumulation and relative area occupied by IIA fibres (7, 10 and 11 m/s, P<0.05) and OGDH activity (10, 11 and 12 m/s, P<0.05). No significant correlations were found between muscle lactate accumulation and CPK or PFK activity.

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Figure 8.18. Peak plasma ammonia concentration (μmol/l) in relation to the change in muscle ATP content (mmol/kg d.m.) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline. Peak ammonia = 120 - 62.2.delta ATP, r = -0.749, P<0.001.

Figure 8.19. Peak plasma uric acid concentration (μmol/l) in relation to the change in muscle ATP content (mmol/kg d.m.) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline. Peak uric acid = 25.5 - 11.7.delta ATP, r = -0.686, P<0.001.

Peak plasma ammonia, umol/l vs Delta ATP, mmol/kg d.m.

Peak plasma uric acid, umol/l vs Delta ATP, mmol/kg d.m.
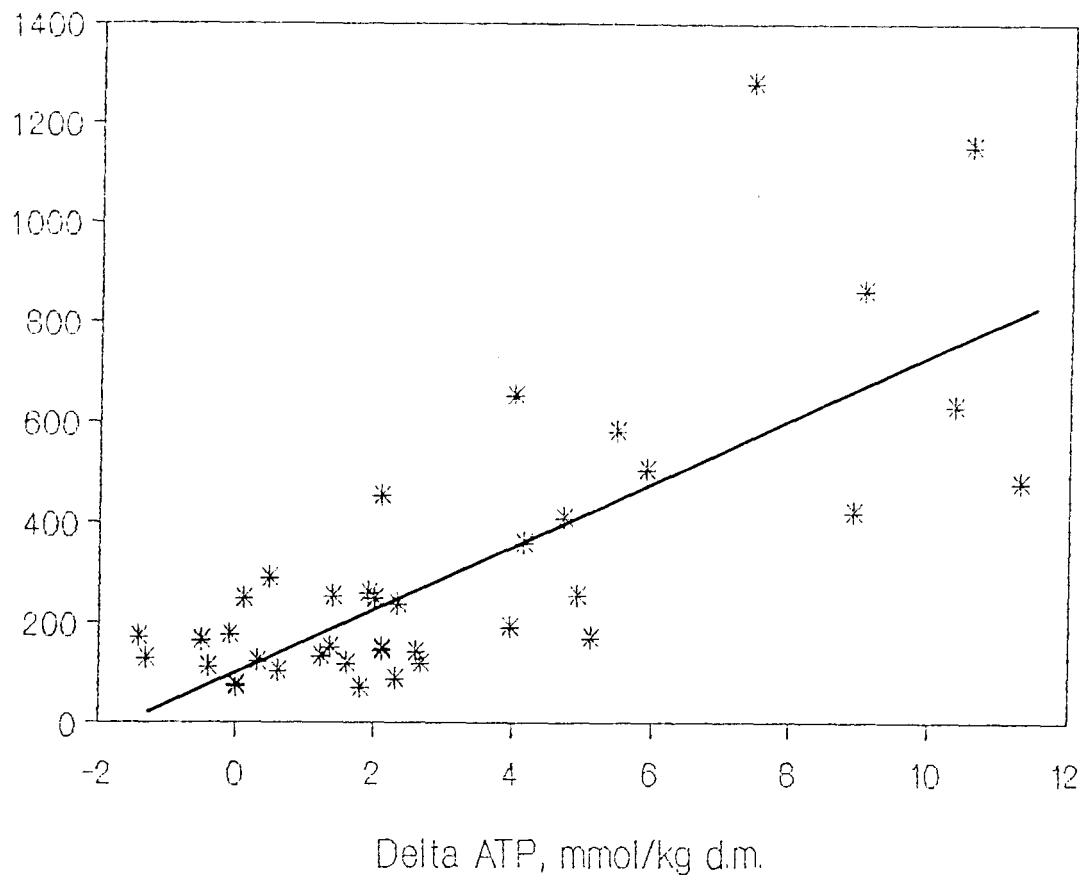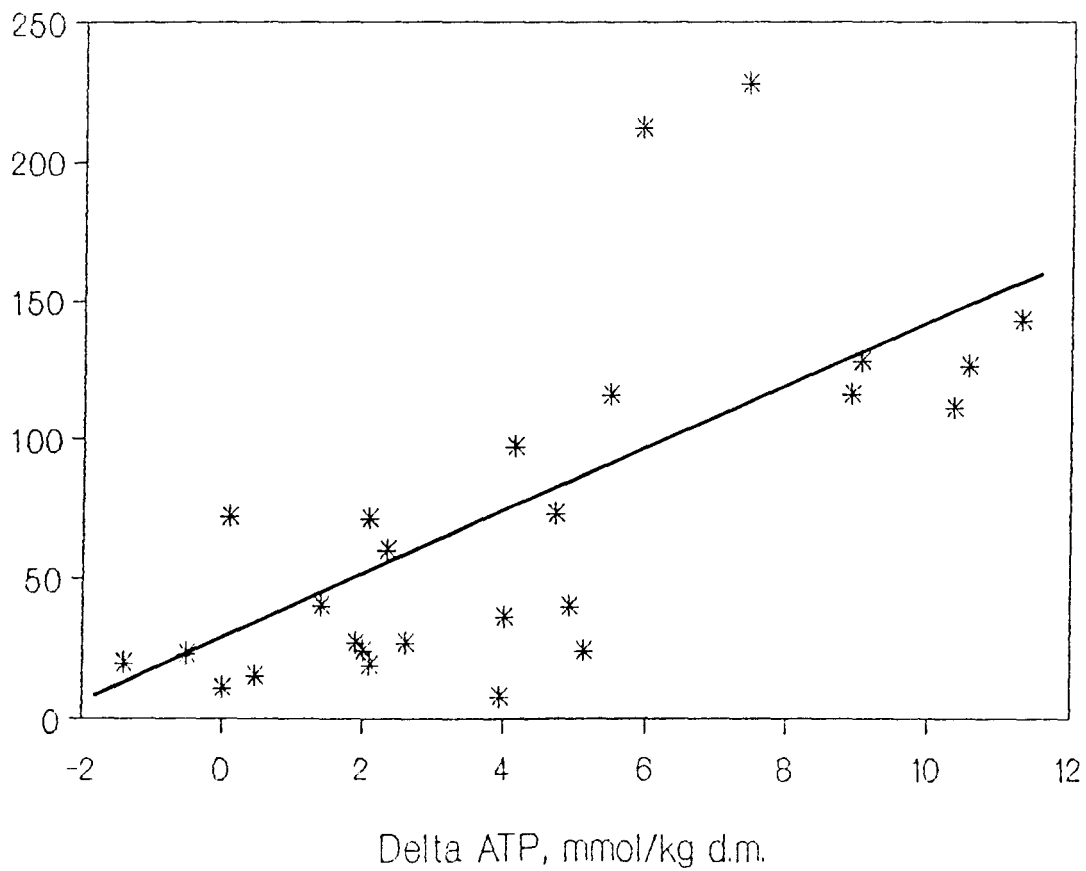
Figure 8.20. Peak plasma hypoxanthine concentration (μmol/l) in relation to the change in muscle ATP content (mmol/kg d.m.) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s on a 5° incline.

Figure 8.21. Ln peak plasma hypoxanthine in relation to the change in muscle ATP content (mmol/kg d.m.) following 2 min treadmill exercise at 6, 7, 8, 9, 10, 11 or 12 m/s. Ln peak hypoxanthine = -1.28 - 0.321.delta ATP, r = -0.813, P<0.001.

Peak plasma
hypoxanthine, umol/l

Delta ATP, mmol/kg d.m.

Ln peak
hypoxanthine

Delta ATP, mmol/kg d.m.
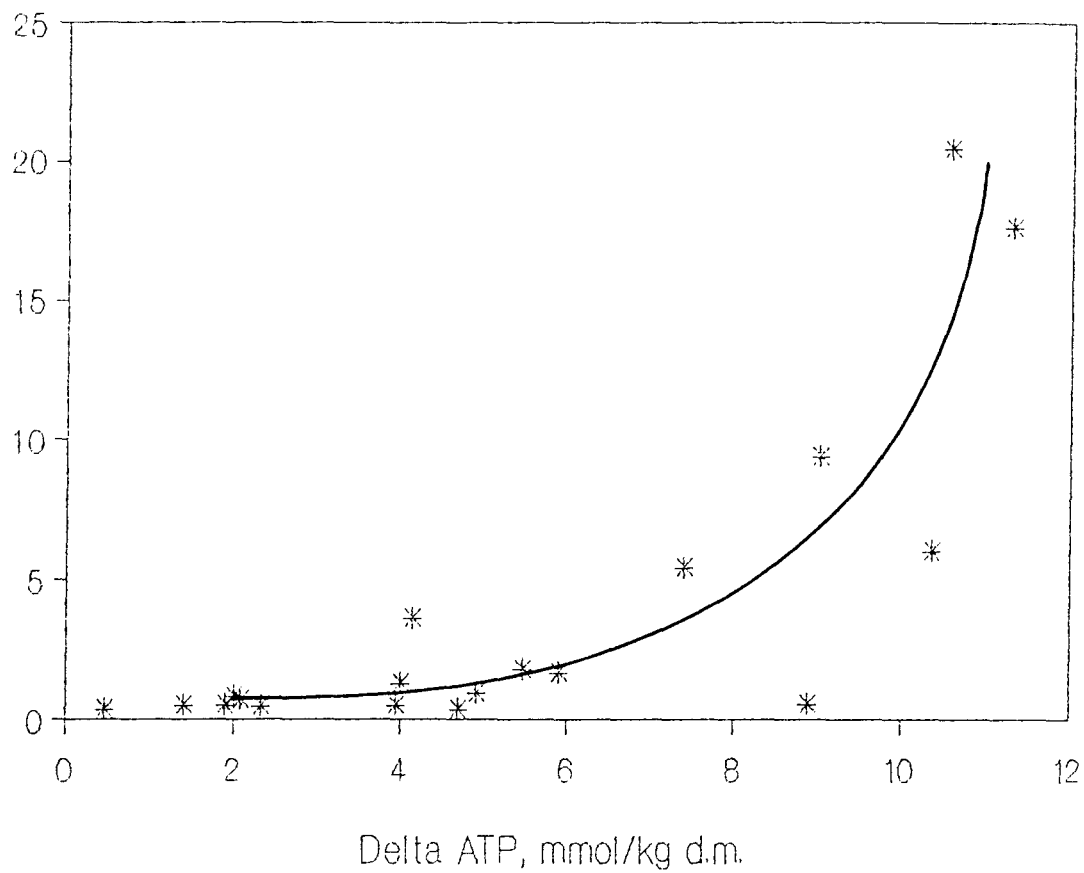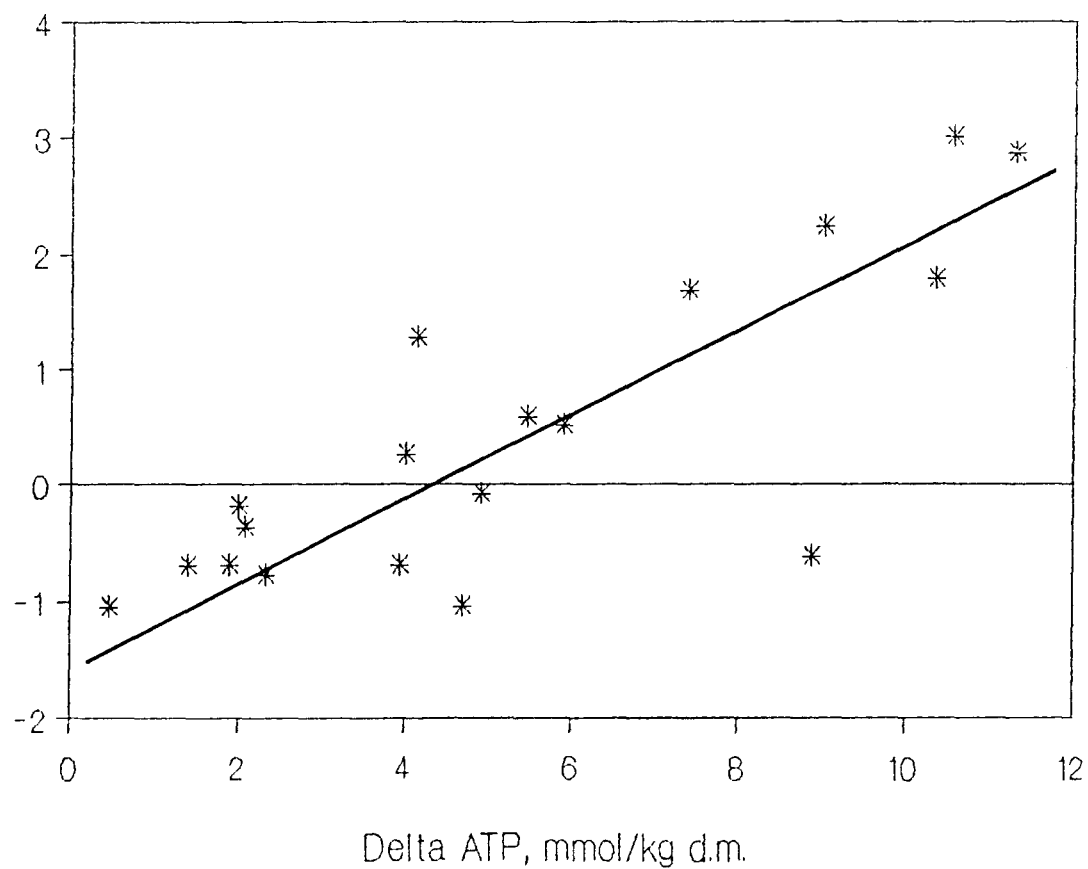
Table 8.18. PFK, LDH, CPK and OGDH activity (μmol/g w.m./min, 25°C) and %LDH-5 isoenzyme content in resting muscle samples taken in the pre-session (PRE) and in the post-session (POST). R and L refer to right and left side.

