The Study of Energy Supply For Exercise In Horses and Factors Influencing Glycogen Resynthesis in Equine Skeletal Muscle

by

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Thesis submitted to the University of Sydney for the degree of Doctor of Philosophy
This thesis is dedicated to my Father a man of great courage
Apart from the personnel listed in the acknowledgements this thesis represents the original work of the author

Allan James Davie
ACKNOWLEDGEMENTS

Those who have walked this path before know the value that one places on the moral support and assistance from those around you. To my loving wife Susan, and daughter Allana, who have been at my side and who gave encouragement and love throughout. To Professor Reuben Rose, Dr David Evans and Associate Professor David Hodgson who gave me the opportunity to work under their guidance, and for their patience and willingness in providing advice, support and friendship. Dr Greg Cooney for his valued assistance in the analysis of muscle glycogen synthetase.

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SUMMARY

The major aims of this investigation were to determine whether the rate of resynthesis of muscle glycogen could be enhanced in the first 24 hours after exercise. In addition, the effects of muscle glycogen depletion were investigated to determine whether there was adverse effects on the physiological responses to low and high intensity exercise.

In the development of protocols for these studies, two methodological issues were investigated. Firstly, the maximum safe glucose polymer dose that could be administered nasogastrically and secondly, the identification of areas of the middle gluteal muscle that could provide reproducible glycogen concentrations and fibre types. Results from these investigations demonstrated that a dose of 3 g.kg⁻¹(bwt) was the upper limit of the dose range for nasogastric administration of a glucose polymer and the provision of a glucose polymer, as a substitute for glucose, did not increase the tolerable dose range for the horse. Further, no difference was observed in the initial absorption phase, up to 2 hours after administration, between the glucose and glucose polymer. For the Standardbred, there was no significant difference in muscle glycogen concentration between muscle biopsy sample locations used. Although there were a variation in the proportion of ST and FT fibres between some of the locations, there were no differences between any of the sites sampled for FTH fibres. The large difference in the coefficient of variation between sites for the same fibre type and between the ST and FT fibres indicate that the muscle distribution of fibres types is not uniform. This variation may present problems in interpreting data if repeated biopsies are taken from different locations.

The investigation into the effects of oral and intravenous glucose administration on muscle glycogen resynthesis after exercise, found that the rate of resynthesis can be increased by the provision of a glucose supplement, but this may be impractical in the field. Muscle glycogen resynthesis following
exercise was enhanced by the administration of glucose intravenously at a dose rate of 6 g.kg$^{-1}$(bwt), but not by provision of a glucose polymer nasogastrically at a dose rate of 3 g.kg$^{-1}$ (bwt) as a single dose, or 1.5 g.kg$^{-1}$(bwt) with a second equivalent dose administered three hours later.

Muscle glycogen synthetase was examined before and immediately after exercise then at 3 and 6 hours post exercise during intravenous infusion of glucose or control (A solution of equivalent volume of polyionic, isotonic fluid). The activity state of muscle glycogen synthetase was increased with a decrease in muscle glycogen concentration, with the activity level in the glucose treatment being higher than the control treatment. This increased activity state during dextrose infusion may be a result of the large insulin response to the infusion of dextrose.

In the Thoroughbred, a decrease in muscle glycogen concentration of 29% before moderate intensity exercise and a 22% decrease before high intensity exercise on the treadmill, did not have an apparent detrimental effect on physiological responses to exercise.

In conclusion, it has been demonstrated that the rate of muscle glycogen resynthesis can be increased by the administration of glucose after exercise. However, difficulty lies in being able to practically administer the dose required to produce an effect on resynthesis. Further, the importance of influencing muscle glycogen resynthesis is questionable, when one considers the large muscle glycogen concentration in the horse and that a reduced muscle glycogen concentration was found not to affect exercise capacity or the physiological responses to moderate and high intensity exercise.
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATPase</td>
<td>adenosine triphosphatase</td>
</tr>
<tr>
<td>APB</td>
<td>alkaline preincubation buffer</td>
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<tr>
<td>AcPB</td>
<td>acid preincubation buffer</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<td>ethylenediamine tetraacetic acid</td>
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<td>FFA</td>
<td>free fatty acid</td>
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<td>FOG</td>
<td>fast twitch oxidative glycogenolytic</td>
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<td>fast twitch</td>
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<td>FTH</td>
<td>fast twitch oxidative</td>
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<td>g</td>
<td>gram</td>
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<td>GIP</td>
<td>gastric inhibitory peptide</td>
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<tr>
<td>g,kg⁻¹</td>
<td>grams per kilogram</td>
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<tr>
<td>G-6-P</td>
<td>glucose-6-phosphate</td>
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<td>H⁺</td>
<td>hydrogen ion</td>
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<td>H₂O</td>
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<td>hydrogen peroxide</td>
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<td>LA</td>
<td>left arm</td>
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<td>m</td>
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<td>m</td>
<td>muscle</td>
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<td>mm</td>
<td>millimetre</td>
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<tr>
<td>mM</td>
<td>millimole</td>
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<tr>
<td>mean±(SEM)</td>
<td>mean±standard error of the mean</td>
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<tr>
<td>MgCl</td>
<td>magnesium chloride</td>
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min = minute
mL = millilitre
mmol.kg⁻¹(dwt) = millimole per kilogram of dry weight
M = mole
mOsm.kg⁻¹ H₂O = milliOsmoles per kilogram water (litre)
m.s⁻¹ = metres per second
NaCl = sodium chloride
NaOH = sodium hydroxide
NADH = nicotinamide adenine dinucleotide (reduced form)
NADH-TR = nicotinamide adenine dinucleotide-tetrazolium reductase
NaF = sodium fluoride
NH₂CH₂COOH = glycine
O₂ = oxygen molecule
°C = degrees celsius
PAS = periodic acid-schiff base
PCV = packed cell volume
PCR = creatine phosphate
PFK = Phosphofructokinase
RA = right arm
RE = run to exhaustion
rpm = revolutions per minute
s = second
SDH = succinate dehydrogenase
SO = slow twitch oxidative
-SH = sulphydryl
Temp = rectal temperature
TP = plasma total protein
μl = microlitre
\( \dot{V} \text{CO}_2 \) = carbon dioxide production
\( \dot{V} \text{O}_2 \) = oxygen uptake
\( \dot{V} \text{O}_2 \text{max} \) = maximum oxygen uptake
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INTRODUCTION

This thesis describes studies of the role of muscle glycogen concentrations in exercising horses, and the effects of exogenous glucose supply, on muscle glycogen resynthesis in equine skeletal muscle after prolonged exercise.

In humans, the importance of muscle glycogen and blood glucose supply during exercise has been highlighted by the work of Ahlborg, Bergström, Ekelund and Hultman (1967); Bergström, Hermansen, Hultman and Saltin (1967a); Costill, Sherman, Fink, Maresh, Witten and Miller (1981); Ivy, Katz, Cutler, Sherman and Coyle (1988a). These studies showed that a high initial skeletal muscle glycogen concentration, and the ingestion of glucose during performance, can improve exercise capacity.

During recovery from exercise, an increased rate of glucose uptake as well as muscle glycogen synthesis occurs, with these processes being controlled by hormones and intracellular metabolites (Blom, Vøllestad and Costill, 1986). In muscle glycogen resynthesis, the reaction catalysed by glycogen synthetase is thought to be the rate limiting step (Conlee, Hickson, Winder, Hagberg and Holloszy, 1978). Two factors influencing the rate of muscle glycogen synthesis after exercise are the muscle’s glycogen concentration and time between the end of exercise and provision of a carbohydrate supplement (Ivy, Lee, Brozinick and Reed, 1988b).

There have been several studies investigating muscle adaptations to training in horses (Lindholm, Bjerneld and Saltin, 1974; Lindholm and Piehl, 1974; Guy and Snow, 1977; Snow, Baxter and Rose, 1981; Hodgson, Rose, DiMauro and Allen, 1985; Hodgson and Rose, 1987; Essén-Gustavsson, McMiken, Karlström, Lindholm, Persson
and Thornton, 1989). However, in the horse the importance of the initial muscle glycogen concentration on performance, and of exogenous glucose for muscle glycogen resynthesis following exercise, have not been investigated.

The principal null-hypotheses investigated in this thesis were

a. That the provision of exogenous glucose immediately after exercise does not affect the rate of skeletal muscle glycogen resynthesis in the horse (Chapters 5 and 6);

and

b. That a reduced skeletal muscle glycogen concentration does not affect endurance or high intensity exercise (Chapters 7 and 8).

Preliminary work was necessary to determine the maximum oral glucose dose that could be administered to the horse without causing intestinal discomfort. These results are presented in Chapter 4.

It was also important to establish the validity of using muscle biopsy samples from different muscle locations as representative of the whole muscle. This was achieved by taking repeated biopsies from different sites of the m. gluteus medius and determining the variability between sites for fibre type and glycogen concentration. These results are presented in Chapter 3.

To provide the study with perspective, a review of the current literature, with a focus on muscle energy supply and physical performance and factors influencing muscle glycogen resynthesis, is included in Chapter 1. Due to the vast amount of literature in the human field, the review has focused on the principal issues that may relate to the horse.
Chapter 1

LITERATURE REVIEW

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1.1 Introduction

There is little information on the importance of an exogenous glucose supply on muscle glycogen resynthesis after exercise in the horse. Most of the literature concerning muscle glycogen resynthesis comes from human and rat investigations. The only information referable to the current study is that by Snow, Harris, Harman and Martin (1987), who examined the provision of exogenous glucose in a study examining the effects of a low, high or normal carbohydrate diet on the rate of muscle glycogen resynthesis following exercise. The review also details issues concerning muscle glycogen and performance in both endurance and high intensity exercise in humans. In addressing this area of study, parallels with the horse have been attempted by considering the depletion levels which have been reported in endurance rides and racing competition.

The final part of the review concerns muscle glycogen resynthesis and specifically the glycogen depletion and repletion rates of muscle fibres. It concludes with the different aspects of resynthesis, with most reference being to the human studies. The work of Ivy and co-workers and Blom and co-workers comprise the majority of contributions to this field of study.
1.2 Fatigue During Exercise

Exercise requires an integration of many systems, each containing many elements, and any factor that upsets this integration could cause fatigue (Brooks and Fahey 1985). They state that the concentrations of potassium (K⁺), sodium (Na⁺) and calcium (Ca²⁺), the compartmentalisation of these ions within the cell, energy producing substrate concentrations, plasma volume, pH, osmolality, temperature and hormones are factors involved in maintenance of cell homeostasis.

Fatigue as defined by Brooks and Fahey (1985) is the inability to maintain a given exercise intensity. The cause of this inability of muscle to maintain a specific level of contraction could lie in either the central nervous system, the final motor neuron, the neuromuscular junction or the muscle (Åstrand and Rodahl, 1970; Saltin and Karlsson, 1971; Brooks and Fahey, 1985; Sahlin, 1986a; Enoka and Stuart, 1992).

1.2.1 Theories of Fatigue

One problem in trying to identify the cause of fatigue is that it is a multidimensional phenomenon and can vary in accordance with the activity itself, the training and physiological status of the individual and the environmental conditions (Brooks and Fahey, 1985). Fatigue may take place at a single site, in the case of a maximal lift, or it may involve many sites when there is depletion of energy supplies, accumulation of waste products, changes in pH, depletion of nervous system transmitters and dehydration (Kraemer, 1983).

In the pursuit of identification of the site of fatigue, most investigations have subdivided fatigue into central and peripheral components. This subdivision is based on
the premise that the resultant decrease in performance is originating from within the central nervous system or within the muscle. Areas such as the sarcolemma (Tibbits, 1988), sarcoplasmic reticulum (Donaldson, 1988), brain and spinal cord (Simonsen, 1971; Bigland-Ritchie, Jones, Hosking and Edwards, 1978) motor nerve (Simonsen, 1971) and the neuromuscular junction (Stephens and Taylor, 1973) have all been addressed in an attempt to identify the site of fatigue.

The onset of fatigue is most often associated with either the accumulation of metabolic by-products such as ($H^+$) and inorganic phosphate or a decline in muscle glycogen. This review will therefore focus only on the changes that occur within the muscle cell with increased activity.

The contraction of a muscle involves the conversion of chemical energy into mechanical work, with adenosine triphosphate (ATP), adenosine di-phosphate (ADP), inorganic phosphate ($P_i$), magnesium ($Mg^{2+}$) and hydrogen ($H^+$) being directly involved in this process. As ATP supply within the muscle is limited, and as it is the only energy form that can be used directly, this necessitates continual rephosphorylation to maintain supply. The energy for the rephosphorylation of ATP comes from creatine phosphate (PCr) and substrate breakdown. The total phosphagen pool is also limited and Karlsson, Diamant and Saltin (1971), showed that the estimated total phosphagen depletion, expressed in oxygen equivalents, was related to the relative work load. This applied not only at submaximal concentrations but also at maximal and supramaximal concentrations.

The PCr supply within the muscle is also limited, and during exercise at 70% $\dot{V}O_2max$, the PCr stores can be depleted with the resultant production of ATP lasting for
only about 30 sec of exercise (Sahlin, 1986a). Exercise of longer duration and lower intensity necessitates the oxidation of carbohydrates and fats within the mitochondria in order to sustain work output. The volume of fat stored within an average person has the capacity for sustaining ATP production for several days and is not generally considered a limiting factor. However, the rate of ATP production from fat oxidation is slow and limits its usage to low intensity activities (Sahlin, 1986a). As the intensity increases above 30% to 50% $\dot{V}O_{2\text{max}}$ the contribution of carbohydrates increases with the contribution of ATP from glycolysis and PCr increasing at intensities above 70-80% $\dot{V}O_{2\text{max}}$. At this high intensity exercise (greater than 80% $\dot{V}O_{2\text{max}}$) the by-products of glycolysis, lactate and hydrogen ions, will accumulate within the muscle. At these intensities PCr will continually decrease to a point where anaerobic energy production cannot meet the energy demands, and the force of muscle contraction will be reduced (Sahlin, 1986a). The accumulation of hydrogen ions in the muscle has been associated with fatigue. In studies on contraction of skinned muscle, it has been reported (Cook and Pate, 1988) that the isometric tension is reduced by approximately 50% as the pH drops from 7 to 6. Further, the maximum velocity of contraction decreases but not to the same degree as tension. The decreased pH has also been associated with fatigue through its inhibitory effects on glycolytic enzymes. Decreased pH has been shown to inhibit both phosphorylase kinase activity and the activity state of phosphofructokinase (PFK) (Hultman, Bergstrom, Spriet and Soderlund, 1988). As PFK is the rate limiting enzyme in the glycolytic pathway, any reduction in its activity will result in a reduction of ATP production.
The importance of the initial muscle glycogen concentration to performance in humans was highlighted by Bergström, Hermansen, Hultman and Saltin (1967a), who found a strong correlation between the initial muscle glycogen concentration and work time during endurance exercise.

In endurance rides, Snow et al. (1981) reported muscle glycogen depletion levels of 56% for an 80 km ride and for rides of 160km, muscle glycogen depletion of more than 70% of ST fibres and substantial depletion of type FT has been reported (Hodgson, Rose and Allen, 1983). In the roads and track component of three day event competitions, decreases in muscle glycogen concentration of 306 mmol.kg\(^{-1}\)(dwt),(546.7-248.7 mmol.kg\(^{-1}\)(dwt)) with a mean rate of utilization of 4.1 mmol.kg\(^{-1}\)(dwt) have been reported (Hodgson, Rose, Allen and DiMauro, 1984a). Lindholm (1979), reported that during a 4 hour slow trot (5m.s\(^{-1}\)) muscle glycogen concentration decreased from a resting level of 476 mmol.kg\(^{-1}\)(dwt) to 192 mmol.kg\(^{-1}\)(dwt). However, when the experiment was repeated with the speed increased to 8.3 m.s\(^{-1}\), muscle glycogen concentration decreased approximately by the same amount (412 to 172 mmol.kg\(^{-1}\)(dwt)).

Costill et al. (1981), examined the effect of the muscle's initial glycogen concentration on a sprint performance in humans, and in contrast to the findings of Bergström et al. (1967) on endurance performance, they found that the muscle's initial glycogen concentration had no effect on either 300 m sprint time or 100 m and 200 m splits. Similar to this, Symons and Jacobs (1989) reported muscle glycogen decreases from 427 to 153 mmol.kg\(^{-1}\)(dwt) without having a significant effect on repeated maximal isokinetic contractions, or on a single maximum isometric contraction. The muscle's initial glycogen concentration seems to be more important as the time period of the exercise
increases with the short term high intensity exercise not so dependent on the muscle glycogen concentrations.

In an endeavour to improve endurance performance, athletes have attempted to increase muscle glycogen storage capacity 2 to 3 times greater than normal. This process is called carbohydrate loading or glycogen supercompensation (Brooks and Fabey, 1985), and involves a combination of diet and exercise. The procedure utilizes high intensity training on a low carbohydrate, high fat/protein diet, followed by low intensity training on a high carbohydrate diet. Problems that have been associated with this process include, increased water retention, which may lead to increased body weight, and decreased vitamin intake (Jette, 1978).

Many studies have confirmed the importance of pre-exercise muscle glycogen concentration and endurance performance (Bergström et al., 1967; Hargreaves, Costill, Coggan, Fink and Nishibata, 1984; Coyle, 1991). In a field experiment conducted by Karlsson and Saltin (1971), involving a 30 km time trial, they reported that the elevated muscle glycogen concentration did not increase the running speed of the athletes at the start of the run, but allowed them to run longer at their optimal speed. Glycogen loading has also been shown to affect the acid-base status of the blood, and may as a result, influence exercise performance (Greenhaff, Gleeson and Maughan, 1988).

The issue of glycogen loading, as it applies to humans, would seem to have little practical application for the racing horse based on the degree of depletion that occurs during such events (Snow and Harris, 1991). Further, Topliff, Potter, Dutson, Kreider and Jessup (1983) and Topliff, Potter, Kreider, Dutson and Jessup (1985), reported no
improvement in performance with increases in muscle glycogen concentration of up to 36% above resting concentrations.

1.3 Glucose and Exercise

1.3.1 Glucose and Metabolism

Metabolism as defined by Brooks and Fahey (1985), is the sum total of metabolic processes occurring in a living organism. For the cell, the main sources of energy are derived from the oxidation of carbohydrates and fats to form carbon dioxide (CO$_2$) and water (H$_2$O) in the presence of oxygen. The oxidative processes of metabolism take place in the mitochondria of the cell and are referred to as aerobic metabolism. The power that can be generated from carbohydrate oxidation is greater than from fat oxidation (Brooks and Fahey, 1985). However, the total storage of carbohydrate in the muscle and liver compared to fat is very limited. In humans, the total carbohydrate stores would provide sufficient energy for approximately only 90 min of exercise while working at an intensity of 70% $\dot{V}O_{2}\text{max}$ (Sahlin, 1986a).

When glucose enters the cell it is immediately phosphorylated to glucose-6-phosphate (G-6-P), which is unable to participate in facilitated diffusion across the cell membrane and therefore effectively traps the glucose within the cell. Once within the cell glucose has two options. It can be converted to glycogen, with the final step mediated by the enzyme glycogen synthetase, or it can enter the glycolytic pathway and be converted
to pyruvate (Ganong, 1981). At low intensity exercise and in the presence of adequate oxygen, pyruvate is converted to Acetyl Co-enzyme A (CoA) and completely oxidised to carbon-dioxide (CO₂) and water (H₂O) via the Krebs cycle and the electron transfer system (Brooks and Fahey, 1985). During high intensity exercise most of the pyruvate formed is reduced forming lactate. The breakdown of one glucose molecule to pyruvate results in the net production of 2 adenosine triphosphate (ATP molecules) (Brooks and Fahey, 1985). One glucose molecule completely oxidised to CO₂ and H₂O produces 38 molecules of ATP (Ganong, 1981).

For triglycerides to be utilized as an energy source they must first be hydrolysed to glycerol and free fatty acids (FFA). The glycerol component is converted to dihydroxy acetone phosphate, an intermediate in the glycolytic pathway. The FFA must be converted to Fatty-acid-CoA, then moved into the mitochondria where 2 carbons are serially split off the fatty acid (beta-oxidation), to form acetyl-CoA which enters the Krebs cycle. The breakdown of 1 molecule of a 6-carbon fatty acid to CO₂ and H₂O generates 44 molecules of ATP (Ganong, 1981).

1.3.2 Blood Glucose Absorption

The primary area for digestion and absorption of carbohydrates, proteins, water and water soluble vitamins and most minerals in the horse is in the small intestine (Evans, Barton, Hintz and Van Vleck, 1977). As there can be a rapid movement of feed through the stomach and small intestine into the large intestine of the horse, caution must be taken when providing supplements such as glucose. This is due to the microbial population of
the large intestines being active in metabolising the glucose, with the possibility of producing a state of alimentary laminitis (Frape, 1986).

Glucose absorption, blood glucose concentration and testing for the functional capacity of the small intestines in the horse, have been examined by several researchers (Alexander, 1955; Jacobs, Norman, Hodgson and Cymbaluk, 1982; Frape, 1986; Mair, Hillyer, Taylor and Pearson, 1991).

Alexander (1955) examined blood and caecal fluid in ponies for glucose concentrations, following the oral administration of glucose. He reported that the absorption of sugars occurred in the upper small intestine and not in the ileum. Peak blood glucose concentration occurred between 1 to 2 hours following the dose, with the blood glucose concentration returning to resting levels by 4 hours after administration. Although no appreciable amount of glucose was found to have accumulated in the caecum, this did not exclude the possibility of glucose reaching the caecum and being fermented quickly.

The absorption of glucose across the intestinal wall is dependent upon an active transport process that appears to involve sodium ions (Crane, 1968 cited in Holmes, 1971; Mair et al., 1991). A carrier molecule, which affects translocation through the membrane, is activated by the reversible combining to it of glucose and sodium. The movement of glucose across the membrane can occur against its own concentration gradient, with the energy for the transport process being provided in part by a concentration gradient of sodium across the membrane. This sodium concentration gradient is maintained through the continual removal of sodium ions from the epithelial cell by the sodium pump (Holmes, 1971).
The chain length of glucose polymers has been considered as a factor affecting absorption. Wahlqvist, Wilmshurst, Murton and Richardson (1978), investigated the role of saccharide chain length in glucose absorption on 6 healthy male volunteers (18-21 years) following ingestion of either a mono-, di-, penta-, or a polysaccharide consisting exclusively of glucose monomeric units. There was no significant difference in absorption rates of the mono-, penta-, or polysaccharide, or between blood glucose concentrations following the ingestion of a monosaccharide or disaccharide. Further, the plasma insulin responses elicited by the mono-, penta-, or polysaccharide were also not significantly different from each other. They concluded that the chain length alone does not appear to influence the rate of glucose absorption across the intestinal wall.

The blood glucose response to an oral dose of glucose shows a repeatable characteristic pattern which is dose dependent and has two phases (Roberts and Hill, 1973; June, Soderholm, Hintz and Butler, 1992). The first phase is representative of continuous absorption of glucose from the small intestine, and lasts for approximately 120 min. The second phase is dependent on the insulin response to the hyperglycaemia induced by the glucose dose. This second phase lasts for approximately 4 hours with a return to resting values occurring by 6 hours. The insulin response promotes the uptake of glucose into the cells for glycogen synthesis and glucose utilisation, but inhibits the continual absorption of glucose. In order to avoid hypoglycaemia, the effects of insulin need to be counteracted. The hormones glucagon, cortisol, epinephrine and norepinephrine are active in this control for maintaining equilibrium of blood glucose concentration (Frape, 1986). An overshoot in the glucose curve below resting level may be witnessed, which Loeb, McKenzie and Hoffsis (1972) explain is due to the delay in the
termination of insulin action. With the cessation of the insulin action, there is a slight rise in blood glucose above resting values.

1.3.3 Laminitis

A major problem that can occur following the administration of glucose to the horse is laminitis. Laminitis is described by Hunt (1991) as among the most devastating and permanently crippling diseases of the horse. Moore, Allen and Clark (1989) and Hunt (1991) in their description of laminitis, state that, if the blood supply to the laminar region is reduced, there is a resultant degeneration of the laminar interdigititation and the distal phalanx separates from the hoof. Hunt (1991), adds that the separation of the distal phalanx usually results from biomechanical influences exerted on the distal phalanx.

Moore, Garner, Berg and Sprouse (1979) administered a carbohydrate overload to two horses and found there was a decrease in caecal pH by 3 hours after the carbohydrate overload. They suggest that the early alterations in caecal pH, lactate concentration and free endotoxin found within the caecum following the carbohydrate overload, are indicative of the involvement of endotoxin in the pathogenesis of acute alimentary laminitis-hypertension in the horse.

Garner, Coffman, Hahn, Hutcheson and Tumbleson (1975) studied the physiology of laminitis by inducing laminitis via the administration of a gruel (80% corn starch and 15% wood cellulose, 17.6 g.kg⁻¹). They reported that the mean lapse in time between administration of the gruel and onset of laminitis was 40 hours. Physiological changes that were found in response to the laminitis were an increased heart rate from 41 beats per min
(bpm) at rest to 58 bpm at 24 and 73 bpm at 40 hours. Rectal temperature increased from 37.7°C during the control period to 39.2°C and 39.8°C at 24 and 40 hours respectively. Packed cell volume (PCV) by 24 hours after carbohydrate administration had increased from 0.33 L.L\(^{-1}\) to 0.36 L.L\(^{-1}\), but had decreased to 0.34 L.L\(^{-1}\) at 40 hours. Total plasma protein (TP) increased from 74 g.L\(^{-1}\) to 83 g.L\(^{-1}\) at 24 hours and 77.8 g.L\(^{-1}\) at 40 hours. The values for serum TP and PCV measured at 24 hours indicated a marked compartmental fluid shift, which in turn indicated an increase in blood viscosity and a tendency toward cardiovascular insufficiency (Garner et al., 1975). They suggested that the early increase in temperature is indicative of an inflammatory response brought about by either an increase in vascular permeability, an endotoxin-induced release of endogenous pyrogens or bacteraemia.

1.3.4 Effects of Diet on Blood Glucose

Glucose metabolism and absorption are influenced by a number of factors. Thompson and Yoon (1984) found that polyphenols such as tannic acid and phytic acid reduced the rate of starch digestibility. Simple carbohydrates have been shown to elicit earlier and higher glucose and insulin responses than complex carbohydrates (Crapo, Reaven, Olefsky and Alto, 1977). Rices with varying degrees of amylose led to differences in the glucose and insulin responses (Goddard, Young and Marcus, 1984). Raw starches high in amylopectin are digested faster than starches high in amylose (Thorne, Thompson and Jenkins, 1983). The amylopectin, being a branched chain compared to amylose, which is a straight chain, is similar to glycogen with the only difference being the number of
glucose molecules per branch. The glucose polymers have the ability to empty from the stomach more rapidly than glucose (Decombaz, Sartori, Arnaud, Thelin, Schurch and Howald, 1985; Nuefer, Costill, Fink, Kirwan, Fielding and Flynn, 1986; Fielding, Costill, Fink, King, Kavoliski and Kirwan, 1987). Maegawa, Kobayashi, Ishibashi, Takata and Shigeta (1986), examined the effects of a high sucrose and high fat diet and found that a high fat diet induced a state of insulin resistance with an impaired glucose metabolism and glucose uptake.

In the horse, Hintz, Hogue, Walker, Lowe and Schryver (1971) found that feeding a high grain diet compared to a forage diet, resulted in more carbohydrates being digested in the small intestines and absorbed as glucose. The forage diet resulted in more carbohydrate being converted to volatile fatty acids in the lower gut. Glucose absorption rates have been reported to be 30% greater for horses fed oats rations as opposed to alfalfa (Argenzio and Hintz, 1972). Rich, Fontenot and Meachan (1981), added fats to an lucerne and grain diet and found that the digestibility of acid detergent fibre or absorption of minerals was not affected by the addition of the fat.

Plasma glucose concentration and/or glucose utilisation rate were considered as factors in the control of meal size, duration and frequency of feeding by Ralston and Baile (1982). They administered intravenously a low glucose dose (0.2 g.\(^{75}\text{kg}\^{-1}\) body weight (bwt)) versus a high glucose dose (1.0 g.\(^{75}\text{kg}\^{-1}\)(bwt)) with water as a control, to examine the effects of blood glucose concentration on feeding behaviour. Each treatment was administered following a 4 hour fast and 5 min prior to allowing free access to feed. They reported that neither dose altered the ponies' immediate feeding response. There was a tendency to prolong the subsequent intermeal interval after the low dose. The high dose
produced an increased first intermeal interval relative to the control, and additionally led to a lower feed consumption in total in the experimental period. They concluded that elevations in plasma glucose or insulin do not immediately generate satiety cues in a fasted pony. However, the blood glucose levels do alter the interval between meals.

1.3.5 Blood Glucose and Insulin Responses to Diet

The effects of both the type and combinations of feeds on blood glucose and insulin responses during exercise in horses were examined by Stull and Rodiek (1988). They reported that changing the grain-forage ratio of a diet resulted in no difference in observed blood glucose concentrations between the diets. While no difference in responses of glucose was found for any of the diets, insulin responses did show marked differences between dietary treatments. It was concluded that these differences in insulin responses may be due to release of gastric inhibitory polypeptide (GIP) from the small intestine, as GIP is a major hormonal mediator of the insulin response in humans and dogs.

Arana, Rodiek and Stull (1989) examined the blood glucose and insulin responses following the feeding of four common grains, (barley, corn, oats and sweet feed), and four different forms of lucerne, (chopped, cubed, long and pelleted). The time to peak glucose concentration varied between grains and lucerne. However, there was no difference in glucose concentration between the grains, nor were there differences between the forms of hay. Insulin concentrations followed the changes in glucose concentrations, with the exception of those horses fed the pelleted hay. Five hours after feeding, those fed pelleted hay showed a higher mean insulin concentration than horses fed cubes. They also reported
that mean peak glucose concentrations for the grains were higher than for the hay. This difference was probably due to grains being higher in soluble carbohydrate than lucerne hay.

1.3.6 Blood Glucose and Performance

Ahlborg et al. (1967) reported that in humans, the elevation of blood glucose by infusion, did not reduce the rate of muscle glycogen utilization during cycling on an ergometer. They suggested that the rate of glycogen breakdown during exercise is approximately the same, irrespective of the quantity of glucose delivered to the muscle. Further support for this finding was provided by Bergström and Hultman (1967a) and Fielding et al. (1987). This constant rate of glycogen usage despite elevated blood glucose concentrations, could indicate that the metabolism of the added glucose is replacing some of the fat metabolism during exercise, instead of replacing the energy derived from muscle glycogen. Despite blood glucose not acting as a substitute for muscle glycogen, studies have shown that the ingestion of carbohydrate during endurance performance can prolong exercise duration. However, the mechanism of how carbohydrates contribute to prolonging performance has only been partly elucidated (Ivy, Miller, Dover, Goodyear, Sherman, Farrell and Williams, 1983; Hargreaves, Costill, Coggan, Fink and Nishibata, 1984; Hargreaves, Costill, Fink, King and Fielding, 1987; Coyle, 1991). Coyle (1991) suggested that as glycogen concentration decreased, the majority of carbohydrate energy was derived from the metabolism of glucose derived directly from the blood.
Hargreaves et al. (1984) were the first to be able to demonstrate a reduced rate of glycogen depletion with carbohydrate feeding during exercise. A solid feed, equivalent to 43 grams of sucrose was repeatedly ingested (at 0, 1, 2, and 3 hours) during a 4 hour exercise period which incorporated periods of endurance and short term high intensity exercise. It was shown that following the carbohydrate feeding, subjects were able to perform significantly longer during the short term high intensity ergometer ride to exhaustion. They suggested that the solid carbohydrate feeding may contribute to muscle metabolism during exercise, thereby reducing the demand on intramuscular glycogen stores. This glycogen sparing would provide glycogen for later use. The possibility of the exogenous carbohydrate contribution to glycogen resynthesis and thereby reducing the net glycogen breakdown was also suggested by Hargreaves et al. (1984). This improvement in performance with carbohydrate supplementation has also been reported in horses (Farris, Hinchliff, McKeever and Lamb, 1994). They gave a continuous intravenous infusion of glucose (3.5 ml.min⁻¹) during treadmill running at 6.0 m.s⁻¹ to exhaustion. Compared to a saline trial, run time was prolonged, with lactate and core temperature both being lower during the glucose infusion.

Fructose and glucose polymers have been considered as a substitute for glucose during exercise, because of their different rates of gastric emptying and differing effects on insulin.