		PFK	LDH	CPK	OGDH	%LDH-5
FR	PRE-R	60.7	531	2494	3.9	64.7
	POST-L	55.8	481	2266	6.8	59.9
	MEAN	58.3	506	2380	5.3	62.3
HR	PRE-R	56.5	677	2047	2.2	68.4
	POST-L	46.7	674	1950	1.9	69.3
	MEAN	51.6	675	1999	2.0	68.9
JW	PRE-R	53.2	566	1882	1.2	81.8
	POST-L	56.5	677	2047	3.0	57.6
	MEAN	54.9	622	1965	2.1	69.7
LB	PRE-L	60.7	531	2494	3.9	64.1
	POST-R	55.8	481	2266	6.8	52.2
	MEAN	58.3	506	2380	5.3	58.2
MR	PRE-L	36.2	375	2044	3.5	60.9
	POST-R	35.1	465	1940	2.6	62.8
	MEAN	35.7	420	1992	3.0	61.9
TB	PRE-L	54.0	613	1790	3.0	68.5
	POST-R	41.0	520	1765	2.3	61.5
	MEAN	47.5	567	1778	2.7	65.0

Table 8.19. Frequency (%), mean area (μm^2) and relative area occupied (%AO) of type I, IIA and IIB fibres in resting muscle samples taken in the pre-session (PRE) and in the post-session (POST). R and L refer to right and left side.

		%I	%IIA	%IIB	Area I	Area IIA	Area IIB	%AO I	%AO IIA	%AO IIB
FR	PRE-R	15.3	54.6	30.9	2267	2413	3611	12.6	47.9	39.5
	POST-L	16.2	53.9	29.9	2155	2542	3229	13.0	51.0	36.0
	Mean	15.8	54.3	30.0	2211	2478	3420	12.8	49.5	37.8
HR	PRE-R	7.6	39.7	52.7	2007	2198	3754	5.1	29.1	65.9
	POST-L	15.3	43.6	41.1	3030	2836	3979	13.9	37.1	49.0
	Mean	11.5	41.7	46.9	2519	2517	3867	9.5	33.1	57.5
JW	PRE-R	14.7	37.3	48.0	3058	3477	3969	12.3	35.5	52.2
	POST-L	25.7	30.3	44.1	2407	3247	4545	17.2	27.3	55.6
	Mean	20.2	33.8	46.1	2733	3362	4257	14.8	31.4	53.9
LB	PRE-L	17.8	53.7	28.6	2200	2640	3269	14.3	51.7	34.1
	POST-R	19.5	57.1	23.4	2009	2504	3017	15.5	56.6	27.9
	Mean	18.7	55.4	26.0	2105	2572	3143	14.9	54.2	31.0
MR	PRE-L	28.2	48.0	23.8	2719	3441	4534	21.9	47.2	30.9
	POST-R	18.6	58.9	22.5	2522	3181	3755	18.6	58.9	22.5
	Mean	23.4	53.5	23.2	2621	3311	4145	20.3	53.1	26.7
TB	PRE-L	9.4	48.8	41.8	2057	2380	3840	6.5	39.2	54.2
	POST-R	15.3	49.1	35.6	2573	2860	3824	12.5	44.4	43.1
	Mean	12.4	49.0	38.7	2315	2620	3832	9.5	41.8	48.7

Table 8.20. Carnosine content (mmol/kg d.m.) and buffering capacity (βm, μmol H$^+$/g d.m., pH 7.1-6.5, 37°C, in resting muscle samples taken in the pre-session (PRE) and in the post-session (POST). R and L refer to right and left side.

		Carnosine	βm
FR	PRE-R	102.0	125.5
	POST-L	107.5	137.5
	Mean	104.8	131.5
HR	PRE-R	102.4	119.3
	POST-L	105.1	121.9
	Mean	103.8	120.6
JW	PRE-R	99.3	101.5
	POST-L	88.1	100.1
	Mean	93.7	100.8
LB	PRE-L	102.4	103.2
	POST-R	105.5	112.8
	Mean	104.0	108.0
MR	PRE-L	77.2	115.4
	POST-R	97.0	131.5
	Mean	87.1	123.5
TB	PRE-L	98.7	114.2
	POST-R	93.9	96.5
	Mean	96.3	105.4

Table 8.21. Relationship between muscle histochemical and enzymic profile and muscle lactate accumulation during exercise.

Speed	%AOIIB v Muscle Lactate	%AOIIA v Muscle Lactate	LDH v Muscle Lactate	%LDH-5 ISO v Muscle Lactate	OGDH v Muscle Lactate
6 m/s	r=0.810 $P<0.05$	ns	ns	ns	ns
7 m/s	r=0.871 $P<0.05$	r=-0.884 $P<0.05$	r=0.931 $P<0.01$	r=0.830 $P<0.05$	ns
8 m/s	ns	ns	ns	ns	ns
9 m/s	ns	ns	ns	ns	ns
10 m/s	ns	r=-0.828 $P<0.05$	ns	r=0.823 $P<0.05$	r=-0.833 $P<0.05$
11 m/s	r=0.853 $P<0.05$	r=-0.909 $P<0.05$	r=0.840 $P<0.05$	r=0.899 $P<0.05$	r=-0.899 $P<0.05$
12 m/s	ns	ns	ns	ns	r=-0.890 $P<0.05$
Mean rank muscle lactate accumulation[a]	r=0.912 $P<0.01$	r=-0.964 $P<0.01$	r=0.855 $P<0.05$	r=0.964 $P<0.01$	r=-0.907 $P<0.02$

(a) Horses were ranked from 1 (lowest muscle lactate) to 6 (highest muscle lactate) at each speed. The mean rank over the seven exercise sessions (i.e. seven speeds) was then calculated for each horse. In the case of JW where there was no 12 m/s session, the rank at 12 m/s was estimated as the mean rank over speeds 6 to 11 m/s.

Mean rank of muscle lactate accumulation over all seven exercise sessions from 6 to 12 m/s (Table 8.21) also showed significant correlations with muscle characteristics as follows: %AOIIB - r=-0.912, P<0.01; %AOIIA - r=0.964, P<0.01; LDH activity - r=-0.855, P<0.05; %LDH-5 Isoenzyme - r=0.964, P<0.01; OGDH activity - r=0.907, P<0.02. As for correlations for individual sessions, no significant correlations were found for PFK and CPK activity (P>0.05).

DISCUSSION

Muscle ATP depletion

The studies in Chapter 6 demonstrated extensive loss of muscle ATP during both high-intensity field exercise and treadmill exercise. In some horses the loss following single gallops amounted to as much as 50% of the resting content. Losses on this scale have rarely been observed in human subjects (Sahlin and Katz, 1988), the exception being in trained athletes exercised maximally (Boobis, Williams and Wooton, 1982; McCartney et al, 1986).

The primary aim of the present study was to establish the relationship between muscle nucleotide loss and work intensity and to investigate the use of blood parameters to assess muscle nucleotide status. As illustrated in Table 8.1, there was no evidence of a progressive loss of ATP within the middle gluteal with increasing work intensity. Instead the ATP content was maintained until a speed was reached beyond which a substantial loss in muscle ATP occurred. Individually, this ranged from 10 to 12 m/s for the six horses used. The loss of muscle ATP coincided with the greatest increase in plasma ammonia, hypoxanthine and uric acid (Figures 8.18-8.20).

Previous studies in man (Harris and Hultman, 1985) and the Thoroughbred horse (Snow, Harris and Gash, 1985) have shown that inosine monophosphate (IMP) is the primary accumulation product resulting from ATP loss during and at the end of exercise. The IMP is retained within the muscle and the majority is resynthesized back to AMP during the first hour of recovery (Chapter 7). However, some of the IMP may be further degraded to inosine and hypoxanthine, which being less polar, are able to cross the cell membrane and pass into the circulation (Harkness, 1988). Uric acid that appears in the blood during the recovery period is derived from the degradation of hypoxanthine by the liver and therefore the appearance of hypoxanthine in the blood precedes that of uric acid.

In agreement with the present study, work by McCreanor and Harkness (1987) and Ketai et al (1987) on the appearance of hypoxanthine in the plasma of human subjects exercising at different work intensities also pointed to the existence of a threshold for muscle ATP loss, rather than a progressive decrease. The lack of any apparent relationship between muscle ATP loss and the appearance of xanthine in plasma is most likely a reflection that the latter is derived not only from the breakdown of hypoxanthine, but also from the breakdown of guanine from guanine nucleotides such as guanosine triphosphate (Harkness, McCreanor and Watts, 1988).

The mechanisms relating to purine nucleotide metabolism have recently been reviewed by Harkness (1988) and Sahlin and Katz (1988). IMP accumulation, representing loss of adenine nucleotide, does not appear to begin until significant amounts of lactate have been accumulated. In man, Harris and Hultman (1985) demonstrated that IMP only began to accumulate when muscle lactate contents exceeded 50 mmol/kg d.m. This has recently been confirmed by Sahlin, Broberg and Ren (1989). In the present study, 10 m/s was the lowest speed at which all of the horses showed a decrease in muscle ATP content following exercise. Mean muscle lactate accumulation at 10 m/s amounted to 76.5±31.0 mmol/kg d.m.. If ATP decrease is considered to be an indirect result of acidosis (see later discussion) the higher threshold for ATP decrease in the horse compared with man, in relation to muscle lactate accumulation, may be due to the higher muscle buffering capacity in the horse.

Observations that IMP accumulation does not occur until significant amounts of lactate have been formed has lead to the suggestion of a direct in vivo effect of pH upon AMP-deaminase, which shows an increase in activity with decreasing pH below 7.0 (Setlow and Lowenstein, 1967; Wheeler and Lowenstein, 1979). Other studies however, have demonstrated adenine nucleotide loss and IMP accumulation in the absence of acidosis in muscle poisoned with iodoacetate (Sahlin et al, 1981; Dudley and Terjung, 1985) and in patients with phosphorylase deficiency (Mineo et al, 1985; Lewis and Haller, 1986; Coakley, Wagenmakers and Edwards, 1989) suggesting that increased levels of AMP are the main stimulus to deamination. The very high K_m of AMP-deaminase for AMP (0.4-1.0 mmol/l: Ronca-Testoni, Ragga and Ronca, 1970; Wheeler and Lowenstein, 1979) compared to the normal concentration of AMP in resting muscle further suggests that substrate availability may be the principal regulator of AMP-deaminase activity.

The basic unit of muscle contraction is the single twitch which results in the breakdown of ATP and the formation of ADP. Initially, each

pulse of ADP released will be rapidly resynthesized back to ATP in the cytoplasm at the expense of phosphocreatine and, to a lesser extent, by 1,3 diphosphoglycerate and phosphoenolpyruvate in glycolysis. Creatine released in this process may then be resynthesized back to phosphocreatine from ATP generated from glycolysis or through oxidation. Pertinent to this view is a central role of the PCr/CPK system in buffering ATP (Meyer, Brown and Kushmerick, 1984). However, it has been shown recently that of the major ATP buffering systems known, the PCr/CPK system is the most sensitive to increasing H^+ (Ellington, 1989). Harris, Sahlin and Hultman (1977) demonstrated that an increase in muscle lactate content with exercise to 120 mmol/kg d.m., corresponding to a fall in muscle pH to arround 6.6 or less, would result in a fall in the equilibrium concentration of PCr to around 10 mmol/kg d.m. Therefore, acute, severe acidosis will reduce the equilibrium concentration of PCr that can be maintained and progressively diminish its capacity to buffer the transient increases in ADP, which as a result will become increased in both amplitude and duration. Increases in ADP will in turn result in increases in AMP via the myokinase reaction.

The importance of AMP deamination appears to be in the displacement of the myokinase reaction in the direction of ADP removal, as accumulation of ADP may adversely affect both actin-myosin cross-bridge detachment and formation in subsequent contractions by competitive inhibition of ATP (Schoenberg and Eisenberg, 1987). This in turn would decrease force production as power output is proportional to the number of cross-bridges formed. Furthermore, increases in ADP may also reduce shortening velocity (Siemankowski and White, 1984). As well as being activated by increases in its substrate, AMP-deaminase activity may also be increased by allosteric modulation by di-adenosine tetraphosphate, which has been suggested to be an intermediate of the myokinase reaction and to increase the affinity of AMP-deaminase for AMP in vitro (Fernández et al, 1984).

In the present study muscle ATP loss and plasma ammonia were found to be highly correlated. This would seem to indicate that the work intensity which results in significant nucleotide loss can be estimated based on blood samples alone. Although the muscle biopsy is practical for repeated use in experimental animals and under laboratory conditions, its regular use for investigation of racehorses in training yards is less acceptable or practical. The results of the present study also indicate that changes in plasma hypoxanthine and uric acid could be used as markers of muscle nucleotide loss. However, the longer delay in time to peak concentrations for these metabolites and the extent of the variation between horses make them less satisfactory and possibly subject to a greater degree of error

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when compared with plasma ammonia.

Previous studies in both the horse (Snow, Harris and Gash, 1985; see also Chapter 6) and man (Hultman, Bergström and McClennan-Anderson, 1967; Hultman and Sjöholm, 1983; Hultman, Spriet and Söderlund, 1987) have indicated a reduction in contractile function with reduction in muscle ATP content. Whilst assessment of performance was not possible in the present study, LB, who was easily the most capable of the six horses used, maintained a high muscle ATP content in all sessions, including following the 12 m/s session. It might be expected that the ability to maintain a high muscle ATP content even at relatively high work-intensities and in the presence of a high lactate load would be desirable in terms of exercise capacity and that horses showing significant ATP depletion at relatively low work intensities would not fall in to the category of good to elite racehorses (see Park et al, 1988). Thus, the finding that ATP depletion and plasma ammonia accumulation are closely related may have important implications for the monitoring of horses in practical and applied situations.

Previous reports of significant correlations between ammonia and lactate in blood in man (Babij, Matthews and Rennie, 1983) and in muscle in the horse (Essén-Gustavsson and Valberg, 1987) are consistent with an association between the increase in the concentration of H^+ and increased AMP-deaminase activity. However, in the present study blood lactate accumulation clearly preceeded that of plasma ammonia. More recently Broberg (1989) demonstrated that although muscle lactate and ammonia release in relation to increasing exercise intensity show very similar kinetics, blood lactate concentration begins to increase at a lower exercise intensity compared to plasma ammonia. This may be due to relatively greater redistribution of ammonia, compared to lactate, between plasma and erythrocytes (Broberg, 1989; see Chapter 3) or uptake by tissues such as non-locomotor muscles during low-intensity exercise (Erikson et al, 1985).

Blood lactate disappearance

The results of the present study indicate that the rate of lactate disappearance from blood decreases with increasing lactate concentration (that is, the rate constant for blood lactate removal was negatively correlated to peak post-exercise lactate concentration). This relationship was first demonstrated in man by Margaria, Edwards and Dill (1933). More recently, this relationship has been confirmed in man by Jorfeldt (1976) and Freund and Zouloumian (1981) and in the dog (Issekutz, Shaw and

Issekutz, 1976).

Following exercise, the blood lactate concentration may begin to fall as a result of redistribution of lactate within body fluids other than blood, for example in muscle relatively inactive during the preceeding exercise. Once the lactate has been distributed throughout the tissues, then the concentration in blood will fall as a result of uptake and utilization of lactate, primarily by liver, heart and muscle.

A large variation was seen between horses in rate constants for blood lactate removal following exercise (expressed as half-time, Table 8.6). For example, following exercise at 11 m/s half-times ranged from 11.0 to 26.9 min, although this is probably due to the large variations in concentration. When the effect of concentration was eliminated, by plotting rate constant against blood lactate concentration, there were still large interindividual differences in the rate of blood lactate disappearance (Table 8.7).

Bonen et al, (1979) demonstrated that lactate removal following exercise could be adequately described by a multiple linear regression model taking into account the intensity of recovery exercise, post-exercise blood lactate concentration and % Type I fibres. In the present study all horses were walked at 1.6 m/s, although this may not correspond to the same absolute work intensity for each horse. However, with the assumption that walking at 1.6 m/s approximates to the same absolute exercise intensity for each horse and with the effect of concentration removed, the differences in blood lactate removal may be explained on the basis of variation in the sum of %AOI and %AOIIA fibres and OGDH activity (r=0.939, P<0.01 and r=0.839, P<0.05, respectively).

Muscle glucose increase

The increases in muscle free glucose content with increasing exercise intensity show a similar trend to that observed in humans following exercise between 40 and 100% VO_2Max (Katz, 1986), although the extent of accumulation appears to be greater in the horse. The highest individual value in the present study was almost 14 mmol/kg d.m. compared to 8.5 mmol/kg d.m. after maximal dynamic exercise in man (Katz, 1986).

Field (1960) estimated that between 8-10% of glycogen degraded appeared as free glucose. Although glycogen was not determined in the present study, in Chapter 6, glycogen degradation was of the order of 150 mmol glucosyl units/kg d.m. when muscle lactate contents of 130 mmol/kg

d.m. were attained following maximal exercise in the field. By comparison, a similar degree of glycogen degradation could reasonably be expected to have occurred in the present study following exercise at 12 m/s with muscle lactate accumulation of 132.9±19.5 mmol/kg d.m. Based on the estimate of Field, this could result in an increase in muscle free glucose of 12-15 mmol/kg d.m., sufficient to account for the all the muscle free glucose increase with exercise at 12 m/s.

Alternatively, the intramuscular glucose may be of extracellular origin and the accumulation within the muscle due to penetration or uptake from the circulation without utilization. Glucose accumulation within the muscle suggests that either the activity of hexokinase is limiting or that the enzyme itself is inhibited. The in vitro activity of hexokinase has been shown to be around 11 mmol/kg d.m./min in the Thoroughbred horse (Guy, 1978) which would be sufficient to phosphorylate all the glucose accumulated at the end of exercise. Thus, the glucose within the muscle probably originates from both glycogen breakdown and glucose uptake, whilst accumulation is most likely due to inhibition of hexokinase by increasing concentrations of glucose 6-P (Crane and Sols, 1953; Colowick, 1973).

The uptake of glucose from the circulation by active muscle groups has been shown to increase in proportion to the exercise intensity in man (Wahren, Felig and Ahlborg, 1971; Katz, 1986). During maximal exercise leg glucose uptake rates as high as 2 mmol/kg d.m./min have been recorded (Katz, 1986). The accumulation of free glucose in muscle during high intensity exercise is probably of both intra and extracellular origin. If the horse were to show glucose uptake rates similar to those observed in man, then following 2 minutes exercise at 12 m/s an extra 4 mmol of glucose per kg dry muscle might be expected from uptake of glucose from the circulation. Combined with the estimate of glucose derived from glycogen breakdown (12-15 mmol/kg d.m.) this would indicate an expected concentration of 16-19 mmol/kg d.m. at the end of exercise. The finding that glucose accumulation at the end of exercise at 12 m/s was lower than this estimate indicates that some glucose was probably utilized during the exercise, presumably in the early stages when glucose 6-P was lower.

As to why muscle glucose uptake should increase with increasing exercise intensity, and a corresponding decrease in the ability of the muscle to utilize glucose, remains unclear. Changes in intracellular calcium and an effect on membrane permeability have been suggested (Holloszy and Narahara, 1967; Clausen, 1980). Furthermore, Galbo, Richter and Sonne (1986) proposed that "fuel mobilization is not always, if ever, accurately adjusted to the energy needs of the working muscles".

The major source of free fatty acids (FFA) for utilization by muscle is adipose tissue, although other sources include circulating lipoproteins and intramuscular tryacylglycerols. The liver also stores tryacylglycerols but these do not appear to be mobilized or utilized during exercise (Bülow, 1988). The increase in plasma FFA at the end of the warm-up and following exercise at 7m/s in the present study is most likely due to mobilization and release of FFA from adipose tissue following activation of hormone sensitive lipase (HSL), principally by increases in adrenaline and noradrenaline and a decrease in the circulating insulin concentration (Galbo, 1983). Mobilization and release of FFA from muscle as the explanation for the plasma FFA increase would seem unlikely as muscle preferentially utilizes exogenous FFA, possibly as a result of inhibition of intracellular lipoprotein lipase (Newsholme and Leech, 1983; Oscai and Palmer, 1983).

The decrease in the circulating FFA concentration following exercise at 7, 9 and 11 m/s indicates uptake or uptake and utilization, predominantly by muscle. Following exercise at 7m/s, the plasma FFA concentration increased over the forty minute recovery period, indicating continued lipolysis and fatty acid release. At 9 and 11m/s the absence of any significant increase in plasma FFA is probably due to inhibition of lipolysis by increases in insulin, prostaglandins E1 and E2 (Hales et al, 1978), 3-hydroxybutyrate and adenosine (Green and Newsholme, 1979). However, adenosine released from working muscles during exercise has been shown to promote vasodilation (Martin and Bockman, 1986) and FFA mobilization during exercise is enhanced by increased blood flow (Bülow and Tøndevold, 1982).

It has also been demonstrated that in isolated, perfused, canine adipose tissue, lactate enhances fatty acid re-esterification without affecting glycerol release (Fredholm, 1977). This is consistent with observations in the present study that glycerol accumulation increased with increasing speed, whereas plasma FFA levels showed the opposite. The trend for plasma FFA to increase at around 30-40 minutes of recovery following exercise at 9 and 11m/s when blood lactate was substantially reduced is also consistent with a lactate mediated suppression of lipolysis.

Correlation of muscle enzyme activities and histochemical parameters with muscle lactate accumulation following exercise indicated a trend for muscle lactate accumulation to be positively related to muscle glycolytic capacity and inversely related to muscle aerobic capacity. The relationships were strengthened in all cases by using the mean rank of muscle lactate accumulation over all seven speeds as opposed to making correlations for each individual speed.

The weakness of relationships at certain speeds, particularly at 8 and 9 m/s, can possibly be explained on the basis of muscle lactate contents in individual horses being much higher than might have been predicted from examination of the overall response to increasing speed. As discussed previously, this may have arisen through excess catecholamine stimulation of muscle lactate production (Stainsby, Sumners and Andrew, 1984), possibly due to excitement or some underlying subclinical condition.

The findings of the present study are in agreement with observations in Standardbred horses following treadmill exercise (Valberg et al, 1985) and in both Thoroughbred and Standardbred horses following racing (Valberg and Essén-Gustavsson, 1987). In the former study, immediate post-exercise blood lactate concentration was found to be positively related to percentage IIB fibres and muscle LDH activity and negatively related to percentage IIA fibres. Muscle lactate following racing (Valberg and Essén-Gustavsson, 1987) was found to be positively related to %IIB fibres and LDH activity, whilst a negative relationship between %IIA fibres and citrate synthase activity was found for Thoroughbreds but not Standardbreds. Relationships between muscle metabolic response to exercise and muscle characteristics have previously been identified in man (Tesch et al, 1985).

In summary, the major finding in the present study was that muscle ATP loss does not appear to occur progressively with increasing exercise intensity, but instead shows a threshold. The point at which muscle ATP loss was first seen in each horse also appeared to coincide with a muscle lactate content of 60-70 mmol/kg d.m. or above. Furthermore, plasma ammonia appears to be a good indicator of muscle ATP status.

CHAPTER 9

General Discussion and Summary

GENERAL DISCUSSION

The studies undertaken in this thesis have demonstrated the high aerobic and glycolytic capacities of the Thoroughbred horse. Chapter 6 demonstrated that single bouts of high-intensity exercise in the horse result in a substantial increase in muscle lactate content and significant decreases in muscle PCr and ATP content. Previously, muscle ATP decrease in the horse had only been demonstrated following high-intensity intermittent exercise (Snow, Harris and Gash, 1985). In Chapter 8, it was further demonstrated that whilst the changes in muscle lactate and PCr showed an approximately linear relationship to increasing exercise intensity (increasing speed), there was a threshold for muscle ATP decrease. This appeared to coincide with muscle lactate contents of around 70 mmol/kg d.m. and a decrease in muscle PCr content to around 35 mmol/kg d.m..

Acidosis as a result of lactic acid accumulation has been implicated as the cause of fatigue due to either direct or indirect effects on the contractile apparatus or through indirect effects on other metabolites and processes taking place within the muscle (see reviews by Mainwood and Renaud, 1984; Sahlin, 1986; Fitts and Metzger, 1988; Vøllestad and Sejersted, 1988). In man, Broberg (1989) demonstrated that TAN decrease (indicating a decrease in muscle ATP content, in the absence of any changes in ADP or AMP) did not occur until an exercise intensity corresponding to approximately 80% VO_2max, when muscle lactate contents were in the region of 50 mmol/kg d.m.. The onset of muscle ATP decrease at apparently lower muscle lactate contents in man compared to the horse may be due to differences in muscle buffering capacity (βm). As shown in Chapter 4, the horse has a higher buffering capacity compared to man and therefore, the accumulation of the same amount of lactate will result in a smaller decrease in pH in equine muscle compared to human muscle.

In spite of a higher βm, muscle ATP decrease appears to be a feature of moderate to high-intensity exercise in the horse and is observed less frequently in man. The exception appears to be in highly trained athletes with a high capacity for lactate production or in ordinary subjects under conditions of extreme metabolic stress (Sahlin, Harris and Hultman, 1975; Naveri et al, 1978; Boobis, Williams and Wooton, 1982; Jacobs et al, 1982; McCartney et al, 1986;). Possibly with the exception of human sprinters, comparison of the fibre composition of horse middle gluteal and human vastus lateralis generally indicates a much higher proportion of type II fibres in the horse (Snow and Harris, 1985; Costill et al, 1976; Mero, 1985; Macková et al, 1986; see also Introduction). Furthermore, comparison of enzyme activities between the Thoroughbred horse and human athletes

specializing in different events generally indicates a much higher glycolytic capacity in the horse, as indicated by higher activities of phosphorylase, PFK and LDH, although this may simply be a reflection of a higher proportion of type II fibres (Essén et al, 1975; Harris et al, 1976; Lowry et al, 1978). In general, it would appear that substantial decreases in muscle ATP content in the Thoroughbred horse are frequently associated with large increases in muscle lactate and the development of pronounced muscle acidosis. How can acidosis be related to a loss of muscle adenine nucleotide, or more specifically, a decrease in the muscle ATP store ?

At the very onset of exercise, calcium release, increased demand for ATP at the cross-bridges and at the Ca^{2+} pumps in the sarcoplasmic reticulum and increases in ADP will all result in a rapid increase in glycolytic flux, somewhere in the region of a 1000 fold increase over the resting rate (Newsholme, 1988). In the few seconds in which it takes to activate glycolysis, PCr prevents a build up in ADP at the cross-bridges. As oxidative phosphorylation is accelerated, glycolytic ATP production and hence lactate production, may be reduced. During high-intensity exercise where the ATP turnover rate is higher than the rate of ATP generation from oxidative phosphorylation, lactate will continue to accumulate. The threshold at which this occurs may vary between different fibre types. For example, at the same workload type IIA fibres may produce lactate at a much lower rate compared to type IIB fibres due to a higher oxidative capacity in the former. Therefore, at the whole animal level the horse with the lowest muscle oxidative capacity might be expected to show lactate accumulation first in relation to increasing workload. It could be hypothesized that this would be the animal with the highest proportion of its muscle occupied by type IIB fibres, although this may not always be the case, as shown by Valberg and Essén-Gustavsson (1987). Using pooled fibres, they demonstrated that the citrate synthase activity in a pool of IIB fibres from one horse may be higher than the activity in type I fibres from another horse.

Similarly, the activity of LDH in type I pools from some horses was close to that in IIB's from other horses. This may indicate that care should be exercised in assessing muscle oxidative or glycolytic potential solely by histochemical differentiation of fibre types. As demonstrated in Chapter 5, relationships between type IIB fibres and LDH (+ve) and OGDH (-ve) or type I fibres and LDH (-ve) and OGDH (+ve) were not found in four out of the six groups. The greatest number of significant correlations between histochemical parameters and LDH and OGDH activity were found in untrained yearling horses. This may indicate that to a large extent the

relationship between fibre composition and enzyme activity is genetically determined but that it can be significantly altered by training.