Decombaz et al. (1985), examined the effects of consuming glucose versus fructose, (from corn starch), on muscle glycogen usage during 45 min of submaximal, followed by 15 min at maximal exercise. They reported that the amount of ingested fructose oxidised following intake was as high as that of glucose, indicating that fructose
was utilised for energy as efficiently as glucose during the exercise period. Further, the resultant lactate, free fatty acids, glycerol concentrations, and heart rates, were similar for the two treatments, with the muscle glycogen concentration before exercise and utilization rate during exercise not being significantly different between the glucose and fructose diets. The finding of fructose being utilized for energy as efficiently as glucose was further supported by the absence of any significant difference in glycogen utilization rate between the glucose and fructose. Jandrain, Pallikarakis, Normand, Pirnay, Lacroix, Mosora, Pachiaudi, Gautier, Schene, Riou and Lefebvre (1993), reported that when glucose and fructose were ingested at a rate of 25g, every 30 min during a 3 hour treadmill run at 45% of $\dot{V}O_2$ max, fructose was metabolically less available than glucose. However, the fructose still maintained normoglycemia as well as glucose.

This lack of a performance enhancement from the ingestion of fructose compared to glucose was also reported by Hargreaves et al. (1985) and Fielding et al. (1987). However, others (Levine, Evans, Cadarette, Fisher and Bullen, 1983; McMurry, Wilson and Kitchell, 1983) have reported improved endurance performance. Levine et al. (1983) examined substrate utilization rates following fructose, glucose or water ingestion during a 30 min treadmill run at 75% $\dot{V}O_2$ max, and found significantly reduced muscle glycogen depletion with the fructose ingestion compared to glucose.
1.4 Muscle Fibre Types and Adaptations to Exercise

1.4.1 Analytical Techniques For Description Of Fibre Types

The needle biopsy technique of tissue extraction has been in use since 1868, when it was used for the investigation of muscular dystrophy (as reported by Bergström, 1975). Today the biopsy technique is used extensively for both research and pathological investigations.

Histochemical techniques of muscle staining enable the identification of specific fibre types. Identification is based on specific enzyme reactions at varying pH values. In addition, an absence or excess of a particular enzyme, or structural changes in the muscle, as a result of morphological or physiological stimuli, can be demonstrated utilizing histochemical techniques (Dubowitz, 1985).

The technique of fibre identification has taken different paths over the years, with both histochemical and biochemical methods being employed. Fibre type is determined by staining for ATPase at varying preincubation pH values. The ATPase enzyme can be used as the marker since the speed of contraction of a muscle is directly proportional to its myosin ATPase activity (Barany, 1967). Muscle fibres possessing high ATPase activity are predominantly fast twitch muscles, and fibres having low ATPase activity are predominantly slow twitch muscle (Guth and Samaha, 1969).

Histochemical staining for ATPase, for identification of specific fibre types, was validated by Padykula and Herman (1955). Specificity of the histochemical reaction for ATPase was examined by considering the relationship of the sulfhydryl(-SH) group to the activity of ATPase and phosphomonoesterases, as -SH groups play a role in activating
ATPase and phosphomonoesterases reacting with the ATP at alkaline pH. Guth and Samaha (1969) reported that the high ATPase fibres were alkali-stable and acid-labile, whereas low ATPase fibres were acid-stable and alkali-labile.

Brooke and Kaiser (1970) demonstrated the presence of three fibre types based on the response of the ATPase reaction, in pre-incubation buffer varying in pH from 4.3 to 4.6 and at pH 9.4. Fibres that stained weakly for ATPase at pH 9.4 were called type I fibres, and fibres that stained strongly for ATPase were called type II fibres. The type II fibres were further subdivided into IIa and IIb in accordance with their stain at pH 4.3 and pH 4.6. The IIa had a weak reaction at pH 4.3 and pH 4.6 and IIb which had a strong reaction at pH 4.6 but weak at pH 4.3. At pH 4.3 the type I fibres stained strongly.

A further subdivision of muscle fibres can be obtained by utilizing the enzyme NADH-tetrazolium reductase (NADH-TR). The oxidation of the reduced co-enzyme NADH can be catalysed by the flavin enzyme tetrazolium reductase (formerly called diaphorases). The hydrogen is taken up and transferred to the tetrazolium salt. The tetrazolium reductases are present in the mitochondria and endoplasmic reticulum of the cell and reflect the capacity of intracellular oxidation (Dubowitz, 1985).

Barnard, Edgerton, Furukawa and Peter (1971) utilized both NADH-tetrazolium reductase and myosin ATPase activity to divide guinea pig muscle into red, white or intermediate types. They suggested that muscle fibres could be classified as fast-twitch red, fast-twitch white and slow-twitch intermediate. Halkjaer-Kristensen and Ingemann-Hansen (1979), examined whether the tetrazolium reductase (NADH-TR) and α-glycerophosphate dehydrogenase reactions in human quadriceps muscle could be used for the quantification of oxidative and glycolytic enzymes in muscle. They used the staining
procedure of Dubowitz and Brooke (1973), for validation of the NADH-TR stain procedure. Their results supported the suggestion that such procedures could be used to quantify oxidative and glycolytic enzymes within a single human muscle fibre.

The fibre type classification systems of Peter, Barnard, Edgerton, Gillespie and Stempel (1972), and Brooke and Kaiser (1970), were examined by Nemeth, Hofer and Pette (1979), for their degree of metabolic heterogeneity. Similarity existed in the type I and slow twitch oxidative (SO) classification, but the subgroups within the type II and the fast twitch classification were not equal. The type IIa fibres were not analogous to the fast twitch-oxidative glycogenolytic (FOG) fibres nor were the IIb fibres to the fast twitch glycogenolytic (PG) fibres.

The validity of using metabolic properties of muscle for classifying fibres was examined by Reichmann and Pette (1982). The activity levels of succinate dehydrogenase (SDH) were correlated with fibre types classified according to Brooke and Kaiser (1970). They reported that it was impossible to classify muscle fibres into types I, IIa and IIb by their metabolic properties, based on the mitochondrial activity of SDH. The significance of preincubation pH on muscle fibre identification was examined by Green, Reichmann and Pette (1982). They did a comparative study of an acid (Brooke and Kaiser, 1970) versus an alkaline (Guth and Samaha, 1970) preincubation method of histochemical staining for myofibrillar ATPase. There was commonality between methods for identification of type I fibres, but this did not hold true for the type II fibres, indicating that the classification systems were not interchangeable. The use of staining techniques for the identification of fibre types in equine skeletal muscle has been demonstrated by Snow (1982).
The various sources of variance and error involved in analysing the fibre composition of a muscle from a single biopsy were evaluated by Blomstrand and Ekblom (1982). They reported that, in characterising a muscle into its fibre types, the accuracy was increased by taking at least two biopsies from the same site and that there was no consistent difference between the right and left sides.

1.4.2 Factors Affecting Fibre Composition Of Muscle

1.4.2.1 Age

The effect of age on muscle fibre composition was investigated in horses by Essén, Lindholm and Thornton (1980) and Kline and Bechtel (1988). Kline and Bechtel (1988) examined biopsies of the m. gluteus medius at a sample depth of 2.5 centimetres (cm) from quarter horses within 24 hours after birth, at 6 months of age and from within a group of mature quarter horses. Highly oxidative fibres decreased with age from birth to 6 months, but there was no significant difference between horses aged 6 months and mature horses in the percentage of fast twitch glycolytic, slow twitch oxidative and fast twitch oxidative fibres. Essén et al. (1980), examined the effect of age on fibre type variability within the m. gluteus medius, vastus lateralis, semitendinosus and the triceps brachii, in 55 Standardbred trotters ranging in age from 2 months to 28 years. The type II fibres predominated, 69% to 87% in each of the four muscles. The m. gluteus medius had a higher TypeI/TypeII ratio in the older horses (10 to 28 years) than in the foals. The proportion of IIA/IIB fibres varied, both between different age groups and between horses
of similar age. Essén et al. (1980) also found changes in the Type IIA/IIB ratio of the m. gluteus medius, triceps and semitendinosus with age and suggested that these changes were the result of the muscles being active and adaptable to the training throughout the lifetime of the horse.

1.4.2.2 Sample Depth

Fibre type has been shown to vary with depth of biopsy and results of studies indicate that variations are widespread. In general, it appears that within the m. gluteus medius muscle, the %FT fibres decrease and the %ST fibres increase with increasing depth. Kline and Bechtel (1988), examined biopsies from the m. gluteus medius from horses of unknown breeding, at depths of 1, 2, 4, 6 and 8 cm below the superficial fascia. Samples from the same depth were not significantly different for the enzymes 3-Hydroxyacyl-CoA dehydrogenase, citrate synthase, lactate dehydrogenase and glycogen phosphorylase or fibre type. However, the enzyme activities and percentage fibre type changed as a function of sampling depth. Fast glycolytic fibres decreased by 65% from the superficial to the deepest site, whilst slow oxidative fibres increased by 400% from the superficial site to the deepest site. Bruce and Turek (1985), studied the variability in depth of sampling within Thoroughbred m. gluteus medius in a 4 year old filly and a 7 year old stallion. Muscle samples of approximately 2 cm cube were taken from the top 3 cm of the muscle (superficial), the deepest 3 cm of the muscle (deep), and 2 samples from the middle portion of the muscle (upper and lower middle), along a straight line joining the
tubae coxae to the head of the femur. An increase in percentage of ST fibres from superficial to deep locations occurred, with the rate of increase being dependent on location. The estimated standard deviation in the percentage of ST fibres from a needle biopsy taken from one of the 2 cm cubes of m. gluteus medius was 2.56%. Van den Hoven, Wensing, Breukink, Meijer and Kruip (1985), examined the variability of depth and site within the triceps brachii, longissimus dorsi, gluteus medius and biceps femoris muscles in a 3 year old Dutch saddle horse. Immediately after death parts of these muscles were dissected. For the m. gluteus medius a transverse slice was dissected at a point 10 cm behind the tubae coxae through the muscle belly. The muscle slices were divided into nine parts to provide three superior, three intermediate and three inferior parts at depths of 1, 5, and 8 cm below the superficial muscle surface. In samples originating from different zones the maximal significant intra muscular difference in percentage of ST fibres for the m. gluteus medius was 36%. The maximal significant differences in percentage of ST fibres in samples originating from the same zone was 30%, which was for the deep zone of m. gluteus medius. Results indicated that the distribution of type I fibres in the cross sections of the m. gluteus medius was not homogeneous.

Further support for fibre type change with depth of biopsy was provided by Lopez-Rivero, Serrano, Diz and Galisteo (1992) and Bruce, Turek and Schurg (1993). Lopez-Rivero et al. (1992) examined biopsies from the gluteus medius at depths of 2, 4, 6 and 8 cm below the fascia and reported significant differences between depths. Bruce et al. (1993), divided the m. gluteus medius of the horse into two regions, dorsal and ventral, based on architectural design, innervation patterns, fibre composition and points of origin and insertion. The dorsal region had a higher percentage of Type I fibres than the ventral
region, with the ventral region having the higher percentage of Type IIA and Type IIB fibres.

1.4.3 Analytical Techniques For Determination of Muscle Glycogen Concentration.

Muscle glycogen concentration can be determined biochemically (Harris Hultman and Nordesjo, 1974) or histochemically (Pearse 1968). One of the early methods for muscle glycogen analysis was based on the O-toluidine method used for the determination of glucose in blood and urine (Hultman, 1967 cited in Harris et al., 1974). However, this method was found to be unsuitable for freeze dried muscle, and Harris et al. (1974), adopted a procedure based on the method of Marshall and Whelan (cited in Harris et al., 1974).

The histochemical technique utilizes the Periodic Acid Schiff (PAS) reaction, for the staining of individual muscle fibres. The intensity of the stain can be subjectively rated (Hodgson, 1984) or photometrically analysed (Edgerton, Essén, Saltin and Simpson, 1975) to provide a quantitative measure.

Halkjaer-Kristensen and Ingemann-Hansen (1979), examined the PAS staining technique and adapted a microphotometric technique for the quantitation of glycogen concentration in single muscle fibres. They reported that the microphotometric technique was a suitable method of quantifying the glycogen concentration in human muscle fibre. The use of microdensitometry for determination of glycogen concentration of equine muscle has also been demonstrated (White and Snow, 1987).
1.4.4 Glycogen Concentration In Skeletal Muscle

Considerable variability in glycogen concentration of the m. gluteus medius has been reported, both within and between horse breeds. Lindholm and Piehl (1974) examined the glycogen concentration in Standardbred trotters varying in age from 0.5 to 8 years. Glycogen concentration ranged from an average of 380 mmol.kg\(^{-1}\) (dwt) (glucose units kg\(^{-1}\) (dry muscle)) for the 0.5 year old horses to 504 mmol.kg\(^{-1}\) (dwt) for the 5-8 year old horses. Lindholm et al. (1974) and Essén-Gustavsson et al. (1989), reported glycogen concentrations of Standardbreds varying from 476-652 mmol.kg\(^{-1}\) (dwt). This compares with 396-606 mmol.kg\(^{-1}\) (dwt) reported for Thoroughbreds (Snow & Guy, 1976; Snow et al., 1987), and of 484-510 mmol.kg\(^{-1}\) (dwt) in endurance horses (Snow et al., 1981; Essén-Gustavsson, Karlström and Lindholm 1984). Values reported for humans are between 324 - 528 mmol.kg\(^{-1}\) (dwt) (Gollnick, Armstrong, Sembrowich, Shepherd and Saltin, 1973; Piehl, 1974; Symons and Jacobs, 1989; Vøllestad, Tabata and Medbø, 1992).

Diurnal variation does not appear to be a factor in the examination of muscle glycogen concentration. Lindholm and Piehl (1974) collected biopsies at 3 hourly intervals during the day and reported no significant difference between the different sampling times.

1.5 Muscle Glycogen and Physical Performance

1.5.1 Effects of Initial Muscle Glycogen Concentration on Performance

The importance of the muscle's initial glycogen concentration on human exercise performance has been highlighted by the work of Bergström et al. (1967). They found a strong correlation between the muscle's initial glycogen concentration and work time.
Since the muscle glycogen concentration is influenced by the type of diet before exercise, then nutritional status is a crucial factor in the ability to sustain prolonged endurance exercise.

Costill et al. (1981), examined the effect of a muscle's initial glycogen concentration on sprint performance. Total time for a 300 m sprint and split times for 100 m and 200 m were recorded. One hour after the sprint trial each subject performed a 30 min treadmill run at 70% \( \dot{V}O_2 \) max. The initial muscle glycogen concentration had no effect on either the 100 m and 200 m split or the 300 m time. This result is in contrast to the data from the endurance performance reported by Ahlborg et al. (1967) and Bergström et al. (1967).

The effects of athletes ingesting a carbohydrate supplement during performance was investigated by Hargreaves, et al. (1984). They found that total glycogen utilization was significantly lower during a solid feeding than during the control. The lower muscle glycogen utilization in the feeding trial was a consequence of the carbohydrate feeding rather than an effect of the lower initial glycogen level. In addition, following the carbohydrate feeding, subjects were able to perform significantly longer during the short term high intensity cycle phase of the test.

Spencer, Yan and Katz (1992), examined the effects of the muscle's initial glycogen concentration on metabolic responses to exercise. The increase in heart rate and \( \dot{V}O_2 \) during exercise were attenuated during the high versus the low initial muscle glycogen concentration trial. They found lactate concentration to be approximately four times higher after exercise in the high initial muscle glycogen group compared to the low.
The higher glycogenolytic rate and lactate content indicate that glycolysis was higher during exercise when the muscle's initial glycogen concentration was high. Also both before and after exercise, the lactate concentration in plasma was greater with the high glycogen treatment group, indicating that the contribution of glycogen to energy production was greater. The results of Spencer et al. (1992), are in contrast to the earlier work of Bergström et al. (1967), who reported that the oxygen uptake during exercise was similar for a fat, a protein and a carbohydrate diet during an exhaustive exercise bout.

Topliff et al. (1983), examined the influence of muscle glycogen concentration in horses, on metabolic responses during exercise on a treadmill at 2.9 m.s\(^{-1}\) to exhaustion. The run was administered before and after a 28 day training phase, at the end of a 5 day glycogen depletion phase, and at the end of a 3 day glycogen repletion phase. They reported that \(\dot{V}O_2\), respiratory rate and HR responses during the exercise were not significantly affected by a reduced initial muscle glycogen concentration. However, the HR and blood lactate concentration at the end of exercise were lower for the glycogen depleted phase compared to the training and glycogen repletion phases.