The advantage of possessing a high percentage of type II fibres, and in particular a high proportion of IIB's, is that they possess a higher shortening velocity and develop more force compared to type I fibres. The sprinters among the animal kingdom are generally endowed with a higher percentage of type II fibres. For example, amongst the various breeds of horse, the Quarterhorse which has been bred to run as fast as possible over a ¼ of a mile, has the highest percentage of type II fibres in its middle gluteal compared with other breeds (Snow, 1983). The high glycolytic capacity of type II fibres, especially type IIB's, results in high or rapid lactate accumulation, but also allows very high rates of ATP turnover to be supported. A high ATP turnover rate is necessary for high power output and therefore, a high capacity to produce lactate is desirable in short duration events.

The consequences of high lactate accumulation have already been reviewed in the Introduction (see Chapter 1). One of the effects of a decrease in muscle pH is to shift the creatine phosphokinase equilibrium in the direction of ATP formation.

$$PCr + ADP + H^+ \rightleftharpoons ATP + Cr$$
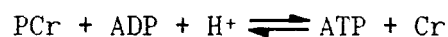
The advantage of this is in the maintenance of a low free ADP content. However, increased H^+ will also result in a lowering of the PCr pool. If it is accepted that PCr is important for spatial as well as temporal buffering of ATP, then a decrease represents a reduced potential to rephosphorylate ADP. With continuing contractions, the pulse of ADP produced with each contraction will become extended in amplitude and time. ADP remaining attached to active sites may compete with ATP and prevent formation of cross-bridges, resulting in a decreased power output (Schoenberg and Eisenberg, 1987). The ADP pulse following each contraction is further attenuated by the myokinase reaction.

$$ADP + ADP \rightleftharpoons ATP + AMP$$

The activity of myokinase in type II fibres is around one order of magnitude lower than that of creatine phosphokinase (Thorstensson et al, 1977) and it can be assumed that the latter is the preferred method for maintaining a low ADP level. Therefore, initially, the removal of the ADP pulse will be essentially via the creatine phosphokinase reaction and

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glycolysis. When the ADP pulse becomes more prolonged and reaches higher concentrations the myokinase reaction will supplement ADP removal.

Accumulation of AMP will slow down the myokinase reaction, again leading to prolonged increases in ADP. AMP can be dephosphorylated to yield adenosine with the release of Pi. This reaction is catalysed by 5'-nucleotidase which has a very low in vitro activity and is therefore not a quantitatively important route for the removal of AMP. High-intensity exercise results in accumulation of H^+, ADP and AMP which have been shown to enhance the activity of AMP-deaminase (Wheeler and Lowenstein, 1979). Due to the low K_m of AMP-deaminase for AMP (see Chapter 8) and the presence of modulating concentrations of ADP and H^+, small increases in AMP will result in activation of AMP-deaminase and the production of IMP. This is seen as a drop in the muscle ATP content due to the tight regulation of ADP and AMP contents. Evidence from a number of studies seems to indicate that during high-intensity exercise the purine nucleotide cycle is not operative to any appreciable extent (Meyer and Terjung, 1979; Katz, Sahlin and Henriksson, 1986; Katz et al, 1986), that is, there is no concurrent AMP deamination and IMP reamination (AMP resynthesis) and as a result there is a decrease in the ATP pool.

A loss of ATP may initially appear undesirable, but it is unlikely that it is a diminishing muscle ATP content that causes fatigue directly. Instead, muscle ATP decrease, representing adenine nucleotide loss, is a reflection of a reduced potential for ADP rephosphorylation and the loss of adenine nucleotide allows the muscle to maintain a low ADP level via the myokinase-AMP-deaminase couplet, facilitating continued work.

Mammalian muscle could therefore be considered to have developed four enzyme systems which all function to keep ADP low: PCr/CPK; myokinase-AMP-deaminase; phosphoglycerate kinase (glycolysis); pyruvate kinase (glycolysis). These systems are initially most active in the order of PCr/CPK, glycolysis, myokinase/AMP-deaminase, although the metabolic changes associated with exercise may alter the relative importance of each system as exercise progresses.

Although AMP-deaminase shows a pH optimum of 6.5, acidosis is not essential for its activation as AMP-deaminase appears to be activated under conditions where the rate of ADP phosphorylation is reduced, i.e. the rate of ATP utilization exceeds the rate of ATP resynthesis. Thus, IMP formation has been shown to occur in relation to glycogen depletion following prolonged submaximal exercise (Norman et al, 1987; Broberg and Sahlin, 1989). Activation of AMP-deaminase in the absence of acidosis was

attributed to increases in ADP and AMP due to an insufficient rate of ATP resynthesis as a result of low muscle glycogen contents. Furthermore, during ischaemia when lactate accumulation and PCr depletion are similar to that seen following high-intensity exercise to fatigue, there is only limited AMP deamination (Larsson and Hultman, 1979; Norman et al, 1988). AMP-deaminase activity has also been demonstrated to occur in relation to hypoxaemia (Sahlin and Katz, 1989).

Premature accumulation of ADP has been implicated in the exercise intolerance observed in patients where either phosphorylase (McArdle's disease, Lewis and Haller, 1986) or PFK (Tauri's disease) are deficient. These patients all exhibit severe muscle pain and fatigue at the onset of moderate to high-intensity exercise, but are generally able to exercise at these intensities if exercise is initiated at a very low workload and the intensity increased slowly. It could be inferred from these observations that at the onset of moderate to high-intensity exercise, following an initial drop in PCr, in the abscence of glycogenolysis to rephosphorylate ADP, ADP accumulates rapidly. These patients also show abnormaly high increases in ammonia, suggesting activation of AMP-deaminase, presumably as a result of the elevated ADP and AMP contents. When work is initiated at a low exercise intensity this presumably allows ADP to be rephosphorylated oxidatively and as the rate of phosphorylation increases, a higher rate of ATP turnover, and hence a higher intensity of exercise, can be supported.

AMP-deaminase deficient patients also fatigue more rapidly during moderate to high-intensity exercise compared to normal controls (Fishbein, Armbrustmacher and Griffin, 1978; Sabina et al, 1980; Sabina et al, 1984). As with McArdle's and Tauri's patients, the premature fatigue may be related to rising ADP levels as a result of a slowing of the myokinase reaction in response to AMP accumulation.

In summary, although the deamination of AMP to IMP during high-intensity exercise is probably the condition under which activation of AMP-deaminase has been most frequently investigated, it is clear that acidosis is not necessary for AMP-deaminase activity. The common theme for activation of AMP-deaminase appears to be transient increases in ADP due to a reduced potential for ATP resynthesis.

A review of the literature indicates that controversy exists regarding the role of different metabolites in the production of fatigue. Of the potential metabolic fatigue agents, H^+, Pi and ADP are the most frequently cited. Fatigue most probably arises through a combination of effects brought about by changes occurring with exercise.

The studies undertaken have investigated some of the metabolic aspects related to exercise in the Thoroughbred horse, with the emphasis on an intensity of exercise comparable to that in racing. Do these studies make any contribution to the general understanding of Thoroughbred race performance ?

Thoroughbred racing in Britain takes place over distances from 5 to 21 furlongs (1000 - 4200 metres) and these distances represent a difference in exercise time of around 1 minute to as long as $4\frac{1}{2}$ minutes. In man, Hermansen and Medbo (1984) demonstrated that the contribution of aerobic metabolism to high-intensity treadmill exercise increased from 22% over 15 sec, to 46% over one minute and up to 59% over 2 min of exercise. It is likely, therefore, that there will be a difference in the aerobic and anaerobic (glycolytic) components of races lasting 1 minute and one lasting for $4\frac{1}{2}$ minutes and that there will be an increase in the importance of the aerobic component as the time of exercise increases also in the horse.

Studies have not yet been undertaken to demonstrate the relative importance of aerobic and anaerobic metabolism in Thoroughbreds racing over different distances. In the study in Chapter 5 (survey) positive relationships were found between Timeform and %IIB, %AOIIB, LDH, %LDH5-isoenzyme and PFK. The horses forming the survey material were raced over distances of between 5 and 14 furlongs, which would seem to indicate that a high proportion of IIB fibres and a high glycolytic capacity is an important factor in determining ability over this range of race distances. As a significant proportion of races are considered to be run tactically in this country, this is perhaps not surprising, as irrespective of the distance the race is generally run at a high but steady pace and the race ends with a sprint for the line over the last 2-3 furlongs.

Thus, it could be speculated the ideal muscle composition is that which allows the horse to maintain racing pace almost entirely aerobically, but with a supply of large IIB fibres that can be recruited towards the end of the race in the sprint for the line. In races run at a very fast speed throughout over distances of around 10f and with an exercise time in excess of two minutes, locomotor muscles high in IIA fibres may be more advantageous. The ideal muscle characteristics will therefore likely vary with the distance of the race (equivalent to exercise duration) and the way in which the race is to be run.

From the studies undertaken in this thesis the following observations can be made:

I   The Thoroughbred horse has both high muscle oxidative and glycolytic capacities as demonstrated by the high activities of various enzymes of oxidative and glycolytic pathways.

II High-intensity exercise in the Thoroughbred horse results in a degree of lactate accumulation infrequently seen in man. The high glycolytic capacity may be a reflection of the apparently higher proportion of type II fibres in the horse compared to man.

III Acidosis as a result of lactate accumulation may be a factor in precipitating loss of muscle adenine nucleotide, although this may not necessarily be a disadvantage under conditions of extreme metabolic stress and may facilitate continued exercise and delay the onset of fatigue.

IV Muscle ATP decrease demonstrates a threshold as opposed to a linear decline with increasing work intensity.

V   The high glycolytic capacity in the Thoroughbred horse is accompanied by a high muscle buffering capacity, both of which are higher when compared to man. This may partly explain the ability of the horse to generate and tolerate higher lactate levels compared to man.

VI Muscle composition may be an important factor in determining ability in the Thoroughbred horse, although the ideal composition for optimum performance over different distances remains unclear.

VII The results of the present thesis indicate that the metabolic stress of high-speed treadmill exercise (12 m/s, 5° incline) as judged by muscle and blood lactate changes, compares closely with the changes seen following maximal gallops in the field over comparable distances.

REFERENCES

Alnaqeeb, M.A. and Goldspink, G. (1986) Changes in fibre type, number and diameter in developing and ageing skeletal muscle. J. Anat. 153, 31-45.

Armstrong, R.B., Saubert, C.W., Seeherman, H.J. and Taylor, C.R. (1982) Distribution of fibre types in locomotory muscles of dogs. Am. J. Anat. 163, 87-98.

Asheim, J.F., Knudsen, O., Lindholm, A., Ruelecker, C. and Saltin, B. (1970) Heart rates and blood lactate concentrations of Standardbred horses during training and racing. J. Am. Vet. Med. Assoc. 157, 304-312.

Asmussen, E. (1950) Pyruvate and lactate content of the blood during and after muscular work. Acta. Physiol. Scand. 20, 125-132.

Astrand, P-O. and Christenssen, E.H. (1964) Aerobic work capacity. In: Oxygen in the Animal Organism. Eds. Dickens, F. and Neil, E. Pergamon Press, London. p 295-314.

Astrand, P-O., Cuddy, T.E., Saltin, B. and Stenberg, J. (1964) Cardiac output during submaximal and maximal work. J. Appl. Physiol. 19, 268-278.

Astrand, P-O., Hultman, E., Juhlin-Dannfelt, A. and Reynolds, G. (1986) Disposal of lactate during and after strenuous exercise in humans. J. Appl. Physiol. 61, 338-343.

Astrand, P-O. and Rodahl, K. (1986) Textbook of Work Physiology: Physiological bases of exercise. McGraw-Hill Book Co., Singapore.

Babij, P., Matthews, S.M. and Rennie, M.J. (1983) Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. Eur. J. Appl. Physiol. 50, 405-411.

Banister, E.W. and Purvis, A.D. (1968) Exercise electrocardiography in the horse by radiotelemetry. J. Am. Vet. Med. Assoc. 152, 1004-1008.

Barlow, D.A., Lloyd, J.M., Hellhake, P. and Seder, J.A. (1984) Equine muscle fiber types: A histological and histochemical analysis of select thoroughbred yearlings. J. Equine Vet. Sci. 4, 60-66.

Bate Smith, E.C. (1938) The buffering of muscle in rigor; protein, phosphate and carnosine. J. Physiol. 92, 336-343.

Bayer, A. (1973) Bewegungsanalysen an Trabrennpferden mit Hilfe der Ungulographie. Zbl. Vet. Med. A 20, 209-221.

Bayly, W.M. (1984) Hypoxemia in exercising horses. Proc. 4th. Annu. Meeting Ass. Equine Sports Med., p 58-73.

Bayly, W.M., Grant, B.D. and Pearson, R.C. (1987) Lactate concentrations in Thoroughbred horses following maximal exercise under field conditions. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 426-437.

Bayly, W.M., Schulz, D.A., Hodgson, D.R. and Gollnick, P.D. (1987) Ventilatory response to exercise in horses with exercise induced hypoxemia. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 172-182.

Beaver, W.L., Wasserman, K. and Whipp, B.J. (1985) Improved detection of the lactate threshold during exercise using log-log transformation. J. Appl. Physiol. 59, 1936-1940.

Benadé, A.J. and Heisler, N. (1978) Comparison of efflux rates of hydrogen and lactate ions from isolated muscles in vitro. Respir. Physiol. 32, 369-380.

Bergmeyer, H.U. (1970) Metoden der Enzymattischem Analyse, Verlag Chemie, Weinheim.

Bergström, J. (1962) Muscle electrolytes in man. Determination by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients and patients with chronic diarrhoea. Scand. J. Clin. Lab. Invest. Suppl. 68, 1-110.

Bergström, J. and Hultman, E. (1966) Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. Nature 210, 309-310.

Bergström, J., Hermansen, L., Hultman, E. and Saltin, B. (1967) Diet, muscle glycogen and physical performance. Acta. Physiol. Scand. 71, 140-150.

Bessman, S.P. and Geiger, P.J. (1981) Transport of energy in muscle: The phosphorylcreatine shuttle. Science, N.Y. 211, 448-452.

Bigland-Ritchie, B., Jones, D.A., Hosking, G.P. and Edwards, R.H.T. (1978) Central and peripheral fatigue in maximum voluntary contractions of human quadriceps muscle. Clin. Sci. Mol. Med. 54, 604-614.

Bigland-Ritchie, B., Jones, D.A. and Woods, J.J. (1979) Excitation frequency and muscle fatigue: electrical responses during human voluntary and stimulated contractions. Exp. Neurol. 64, 414-427.

Bigland-Ritchie, B., Dawson, N.J., Johansson, R.S. and Leppold, O.C.J. (1986) Reflex origin for the slowing of motorneurone firing rates in fatigue of human voluntary contractions. J. Physiol. (Lond.) **379**, 451-459.

Bigland-Ritchie, B., Furbush, F. and Woods, J.J. (1986) Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. J. Appl. Physiol. **61**, 421-429.

Bockman, E. and McKenzie, J. (1983) Tissue adenosine content in active soleus and gracilis muscles of cats. Am. J. Physiol. **244**, H552-H559.

Bonen, A., Campbell, C.J., Kirby, R.L. and Belcastro, A.N. (1979) A multiple regression model for blood lactate removal in man. Pflügers Arch. **380**, 205-210.

Boobis, L.H., Williams, C. and Wooton, S.A. (1982) Human muscle metabolism during brief maximal exercise. J. Physiol. (Lond.), **338**, 21-22.

Boutilier, R.G., Emilio, M.G. and Shelton, G. (1986) Aerobic and anaerobic correlates of mechanical work by gastrocnemius muscles of the aquatic amphibian Xenopus Laevis. J. Exp. Biol. **122**, 223-235.

Broberg, S. and Sahlin, K. (1988) Hyperammonemia during prolonged exercise: effect of glycogen depletion. J. Appl. Physiol. **65**, 2475-2477.

Broberg, S. (1989) Adenine nucleotide degradation and ammonia formation in human skeletal muscle during exercise. Ph.D. Thesis, Karolinska Institute, Stockholm, Sweden.

Brooke, M.H. and Kaiser, K.K. (1970) Muscle fibre types: How many and what kind? Arch. Neurol. **23**, 369-379.

Brooks, G.A. and Gaesser, G.A. (1980) End points of lactate and glucose metabolism after exhausting exercise. J. Appl. Physiol. **49**, 1057-1069.

Brown, D.H. and Cori, C.F. (1961) Animal and plant polysaccharide phosphorylase. In: The Enzymes. Eds. Boyer, P.D., Lardy, H. and Myrbäck, K. Academic Press, New York-London.

Bruce, V. and Turek, R.J. (1985) Muscle fibre variation in the gluteus medius of the horse. Equine Vet. J. **17**, 317-321.

Bülow, J. and Tøndvold, E. (1982) Blood flow in different adipose tissue depots during prolonged exercise in dogs. Pflügers Arch. **392**, 235-238.

241

Bülow, J. (1988) Lipid mobilization and utilization. In: Principles of Exercise Biochemistry, Ed. Poortmans, J.R. Karger, Basel. p 140-163.

Buono, M.J., Clancy, T.R. and Cook, J.R. (1984) Blood lactate and ammonium ion accumulation during graded exercise in humans. J. Appl. Physiol. 57, 135-139.

Buono, M.J. and Yeager, J.E. (1986) Intraerythrocyte and plasma lactate concentrations during exercise in humans. Eur. J. Appl. Physiol. 55, 326-329.

Burke, R.E., Levine, D.N., Tsairis, P. and Zajac, F.E. (1973) Physiological types and histochemical profiles in motor units of the cat gastrocnemius. J. Physiol. (Lond.) 234, 723-748.

Burton, R.F. Intracellular buffering.(1978) Respir. Physiol. 33, 51-58.

Carlson, G.P. and Mansman, R.A. (1974) Serum electrolytes and plasma protein alterations in horses used for endurance rides. J. Am. Vet. Med. Assoc. 165, 262-264.

Carlson, G.P. and Harrold, D.R. (1977) Relationship of protein concentration and water content of equine serum and plasma samples. Vet. Clin. Pathol. 6, 18-20.

Carlsson, L.A., Fröberg, S. and Persson, S.G. (1965) Concentration and turnover of free fatty acids of plasma and concentration of blood glucose during exercise. Acta. Vet. Scand. 63, 434-441.

Castellini, M.A. and Somero, G.N. (1981) Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. J. Comp. Physiol. 143, 191-198.

Catling, S. (1978) Studies on the haematology of exertion in the horse. Ph.D. Thesis, Cambridge.

Cavendish, W. (Duke of Newcastle) (1667) A new method and extraordinary invention to dress horses, and work them according to nature. Thomas Milbourn, London.

Chapman, A.E. and Caldwell, G.E. (1983) Kinetic limitations of maximal sprinting speed. J. Biomech. 16, 79-83.

Chase, P.B. and Kusmerick, M.J. (1988) Effects of pH on contraction of rabbit fast and slow skeletal muscle fibres. Biophys. J. 53, 935-946.

Chasiotis, D. (1983) The regulation of glycogen phosphorylase and glycogen breakdown in human skeletal muscle. Acta. Physiol. Scand. Suppl. **518**.

Cheetham, M.E., Boobis, L.H., Brooks, S. and Williams, C. (1986) Human muscle metabolism during sprint running. J. Appl. Physiol. **61**, 54-60.

Cheetham, M.E. (1988) Effect of training on the metabolic responses to treadmill sprinting in man. Ph.D. Thesis, Loughborough.

Clausen, J.P. (1973) Muscle blood flow during exercise and its significance for maximal performance. In: Limiting Factors of Physical Performance. Ed. Keul, J. Thieme Verlag, Stuttgart. p 253-266.

Clausen, T. (1980) The role of calcium in the activation of the glucose transport system. Cell Calcium. **1**, 311-325.

Coakley, J.H., Wagenmakers, A.J.M. and Edwards, R.H.T. (1989) Excessive ammonia production during cycle ergometry in McArdle's disease. Clin. Sci. **76** (Suppl. 25), 49P.

Colowick, S.P. (1973) The Hexokinases. In: The Enzymes 9, Ed. Boyer, P.D. Academic Press, New York. p 1-48.

Constable, S.H., Favier, R.J. and Holloszy, J.O. (1986) Exercise and glycogen depletion: effects on ability to activate muscle phosphorylase. J. Appl. Physiol. **60**, 1518-1523.

Cooke, R. and Pate, E. (1985) The effects of ADP and phosphate on the contraction of muscle fibres. Biophys. J. **48**, 789-798.

Costill, D.L., Daniels, J., Evans, W., Fink, W., Krahenbuhl, G. and Saltin, B. (1976) Skeletal muscle enzymes and fibre composition in male and female track athletes. J. Appl. Physiol. **40**, 49-54.

Crane, R.K. and Sols, A. (1953) The association of hexokinase with particular fractions of brain and other tissue homogenates. J. Biol. Chem. **203**, 273-292.

Crush, K.G. (1970) Carnosine and related substances in animal tissues. Comp. Biochem. Biophys. **34**, 3-30.

Dahlberg, E. (1983) Estimation of blood contamination of tissue extracts. Anal. Biochem. **130**, 108-113.

Daniel, S.S., Morishima, H.O., James, L.S. and Adamsons, K. (1964) Lactate and pyruvate gradients between red blood cells and plasma during acute asphyxia. J. Appl. Physiol. 19, 1100-1104.

Danforth, W.H. (1965) Activation of glycolytic pathway in muscle. In: Control of Energy Metabolism. Eds. Chance, B. and Estabrook, R.W. Academic Press, New York.

Davey, C.L. (1960) The significance of carnosine and anserine in striated muscle. Arch. Biochem. Biophys. 89, 303-308.

Davies, C.T.M., Knobbs, A.V. and Musgrove, J. (1970) The rate of lactic acid removal in relation to different baselines of recovery exercise. Int. Z. Angew. Physiol. Einschl. Arbeitsphysiol. 28, 155-161.

Dawson, M.J., Gadian, D.G. and Wilkie, D.R. (1978) Muscular fatigue investigated by phosphorus nuclear magnetic resonance. Nature (Lond.) 274, 861-866.

Dawson, M.J., Smith, S. and Wilkie, D.R. (1986) The [$H_2PO_4^{-1}$] may determine cross-bridge cycling rate and force production in living fatiguing muscle. Biophys. J. 49, 268a.

DeLanne, R., Barnes, J.R. and Brouha, L. (1958) Changes in concentration of plasma protein fractions during muscular work and recovery. J. Appl. Physiol. 13, 97-104.

Deuel, N. and Lawrence, L.M. (1985) Effects of velocity on gallop limb contact variables. Proc. Equine Nutr. Physiol. Symp. 9, 254-259.

Devadatta, S.C. (1935) Distribution of lactate between the corpuscles and the plasma in blood. Q. J. Exp. Physiol. 24, 295-303.

DiPrampero, P.E. (1986) Central and peripheral factors limiting VO$_2$max in humans. In: Biochemical Aspects of Physical Exercise. Eds. Benzi, G., Packer, L. and Siliprandi, N., Elsevier Science Publishers B.V., Amsterdam. p 63-71.

Dobson, G.P., Yamamoto, E. and Hochachka, P.W. (1986) Phosphofructokinase control in muscle: nature and reversal of pH-dependent inhibition. Am. J. Physiol. 250, R71-R76.

Dodd, S., Powers, S.K., Callender, T. and Brooks, C. (1984) Blood lactate disappearance at various intensities of recovery exercise. J. Appl. Physiol. 57, 1492-1465.

Donaldson, S.K.B., Hermansen, L. and Bolles, L. (1978) Differential, direct effects of H^+ on Ca^{2+}-activated force of skinned fibres from the soleus, cardiac and adductor magnus muscles of rabbits. Pflügers Arch. 376, 55-65.

Donaldson, S.K.B. (1983) Effect of acidosis on maximum force generation of peeled mammalian skeletal muscle fibres. In: Biochemistry of Exercise. Eds. Knuttgen, H.G., Vogel, J.A. and Poortmans, J. Human Kinetics Publishers, Champaign, Illinois. p 126-133.

Dudley, G.A. and Terjung, R.L. (1985) Influence of aerobic metabolism on IMP accumulation in fast-twitch muscle. Am. J. Physiol. 248, C37-C42.

Dusek, J., Ehrlein, H.J., von Engelhardt, W. and Hornicke, H. (1970) Bezeihungen zwischen Trittlange, Trittfrequenz und Geschwindigkeit bei Pferden. Z. Tierzuchtg. Zuchtg. Biol. 87, 177-188.

Edwards, R.H.T. (1981) Human muscle function and fatigue. In: Human muscle fatigue: Physiological mechanisms. Proceedings of the Ciba Foundation No.82. Eds. Porter, R. and Whelan, J. Pitman Medical Ltd, London. p 1-18.

Ehrlein, H.J., Hörnicke, H., Engelhardt, W.v. and Tolkmitt, G. (1973) Heart frequency during standardized exercise as a measure of the working capacity of horses. Zentralbl. Veterinärmed. A. 20, 188-208.

Ellington, W.R. (1989) Phosphocreatine represents a thermodynamic and functional improvement over other muscle phosphagens. J. Exp. Biol. 143, 177-194.

Ericson, A. and de Verdier, C-H. (1972) A modified method for the determination of 2,3-diphosphoglycerate in erythrocytes. Scand. J. Clin. Lab. Invest. 29, 85-90.

Eriksson, L.S., Broberg, S., Bjorkman, O. and Wahren, J. (1985) Ammonia metabolism during exercise in man. Clin. Physiol. 5, 325-336.

Essén, B., Jansson, E., Henriksson, J., Taylor, A.W. and Saltin, B. (1975) Metabolic characteristics of fibre types in human skeletal muscle. Acta. Physiol. Scand. 95, 153-165.

Essén, B. (1978) Studies on the regulation of metabolism in human skeletal muscle using intermittent exercise as an experimental model. Acta. Physiol. Scand. Suppl. 454.

Essén-Gustavsson, B. (1986) Activity- and inactivity-related muscle adaptation in the animal kingdom. In: Biochemistry of Exercise. Ed. Saltin, B. Human Kinetics Publishers, Champaign, Illinois. p 435-444.

Essén-Gustavsson, B., Lindholm, A., McMiken, D., Persson, S.G.B. and Thornton, J. (1983) Skeletal muscle characteristics of young Standardbreds in relation to growth and training. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J.. Granta Editions, Cambridge. p 200-217.

Essén-Gustavsson, B., Lindholm, A., McMiken, D. and Persson, S.G.B. (1987) Skeletal muscle characteristics of young Standardbreds in relation to growth and early training. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 200-210.

Essén-Gustavsson, B. and Valberg, S. (1987) Blood and muscle ammonia concentrations in horses during treadmill work and after racing. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 456-463.

Evans, B.W. and Cureton, K.J. (1983) Effect of physical conditioning on blood lactate disappearance after supramaximal exercise. Br. J. Sports Med. 17, 40-45.

Evans, D.L. and Rose, R.J. (1987) Maximum oxygen uptake in racehorses: Changes with training state and prediction from submaximal cardiorespiratory measurements. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 52-67.

Evans, D.L. and Rose, R.J. (1988) Determination and repeatability of maximum oxygen uptake and other cardiorespiratory measurements in the exercising horse. Equine Vet. J. 20, 94-98.

Fabiato, A. and Fabiato, F. (1978) Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. J. Physiol. (Lond.) 276, 233-255.

Fernández, A., Costas, M.J., Sillero, M.A.G. and Sillero, A. (1984) Diadenosine tetraphosphate activates AMP deaminase from rat muscle. Biochem. Biophys. Res. Commun. 121, 155-161.

Field (1960) Glycogen deposition diseases. In: The metabolic basis of inherited disease. McGraw-Hill, New York. p 156-207.

Fishbein, W.N., Armbrustmacher, V.W. and Griffin, J.L. (1978) Myoadenylate deficiency. Science, Wash.DC., 200, 545-548.

Fitts, R.H. and Holloszy, J.O. (1976) Lactate and contractile force in frog muscle during development of fatigue and recovery. Am. J. Physiol. 231, 430-433.