Symons and Jacobs (1989), considered whether muscle glycogen concentration had to be reduced to some critical level before performance was adversely affected. They examined whether intramuscular glycogen concentrations below approximately 220 millimole (mmol) glucose units kg\(^{-1}\) (dwt) would have an adverse effect on short term, high intensity exercise. Subjects performed a submaximal isometric contraction, plus a muscle fatigue test, followed by an exercise protocol designed to deplete the muscle glycogen concentration. For two days following the exercise they consumed low
carbohydrate diet, then on the fourth day repeated the submaximal and maximal exercise and fatigue test. Muscle glycogen concentration decreased from 427 to 153 mmoLkg\(^{-1}\) (dwt) without having a significant effect on either repeated maximal isokinetic muscle contractions or a single maximum isometric contraction.

Wootton and Williams (1984), reported that high intensity performance, despite large differences in initial muscle glycogen concentrations, was not affected, nor were blood lactate responses influenced by the initial muscle glycogen concentrations.

In contrast to that reported for humans (Costill et al., 1981; Symons and Jacobs, 1989; Spencer et al., 1992), Topliff et al. (1985), reported that a decreased muscle glycogen concentration does affect the degree of relative work performed by the horse. Relative work represented the distance the horse was able to drag a sled, times the weight of the horse plus sled. They compared the relative work performed by each horse following a 28 day training phase, a 5 day glycogen depletion phase and a 3 day glycogen repletion phase. Glycogen depletion was obtained by feeding horses a high fat/protein diet in combination with low and high intensity exercise.

There was a significant difference in relative work performed between the glycogen depletion phase, and the end of the 28 day training phase and the 3 day glycogen repletion phase. However, there was no significant difference between the end of training and the glycogen resynthesis phases (Topliff et al., 1985).
1.5.2 Muscle Glycogen Depletion and Repletion Patterns In Humans

The selective activation and rate of glycogen usage of muscle fibres is governed by the intensity, duration and type of exercise. However, the literature is not in total agreement in relation to the fibre recruitment pattern and glycogen usage rates of the selective fibres. These differences may as suggested by Vøllestad, Odd Vaage and Hermansen (1984), be due to differences in methodology of glycogen measurement, protocols and mode of exercise utilized for depletion.

Reported muscle glycogen depletion levels in humans following endurance exercise, vary from 40% following a 16.1 kilometre (km) run, to 86% following a 2 hour run (Ahlborg et al., 1967; Costill, Sparks, Gregor and Turner, 1971; Gollnick et al., 1973; Essén and Henriksson, 1974). Gollnick et al. (1973), after having subjects exercise for six one min bouts at 68% $\dot{V}O_2$ max, with a 10 min rest between bouts, found a 20% reduction in muscle glycogen concentration following the first bout, with this decreasing to about 63% by the 6th bout. Following the first bout there was no change in intensity of Periodic Acid Schiff (PAS) reaction staining for ST fibres with FT staining moderately. Following the third bout, 11% of the ST fibres stained moderately with this increasing to 9% staining negative, and 13% light after the sixth bout. The FT by comparison, had 54% stained moderate and 14% light after the third bout with this increasing to 26% light and 12% negative by the sixth bout. The change in percentage glycogen contribution from the ST and FT fibres with increasing exercise bouts, indicates that at the commencement of the bouts, the FT were the major glycogen contributors with ST becoming more important as exercise continued.
This fibre specific contribution was also shown in the work of Essén and Henriksson (1974) and Piehl (1974). Essén and Henriksson (1974), reported that during a 2 hour exercise period, by 40 min the FT and ST fibres had decreased their glycogen concentration by 59% and 57% respectively, with this increasing to 70% and 74% respectively by 80 min. At the completion of the 2 hours, the FT fibres had decreased by 29% whereas the ST had decreased by 86% of their respective resting values. Piehl (1974), found that after bouts of both endurance and sprint exercise in which the total glycogen had decreased by approximately 82%, the percentage in fibres showing either negative or light staining was 95% for ST and 65% for FT fibres.

This variability in rate of muscle glycogen reduction between fibre types with duration and intensity of exercise was further supported by the work of Vøllestad et al. (1984) and again by Vøllestad et al. (1992). However, Vøllestad et al. (1992), found that when the FT fibres were examined individually as IIA, IIAB and IIB, the depletion rate of the IIA fibres was similar to that of ST fibres with the IIAB and IIB being different. In their early work Vøllestad et al. (1984), examined the glycogen depletion pattern of fibres during sets of 20 min runs at 75% \( \dot{V}O_2 \text{max} \) until exhaustion. At the completion of the first 20 min of work there was a 78% and 67% reduction in type I and IIA fibres respectively, with the IIAB and IIB fibres remaining unchanged. During the next four 20 min bouts, glycogen staining intensity for the IIAB and IIB fibres respectively reached 45% and 62% of pre-exercise staining intensity. This finding of a similar rate of depletion with the ST and IIA fibres, they state, could be due to improved measuring techniques for glycogen concentration (optical density technique), compared to the subjective rating technique utilized in earlier studies. They further outline that as both ST
and IIA fibres had similar rates of glycogen depletion, they were both activated at the commencement of the exercise period with the IIAB and IIB not being recruited until later.

In examining the effects of high intensity exercise on muscle depletion rates Vøllestad et al. (1992), found that during exercise bouts at maximum effort for periods of 30 seconds (s) to 2-3 min duration the decline in muscle glycogen was 30 to 35% less in type I fibres compared to type II fibres, with no difference between the subgroups of type II fibres.

1.5.3 Muscle Glycogen Depletion and Repletion Patterns In Horses

The pattern of glycogen usage and selective fibre depletion in humans, with varying intensities of exercise, has also been demonstrated for horses (Essén-Gustavsson et al., 1984). They found that following a 100 km endurance ride approximately 90% of type I fibres stained low and 69% of type II fibres stained medium for glycogen. Regarding total glycogen used with exercise, Snow et al. (1981) reported a decrease of 56% in muscle glycogen content following an 80 km endurance ride with ST fibres showing the greatest depletion.

For high intensity exercise, Lindholm (1974) reported that when trotting speed was increased from approximately 5 m.s⁻¹ to 12.5 m.s⁻¹ the rate of glycogen usage increased from 0.3 mmol.kg⁻¹ to 14 mmol.kg⁻¹ min. This rate of glycogen usage is similar to that reported by Hodgson (1984) of 15.4 mmol.kg⁻¹ during a graded exercise test and that of Snow and Harris (1991) in which they found similar glycogen depletion rates
following a 1000 m and 1600 m gallop, and Snow, Harris and Gash (1985) following four 620 m gallops. However, this rate of glycogen usage appears to be very variable within a species as Snow and Harris (1991) reported a range of glycogen concentrations from a 15 mmol.kg⁻¹ gain to a 265 mmol.kg⁻¹ loss following exercise. They indicate that this large variation in glycogen usage rate between horses may be attributable to variance between muscle biopsy sites used for the pre and post exercise samples.

1.6. Muscle Glycogen Resynthesis Following Exercise

Studies have shown that the rate of muscle glycogen resynthesis following exercise is affected by factors such as the type of fibres depleted (Terjung, Baldwin, Winder and Holloszy, 1974), glucose dose administered after exercise (Blom et al., 1986; Blom, Høstmark, Veage, Kardel and Maehlum, 1987), the timing of the administration of the supplement after exercise (Ivy et al., 1988a), the activity state of glycogen synthetase and the initial glycogen concentration (Danforth, 1965; Larner, Villar-Palasi, Goldberg, Bishop, Huijing, Wenger, Sasko and Brown, 1967; Bergstrom, Hultman and Roch-Norlund, 1972; Kochan, Lamb, Lutz, Perrill, Reimann and Scholender, 1979).

In humans, rates of muscle glycogen resynthesis of 23 mmol.kg⁻¹.h⁻¹(dwt), during the first 2 hours of recovery, following the provision of 3.0 g.kg⁻¹ of glucose (Ivy, Lee, Brozinick and Reed, 1988b), 30.8 mmol.kg⁻¹.h⁻¹(dwt) following the ingestion of 2 g.kg⁻¹ of a 25% carbohydrate solution (Ivy et al., 1988a) and 23 mmol.kg⁻¹.h⁻¹(dwt) with the
ingestion of 0.7g.kg⁻¹ of glucose every 2 hours for 8 hours (Blom et al., 1987) have been reported.

In horses, muscle glycogen resynthesis rates of 12.5 mmol.kg⁻¹.h⁻¹(dwt) were reported by Snow et al. (1987) for the first 8 hours, following a high carbohydrate diet plus infusion, and 5.6 mmol.kg⁻¹.h⁻¹(dwt) and 7.8 mmol.kg⁻¹.h⁻¹(dwt) by Hodgson (1984) for the 20-24 hour period following a 160 km endurance ride and for the first 4 hours immediately following a treadmill exercise test.

1.6.1 Effects of Fibre Type on Muscle Glycogen Resynthesis

Based on differences in the enzymatic capacity of the different muscle fibre types to convert glucose to glycogen, Terjung, et al. (1974) proposed that differences could exist between specific muscle fibres in relation to their capacity for glycogen resynthesis. They reported that in rats, fast twitch oxidative muscle had the most rapid repletion of glycogen stores, with a return to the resting concentrations by 1 to 2 hours after exercise. Fast twitch glycolytic muscle only attained 74% of the resting level by 4 hours after exercise. Slow twitch red muscle was intermediate between that of the fast twitch oxidative and the FT glycolytic muscle fibre. The highest rate of glycogen synthesis occurred between 30 to 60 min after exercise with the fast twitch oxidative being approximately three times faster than the fast twitch glycolytic. The findings that fast twitch oxidative muscle fibres have the fastest rate of glycogen resynthesis was also reported for rats by Conlee et al. (1978). They reported that this was followed by slow-twitch oxidative muscle, with fast twitch glycolytic muscle having the slowest rate of
glycogen repletion in rats. Piehl (1974) examined muscle glycogen repletion in humans over a 46 hour period following a depletion run. Fast twitch fibres also displayed the fastest rate of muscle glycogen resynthesis at 5 and 24 hours after depletion runs, with all fibres having returned to pre-exercise concentrations by 46 hours.

Conlee et al. (1978) suggest that the differences in glycogen repletion rates between muscle fibres are the result of a combination of the initial muscle glycogen concentrations and glycogen synthetase activity. They report a strong inverse relationship between the percentage of glycogen synthetase in the I form and muscle glycogen concentration. Further, a linear relationship exists between total glycogen synthetase I activity and the rate of glycogen synthesis. Conlee et al. (1978) also showed that fast twitch red fibres, which have the highest total glycogen synthetase I activity, displayed the most rapid rate of glycogen synthesis, while fast twitch white, which had the slowest rate of glycogen synthesis, had the lowest total glycogen synthetase I activity. Slow twitch red muscle which had an intermediate total glycogen synthetase activity had an intermediate rate of glycogen synthesis.

The activity state of the different fibres with reference to glycogen synthetase as reported by Conlee et al. (1978), provides an explanation for varying degrees of increased glycogen concentrations between fibres, as reported in Richter, Hansen and Hansen (1988). They found the perfused muscle of fed rats could be increased almost 2-fold in fast twitch white fibres, 3-fold in slow twitch red fibres and 3.5-fold in fast twitch red fibres during prolonged perfusion with a maximally stimulating insulin concentration and a moderately high glucose concentration.
1.6.2 Glycogen Synthetase

Glycogen synthetase is the rate limiting enzyme in skeletal muscle glycogen resynthesis (Kochan et al., 1979). It has been demonstrated that Glycogen synthetase exists in two forms, a D (dependent) or phosphorylated form and an I (independent) dephosphorylated form (Lehninger, 1975; Larner, 1990). The D form is inactive in the presence of G-6-P, with the I form being active in the presence of G-6-P. The conversion from the physiological active I form to the less active D form requires phosphorylation of the enzyme by protein kinase (Soderling, Jett, Hutson and Khatra, 1977).

Bergström et al. (1972) compared the effects of a diet high in carbohydrate with carbohydrate administered by infusion on muscle glycogen resynthesis. The percentage of glycogen synthetase I activity in the exercised muscle increased concomitant with the fall in glycogen content, whereas the percentage of synthetase I activity in the resting leg was unchanged. In addition, the glycogen synthetase in the I form decreased as the glycogen content in the muscle returned to normal. They proposed that an inverse relationship existed between glycogen content and synthetase I activity at normal and low glycogen concentrations. This relationship between glycogen concentration and synthetase I activity is the result of both the synthetase and the phosphatase being bound to glycogen in the cell. A decrease in glycogen stores would result in the release of both the synthetase and phosphatase molecules resulting in an increase in the I form of the synthetase (Larner et al., 1967). Bak and Pedersen (1990) further add that following exercise glycogen synthetase is activated as it becomes more sensitive to the allosteric activator G-6-P and thus becomes stimulated in favour of glycogen synthesis.
Kochan et al. (1979) found that glycogen synthetase activity ratio (D to I) increased dramatically (10 fold) immediately after exercise. In comparing exercised with non exercised legs, they found that after 24 and 48 hours of recovery, activity ratios in exercised and non exercised muscle were significantly higher than in the corresponding pre-exercise control. By 96 hours, activity ratio returned to pre-exercise concentrations in both muscles as glycogen had increased above resting concentrations. The Fractional Velocity, as an alternative method of expressing the activation state of glycogen synthetase was proposed by Kochan et al. (1979). Fractional Velocity is the velocity of the enzyme at any concentration of G-6-P divided by the velocity at saturation levels of G-6-P. Yan, Spencer, Bechtel and Katz (1993) reported a decreased fractional velocity from rest to fatigue, with it increasing to above resting values by 1 min after exercise.

Studies on humans have shown that training can increase the activity of glycogen synthetase by 35% (Piehl et al., 1974), and this increase in glycogen synthetase is a factor responsible for the increase in muscle glycogen witnessed with training. However, reports of increases in glycogen synthetase activity with training are conflicting, with greater increases having been reported for both FT and ST fibres (Engel, 1962; St George Stubbs and Blancher 1965). For the horse, Snow and Guy (1979) reported that both training and detraining had little effect on the activity state of glycogen synthetase.

1.6.3 Effects of Diet on Muscle Glycogen Resynthesis

The major limiting factor in glycogen synthesis according to Fell, Terblanche, Ivy, Young and Holloszy (1982), is the ability of muscle to synthesise glycogen rather
than availability of substrate. They indicate that a low muscle glycogen content following exercise is a major stimulus for increasing the rate of glucose transport into the muscle, in addition to an increased rate of glycogen synthesis.

A prime regulator of glycogen resynthesis is the activity state of glycogen synthetase (Danforth, 1965; Bergström et al., 1972 and Bak and Pedersen, 1990), which in turn is regulated in part by the muscles' concentration of glycogen and glucose-6-phosphate (G-6-P). As G-6-P concentration is affected by the available blood glucose, it can be postulated that the diet which follows exercise could be of paramount importance in maximising muscle glycogen resynthesis.

In humans and rats, both the type of substrate, volume consumed and time of consumption following exercise have been examined in reference to maximising the rate of muscle glycogen resynthesis. Young, Garthwaite, Bryan, Cartier and Holloszy (1983) fed rats a carbohydrate free diet following exercise. This did not completely prevent an increase in muscle glycogen concentrations, but resulted in muscle glycogen concentrations similar to those found in fasting or fat fed non-exercised rats. Saitoh, Shimomura and Suzuki (1993) examined the effects of a high carbohydrate intake (3g.kg⁻¹.bwt⁻¹) during recovery, in rats previously fed a high fat or carbohydrate diet. They reported a positive relationship between the pre-exercise muscle glycogen concentration and rate of glycogen repletion.

Maehlum, Hostmark and Hermansen (1977) reported that the most pronounced increase in glycogen repletion was seen in the course of the first 4 hours of recovery, with a mean rate of glycogen synthesis during this period of 7.2±0.7 mmol.kg⁻¹.h⁻¹.
Blom et al. (1986) and Blom et al. (1987) examined the type of sugar, total dosage, and the timing of providing a supplement after exercise on muscle glycogen resynthesis rates in humans. The increase in muscle glycogen resynthesis corresponded with the amount of carbohydrate ingested, but there was an upper limit to the glucose dose on muscle glycogen resynthesis. Increasing the glucose load from 0.35 g.kg\(^{-1}\) to 0.7 g.kg\(^{-1}\) every 2 hours more than doubled the rate of resynthesis. However, when each of the glucose loads was again doubled to 1.40 g.kg\(^{-1}\) there was no further increase in the rate of muscle glycogen synthesis. Their results from the different sugar sources indicate that glucose and sucrose ingestion have a significantly stronger effect on post-exercise muscle glycogen synthesis than fructose ingestion. The lower rate of resynthesis of glycogen during fructose infusion has been attributed to the difference in insulin activity, a lower blood glucose concentration following the fructose infusion (Bergström and Hultman, 1967b) and to different handling processes of the various sugars in the body, as fructose metabolism takes place predominantly in the liver.

The effects of simple sugars have been compared to polysaccharides (complex carbohydrates, starches), as a stimulus for glycogen resynthesis (Costill et al., 1981). They found that the type of carbohydrate, simple or complex, had no differential effect on the change in muscle glycogen during the 24 hours after exercise. However, the next 24 hours resulted in a significant increase in the storage rate for the complex carbohydrate diet. An elevated serum insulin concentration, which occurred as a result of the complex carbohydrate diet as compared to the simple sugars was suggested to be responsible for the enhanced muscle glycogen storage during the second 24 hour period.
The effect of timing of feeding after exercise was examined by Ivy et al. (1988a) and Ivy et al. (1988b). They provided a carbohydrate supplement dose either immediately after exercise or after a 2 hour delay. The muscle glycogen resynthesis rate in the first 2 hours of recovery was three times faster in the immediate than in the delayed feed group. In the second 2 hours of recovery, the rate of glycogen storage slowed 44% for the immediate feed group but increased 67% for the delayed feeding group. However, the rate of glycogen storage for the delayed feeding group in the second 2 hour period, was 45% slower than that which occurred in the immediate feeding treatment during the first two hours after exercise.

Ivy et al. (1988b) further reported that the rate of muscle glycogen storage was the same whether the subjects ingested a high or low carbohydrate supplement, indicating, as reported by Blom et al. (1987), that there exists an upper limit to the carbohydrate ingested effects on glycogen resynthesis. The rate of resynthesis was greatest in the initial 2 hours with a 25% decline occurring in the second 2 hours of recovery. This decline in the second 2 hour period Ivy et al. (1988a) state could be due to a number of factors, namely that there is an initial rapid decline in blood flow, followed by a more gradual decline back to the resting rate following exercise, and an increased muscle insulin sensitivity which also decreases over time. In addition, a decline in the activity of glycogen synthetase occurs, resulting in a reduction in the rate at which glucose could be processed through the glycogen synthetic pathway.

The effects of gastric emptying rates on muscle glycogen resynthesis were examined by Reed, Brozinick, Lee and Ivy (1989). Three methods of administration were compared: a solid, liquid and intravenous glucose infusion, each at a dose of 3 g·kg⁻¹
1(bwt). The rates of glycogen resynthesis were similar for the three treatments, even though blood glucose concentrations in the intravenous treatment were higher, suggesting that entry of carbohydrate into the circulation would not be a limiting factor.

The addition of a protein supplement to a carbohydrate diet has been shown to affect the glycogen resynthesis rate (Zawadzki, Yaspelkis and Ivy, 1992). Subjects were provided with either a carbohydrate, carbohydrate plus protein or protein supplement immediately and two hours post exercise. Muscle glycogen concentration of the carbohydrate and carbohydrate plus protein treatments were not significantly different at 4 hours after exercise. However, the rate of resynthesis for the carbohydrate plus protein treatment was 38% faster than that which occurred during the carbohydrate treatment. They emphasise, however, that the rate of resynthesis was similar to that reported following standard carbohydrate supplements. They suggest that the protein addition may only be beneficial when carbohydrate intake is low. The addition of the protein resulted in a synergistic insulin response, which could result in an increased clearance of glucose by the muscle due to the increased plasma insulin response.

There have been few studies of factors influencing muscle glycogen resynthesis in the horse, with the only referable one to the current study being that by Snow et al. (1987). They examined the effects of a low, high and normal diet on muscle glycogen resynthesis following exercise in Thoroughbred horses. In addition to the high carbohydrate diet, the horses were given glucose intravenously at a constant rate over 6 hours. The greatest resynthesis of muscle glycogen was in the first 8 hours, with the low carbohydrate group having little further increase after this. During the first 28 hours the repletion rates for the normal and high carbohydrate groups were similar but both were
higher than the low carbohydrate diet and only at 28 hours was the difference between
the low and high/normal diets significant.

1.6.4 Glucose Uptake After Exercise

Utilizing the rat's hindlimb to study the initial stages of glucose uptake after
exercise, Ivy and Holloszy (1981) found that the permeability of the cell membrane to
glucose was elevated and remained markedly elevated for 1 hour after exercise. They
concluded that as muscle glycogen concentration approached its upper limit, the rate-
limiting step for glucose uptake may shift from transport to conversion of glucose to
glycogen. This mechanism involved a slowing of glycogen synthesis as the percentage of
glycogen synthetase in the I form decreased with high glycogen concentrations (Ivy and

Ivy et al. (1988b) found that in humans following a high carbohydrate
supplement immediately after exercise, the blood glucose concentration increased to
above the pre-exercise concentration by 15 min after exercise and continued to rise for
up to one hour after exercise. Even though concentrations began to decrease after this,
they were still above the pre-exercise level at the end of the 4 hour recovery period. A
similar pattern was observed following the administration of a low carbohydrate
treatment, but the blood glucose did not rise above the pre-exercise level until 30 min
after exercise. When food was withheld in the recovery period, blood glucose increased
minimally but still remained below the pre-exercise concentration during the recovery
period.
1.6.5 Role of Insulin

In horses and humans, exercise has been shown to produce a reduction in plasma insulin concentration (Snow and Rose 1981; Church, Evans, Lewis and Rose 1987; McArdel, Katch and Katch, 1991) with a rise immediately post exercise. These responses are the result of an inhibitory effect by increasing levels of alpha-adrenergic input to the beta cells of the pancreas during exercise, with a withdrawal of the input following exercise (Church et al., 1987; McArdel et al., 1991).

It is known that exercise results in an increased muscle insulin sensitivity in the active musculature (Richter, Ploug and Galbo, 1985). However, in the presence of glucose this increase in insulin sensitivity and glucose transport decays over time.

Rodnick, Reaven, Azhar, Goodman and Mondon (1990) report that in rats, insulin stimulated glucose uptake is enhanced in muscle with training. The improvement in insulin stimulated glucose uptake following training was associated with increased glycogen deposition in fast twitch muscle but not in slow twitch muscle. An improved insulin sensitivity in hyperinsulinaemic ponies has been shown following an improvement in the exercise capacity of ponies (Freestone, Beadle, Shoemaker, Bessin, Wolfsheimer and Church, 1992).

Ivy et al. (1988b) observed that immediately following an exercise period, insulin concentration during a high glucose dose treatment peaked at 3.5 hours of recovery, at a level that was 10 times higher than the pre-exercise concentration. Insulin responses to a lower dose treatment followed a similar pattern, although significantly lower.
Insulin responses in humans following a 2 hour exercise period, during which subjects received similar amounts of carbohydrate relative to subject body weight, as either a liquid or a solid carbohydrate feeding, or an intravenous glucose infusion, were examined by Reed et al. (1989). The plasma insulin concentrations were similar for the three treatments both before and during exercise. However, by 150 and 180 min after exercise the insulin response of the liquid treatment was significantly higher than that for the solid treatment, with the mean concentrations for the recovery period being significantly different.

A synergistic insulin response has been shown to occur with the addition of protein to a carbohydrate supplement, resulting in a faster rate of muscle glycogen resynthesis compared with the carbohydrate treatment alone (Zawadzki et al., 1992).

1.6.6 Effects of Training on Muscle Glycogen Resynthesis

In rats, the effects of exercise plus the feeding of a high carbohydrate diet over the ensuing 18 hours, increased muscle glycogen concentration approximately 30% above that seen in non-exercised animals fed the same diet (Young et al., 1983).

James and Kraegen (1984) examined the effects of training intensity on glycogen regulation in specific fibres of rats. They reported that regardless of the intensity of training, the glycogen content in the fast twitch extensor digitorum longus and liver was unaffected. However, there was a training effect for the intermediate muscle (soleus). This increase in glycogen content was coupled with an increase in glycogen synthetase and phosphorylase activities in the intermediate muscle. The
increase in synthetase and phosphorylase activities in the soleus was proportional to the
degree of training, emphasising the importance of the intensity of the training program
on stimulating changes.

Piehl, Adolfsson and Nazar (1974), found that following training at 70-80% 
\( \dot{V}O_2 \) max, plus the addition of short supramaximal work bouts to the point of
exhaustion, there was a 35% and 18% increase respectively in the activity of glycogen
synthetase and hexokinase.

Training therefore seems to induce a local increase in muscle glycogen
independently of possible dietary adjustments. The mechanism behind the increase in
muscle glycogen resulting from training involves an increased glycogen synthetase
activity.

1.7 Conclusion

In humans, endurance performance is improved by increasing the muscle's initial
glycogen concentration (Bergström et al., 1967a) and by the provision of a carbohydrate
supplement during exercise (Hargreaves et al., 1984).

Resynthesis of muscle glycogen post exercise in humans has been shown to be
affected by factors such as the activity state of glycogen synthetase (Danforth, 1965;
Larner et al., 1967; Bergström et al., 1972; Kochan et al., 1979), the glucose dose
administered and the timing of the administration of the dose post exercise (Blom et al.,
1986; Blom et al., 1987; Ivy et al., 1988a)
In horses, there is little information examining the importance of an exogenous glucose supply on muscle glycogen resynthesis after exercise, or of the importance of the muscle's initial glycogen concentration on metabolic responses to exercise. The only information referable to these areas are a study by Snow et al. (1987) on resynthesis, and by Topliff et al. (1983) and Topliff et al. (1985) on glycogen overloading and performance.

An understanding of whether glucose supplementation post exercise can increase the rate of muscle glycogen resynthesis would have a practical application in assisting to optimise performance in horses competing on successive days in endurance events. In addition, an understanding of the role of muscle glycogen on endurance and high intensity exercise may be of assistance in developing nutritional strategies for horses.
Chapter 2

GENERAL MATERIALS AND METHODS

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2.1 Introduction

In this chapter the materials and methods used in experiments are described in full. In the following chapters, only a brief outline of each procedure is made, however, reference to the authors of the original procedures and appropriate sections of this chapter are provided.

All experimental procedures were approved by the University of Sydney Animal Care and Ethics Committee.

2.2 Horses

Both Standardbred and Thoroughbred horses were used for the experiments. Selection of horses for the experiments was based on availability. All horses were purchased from the sale yards and were used exclusively for experimental purposes. Daily nutritional intake during the exercise studies consisted of approximately 2.0kg pellets, (Coprice, Leeton, Australia) 200 g lucerne, 800 g chaff, 500 g sweet feed, (Stable King Working Horse Mix, Fielders Ltd, Sydney, Australia) and a table spoon of salt for the morning and night feeds, with 2 kg of lucerne hay at noon. This provided a total intake of 11 kg or approximately 2.3% body weight. Examinations for lameness were conducted on all horses prior to the commencement of experiments. All horses were housed in yards.
2.3 Bodyweight Measurement

Electronic scales (Model KT2000R, Kinetic Technology, Queensland, Australia) with an accuracy of ±1.0 kg were used for weighing horses before each exercise test.

2.4 Blood Collection Procedure

2.4.1 Catheterisation of Horses

An area of the neck surrounding the site for the placement of the catheter into the jugular vein was shaved, scrubbed with povidone iodine solution (PCV-iodine 75 mg.ml⁻¹ Apex Lab. Sydney, Australia) and disinfected with 70% alcohol and tincture of iodine. The area was desensitised by the injection of 1mL of 2% prilocaine (Delta Veterinary Laboratories Pty. Ltd. Sydney) subcutaneously into the site for the placement of the catheter. A 14 gauge catheter (Angiocath, Becton Dickinson, Vascular Access, Utah, USA) was flushed with sterile heparinised saline solution (4IU heparin.ml⁻¹) prior to being inserted through the skin into the jugular vein. An extension tube (Cat No. 72-035, Tuta Laboratories, Sydney, Australia), with a three way tap was connected to the catheter and secured to the skin with Super Glue (Sellys, Australia). The tube was taped to the horse’s mane to allow freedom of movement without dislodging the catheter. Catheter flow was maintained by occasional irrigation with sterile heparinised saline (4 IU.ml⁻¹).
2.4.2 Blood Collection

All blood samples were collected from the jugular vein. Prior to the drawing of a sample, 15 mL of waste was drawn into a 20 mL syringe. Following the taking of the sample, an equal volume of sterile heparinised saline was injected back into the jugular vein to replace the volume of blood removed.

Samples for glucose assays were collected into chilled 5 mL evacuated tubes containing potassium oxalate and sodium fluoride (Becton Dickinson Rutherford, New Jersey) and those for insulin collected into chilled 5 mL evacuated tubes (Becton Dickinson Rutherford, New Jersey) containing lithium heparin. Samples for white blood cell counts (WBC), packed cell volume (PCV) and total protein (TP) were collected into chilled 5mL evacuated tubes containing ethylene diamine tetraacetic acid (EDTA, Becton Dickinson Rutherford, New Jersey). All tubes were chilled by keeping them on ice prior to collection of blood and were immediately placed back on ice following the collection of the sample.

The blood for glucose and insulin was centrifuged (Beckman GPR Centrifuge, USA) for 15 min at 3000 revolutions per minute (rpm) and plasma decanted into 5 mL disposable containers and stored at -20°C until assays were conducted.

2.5 Blood Analysis Procedures

2.5.1 Osmolality

Osmolality of the plasma was measured with an Osmometer (3W2 Advanced
Instruments, Massachusetts, USA) which utilizes the freeze up depressor method. Readings were reported in the units milliOsmoles per kilogram water (1 litre) (mOsm/kg H₂O).

Two hundred microlitres of plasma was pipetted into a 5 mL glass tube using a Gilson pipette. The tube was then placed into the plunger head and lowered into the cooling chamber. When the sample was cooled to the required temperature a reading was displayed and recorded. Standards of 100 and 500 (mOsm/kg H₂O) were run following every sixteenth sample with mean±(SEM) for each being 107±0.99 and 503±1.5 respectively.

2.5.2 White Blood Cell Counts

White blood cell counts (WBC) were done according to the procedure of Dacie and Lewis (1975). A 1 in 20 dilution was made by adding 20 microlitres of blood to 0.38 ml of the staining fluid. Acetic acid coloured with toluidine blue was used for staining. The solution was vortexed and a small volume pipetted out and placed under a cover slip on a counting chamber (Haemocytometer Spencer, Germany). Care was taken not to overfill the chamber. Approximately 2 min was allowed between filling the chamber and counting to allow for settling of the cells. The red cells were lysed by the staining fluid but the white cells remained intact, with their nuclei stained.

The mean of the 4 chambers counted was taken. The mean was multiplied by 200 x 10⁶ to convert from a 0.1 microlitre volume to number of cells per litre (10⁹.L⁻¹)
2.5.3 Plasma Total Proteins

Total plasma protein was measured using a refractometer (Cambridge Inst. Inc. Buffalo, NZ, USA). A small volume of plasma was placed onto the cover lid of the instrument. The instrument was then held up to a natural light source and the protein value read. Duplicate samples were taken for each period and the mean of the two samples recorded.

2.5.4 Packed Cell Volume

Blood was drawn into a heparinised haematocrit tube and spun in a microhaematocrit centrifuge for 15 min. The tube was placed onto a grid and the percentage of cells to plasma read. Duplicate samples were done for each time period and the mean of the two readings taken.

2.5.5 Glucose Concentration

Plasma glucose concentration was measured using an automatic analyser (YSI Model 2300, Yellow Springs Instruments, Columbus, OH, USA). The process involved the substrate reacting with the enzyme glucose oxidase, producing hydrogen peroxide (H₂O₂). The hydrogen peroxide was in turn oxidised at a platinum anode, producing electrons. An equilibrium was achieved when the rate of hydrogen peroxide production and the rate at which it leaves the reaction area are equivalent and is indicated by a steady
state response. The electron flow is linearly proportional to the steady state hydrogen peroxide concentration and therefore to the concentration of glucose.

Repeatability of measures was determined by running glucose standards (4 mmol and 10 mmol) 10 times and calculating the co-efficient of variations (CV). The CV values were 2.7% and 5.9% respectively for the 4 mmol and 10 mmol standards. Standards were run daily before the measurement of samples.

2.5.6 Insulin Assay

Plasma insulin concentration was measured by radioimmunoassay, using a commercial kit (Medgenic Diagnostics, Brussels, Belgium).

The principle of this test was based upon the existence of competition between a labelled and an unlabelled antigen for specific antibodies. In the presence of an increasing quantity of unlabelled antigen, more unlabelled antigen-antibody association and less labelled antigen-antibody association will occur. After an incubation period the antibody bound antigen was separated from the free unbound reactants and the bound radioactivity was measured on a gamma counter.