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Fitts, R.H. and Holloszy, J.O. (1977) Contractile properties of rat soleus muscle: effects of training and fatigue. Am. J. Physiol. 2, C86-C91.

Fitts, R.H. and Holloszy, J.O. (1978) Effects of fatigue and recovery on contractile properties of frog muscle. J. Appl. Physiol. 45, 899-902.

Fitts, R.H. and Metzger, J.M. (1988) Mechanisms of muscular fatigue. In: Principles of Exercise Biochemistry. Ed. Poortmans, J.R. Karger, Basel. p 212-229.

Foster, C.V.L., Harman, J., Harris, R.C. and Snow, D.H. (1986) ATP distribution in single muscle fibres before and after maximal exercise in the Thoroughbred horse. J. Physiol. (Lond.) 378, 64P.

Fox, E.L. and Mathews, D.K. (1981) The physiological basis of physical education and athletics. CBS College Publishing, USA.

Fox, G., Henckel, P., Juel, C., Falk-Rønne, J. and Saltin, B. (1987) Skeletal muscle buffer capacity changes in Standardbred horses: effects of growth and training. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 341-347.

Fredholm, B.B. (1971) The effect of lactate in canine subcutaneous adipose tissue in situ. Acta. Physiol. Scand. 81, 110-123.

Freund, H. and Gendry, P. (1978) Lactate kinetics after short strenuous exercise in man. Eur. J. Appl. Physiol. 39, 123-135.

Freund, H. and Zouloumian, P. (1981) Lactate after exercise in man: I. Evolution kinetics in arterial blood. Eur. J. Appl. Physiol. 46, 121-133.

Galbo, H. (1983) Hormonal and metabolic adaptation to exercise. Thieme, Stuttgart.

Galbo, H., Richter, E.A. and Sonne, B. (1986) On the accuracy of fuel mobilization in exercise. In: Biochemistry of Exercise. Ed. Saltin, B. Human Kinetics Publishers, Champaign, Illinois. Ed. Saltin, B. p 223-226.

Garnett, R.A.F., O'Donovan, M.J., Stephens, J.A. and Taylor, A. (1978) Motor unit organization of human medial gastrocnemius. J. Physiol. (Lond.) 287, 33-43.

Gass, G.C., Rogers, S. and Mitchell, R. (1981) Blood lactate concentration following maximum exercise in trained subjects. Br. J. Sports Med. 15, 172-176.

Gillespie, J.R. (1975) The role of the respiratory system during exertion. J. S. Afr. Vet. Assoc. 45, 305-309.

Gisolfi, C., Robinson, S. and Turrell, E.S. (1966) Effects of aerobic work performed during recovery from exhausting work. J. Appl. Physiol. 21, 1767-1772.

Gollnick, P.D., Armstrong, R.B., Saubert, C.W., Piehl, K. and Saltin, B. (1972) Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J. Appl. Physiol. 33, 312-319.

Gollnick, P.D., Armstrong, R.B., Saltin, B., Saubert, C.W., Sembrowich, W.L. and Sheperd, R.E. (1973a) Effect of training on enzyme activity and fibre composition of human skeletal muscle. J. Appl. Physiol. 36, 107-111.

Gollnick, P.D., Armstrong, R.B., Sembrowich, W.L., Sheperd, R.E. and Saltin, B. (1973b) Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise. J. Appl. Physiol. 34, 615-618.
Gregg, I. and Nunn, A.J. (1973) Peak expiratory flow in normal subjects. Br. Med. J. 3, 282-p.
Greenhaff, P.L. (1988) Diet, acid-base status and metabolism during high-intensity exercise in man. Ph.D. Thesis, University of Aberdeen.
Gunn, H.M. (1978a) Differences in the histochemical properties of different breeds of horses and dogs. J. Anat. 127, 615-634.

Gunn, H.M. (1978b) The proportion of muscle, bone and fat in two different types of dog. Res. Vet. Sci. 24, 277-282.

Gunn, H.M. (1987) Muscle, bone and fat proportions and muscle distribution of Thoroughbreds and other horses. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 253-264.

Guy, P.S. and Snow, D.H. (1977) The effect of training and detraining on muscle composition in the horse. J. Physiol. (Lond.) 269, 33-51.

Guy, P.S. (1978) Factors influencing muscle fibre composition in the horse. Ph.D. Thesis, Glasgow.

Hales, S. (1733) Experiments made on the blood and blood vessels of animals. In: Statistical essays containing haemostatics (II). Innys and Manby, London.

Hales, E.N., Luzio, J.P. and Siddle, K. (1978) Hormonal control of adipose tissue lipolysis. Biochem. Soc. Symp. 43, 97-135.

Hall, W.C. and Brody, S. (1934) The energy cost of horizontal walking in cattle and horses of various ages and body weights. Mo. Univ. Agric. Res. Stat. Bull. 208, 3-16.

Hambleton, P.L., Slade, L.M., Hamar, D.W., Kienholz, E.W. and Lewis, L.D. (1980) Dietary fat and exercise conditioning effect on metabolic parameters in the horse. J. Anim. Sci. 51, 1330-1339.

Harkness, R.A. (1988) Hypoxanthine, xanthine and uridine in body fluids, indicators of ATP depletion. J. Chromatogr. 429, 255-278.

Harkness, R.A., McCreanor, G.M. and Watts, R.W.E. (1988) Lesch-Nyhan syndrome and its pathogenesis: purine concentrations in plasma and urine with metabolite profiles in CSF. J. Inher. Metab. Dis. 11, 239-252.

Harris, R.C., Hultman, E. and Nordesjö, L-O. (1974) Glycogen, glycolytic intermediates and high energy phosphates in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand. J. Clin. Lab. Invest. 33, 109-120.

Harris, R.C., Edwards, R.H.T., Hultman, E., Nordesjö, L.O., Nylind, B. and Sahlin, K. (1976) The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. Pflügers Arch. 367, 137-142.

Harris, R.C., Sahlin, K. and Hultman, E. (1977) Phosphagen and lactate contents of m. quadriceps femoris of man after exercise. J. Appl. Physiol. 43, 852-857.

Harris, R.C. (1981) Muscle energy metabolism in man in response to isometric contraction: a biopsy study. M.Sc. Thesis, University of Wales.

Harris, R.C., Sahlin, K. and Hultman, E. (1981) Metabolic recovery in human muscle following isometric contraction to fatigue. Pflügers Arch. 389, 277-282.

Harris, R.C. (1985) Muscle fatigue: the other side of the performance coin. Equine Vet. J. 17, 409-411.

Harris, R.C. and Hultman, E. (1985) Adenine nucleotide depletion in muscle in response to intermittent stimulation in situ. J. Physiol. (Lond.) 365, 73.

Harris, R.C., Katz, A., Sahlin, K. and Snow, D.H. (1989) Effect of freeze-drying on measurements of pH in biopsy samples of the middle gluteal muscle in the horse: comparison of muscle pH to the pyruvate and lactate content. Equine Vet. J. 21, 45-47.

Hering (1829) Cited by Woodcock, J.P. (1983) Noninvasive blood flow measurement. CRC Crit. Rev. Biomed. Eng. **12**, 201-235.

Hermansen, L. (1981) Muscle fatigue during maximal exercise of short duration. Med. Sport. **13**, 45-52.

Hermansen, L. and Osnes, J.B. (1972) Blood and muscle pH after maximal exercise in man. J. Appl. Physiol. **32**, 304-308.

Hermansen, L. and Vaage, O. (1977) Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am. J. Physiol. **233**, E422-E429.

Hermansen, L. and Medbo, J.I. (1984) The relative significance of aerobic and anaerobic processes during maximal exercise of short duration. Med. Sport Sci. **17**, 56-67.

Hickson, R., Hagberg, J. and Conlee, R., Jones, D.A., Ehsani, A.A. and Winder, W.W. (1979) Effect of training on hormonal responses to exercise in competitive swimmers. Eur. J. Appl. Physiol. **41**, 211-219.

Hodgson, D.R., Rose, R.J. and Allen, J.R. (1983) Muscle glycogen depletion and repletion patterns in horses performing various distances of endurance exercise. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J.. Granta Editions, Cambridge. p 229-236.

Hodgson, D.R., Rose, R.J., Allen, J.R. and Dimauro, J. (1984) Glycogen depletion patterns in horses performing maximal exercise. Res. Vet. Sci. **36**, 169-173.

Hodgson, D.R., Rose, R.J., Dimauro, J. and Allen, J.R. (1985) Effects of a submaximal treadmilltraining programme on histochemical properties, enzyme activities and glycogen utilisation of skeletal muscle in the horse. Equine Vet. J. **17**, 300-305.

Hodgson, D.R., Rose, R.J., Dimauro, J. and Allen, J.R. (1986) Effects of training on muscle composition in horses. Am. J. Vet. Res. **47**, 12-15.

Högberg, P. (1952) How do stride length and stride frequency influence the energy output during running? Arbeitsphysiol. **14**, 437-p.

Hohorst, H.S. (1963) L-(+)-lactate. In: Methods of Enzymatic Analysis. Ed. Bergmeyer, H.U. Academic Press, New York. p 266-270.

Hoppeler, H. (1986) Muscle aerobic potential in the animal kingdom. In: Biochemistry of Exercise. Ed. Saltin, B. Human Kinetics Publishers, Champaign, Illinois. p 417-434.

Hoppeler, H., Jones, J.H., Lindstedt, S.L., Claassen, H., Longworth, K.E., Taylor, C.R., Straub, R. and Lindholm, A. (1987a) Relating maximal oxygen consumption to skeletal muscle mitochondria in horses. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 278-289.

Hoppeler, H., Kayar, S.R., Claassen, H., Uhlmann, E. and Karas, R.H. (1987b) Adaptive variation in the mammalian respiratory system in relation to energetic demand: III. Skeletal muscles: setting the demand for oxygen. Respir. Physiol. 69, 27-46.

Hörnicke, H., Weber, M. and Schweiker, W. (1987) Pulmonary ventilation in Thoroughbred horses at maximum performance. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 216-224.

Huckabee, W.E. (1956) Control of concentration gradients of pyruvate and lactate across cell membranes in blood. J. Appl. Physiol. 9, 163-170.

Hultman, E., Bergström, J. and McLennan-Anderson, N. (1967) Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with musscular work in man. Scand. J. Clin. Lab. Invest. 19, 56-66.

Hultman, E. and Sahlin, K. (1980) Acid-base balance during exercise. In: Exercise and Sports Science Reviews. Eds. Hutton, R.S. and Miller, D. Franklin Institute Press, Philadelphia. p 41-128.

Hultman, E. and Sjöholm, H. (1983) Energy metabolism and contraction force of human skeletal muscle in situ during electrical stimulation. J. Physiol. (Lond.) 345, 525-532.

Hultman, E. and Sjöholm, H. (1986) Biochemical causes of fatigue. In: Human Muscle Power. Eds. Jones, N.L., McCartney, N. and McComas, A.J. Human Kinetics, Champaign, Illinois. p 215-238.

Hultman, E., Spriet, L.L. and Söderlund, K. (1987) Energy metabolism and fatigue in working muscle. In: Exercise: Benefits, Limits and Adaptations. Eds. Macleod, R., Maughan, M., Nimmo, M., Reilly, T. and Williams, C. E & F.N. Spon, London and New York. p 63-84.

Hultman, E. and Harris, R. (1988) Carbohydrate Metabolism. In: Principles of Exercise Biochemistry. Karger, Basel. p 78-119.

Issekutz, B., Shaw, W.A.S. and Issekutz, A.C. (1976) Lactate metabolism in resting and exercising dogs. J. Appl. Physiol. 40, 312-319.

Iwaoka, K., Hatta, H., Atomi, Y. and Miyashita, M. (1988) Lactate, respiratory compensation thresholds and distance running performance in runners of both sexes. Int. J. Sports Med. 9, 306-309.

Jacobs, I., Bar-Or, O., Karlsson, J., Dotan, R., Tesch, P., Kaiser, P. and Inbar, O. (1982) Changes in muscle metabolites in females with 30-S exhaustive exercise. Med. Sci. Sports. Exerc. 14, 457-460.

Jansson. E., Dudley, G.A., Norman, B. and Tesch, P.A. (1987) ATP and IMP in single human fibres after high intensity exercise. Clin. Physiol. 7, 337-345.

Johnson, R.E., Edwards, H.T., Dill, D.B. and Wilson, J.W. (1945) Blood as a physicochemical system. XIII. The distribution of lactate. J. Biol. Chem. 157, 461-473.

Jorfeldt, L. (1970) Metabolism of L(+)-lactate in human skeletal muscle during exercise. Acta. Physiol. Scand. Suppl. 338.

Jorfeldt, L. and Wahren, J. (1971) Leg blood flow during exercise in man. Clin. Sci. 41, 459-473.

Jorfeldt, L., Juhlin-Dannfelt, A. and Karlsson, J. (1978) Lactate release in relation to tissue lactate in human skeletal muscle during exercise. J. Appl. Physiol. 44, 350-352.

Joyce, H. and Poortmans, J.R. (1970) Haematocrit and serum proteins during arm exercise. Med. Sci. Sports Exerc. 2, 187-190.

Judson, G.J. and Mooney, G.J. (1983) Body water and water turnover rate in Thoroughbred horses in training. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J., Granta Editions, Cambridge. p 371-376.

Judson, G.J., Frauenfelder, H.C., and Mooney, G.J. (1983) Plasma biochemical changes in Thoroughbred racehorses following submaximal and maximal exercise. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J.. Granta Editions, Cambridge. p 354-361.

Karas, R.H., Taylor, C.R., Rösler, K. and Hoppeler, H. (1987) Adaptive variation in mammalian respiratory system in relation to energetic demand: V. Limits to oxygen transport by the circulation. Respir. Physiol. 69, 65-79.

Karlsson, J. and Saltin, B. (1970) Lactate, ATP and CP in working muscles during exhaustive exercise in man. J. Appl. Physiol. 29, 598-602.

Karlsson, J., Nordesjö, L.O., Jordfeldt, L. and Saltin, B. (1972) Muscle lactate, ATP and CP levels during exercise after physical training in man. J. Appl. Physiol. 33, 199-203.