Method;

a. 100 μL of standards (0-5-10-20-50-100-200-500 μU.ml\(^{-1}\)), controls (high and low) and samples were dispensed into labelled tubes in duplicate:
b. The standard with zero concentration of insulin as used for non-specific binding
determination:

c. 100 µL of $^{125}$I-Insulin was added to each tube:

d. 100 µL of insulin antiserum was added to each tube, except those used for
determination of total count and non-specific binding:

e. 100 µL of NSB buffer (1 mL phosphate buffer (0.05 M, pH 7.5) containing
0.024% sodium merthiolate and 0.5% of bovine serum albumin) was added to the
tubes for non-specific determination:

f. Tubes were covered with plastic wrap and allowed to incubate for 90 min at
room temp:

g. 1 mL of solution containing a second antibody plus polyethylene glycol (DA-
PEG solution) was added to every tube, except those used for total counts. Tubes
were shaken gently for approximately 20 s:

h. Samples were allowed to incubate for 20 min at room temp:

i. The samples were then centrifuged for 15 min at 1500 g (Beckman GPR,
USA):

j. The supernatant was decanted and tubes inverted to drain onto blotting paper.
Care was taken not to disturb the precipitate:

k. The radioactivity of each sample was then determined by counting on a gamma
counter for 60 s (LKB (Wallac) 1272 Clinigamma, Finland):

l. Calculation of bound radioactivity as a percentage of the binding was
determined at the zero standard point.
Intra-assay variability was determined by repeating assays of a low and high human insulin standards 12 times each. Inter-assay variability was determined by running an equine plasma sample of known concentration with each assay. The coefficient of variation for the low and high human insulin standards ranged from 7.4%-11.9% and 4.4%-10.9% respectively. Intra and inter-assay coefficient of variations were 8.7% and 6.8% respectively.

The specificity of the assay was evaluated by running dilution curves of a human standard and a sample of equine plasma. Sample and standard were diluted by 20, 40, 60 and 80%. Parallelism between insulin concentrations in serial dilutions of human insulin standards and equine plasma was demonstrated (See appendix B).

2.6 Calculation of Area Under The Curve

Area under curves was calculated by digitizing the perimeter and using a computer programme (CADD, Generic 6.0, Digitizer A4-Teck Model KD1212, Autodeck, Pty. Ltd. Nth Ryde NSW, Australia). Time was on the X-axis and glucose or insulin concentrations on the Y-axis. Units are in square millimeters (sq.mm)
2.7 Muscle Biopsy

2.7.1 Muscle Biopsy Sites

Biopsies were taken from the m. gluteus medius. The site for biopsy was clipped and marked for the location of the biopsy incisions. Locations were identified by drawing lines between several anatomical landmarks. A line was drawn from the tuber coxae to the trochanter major of the femur, whilst a second line was drawn vertically from the first line to the tuber ischii. An additional landmark was where this line crossed the border of the sacro-sciatic ligament/semitendinosus. A third line was drawn from the tuber sacrale to the tuber coxae and a point 20 cm along this line was marked. A line was then drawn from this point to the mark on the sacro-sciatic ligament. The area enclosed within these lines was then divided into sections for the biopsy sites (see Figure 2.1).

2.7.2 Muscle Biopsy Procedure

Muscle biopsies were taken using the needle biopsy technique of Bergström (1962) as modified by Lindholm and Piehl (1974). The biopsy site was clipped, scrubbed with povidone iodine and disinfected with 70% alcohol and tincture of iodine. The area was desensitised by the injection of 1 mL of 2% prilocaine (Delta Veterinary Laboratories Pty. Ltd., Sydney) subcutaneously into the site using a 25 gauge needle. Care was taken not to inject into the muscle.
The biopsy needle (Stille, Germany) consisted of three parts;

a. 6 mm diameter outer needle with a pointed tip and a window opening:

b. an inner sharp-edged cutting cylinder:

c. a stilette which fitted into the cutting cylinder to enable removal of the specimen from the needle.
Figure 2.1 Diagram of the m. glutaeus medius of the horse, showing the 8 sites utilized for taking muscle biopsy samples.
The biopsy needle was inserted to a depth of 8 cm and four quick cuts were made. When retracting the needle two fingers were placed on either side of the incision to reduce tissue damage. A clean swab was placed over the site to prevent any infection.

The stilette was used to extract a cylindrical piece of muscle from the needle which was placed onto a piece of aluminium foil. The sample was examined under a dissecting microscope and, using two 25 gauge needles, the muscle sample was divided into histochemical and biochemical sections. Consistency of sampling depth was maintained by the use of markers on the biopsy needle. Biopsy needles were immediately washed after each biopsy and sterilised by either soaking in Cidex activated dialdehyde solution (Johnson and Johnson, Sydney), when frequent use was required or alternatively by autoclaving.

Muscle fibres for histochemistry were aligned vertically and mounted onto cork blocks (4x4 mm) using OCT embedding medium (Ames Tissue-Tek), and frozen in chilled isopentane (2 methyl butane, BDH Chemicals, Port Fairy Victoria, Australia) cooled in liquid nitrogen.

The remaining muscle from each biopsy sample was placed into 2 mL plastic ampoules (Bacto Laboratories, Liverpool, England) and stored in liquid nitrogen storage containers (MVE Cryogenics, CIG, Sydney, Australia) at -196°C for later biochemical analysis.
2.7.3 Freeze Drying

All samples before freeze drying were dissected free of connective tissue, fat or blood. To prevent any thawing of the muscle, the dissecting stainless steel tray (12 cm x 12 cm x 1 cm), instruments, samples and the stainless steel freeze drying block were placed on dry ice prior to and during the dissection process. The freeze drying block was 125 mm x 60 mm x 18 mm with 10 storage holes, and a perspex lid held in place by two screws. The muscle samples were dried overnight in a freeze drier (Dynavac Model ED16/M high voltage system, Burwood, Victoria, Australia), then stored in Eppendorf Micro-test tubes (3810) at -20°C.

2.7.4 Glucose Extraction for Glycogen Concentration

Muscle glycogen concentrations were determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al. (1987). The freeze dried sample was examined under a dissecting microscope and again dissected free of any blood or fibrous tissue. The dried muscle sample (2-4 mg) was weighed (Sartorius analytical balance, Selby's Scientific, Sydney, Australia, (.0001 mg)) and placed into a 1.5 mL screw top conical tube (Alltech Pty. Ltd., Homebush, Australia).
Method:

a. 250 µL of 2 M KOH was added and the muscle solubilised at 50°C for 10 min in a water bath (Gallenkamp PN 4BIR-M England):

b. tubes were allowed to cool and 250 µL of 3 M HCl was added to hydrolyse the glycogen to glucose:

c. tubes were placed in a water bath (Grant W28, Instruments, Cambridge, England) set at 100°C for one hour:

d. tubes were weighed pre and post water bath to ensure no leakage had occurred:

e. tubes were cooled and 90 µL of KOH was added. The volume of KOH added was slightly below that needed for total neutralisation to ensure that the solution does not become too alkaline thereby destroying any glucose:

f. tubes were vortexed and centrifuged (Orgital 400 Clements Phoenix Scientific, Australia) at 3000 rpm for 20 min:

g. the supernatant was pipetted off and stored in Eppendorf Micro-test tubes (3810) at -20°C.

All samples were done in duplicate.

The concentration of glucose in the extracted sample was determined by fluorescence, utilizing the method referenced in Essén and Henriksson (1974).

The reactivation solution was made up of:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>50 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris. HCl</td>
<td>100 mM</td>
</tr>
<tr>
<td>0.1 M ATP</td>
<td>0.3 mM</td>
</tr>
</tbody>
</table>
0.1 M MgCl 6.0 μM 300 μL
0.1 M NAD 0.05 mM 25 μL
10% BSA 0.26 % 130 μL
2 mg mL⁻¹ G-6-P, DH 1 μg mL⁻¹ 35 μL

The solution was adjusted to pH 8 by adding 1 M HCl or 1 M NaOH (PLM83 Autocal pH meter, Radiometer, Copenhagen). A volume of 2.95 mL of reactivation solution was added to each glass tube. To the 2.95 mL of reactivated solution was added 40 μL of sample. All samples were then vortexed, wiped to remove any fingerprints and the initial fluorescence was read (Perkin-Elmer 1000M, Fluorimeter). Four μL of hexokinase (1500 U mL⁻¹) was added to each glass tube and allowed to incubate for 60 min.

A control and one sample were checked at intervals of approximately 10-15 min after 30 min, to ascertain when the reaction was completed. The final fluorescence of each sample was read. All samples were vortexed just prior to being placed into the fluorimeter. A standard curve was run with each assay consisting of 0, 2.5, 5.0, 7.5, 10 μL of a 10 mM glucose standard giving final concentrations of 10, 25, 50, 75 and 100 nM glucose. All standards were run in duplicate.

Final glucose concentration was calculated as follows:

a. Subtract the initial fluorescence from the final fluorescence:
b. Determine nMoles glucose in assay volume (20 μL). A regression equation was calculated from standards. The X-coefficient was multiplied by (final fluorescence-initial fluorescence) to determine glucose in assay volume (20 μL):

c. To determine total glucose in muscle extract (nmoles in assay volume /20 *[590]). 590 represents total volume added in μL.

d. Convert answer to nmoL glucose.mg⁻¹ (divided by sample weight mg).

2.7.5 Histochemistry:

Serial muscle transverse sections (10 μm) for staining were cut on a cryostat microtome (Reichert-Jung Model 2700 Frigocut Selbys Scientific, Sydney) which was pre-cooled to -20°C. Sections were mounted on cover slips and placed into staining jars (Columbia jars) which were kept at -20°C.

Serial sections were then pre-incubated at both pH 4.3 and 10.3 for identification of slow twitch (ST) and fast twitch (FT) fibres (Brooke and Kaiser, 1970). To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff, Shin and Drucker (1961), and the fast twitch fibres subdivided into high oxidative (FTH) and low oxidative (FT). The identification of muscle fibre glycogen concentration was carried out using the Periodic Acid Schiff (PAS) reaction as described by Pearse (1968).
2.7.5.1 Method for Myosin Adenosine Triphosphatase (Myosin ATPase)

The method was based on the procedure described by Brooke and Kaiser (1970) and modified for equine skeletal muscle by Essén, Lindholm and Thornton (1980). All staining was carried out in staining jars.

**Alkaline Preincubation Buffer (APB)**

- glycine (NH$_2$CH$_2$COOH)  2.253 g
- calcium chloride (CaCl$_2$2H$_2$O)  2.4 g
- sodium chloride (NaCl)  1.755 g
- distilled water  300 mL
- 0.1 M sodium hydroxide  270 mL

**APB Stain:**

a. Samples were pre-incubated in APB (pH 10.3) for 5 min in an agitating water bath (37°C) (Gallenkamp, PN 4 BIR-M, England):

b. Samples were rinsed 5 times with distilled water, then the staining jars inverted to allow excess water to drip off:

c. Samples were then incubated (Gallenkamp, England) in ATPase (pH 9.4) for 30 min at 37°C in an agitating bath.
ATPase Incubation Buffer (APB).

Adenosine 5'Triphosphate (ATP disodium salt, from equine muscle, Sigma Chemical Co, St Louis, USA) 0.017 g per 10 mL

d. Rinsed 5 times with distilled water and inverted to allow to drain on absorbent paper:

e. 1% calcium chloride was added to staining jars and samples immersed for 3 min then rinsed 5 times:

f. Samples immersed in 2% cobalt chloride for 3 min, rinsed 5 times and the staining jars inverted onto absorbent paper and allowed to drain:

g. Samples were then immersed for 1 min in 10% ammonium sulphide, rinsed and inverted onto absorbent paper to drain:

h. Cover slips were mounted on slides using a Aquamount and stored at -20°C.

For the determination of three fibre types using ATPase staining an acid pre-incubation period was used.

Acid Preincubation Buffer (AcPB)

Sodium acetate (CH₃COONa.3H₂O) 3.90 g

Potassium chloride (KCl) 3.70 g

distilled water 500 ml
Method:

The 4.3 pH staining procedure was as per 10.3 pH procedure except that step one involved a pre-incubation period consisting of 1.5 min in an acid (4.3 pH) solution at 25°C.

Also used for the identification of the three fibre types was step one with a 4.6 pH and a pre-incubation period of 3 min.

All solutions were made fresh, warmed to their incubation and pre-incubation temperatures and pH adjusted immediately prior to each staining.

2.7.5.2 Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR)

Histochemical classification of the subtypes of FT fibres into high and low oxidative was performed according to the technique of Novikoff et al. (1961).

Method:

a. Muscle sections were incubated (Gallenkamp PN 4 BIR-M, England) for 30 min at 37°C:

b. Rinsed 10 times in distilled water:

c. Staining jars inverted and samples allowed to drip dry:

d. Cover slips were mounted onto slides using Aquamount and stored at -20°C.

All solutions were made fresh.
Incubation Solution:

*3.2 mg NADH (NADH disodium salt grade one, Boehringer Mannheim, West Germany).

*8 mg P-Nitroblue tetrazolium chloride.

*10 mL of MOPS buffer (2 mM MOPS buffer[3-(N-Morpholino) Propane Sulphonic Acid]).

Photomicrograph of a transverse section of a biopsy sample from the m. gluteus medius illustrating histochemical stains for NADH-TR and for ATP(ase) at pre-incubation pH of 10.3 and 4.6 are shown in Figure 2.2.
Figure 2.2 Photomicrograph of a transverse section from the m. gluteus medius illustrating histochemical stains of serial muscle sections for muscle fibre types using the staining techniques:

<table>
<thead>
<tr>
<th></th>
<th>ATPase 10.3</th>
<th>ST</th>
<th>FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>ATPase 4.6</td>
<td>Type I</td>
<td>Type IIa</td>
</tr>
<tr>
<td>c</td>
<td>NADH-TR</td>
<td>ST</td>
<td>FTH</td>
</tr>
</tbody>
</table>

![Photomicrograph of a transverse section from the m. gluteus medius illustrating histochemical stains of serial muscle sections for muscle fibre types using the staining techniques.]
2.7.5.3 Periodic Acid Schiff (PAS) Reaction

Muscle sections (20 μm) were cut on the cryostat microtome at -20°C, fixed in Carnoy's fixative for a minimum of 10 min (Hodgson, 1984) and stained according to the procedure described by Pearse (1968). Figure 2.3 shows a typical staining pattern.

Method:

a. 10 min incubation period in PAS solution at 37°C agitated

b. Rinsed 15 times with distilled water.

c. 10 min incubation period in Schiff's reagent at 37°C agitated.

d. Staining jar inverted and samples allowed to drain.

e. Cover slips were mounted onto glass slides using Aquamount (Gurr, BDH Chemicals, England) and stored at -20°C.

Carnoy's Fixative

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.2 mL</td>
</tr>
<tr>
<td>Ethanol (Absolute)</td>
<td>6.4 mL</td>
</tr>
</tbody>
</table>

The Carnoy's fixative was made fresh for each staining.

Schiff's Reagent

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic fuschin</td>
<td>1 g</td>
</tr>
<tr>
<td>Anhydrous potassium metabisulphide</td>
<td>2 g</td>
</tr>
<tr>
<td>1M hydrochloric acid</td>
<td>10 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>200 mL</td>
</tr>
</tbody>
</table>
The solution was boiled then allowed to cool, wrapped in silver foil to protect it from the light and stored overnight in a refrigerator at 4°C.

Activated charcoal was added and allowed to incubate for 1 hour. The solution was filtered, wrapped in silver foil and stored at 4°C.
Figure 2.3 Photomicrograph of a transverse section from the m. gluteus medius illustrating muscle fibre glycogen concentration using the PAS stain.

Glycogen concentration of fibres was graded into five categories (0 to ++++) based on intensity of stain (Hodgson et al., 1983).

Grading: A 0  B +  C ++  D +++  E ++++
2.7.6 Glycogen Synthetase:

The protocol was modified from Thomas, Schlender and Larner (1968) and Nuttal and Gannon (1989). Muscle glycogen synthetase was determined on freeze dried muscle. The degree of radioactivity turned into glycogen from UDP-glucose C\textsuperscript{14} was measured in the absence and presence of varying concentrations of G-6-P (nmol of glucose into glycogen per min per milligram of tissue (dry) (nmol.min\textsuperscript{-1} mg\textsuperscript{-1}). The total glycogen synthetase activity (I+D) was determined in the presence of 10 nmol G-6-P with activity of I being determined in the absence of G-6-P.

Method:

a. 5 mg of dried muscle tissue was added to 500 mL of cold diluted extraction buffer and homogenised:

b. assay mixture buffers:

- extraction buffer concentration
  - 100 mM imidazole
  - 50 mM NaF
  - 40 mM EDTA
  - pH 7.0

- UDPG 200 mM
- 10% glycogen
- \textsuperscript{14}C-UDPG
G-6-P 200 mM

or water (+/- G-6-P)

c. To 50 μL of assay mixture (+ or - G-6-P) was added 50 μL of tissue extract:

d. Both mixtures were kept at 30°C in a waterbath:

e. At regular intervals a 50 μL aliquot was removed from the reaction mixture and spotted onto a 1.5 cm² piece of filter paper:

f. The paper was dropped immediately into a stirring bath of 70% ethanol and left for approximately 20 mins:

g. The paper was then added to a bath of acetone for a few mins, removed and left to dry:

h. Once dry the paper was placed in a scintillation vial to which scintillation fluid was added:

i. A scintillation counter then measured the radioactivity of each sample in order to calculate the μmol of UDPG incorporated into glycogen per min per gram of tissue (the rate of glycogen synthesis).

2.7.7 Glucose-6-phosphate (G-6-P)

G-6-P concentrations were determined by observed changes in NADH equivalents in vitro, using fluorimetry. Assays were performed in 5 mL borosilicate tubes using a Perkin-Elmer 1000 M ratio filter fluorimeter (Perkin-Elmer, Glen Waverly, Victoria, Australia) fitted with 340 nm and 454 nm excitation and emission filters respectively.
All fluorescence readings were made by reference to a standard quinine sulphate solution.

**Fluorimeter Standardisation:**

**Preparation of quinine sulphate standard:**

Quinine sulphate was prepared in 5 mM H$_2$SO$_4$ to yield a 0.05 mg.mL$^{-1}$ stock solution:

Two standards were prepared, one for the 20x sensitivity, and one for the 50x sensitivity.

The stock solution was diluted:

10 μL in 3.05 mL 5 mM H$_2$SO$_4$ for the x20

4 μL in 3.05 mL 5 mM H$_2$SO$_4$ for the x50

The standards were placed in the fluorimeter, and their fluorescence adjusted (with 5 mM H$_2$SO$_4$) until within 10 units of the maximum fluorescence for their respective sensitivities.

Standards were left for 24 hours to allow them to stabilise.

**NADH Standard Curve**

1. A 5 mM NADH solution was prepared (36 mg NADH in 10 ml carbonate buffer). (NADH disodium salt, Grade One, Boehringer Mannheim, W. Germany).

Carbonate buffer: 0.08 M Na$_2$CO$_3$ (0.848 g/100 ml)

0.02 M NaHCO$_3$ (0.168 g/100 ml)
The solution was heated to 60°C for 10 min to denature any NAD.

2. A Pye Unicam SP6-350 visible spectrophotometer (Phillips, Australia) was used to measure the absorbence of the NADH standard at 340 nm.

3. The NADH concentration of the standard was then calculated: concentration (mM) = absorbence/6.270/ dilution factor.

An NADH standard curve was prepared from the fluorescence verses NADH concentrations at the sensitivity and scale expansion settings determined by the quinine sulphate standard.

1. The 5 mM NADH solution was diluted to 0.1 mM (by weight) using the concentrations determined from the spectrophotometer.

2. Tubes for the spectrometer were prepared as listed:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Sensitivity</th>
<th>50 mM (TEA mL)</th>
<th>0.1 mM NADH (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>x20 &amp; x50</td>
<td>3.05</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>x20 &amp; x50</td>
<td>3.04</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>x20 &amp; x50</td>
<td>3.03</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>x20 &amp; x50</td>
<td>3.02</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>x20</td>
<td>3.01</td>
<td>40</td>
</tr>
<tr>
<td>6.</td>
<td>x20</td>
<td>3.00</td>
<td>50</td>
</tr>
<tr>
<td>7.</td>
<td>x50</td>
<td>3.045</td>
<td>5</td>
</tr>
<tr>
<td>8.</td>
<td>x50</td>
<td>3.035</td>
<td>15</td>
</tr>
<tr>
<td>9.</td>
<td>x50</td>
<td>3.025</td>
<td>25</td>
</tr>
</tbody>
</table>
The x20 quinine sulphate standard was placed in the fluorimeter and the readout adjusted to within 10 units of the full scale. The initial fluorescence (F) for tubes 1 to 6 was determined. The x 20 standard was replaced with the x 50 standard and the procedure repeated determining initial F for tubes 1 to 4, 7, 8 and 9.

3. The quantities of 0.1 mM NADH standard indicated were added. Tubes were then mixed and incubated for 30 min.

4. Contents were again mixed and the final fluorescence reading obtained at the indicated sensitivities.

5. A graph of changes of F versus nmol NADH was constructed. The calibration factor (slope of the line) was determined by regression analysis.

Muscle glucose-6-phosphate concentrations were determined on freeze dried muscle.

a. The freeze dried muscle was dissected free of any blood or fibrous tissue.

b. The dried muscle sample was weighed and homogenised at -10°C in an extraction medium of 3 M perchloric acid.

c. The supernatant was neutralized and then centrifuged.

Neutralizing solution

3 M KOH, 0.4M HCl 0.4M imidazole

d. 6M NaOH was added to denature the muscle myokinase.

e. 6M HCl was added to neutralise the extract.
The concentration of G-6-P in the extracted muscle was determined fluormetrically (Lowry and Passonneau, 1972).

Reactivation solution:

25 mM Tris.HCl
12.5 mM MgCl₂
1 mM NAD
50 mM D-glucose
20 μg.mL⁻¹ G-6-P-DH
25 mM DTT

The solution was adjusted to pH 7.8.

Reaction: G-6-P + NAD = (G-6-P) - DH = > Gluocolactone + NADH

f. 3.02 mL of reactivation solution was added to each glass tube and the initial fluorescence read.

g. To the 3.02 mL of reactivation solution was added 20μL of sample.

h. The sample was mixed and allowed to incubate for 10 min then the final fluorescence read.

G-6-P concentration was calculated from:

(F final - F initial) x calibration factor = nmol NADH = nmol G-6-P in 20 μL muscle extract.
2.8 Photography

All histochemical sections were photographed using an Olympus BHB binocular microscope, incorporating an Olympus Photomicrograph System PM-10-A (Olympus Optical Co Ltd, Japan). Kodak Ektachrome 25 print film was used for all photographic shots. A x 25 magnification was used for photographing muscle biopsy slides.

2.9 Treadmill

A treadmill (Kagra Mustang 2000, Switzerland) was used for both training and testing of horses. It had a speed range capability of 0 to 17 m.s\(^{-1}\) and incline range from 0 to 11%. The speed did not vary by more than ±0.2 m.s\(^{-1}\) at any speed when horses were exercising on the treadmill. The speed and distance readout were checked by measuring the treadmill belt length and timing a set number of belt revolutions.

Two 120 Watt fans mounted above and in front of the horses forced air onto the horses to assist in cooling during exercise.

2.10 Heart Rate Measurement

Telemetry electrocardiography was used to measure heart rate. Areas of skin for electrode placement were shaved. Adhesive spray (Vi-drape, Deseret Co. USA) was applied to the skin to assist in holding the electrodes (Red Dot, 3M, Sydney, Australia) in
place. Leads were placed in a lead II configuration with the left arm (LA) and right leg (RL) leads placed vertically approximately 10 cm apart on the left wither and the right arm (RA) lead placed on the left ventral thorax. Two pieces of sponge rubber (15 x 15 x 5 cm.) were placed on top of the electrodes and held in place with an elasticised girth strap. A leather harness was placed around the girth and the telemetry transmitter was placed in a pouch on the harness. A telemetry ECG receiver (Danica Electronik, Type CC14, Copenhagen, Denmark), was used for the recording of the ECG. Paper speed was 25 mm.s\(^{-1}\). Heart rate was calculated by counting the number of R waves in a 15 s period.

2.11 Respiratory Gas Collection and Analysis

An open flow respiratory gas collection system was used for gas collection during experiments. The system consisted of a mask, flexible airway tubing, a gas mixing device, a section of rigid airway tubing containing a venturi and pressure transducer, flow controlling baffle, vacuum motor and a gas sampling system. The apparatus was similar to that described by Seichman and Morris (1989) and Evans and Rose (1988).
2.11.1 Gas Collection Mask

The mask was cone-shaped to fit over the horse’s nose. It was constructed of plastic and weighed 2.5 kg with a volume of 25 L. There were 2 holes 7 cm diameter, and 3 holes 3 cm diameter, in the side of the mask to allow air to be drawn into the mask. The mask was held in position by an elastic strap that was placed over the horse’s head.

2.11.2 Flexible Airway Tubing

The mask was connected to rigid airway tubing by a 4 m length of 150 mm diameter flexible lightweight plastic tubing (Multiflex, Dunlop, Australia). The flexible tubing allowed for horse movement both back and forth on the treadmill while at the same time still ensuring that the face mask stayed in the correct position on the horse’s head.

2.11.3 Mixing Device

A fan was positioned at the junction of the flexible and rigid airway tubes to ensure adequate mixing of the expired gases with the air that was drawn into the system.

2.11.4 Rigid Airway Tubing

Rigid PVC pipe (5 m long and 15 cm diameter) connected the flexible tubing to a vacuum motor. Mounted within the tubing were a venturi, a differential pressure
transducer (Model DP 45-22, Validyne Engineering Corp, Northbridge, USA), a nitrogen inlet valve, a thermocouple and a baffle plate. The transducer measured the change in pressure across the venturi, with the change in pressure being proportional to the air flow through the system. The output of the transducer was digitally displayed on a voltmeter (Model CD 379, Validyne Engineering Corp).

Air flow was calibrated using nitrogen dilution. A nitrogen inlet valve allowed nitrogen to be introduced into the system during flow. The nitrogen flow was measured by a nitrogen flowmeter (Model No.10A4555X, Fischer Porter Co. Warminster, USA). During exercise the consistency of gas flow rate was monitored by the output from a differential pressure transducer. A thermocouple was mounted inside the rigid pipe to measure the temperature of the gas.

Air flow could be varied by adjusting a baffle within the rigid pipe. The baffle rotated and opened or closed the lumen of the pipe thus altering the gas flow. Flow rates varying up to 8000 L.m\(^{-1}\) during intense exercise were used to ensure that all of the expired air was drawn into the mask.

2.11.5 Vacuum Motor

Air flow through the system was generated by a 750 W vacuum motor (CMG Electric, Ringwood, Australia). A smaller electric vacuum motor was used to draw off a sample of the main air stream for gas sample collection.
2.11.6 Gas Sampling System

Gas samples were collected into sample bags during the last 15 sec of each workload. Thirty seconds before a sample was collected the bag was flushed with gas from the system. A sample of gas was drawn out of the bag by a small membrane pump (ASF, Munich, Germany). The sample then passed into a dehumidifier (Model DH-106-1 Komatsu Electronics Inc. Japan). Two flow meters (Model A-125-5 and A-250-1, Porter Instrument Co, Hatfield, USA) and a needle valve (Nupro Co, Willoughby, USA) ensured the correct gas flow to the analysers.

2.11.7 Gas Analysis

The oxygen concentration of the gas sample was measured using a paramagnetic oxygen analyser (Model 1100A, Servomex, Crowborough, UK). The analyser was zeroed using pure nitrogen (CIG, Sydney, Australia) daily, and spanned against room air before each test. A precision gas with a known oxygen concentration (CIG., Sydney, Australia) was used to check the calibration procedure and the linearity of the analyser.

Carbon dioxide concentration was measured using an infra-red analyser (Model 01400 A3, Servomex, Crowborough, UK). The analyser was zeroed with the pure nitrogen and calibrated using a special gas mixture with a known carbon-dioxide concentration (CIG., Sydney, Australia) each day.
2.11.8 Barometric Pressure

The barometric pressure was measured with a barometer (A.L.Franklin Pty. Ltd. Sydney, Australia) during each exercise test.

2.11.9 Equations for Determination of Oxygen Uptake and Carbon Dioxide Production (Jones and Campbell, 1982; Evans, 1987).

1. $\dot{V}_E$ (L.min$^{-1}$) ATPS = m$^3$.hour x 1000/60

2. PH$_2$O = 10 - 0.3592T + 0.03775 T2, T = atmospheric temperature (°C) measured at the flow metre.

3. $\dot{V}_E$ (L.min$^{-1}$) STDP = $\dot{V}_E$ ATPS (L.min$^{-1}$) x (P$_B$ - PH$_2$O)/ (760(1 + 0.00367 T)) STDP standard temp pressure dry.

4. Oxygen consumption ($\dot{V}$ O$_2$) L.min$^{-1}$ STPD = $\dot{V}$ E (L.min$^{-1}$) STPD x (0.265 (1 - F$_{E}$O$_2$ - F$_{E}$CO$_2$) -F$_{E}$O$_2$)). F$_{E}$ is fraction expired.

5. Carbon dioxide ($\dot{V}$ CO$_2$) L.min$^{-1}$ = $\dot{V}_E$ (L.min$^{-1}$) STPD x F$_{E}$CO$_2$.

6. Respiratory exchange ratio (R) = $\dot{V}$ CO$_2$ / $\dot{V}$ O$_2$ STPD

2.12 Determination of Exercise Intensities for Training and Testing:

For both maximal and sub-maximal testing the treadmill was set at 10% slope. All horses had been given at least three acclimatizing runs before any testing was conducted.
For the submaximal test each horse ran for 3 min at 3 m.s⁻¹ then 2 min at 5, 7, and 9 m.s⁻¹.

For the \( \dot{V}O_2\)max test they ran for 3 min at 4 m.s⁻¹, 2 min at 6 m.s⁻¹ then one min at 8, 10, 11, and 12 m.s⁻¹ or until unable to maintain pace with the speed of the treadmill. Gas samples for the calculation of oxygen uptake (\( \dot{V}O_2 \)) and carbon-dioxide production (\( \dot{V}CO_2 \)) were collected during the last 15 s of each work load.

2.13 Training Programme:

All training was carried out with the treadmill slope set at 10%. Horses were trained six days per week with the sixth day being light exercise of 1000 m at 2 m.s⁻¹. Training programmes for Thoroughbreds and Standardbreds were different due to the differing experimental protocols.

2.13.1 Thoroughbred Training Programme

All horses did a preliminary warm-up of 1000 m at 2 m.s⁻¹. Horses were randomly allocated to two groups:

a. Low intensity training
b. High intensity training

The intensity of the training was based on plasma lactate concentrations. The high intensity group worked at an intensity that would produce a plasma lactate concentration
of between 4-8 mmol.L⁻¹. The slow group trained over the same distance but at an intensity that was 50% of that of the high intensity group. Training distance in week one was 1600 m with distance being increased by 800 m every second week.

<table>
<thead>
<tr>
<th>Programme</th>
<th>Distance (m)</th>
<th>Speeds (m.s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Week 1</td>
<td>1600</td>
<td>8.5</td>
</tr>
<tr>
<td>Week 2</td>
<td>1600</td>
<td>8.5</td>
</tr>
<tr>
<td>Week 3</td>
<td>2400</td>
<td>9.0</td>
</tr>
<tr>
<td>Week 4</td>
<td>2400</td>
<td>9.0</td>
</tr>
<tr>
<td>Week 5</td>
<td>Exercise Testing Week</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>3000</td>
<td>9.0</td>
</tr>
<tr>
<td>Week 7</td>
<td>3000</td>
<td>9.0</td>
</tr>
<tr>
<td>Week 8</td>
<td>3600</td>
<td>9.5</td>
</tr>
<tr>
<td>Week 9</td>
<td>3600</td>
<td>9.5</td>
</tr>
<tr>
<td>Week 10</td>
<td>Exercise Testing Week</td>
<td></td>
</tr>
</tbody>
</table>

All horses performed the same exercise in weeks 11-16

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Distance (m)</th>
<th>Speeds (m.s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-16</td>
<td>1000</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>4.2</td>
</tr>
</tbody>
</table>

30 min run at 50% VO₂ max on Fridays
2.13.2 Standardbred Training Programme

All horses used in the glycogen resynthesis studies were trained for seven weeks prior to the study.

Horses were given a 1000 m warm-up at 3 m.s⁻¹ at the beginning of each training session.