Kasvinsky, P.J. and Meyer, W.L. (1977) The effect of pH and temperature on the kinetics of native and altered glycogen phosphorylase. Arch. Biochem. Biophys. 181, 616-631.

Katz, A. (1986) Regulation of lactic acid production during muscle contraction. Ph.D. Thesis, Huddinge University Hospital, Sweden.

Katz, A., Broberg, S., Sahlin, K. and Wahren, J. (1986) Muscle ammonia and amino acid metabolism during dynamic exercise in man. Clin. Physiol. 6, 365-379.

Katz, A., Sahlin, K. and Henriksson, J. (1986) Muscle ammonia metabolism during isometric contraction in humans. Am. J. Physiol. 250, C834-C840.

Kawai, M., Guth, K., Winnikes, K., Haist, C. and Ruegg, J.C. (1987) The effect of inorganic phosphate on the ATP hydrolysis rate and tension transients in chemically skinned rabbit psoas fibres. Pflügers Arch. 408, 1-9.

Keenan, D.M. (1979) Changes of blood metabolites in horses after racing, with particular reference to uric acid. Aust. Vet. J. 55, 54-57.

Ketai, L.H., Simon, R.H., Kreit, J.W. and Grum, C.M. (1987) Plasma hypoxanthine and exercise. Am. Rev. Respir. Dis. 136, 98-101.

Kiley-Worthington, M. (1987) The behaviour of horses in relation to management and training. J.A. Allen, London.

Kline, K.H., Lawrence, L.M., Novakofski, J. and Bechtel, P.J. (1987) Changes in muscle fibre type variation within the middle gluteal of young and mature horses as a function of sampling depth. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 271-277

Krebs, E.G., Graves, D.J. and Fischer, E.H. (1959) Factors affecting the activity of muscle phosphorylase b kinase. J. Biol. Chem. 234, 2867-2873.

Krebs, E.G., Love, D.S., Bratwold, G.E., Trayser, K.A., Meyer, W.L. and Fischer, E.H. (1964) Purification and properties of rabbit skeletal muscle phosphorylase b kinase. Biochemistry 3, 1022-1033.

Kubo, K., Senta, T. and Sugimoto, O. (1974) Relationship between training and heart size in the Thoroughbred racehorse. Exp. Rep. Equine Hlth. Lab. 11, 87-93.

Kubo, K., Takagi, S., Murakami, M. amd Kai, M. (1984) Heart rate and blood lactate concentration of horses during maximal work. Bull. Equine Res. Inst. 21, 39-45.

Lamb, D.R. (1984) Physiology of Exercise: Responses and adaptations. MacMillan Publishing Co., New York. p 115.

Larsson, J. and Hultman, E. (1979) The effect of long-term arterial occlusion on energy metabolism of the human quadriceps muscle. Scand. J. Clin. Lab. Invest. 39, 257-264.

Leach, D.H. and Cymbaluk, N.F. (1986) Relationships between stride length, stride frequency, velocity and morphometrics of foals. Am. J. Vet. Res. 47, 2090-2097.

Leach, D.H. (1987) Locomotion of the athletic horse. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 516-535

Leonard, J.I., Leach, C.S. and Rambaut, P.C. (1983) Quantitation of tissue loss during prolonged space flight. Am. J. Clin. Nutr. 38, 667-679.

Lewis, S.F. and Haller, R.G. (1986) The pathophysiology of McArdles disease: clues to regulation in exercise and fatigue. J. Appl. Physiol. 61, 391-401.

Lindholm, A. and Piehl, K. (1974) Fibre composition, enzyme activity and concentration of metabolites and electrolytes in muscles of Standardbred horses. Acta. Vet. Scand. 15, 287-309.

Lindholm, A., Essén-Gustavsson, B., McMiken, D., Persson, S. and Thornton, J.R. (1983) Muscle histochemistry and biochemistry of Thoroughbred horses during growth and training. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J. Granta Editions, Cambridge. p 211-217.

Lindstedt, S.L., Wells, D.W., Jones, J.H., Hoppeler, H. and Thronson, H.A. (1988) Limitations to aerobic performance in mammals: Interaction of structure and demand. Int. J. Sports Med. 9, 210-217.

Lortie, G., Simoneau, J.A., Hand, P., Boulay, M.R. and Bouchard, C. (1985) Relationships between skeletal muscle characteristics and aerobic performance in sedentary and active subjects. Eur. J. Appl. Physiol. 54, 471-475.

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Lowry, C.V., Kimmey, J.S., Felder, S., Chi, M.M.Y., Kaiser, K.K., Passoneau, P.N., Kirk, K.A. and Lowry, O.H. (1978) Enzyme patterns in single human muscle fibres. J. Biol. Chem. 253, 8269-8277.

Lowry, O.H., Passoneau, J.V., Hasselberger, F.X. and Schulz, D.W. (1964) Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J. Biol. Chem. 239, 18-30.

Macková, E.V., Melichna, J., Novák, J., Fric, J. and Sprynarova, S. (1986) Histochemical and metabolic characteristics of athletes of different kinds of sports. In: Physiological Biochemistry of Exercise and Training. Eds. Tsopanakis, A. and Poortmans, J. Eptalofos, S.A., Athens. p 155-160.

Mainwood, G.W. and Renaud, J.M. (1984) The effect of acid-base balance on fatigue of skeletal muscle. Can. J. Physiol. Pharmacol. 63, 403-416.

Manohar, M. (1987) Furosemide and systemic circulation during severe exercise. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 132-147.

McCartney, N., Spriet, L.L., Heigenhauser, G.J.F., Kowalchuck, J.M., Sutton, J.R., and Jones, N.L. (1986) Muscle power and metabolism in maximal intermittent exercise. J. Appl. Physiol. 60, 1164-1169.

McCutcheon, L.J., Kelso, T.B., Bertocci, L.A., Hodgson, D.R., Bayly, W.M. and Gollnick, P.D. (1987) Buffering and aerobic capacity in equine muscle: variation and the effect of training. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 348-358.

Margaria, R., Edwards, H.T. and Dill, D.B. (1933) The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. Am. J. Physiol. 106, 689-715.

Martin, S.E. and Bockman, E.L. (1986) Adenosine regulates blood flow and glucose uptake in adipose tissue in dogs. Am. J. Physiol. 250, H1127-H1135.

Mason, D.K. and Kwok, H.W. (1977) Some haematological and biochemical parameters in racehorses in Hong Kong. Equine Vet. J. 9, 96-99.

Mero, A., Luhtanen, P., Viitasalo, J.T. and Komi, P.V. (1981) Relationships between maximal running velocity, muscle fibre characteristics, force production and force relaxation of sprinters. Scand. J. Sports Sci. 3, 16-22.

Mero, A. (1985) Relationships between muscle fibre characteristics, sprinting and jumping of sprinters. Biol. Sport. 2, 155-161.

Mero, A. and Komi, P.V. (1985) Effects of supramaximal velocity on biomechanical variables in sprinting. Int. J. Sport Biomech. 1, 240-252.

Meyer, R.A. and Terjung, R.L. (1979) Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. Am. J. Physiol. 237, C111-C118.

Meyer, R.A., Dudley, G.A. and Terjung, R.L. (1980) Ammonia and IMP in different skeletal muscle fibres after exercise in rats. J. Appl. Physiol. 49, 1037-1041.

Meyer, R.A., Brown, T.R. and Kushmerick, M.J. (1984) Phosphorus nuclear magnetic resonance of fast and slow twitch muscle. Am. J. Physiol. 248, C279-C287.

Meyer, R.A., Sweeney, H.L. and Kushmerick, M.J. (1984) A simple analysis of the " phosphocreatine shuttle ". Am. J. Physiol. 15, C365-C377.

Mineo, I., Kono, N., Shimizu, T., Hara, N., Yamada, Y., Somi, S., Nonaka, K. and Rarui, S. (1985) Excess purine degradation in exercising muscle of patients with glycogen storage disease types V and VII. J. Clin. Invest. 76, 556-560.

Mumford, J.A. and Rossdale, P.D. (1980) Virus and its relationship to the 'poor performance' syndrome. Equine Vet. J. 9, 178-180.

Munro, H.N. and Allison, J.B. (Eds.) (1969) Mammalian protein metabolism vol.3., Academic Press, New York. p 133-182.

Mutch, B.J.C. and Banister, E.W. (1983) Ammonia metabolism in exercise and fatigue: a review. Med. Sci. Sports. Exerc. 15, 41-50.

Nachlas, M.M., Walker, D.G. and Seligman, A.M. (1957) Cytochemical demonstration of succinic dehydrogenase by the use of p-Nitrophenyl substituted diterazole. J. Histochem. Cytochem. 5, 420-436.

Nakamura, Y. and Schwartz, A. (1970) Possible control of intracellular calcium metabolism by H^+: Sarcoplasmic reticulum of cardiac and skeletal muscle. Biochem. Biophys. Res. Commun. 41, 830-836.

Nakamura, Y. and Schwartz, A. (1972) The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J. Gen. Physiol. 59, 22-32.

Naveri, H., Rehunen, S., Kuoppasalmi, K., Tulikoura, I. and Harkonen, M. (1978) Muscle metabolism during and after strenuous intermittent running. Scand. J. Clin. Lab. Invest. 38, 329-336.

Naylor, J.M., Kronfeld, D.S., Freeman, D.E. and Richardson, D. (1984) Hepatic and extrahepatic lactate metabolism in sheep: effects of lactate loading and pH. Am. J. Physiol. **247**, E747-E755.

Newsholme, E. and Start, C. (1973) Regulation in metabolism. John Wiley & Sons, London. p 98.

Newsholme, E. (1988) Application of knowledge of metabolic integration to the problem of metabolic limitations in sprints, middle distance and marathon running. In: Principles of Exercise Biochemistry. Ed. Poortmans, J.R. Karger, Basel. p 194-211.

Nimmo, M.A. and Snow, D.H. (1983) Changes in muscle glycogen, lactate and pyruvate concentration in the Thoroughbred horse following maximal exercise. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J. Granta Editions, Cambridge. p 237-244.

Norman, B., Sollevi, A., Kaijser, L. and Jansson, E. (1987) ATP breakdown products in human skeletal muscle during prolonged exercise to exhaustion. Clin. Physiol. **7**, 503-510.

Norman, B., Dudley, G., Heden, P., Sollevi, A., Tesch, P. and Jansson, E. (1988) IMP accumulation in human skeletal muscle during stress. Can. J. Sport Sci. **13**, 26P.

Nosek, T.M., Fender, K.Y. and Godt, R.E. (1987) It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibres. Science, Wash.DC. **236**, 191-193.

Oscai, L.B. and Palmer, W.K. (1983) Cellular control of triacylglycerol metabolism. Exerc. Sport Sci. Rev. **11**, 1-23.

Padykula, H.A. and Herman, E. (1955) The specificity of the histochemical method for adenosine triphosphatase. J. Histochem. Cytochem. **3**, 170-195.

Park, J.H., Brown, R.L., Park, C.R., Cohn, M. and Chance, B. (1988) Energy metabolism of the untrained muscle of elite runners as observed by ^{31}P magnetic resonance spectroscopy: Evidence suggesting a genetic endowment for endurance exercise. Proc. Natl. Acad. Sci. USA **85**, 8780-8784.

Parkhouse, W.S., McKenzie, D.C., Hochochka, P.W., Mommsen, T.P., Ovalle, W.K., Shinn, S.L. and Rhodes, E.C. (1983) The relationship between carnosine levels, buffering capacity, fibre type and anaerobic capacity in elite athletes. In: Biochemistry of Exercise. Eds. Knuttgen, H., Vogel, J. and Poortmans, J. Human Kinetics Publishers. Champaign, Illinois, p 275-278.

Parkhouse, W.S., McKenzie, D.C., Hochachka, P.W. and Ovalle, W.K. (1985) Buffering capacity of deproteinized human vastus lateralis muscle. J. Appl. Physiol. **58**, 14-17.

Persson, S.G.B. (1967) On blood volume and working capacity in horses. Acta. Vet. Scand. Suppl. **19**.

Persson, S.G.B. (1968) Blood volume, state of training and working capacity of race horses. Equine Vet. J. **1**, 52-62.

Piehl, K. (1974) Glycogen storage and depletion in human skeletal muscle fibers. Acta. Physiol. Scand. Suppl. **402**

Pösö, A.R., Soveri, T. and Oksanen, H.E. (1983) The effect of exercise on blood parameters in Standardbred and Finnish-bred horses. Acta Vet. Scand. **24**, 170-184.

Pratt, G.W. (1983) Remarks on gait analysis. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J. Granta Editions, Cambridge. p 245-262.

Randall, J. and Morris, T. (1985) Horse Racing: The Records. Guinness Superlatives Ltd., Great Britain.

Ratzlaff, M.H., Shindell, R.M. and White, K.K. (1985) The interrelationships of stride lengths and stride times to velocities of galloping horses. J. Equine Vet. Sci. **5**, 279-283.

Ricciardi, L., Bucx, J.J.J, and Terkeurs, H.E.D.J. (1986) Effects of acidosis on force - sarcomere length and force velocity relations of rat cardiac muscle. Cardiovascular Res. **20**, 117-123.

Rice, C.L., Pettigrew, F.P., Noble, E.G. and Taylor, A.W. (1988) The fibre composition of skeletal muscle. In: Principles of Exercise Biochemistry. Ed. Poortmans, J.R. Karger, Basel. p 22-39.

Richter, E.A. and Galbo, H. (1986) High glycogen levels enhance glycogen breakdown in isolated contracting skeletal muscle. J. Appl. Physiol. **61**, 827-831.

Ronca-Testoni, S., Raggi, A. and Ronca, G. (1970) Muscle AMP aminohydrolase. III. A comparitive study on the regulatory properties of skeletal muscle enzymes from various species. Biochem. Biophys. Acta. **198**, 101-112.

Rose, R.J. and Davis, P.D. (1977) Treatment of atrial fibrillation in three racehorses. Equine Vet. J. **9**, 68-71.

Rose, R.J., Pardue, R.A. and Hensley, W. (1977) Plasma biochemistry alterations during an endurance ride. Equine Vet. J. 9, 122-126.

Rose, R.J. and Evans, D.L. (1987) Cardiovascular and respiratory function in the athletic horse. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 1-24.

Rose, R.J., Hodgson, D.R., Kelso, T.B., McCutcheon, L.J., Reid, T.A., Bayly, W.M. and Gollnick, P.D. (1988) Maximum O_2 uptake, O_2 debt and deficit, and muscle metabolites in Thoroughbred horses. J. Appl. Physiol. 64, 781-788.

Rost, R. (1982) "The Athlete's Heart", Eur. Heart. J. 3(Suppl.A), 193-198.

Sabina, R.L., Swain, J.L., Patten, B.M., Ashizawa, T., O'Brien, W.E. and Holmes, E.W. (1980) Disruption of the purine nucleotide cycle. A potential explanation for muscle dysfunction in myoadenylate deaminase deficiency. J. Clin. Invest. 66, 1419-1423.

Sabina, R.L., Swain, J.L., Bradley, W.G. and Holmes, E.W. (1984a) Quantitation of metabolites in human skeletal muscle during rest and exercise: a comparison of methods. Muscle Nerve, 7, 77-82.

Sabina, R.L., Swain, J.L., Olanow, C.W., Bradley, W.G., Fishbein, W.N., DiMauro, S. and Holmes, E.W. (1984b) Myoadenylate deaminase deficiency. Functional and metabolic abnormalities associated with the disruption of the purine nucleotide cycle. J. Clin. Invest. 73, 720-730.

Sahlin, K., Harris, R.C. and Hultman, E. (1975) Creatine kinase equilibrium and lactate compared with muscle pH in tissue samples obtained after isometric exercise. Biochem. J. 152, 173-180.

Sahlin, K., Harris, R.C., Nylind, B. and Hultman, E. (1976) Lactate content and pH in muscle samples obtained after dynamic exercise. Pflügers Arch. 367, 143-149.

Sahlin, K. (1978) Intracellular pH and energy metabolism in skeletal muscle of man. Acta. Physiol. Scand. Suppl. 455.

Sahlin, K., Alvestrand, A., Brandt, R. and Hultman, E. (1978) Intracellular pH and bicarbonate concentration in human muscle during recovery from exercise. J. Appl. Physiol. 45, 474-480.

Sahlin, K., Edström, L., Sjöholm, H. and Hultman, E. (1981) Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. Am. J. Physiol. 240, C121-C126.

Sahlin, K. (1983) Effect of acidosis on energy metabolism and force generation in skeletal muscle. In: Biochemistry of Exercise. Eds. Knuttgen, H.G., Vogel, J.A. and Poortmans, J. Human Kinetics Publishers, Champaign, Illinois. p 151-160.

Sahlin, K. (1986) Metabolic changes limiting muscle performance. In: Biochemistry of Exercise. Ed. Saltin, B. Human Kinetics Publishers, Champaign, Illinois. p 323-343.

Sahlin, K. and Katz, A. (1988) Purine nucleotide metabolism. In: Principles of Exercise Biochemistry. Ed. Poortmans, J.R. Karger, Basel. p 120-139.

Sahlin, K., Broberg, S. and Ren, J.M. (1989) Formation of inosine monophosphate (IMP) in human skeletal muscle during incremental dynamic exercise. Acta. Physiol. Scand. 136, 193-198.

Sahlin, K. and Katz, A. (1989) Hypoxaemia increases the accumulation of inosine monophosphate (IMP) in human skeletal muscle during submaximal exercise. Acta. Physiol. Scand. 136, 199-203.

Saltin, B. and Hermansen, L. (1967) Glycogen stores and prolonged severe exercise. Symposia of the Swedish Nutrition Foundation V. Ed. Blix, G. p 32-46.

Saltin, B. and Gollnick, P.D. (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: Handbook of Physiology, Eds. Peachey, L.D., Adrian, R.H. and Geiger, S.R. Sect. 10: Skeletal Muscle. Williams and Wilkins, Bethesda/Baltimore. p 555-663.

Savabi, F., Geiger, P.J. and Bessman, S.P. (1984) Myofibrillar end of the creatine phosphate energy shuttle. Am. J. Physiol. 247, C424-C432.

Schädler, M. (1967) Proportionale Aktivierung von ATPase-Aktivät und Kontraktionsspannung durch Calciumionen in isollierten contractilen Strukturen verschiedener Muskelarten. Pflügers Arch. 296, 70-90.

Schäfer, M. (1975) The language of the horse: Habits and forms of expression. Kaye and Ward, London. p 134-155.

Schoenberg, M. and Eisenberg, E. (1987) ADP binding to myosin cross-bridges and its effect on the cross-bridge detachment rate constants. J. Gen. Physiol. 89, 905-920.

Sejersted, O.M., Medbø, J.I., Orheim, A. and Hermansen, L. (1984) Relationship between acid-base status and electrolyte balance after maximal work of short duration. In: Physiological Chemistry of Training and Detraining. Eds. Marconnet, P., Poortmans, J. and Hermansen, L. Karger, Basel. p 40-55.

Seo, Y. (1984) Effects of extracellular pH on lactate efflux from frog sartorius muscle. Am. J. Physiol. 247, C175-C181.

Setlow, B. and Lowenstein, J.M. (1967) Adenylate deaminase. Purification and some regulatory properties of the enzyme from calf brain. J. Biol. Chem. 242, 607-615.

Siemankowski, R.F. and White, H.D. Kinetics of the interaction between actin, ADP and cardiac myosins. J. Biol. Chem. 251, 5045-5053.

Sjøgaard, G. (1983) Electrolytes in slow and fast muscle fibres of humans at rest and with dynamic exercise. Am. J. Physiol. 245, R25-R31.

Snow, D.H. and Guy, P.S. (1976) Percutaneous needle muscle biopsy in the horse. Equine Vet. J. 8, 87-93.

Snow, D.H. and Guy, P.S. (1979) The effect of training and detraining on several enzymes in horse skeletal muscle. Arch. Int. Physiol. Biochem. 87, 87-93.

Snow, D.H., Baxter, P. and Rose, R. (1981) Muscle fibre composition and glycogen depletion in horses competing in an endurance ride. Vet.Rec. 108, 374-378.

Snow D.H. and Guy, P.S. (1981) Fibre type and enzyme activities of the gluteus medius in different breeds of horse. In: Biochemistry of Exercise. Eds. Poortmans, J. and Niset, G. University Park Press, Baltimore. p 275-282.

Snow, D.H. (1983) Skeletal muscle adaptations: A review. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J. Granta Editions, Cambridge. p 160-183.

Snow, D.H., Ricketts, S.W. and Mason, D.K. (1983) The haematological response to racing and training exercise in Thoroughbred horses, with particular reference to the leucocyte response. Equine Vet. J. 15, 149-154.

Snow, D.H. and Harris, R.C. (1985) Thoroughbreds and Greyhounds: Biochemical adaptations in creatures of nature and of man. In: Circulation, Respiration and Metabolism. Ed. Gilles, R. Springer-Verlag, Berlin, Heidelberg. p 226-239.

Snow, D.H., Harris, R.C. and Gash, S.P. (1985) Metabolic response of equine muscle to intermittent maximal exercise. J. Appl. Physiol. 58, 1689-1697.

Somero, G.N. (1981) pH-temperature interactions on proteins: principles of optimal pH and buffer system design. Mar. Biol. Lett. 2, 163-178.

Spriet, L.L., Söderlund, K., Bergström, M. and Hultman, E. (1987) Skeletal muscle glycogenolysis, glycolysis and pH during electrical stimulation in man. J. Appl. Physiol. 62, 616-621.

Staddon, G.E., Weaver, B.M.Q. and Lunn, C.E.M. (1984) A "standard horse" for use in physiologically based mathmatical modelling. Equine Vet. J. 16, 189-191.

Steel, J.D. (1963) Studies on the electrocardiogram of the racehorse. Australian Medical Publishing Company, Sydney, Australia.

Stewart, J.H., Rose, R.J., Davis, P.E. and Hoffman, K. (1983) A comparison of electrocardiographic findings in racehorses presented either for routine examination or poor racing performance. In: Equine Exercise Physiology I. Eds. Snow, D.H., Persson, S.G.B. and Rose, R.J. Granta Editions, Cambridge. p 135-143.

Sullivan, T. and Armstrong, R. (1978) Rat locomotory muscle fibre activity during trotting and galloping. J. Appl. Physiol. 44, 358-363.

Sutton, J.R., Toews, C.J., Ward, G.R. and Fox, I.H. (1980) Purine metabolism during strenuous muscular exercise in man. Metabolism 29, 254-260.

Tanokura, M., Tasumi, M. and Miyazawa, T. (1976) 1H nuclear magnetic resonance studies of histidine-containing di- and tripeptides. Estimation of the effects of charged groups on the pk_a value of the imidazole ring. Biopolymers 15, 393-401.

Taylor, C.R., Maloiy, G.M.O., Weibel, E.R., Longman, V.A., Kamau, J.M.Z., Seeherman, H.J. and Heglund, N.C. (1980) Design of the mammalian respiratory system. III.Scaling maximum aerobic capacity to body mass: wild and domestic mammals. Respir. Physiol. 44, 25-37.

Tesch, P. (1980) Muscle fatigue in man: with special reference to lactate accumulation during short term intense exercise. Acta. Physiol. Scand. Suppl. 480

Tesch, P.A. and Wright, J.E. (1983) Recovery from short-term intense exercise: its relation to capillary supply and blood lactate concentration. Eur. J. Appl. Physiol. Occup. Physiol. 52, 98-103.

Tesch, P.A., Wright, J.E., Vogel, J.A., Daniels, W.L., Sharp, D.S. and Sjödin, B. (1985) The influence of muscle metabolic characteristics on physical performance. Eur. J. Appl. Physiol. 54, 237-243.

Thomas, D.P. and Fregin, G.F. (1981) Cardiorespiratory and metabolic responses to treadmill exercise in the horse. J. Appl. Physiol. 50, 864-868.

Thomson, J.A., Green, H.J. and Houston, M.E. (1979) Muscle glycogen depletion patterns in fast twitch fibre subgroups of man during submaximal and supramaximal exercise. Pflügers Arch. 379, 105-108.

Thorstensson, A., Sjödin, B., Tesch, P., Karlsson, J. (1977) Actomyosin ATPase, myokinase, CPK and LDH in human fast and slow twitch muscle fibres. Acta. Physiol. Scand. 99, 225-229.

Valberg, S. (1986) Skeletal muscle metabolic responses to exercise in the horse. Effects of muscle fibre properties, recruitment and fibre composition. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Valberg, S. and Essén-Gustavsson, B. (1987) Metabolic response to racing determined in pools of type I, IIA and IIB fibres. Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 290-301.

Viru, A. (1984) The mechanism of training effects: a hypothesis. Int. J. Sports Med. 5, 219-227.

Vøllestad, N.K., Sejersted, O.M., Bahr, R., Woods, J.J. and Bigland-Ritchie, B. (1988) Motor drive and metabolic responses during repeated submaximal contractions in humans. J. Appl. Physiol. 64, 1421-1427.

Vøllestad, N.K. and Sejersted, O.M. (1988) Biochemical correlates of fatigue. Eur. J. Appl. Physiol. 57, 336-347.

Wahren, J., Felig, P., Ahlborg, G. and Jorfeldt, L. (1971) Glucose metabolism during leg exercise in man. J. Clin. Invest. 50, 2715-2725.

Wheeler, T.J. and Lowenstein, J.M. (1979) Adenylate deaminase from rat muscle. Regulation by purine nucleotides and orthophosphate in the presence of 150 mM KCL. J. Biol. Chem. 254, 8894-8999.

White, M.G. and Snow, D.H. (1987) Quantitative histochemical study of glycogen depletion in the maximally exercised Thoroughbred. Equine Vet. J. 19, 67-69.

Wilkie, D.R. (1980) Shortage of chemical fuel as a cause of fatigue: Studies by nuclear magentic resonance and bicycle ergometry. In: Human muscle fatigue: physiological mechanisms. Proceedings of the Ciba Foundation no.82. Eds. Porter, R. and Whelan, J. Pitman Medical Ltd, London. p 102-114.

Wilkie, D.R. (1986) Muscular fatigue: effects of hydrogen ions and inorganic phosphate. Fed. Proc. **45**, 2921-2923.

Wilmore, J.H., Brown, C.H. and Davis, J.A. (1977) Body physique and composition of the female distance runner. Ann. N.Y. Acad. Sci. 301, 764-776.

Wilson, J.R., McCully, K.K., Mancini, D.M., Boden, B. and Chance, B. (1988) Relationship of muscular fatigue to pH and diprotonated Pi in humans: A ^{31}P-NMR study. J. Appl. Physiol. **64**, 2333-2339.

Wilson, R.G., Thornton, J.R., Inglis, S. and Ainscow, J. (1987) Skeletal muscle adaptation in racehorses following high-intensity training. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 367-374.

Woakes, A.J., Butler, P.J. and Snow, D.H. (1987) The measurement of respiratory airflow in exercising horses. In: Equine Exercise Physiology II. Eds. Gillespie, J.R. and Robinson, N.E. Edwards Brothers, Ann Arbor, Michigan. p 194-205.

Wood, C.H., Ross, T.T., Armstrong, J.B. and Hall, D.C. (1988a) Variations in muscle fibre composition between successfully and unsuccessfully raced Quarter horses. J. Equine Vet. Sci. **8**, 217-220.

Wood, C.H., Ross, T.T., Armstrong, J.B. and Hall, D.C. (1988b) Homogeneity of muscle fibre composition in the M.Gluteus medius of the horse. J. Equine. Vet. Sci. **8**, 294-296.

Wright, H. (1986) Encyclopedia of flat racing. Robert Hale Ltd, London, p 145-151.

Wu, M-J., Feldman, B.F., Zinkl, J.G. and Jain, M.C. (1983) Using red blood cell creatine concentration to evaluate the equine erythropoietic response. Am. J. Vet. Res. **44**, 1427-1432.

Zuntz, N. (1898) Untersuchungen uber den Stoffwechsel des Pferdes bei Ruhe und Arbeit. Landwirtschaftl Jb. **27** (Suppl. III). p 1-438.

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