**Programme:**

- Week 1 1600 m at 4 m.s⁻¹
- Week 2 1600 m at 4 m.s⁻¹
- Week 3 2400 m at 4 m.s⁻¹
- Week 4 3000 m at 4 m.s⁻¹
- Week 5 3000 m at 5 m.s⁻¹
- Week 6 3000 m at 5 m.s⁻¹ plus 840 m at 7 m.s⁻¹
- Week 7 3000 m at 5 m.s⁻¹ plus 840 m at 7 m.s⁻¹
Chapter 3

VARIATION OF FIBRE TYPE AND GLYCOGEN CONCENTRATION OF THE GLUTEUS MEDIUS MUSCLE OF THE STANDARDBRED HORSE

3.1 Introduction

3.2 Materials and Methods

3.2.1 Muscle Biopsy Sites

3.2.2 Muscle Biopsy Procedure

3.3 Statistics

3.4 Results:

3.5 Discussion:
3.1 Introduction

It has been established that, both histochemically and biochemically, muscle fibre types can vary considerably within a single skeletal muscle (Lindholm and Piehl, 1974; Bruce and Turek, 1985; van den Hoven et al., 1985; Kline, Lawrence, Novakofski and Bechtel, 1987; Kline and Bechtel, 1988; Bruce et al., 1993). As a result of this variability, a single biopsy sample taken from a muscle may not necessarily be representative of the whole muscle. In addition to the variability within a single muscle, the percentage of individual muscle fibre types, and the fibre type ratio, have been reported to change with both age (Kline and Bechtel, 1988; Essén et al., 1980) and sample depth (Bruce and Turek, 1985; van den Hoven et al., 1985).

Variability in muscle fibre types has also been reported in humans. Lexell, Taylor, Sjostrom (1985) found considerable variation in the proportion of fibre types within the vastus lateralis muscle. They recommended that complete counting from three biopsies of at least 150 fibres from different depths is required to provide a reduction in the sampling error.

Variability in glycogen concentration of the m. gluteus medius has been reported, both within and between horse breeds (Lindholm et al., 1974; Lindholm and Piehl, 1974; Snow & Guy, 1976; Snow et al., 1981; Essén-Gustavsson et al., 1984; Snow et al., 1987; Essén-Gustavsson et al., 1989).

Diurnal variation does not appear to be a factor in the examination of muscle glycogen concentration. Lindholm and Piehl (1974) collected biopsies at 3 hourly intervals
during the day and reported no significant difference in the glycogen concentration between the different sampling times.

Although studies have examined variation in site and depth through the muscle belly (van den Hoven et al., 1985; Bruce and Turek, 1985; Lopez-Rivero, Serrano, Diz and Galisteo, 1992b; Bruce et al., 1993), these studies have been limited in that they have been restricted to a specific zone lying across the muscle belly, and may not be representative of the whole muscle. Therefore, fibre type and glycogen concentration variability within other areas of the m. gluteus medius of the horse still need to be examined.

This investigation was designed to establish the degree of variability of fibre types and glycogen concentration within the m. gluteus medius of the Standardbred horse. The results provide the foundation for the investigations into glycogen resynthesis which were dependent upon taking multiple biopsies from this muscle.

3.2 Materials and Methods

Six Standardbred geldings of age 6.6±0.7 years mean±(SEM) were used in the study. Mean body weight of the group was 457±9 kg.
3.2.1 Muscle Biopsy Sites

Biopsies were taken from the right and left m. gluteus medius. The right side was divided into 8 testing sites and designated "8-site" and the left side divided into 5 sites ("5-site"). The sites for biopsy were clipped and marked for locations. Locations were identified by drawing lines between several anatomical landmarks. For the right side a line was drawn from the tuber coxae to the trochanter major of the femur, whilst a second line was drawn vertically from the first line to the tuber ischii. An additional landmark was where this line crossed the border of the sacro-sciatic ligament/semitendinosis. A third line was drawn from the tuber sacrale to the tuber coxae and a point 20 cm along this line was marked. A line was then drawn from this point to the mark on the sacro-sciatic ligament. The area enclosed within these lines was then divided into sections for the "8-sites" (see Figure 2.1). For the "5-sites" the left m. gluteus medius area was clipped and marked as for the right m. gluteus medius. The mid-point of the vertical line that dissected the line joining the tuber coxae and trochanter major was used as the reference point (Point 4 in Figure 2.1). Four points were taken within 1cm of the mid-point of this line. The midpoint was taken as the first site and the second as the mark towards the line joining the greater trochanter to the tuber ischii, with the third to fifth sites then taken in an anticlockwise direction. Biopsy samples were always taken in the same order in accordance with the site numbering (see Figure 2.1).
3.2.2 Muscle Biopsy Procedure

The muscle biopsies were taken using the needle biopsy technique of Bergstrom (1962) as modified by Lindholm and Pichl (1974).

Muscle glycogen concentration was determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al., (1987). To determine muscle fibre types, muscle sections (10μm) were cut on a cryostat microtome and reactions for myosin ATPase were conducted. Sections were preincubated at pH 4.3 and pH 10.3 for identification of slow and fast twitch fibre types (Brooke and Kaiser, 1970). The pH 4.3 preincubation was used for confirmation of fast twitch fibres for the NADH-TR stain. To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff et al. (1961) and the fast twitch fibres subdivided into high oxidative (FTH) and low oxidative (FT) on the basis of their staining intensity.

3.3 Statistics

All results are expressed as mean±SEM. The effect of site and horse were analysed by analysis of variance. Where F values were significant, a post hoc test of least significant difference was used. The level of statistical significance used was p<0.05.
3.4 Results

Mean percentages of ST and FT fibres, as determined by the pH 10.3 stain, for the "8-sites" are presented in Table 3.1. There was a significant effect of site for ST and FT distribution (p<0.05). Mean percentages of ST, FTH and FT fibres, as determined by the NADH-TR stain, for the 8 sites are presented in Table 3.2. There was a significant difference between sites 2 and 3, 2 and 6 and 3 and 5 for ST and 3 and 5 for FT, but no significant effect of site for FTH fibres (p<0.05).

For the "5-site" fibre types, due to damage to one horses samples, only results for 5 horses are presented.

Mean percentages of ST, and FT fibres for the "5-sites", as determined by the pH 10.3 stain are presented in Table 3.3. There was no significant effect of site (p<0.05).

Mean percentages of ST, and FTH and FT fibres for the "5 sites", as determined by the NADH-TR stain, are presented in Table 3.4. There was no significant effect of site (p<0.05).

Mean percentages, ranges and coefficient of variation (CV) of ST, FTH and FT fibres for 5 and 8 sites, as determined by pH 10.3 and NADH-TR stains are presented in Tables 3.5 - 3.8. The CV for the percentage of ST fibres were about 3 times that for the FT fibres. The CV between sites for each horse is presented in Table 3.9.

Mean muscle glycogen concentration for the "8-site" and "5-site" were 635 mmol.kg\(^{-1}\)(dwt) and 595 mmol.kg\(^{-1}\)(dwt) respectively. Mean glycogen concentrations for the "8-site" and "5-site" are presented in Tables 3.91. There was no significant effect of site for either the eight or five site biopsies (p<0.05).
Table 3.1 Percentage fibre types (means±SEM) for ST and FT fibres for 8 sites in the right m. gluteus medius of Standardbred geldings, as determined from the staining for myofibrillar ATPase following preincubation at pH 10.3 (N=6).

<table>
<thead>
<tr>
<th>Site</th>
<th>Fibre</th>
<th>Type%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>FT</td>
</tr>
<tr>
<td>1</td>
<td>22.6±4.6</td>
<td>77.4±4.6</td>
</tr>
<tr>
<td>2</td>
<td>9.9±0.6&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>90.1±0.6&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>26.1±2.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>73.9±2.7&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>23.6±3.4</td>
<td>76.4±3.4</td>
</tr>
<tr>
<td>5</td>
<td>12.7±1.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>87.3±1.9&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>29.4±1.4&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>70.6±1.4&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>23.7±3.9</td>
<td>76.3±3.9</td>
</tr>
<tr>
<td>8</td>
<td>18.3±4.4</td>
<td>81.7±4.4</td>
</tr>
</tbody>
</table>

Mean±(SEM) 20.8±2.9 79.2±2.9

Note: Means in the same column sharing the same superscript were significantly different (p<0.05).

ST - slow twitch fibres    FT - fast twitch fibres
Table 3.2 Percentage fibre types (mean±SEM) for ST, FTH and FT fibres for 8 sites in the right m. gluteus medius of Standardbred geldings, as determined from the combination of myosin ATPase and NADH-TR stains (N=6).

<table>
<thead>
<tr>
<th>Site</th>
<th>Fibre</th>
<th>Type%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>FTH</td>
</tr>
<tr>
<td>1</td>
<td>16.9±4.1</td>
<td>64.7±3.7</td>
</tr>
<tr>
<td>2</td>
<td>8.4±0.8</td>
<td>69.7±2.5</td>
</tr>
<tr>
<td>3</td>
<td>28.3±3.1</td>
<td>55.6±2.2</td>
</tr>
<tr>
<td>4</td>
<td>20.5±2.5</td>
<td>60.1±3.3</td>
</tr>
<tr>
<td>5</td>
<td>9.7±1.6</td>
<td>63.1±2.0</td>
</tr>
<tr>
<td>6</td>
<td>24.4±2.1</td>
<td>57.9±1.6</td>
</tr>
<tr>
<td>7</td>
<td>22.8±4.7</td>
<td>53.5±4.2</td>
</tr>
<tr>
<td>8</td>
<td>17.0±5.8</td>
<td>62.0±6.6</td>
</tr>
</tbody>
</table>

Mean±(SEM) 18.5±3.1  60.9±3.3  20.6±2.2

Note: Means in the same column sharing the same superscript were significantly different (p<0.05).

ST - slow twitch fibres  FT - fast twitch fibres
Table 3.3 Percentage fibre types (mean±SEM) for ST and FT fibres for 5 sites in the left m. gluteus medius of Standardbred geldings, as determined from the staining for myofibrillar ATPase following preincubation at pH 10.3 (N=5).

<table>
<thead>
<tr>
<th>Site</th>
<th>Fibre</th>
<th>Type%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>FT</td>
</tr>
<tr>
<td>1</td>
<td>26.2±4.2</td>
<td>73.8±4.2</td>
</tr>
<tr>
<td>2</td>
<td>27.2±4.3</td>
<td>72.8±4.3</td>
</tr>
<tr>
<td>3</td>
<td>25.8±6.3</td>
<td>74.2±6.3</td>
</tr>
<tr>
<td>4</td>
<td>32.2±2.8</td>
<td>67.8±2.8</td>
</tr>
<tr>
<td>5</td>
<td>34.8±2.8</td>
<td>65.2±2.8</td>
</tr>
</tbody>
</table>

Mean±(SEM) 29.3±4.1 70.7±4.1

Note: ST - slow twitch fibres
      FT - fast twitch fibres
Table 3.4 Percentage fibre types (mean±SEM) for ST, FTH and FT fibres for 5 sites in the left m. gluteus medius of Standardbred geldings, as determined from the combination of myosin ATPase and NADH-TR stains (N=5).

<table>
<thead>
<tr>
<th>Site</th>
<th>Fibre</th>
<th>Type%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>FTH</td>
</tr>
<tr>
<td>1</td>
<td>24.3±4.4</td>
<td>60.0±3.0</td>
</tr>
<tr>
<td>2</td>
<td>23.4±3.6</td>
<td>63.0±2.2</td>
</tr>
<tr>
<td>3</td>
<td>22.2±5.3</td>
<td>63.2±3.9</td>
</tr>
<tr>
<td>4</td>
<td>26.4±3.3</td>
<td>60.0±3.3</td>
</tr>
<tr>
<td>5</td>
<td>32.8±2.5</td>
<td>58.4±1.5</td>
</tr>
</tbody>
</table>

Mean±(SEM)  25.8±3.8  60.9±2.5  13.3±2.4

Note:  
ST - slow twitch fibres  
FT - fast twitch glycolytic  
FTH - fast twitch oxidative
Table 3.5 Percentage fibre types (mean±SEM), range and coefficient of variation (CV) for ST and FT fibres for 8 sites in the right m. gluteus medius of Standardbred geldings, as determined from the staining for myofibrillar ATPase following preincubation at pH 10.3 (N=6).

<table>
<thead>
<tr>
<th>Site</th>
<th>ST Mean±(SEM)</th>
<th>Range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.6±4.6</td>
<td>11.1 - 35.4</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>9.9±0.6</td>
<td>8.5 - 12.4</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>26.1±2.7</td>
<td>18.1 - 32.8</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>23.6±3.4</td>
<td>13.6 - 37.2</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>12.7±1.9</td>
<td>6.0 - 19.6</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>29.4±1.4</td>
<td>24.9 - 36.1</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>23.7±3.9</td>
<td>8.3 - 30.6</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>18.3±4.4</td>
<td>9.2 - 36.6</td>
<td>54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>FT Mean±(SEM)</th>
<th>Range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77.4±4.6</td>
<td>64.6 - 88.9</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>90.1±0.6</td>
<td>87.6 - 91.8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>73.9±2.7</td>
<td>67.1 - 81.9</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>76.4±3.4</td>
<td>62.7 - 86.4</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>87.3±1.9</td>
<td>80.4 - 94.0</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>70.6±1.4</td>
<td>70.2 - 75.1</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>76.3±3.9</td>
<td>69.3 - 91.7</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>81.7±4.4</td>
<td>63.3 - 90.8</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: ST - slow twitch fibres
FT - fast twitch fibres
Table 3.6 Percentage fibre types (mean±SEM), range and coefficient of variation (CV) for ST, FTH and FT fibres for 8 sites in the right m. gluteus medius of Standardbred geldings, as determined from the combination of myosin ATPase and NADH-TR stains (N=6).

<table>
<thead>
<tr>
<th>ST Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.9±4.1</td>
<td>10.2 - 34.8</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>8.4±0.8</td>
<td>6.3 - 12.3</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>28.3±3.1</td>
<td>19.5 - 34.9</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>20.5±2.5</td>
<td>11.4 - 26.5</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>9.7±1.6</td>
<td>3.2 - 12.7</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>24.4±2.1</td>
<td>16.7 - 32.8</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>22.8±4.7</td>
<td>6.6 - 29.7</td>
<td>41</td>
</tr>
<tr>
<td>8</td>
<td>17.0±5.8</td>
<td>7.0 - 36.4</td>
<td>68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FTH Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64.7±3.7</td>
<td>49.1 - 71.6</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>69.7±2.5</td>
<td>58.5 - 77.7</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>55.6±2.2</td>
<td>49.6 - 63.6</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>60.1±3.3</td>
<td>46.9 - 68.3</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>63.1±2.0</td>
<td>56.4 - 69.1</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>57.9±1.6</td>
<td>52.1 - 63.3</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>53.5±4.2</td>
<td>42.7 - 66.0</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>62.0±6.6</td>
<td>44.5 - 79.6</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FT Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.4±1.1</td>
<td>15.6 - 21.4</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>21.9±2.4</td>
<td>15.7 - 32.5</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>16.1±2.2</td>
<td>9.4 - 24.7</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>19.4±2.3</td>
<td>13.0 - 26.5</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>27.2±2.1</td>
<td>18.6 - 30.8</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>17.7±1.7</td>
<td>11.5 - 23.3</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>23.7±2.1</td>
<td>17.1 - 22.5</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>21.0±3.9</td>
<td>13.4 - 34.7</td>
<td>39</td>
</tr>
</tbody>
</table>

Note: ST - slow twitch fibres  FTH - Fast twitch high oxidative
      FT  - Fast twitch low oxidative
Table 3.7  Percentage fibre types (mean±SEM), range and coefficient of variation (CV) for ST and FT fibres for 5 sites in the left m. gluteus medius of Standardbred geldings, as determined from the staining for myofibrillar ATPase following preincubation at pH 10.3 (N=5).

<table>
<thead>
<tr>
<th>ST</th>
<th>Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.2±4.2</td>
<td>16.1 - 39.6</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27.2±4.3</td>
<td>9.3 - 36.2</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25.8±6.3</td>
<td>8.8 - 46.5</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32.2±2.8</td>
<td>22.7 - 38.3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>34.8±2.8</td>
<td>24.6 - 42.1</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FT</th>
<th>Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73.8±4.2</td>
<td>60.4 - 83.8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72.8±4.3</td>
<td>63.8 - 90.7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>74.2±6.3</td>
<td>53.5 - 91.2</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>67.8±2.8</td>
<td>61.6 - 77.3</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>65.2±2.8</td>
<td>57.9 - 75.4</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Note:  ST - slow twitch fibres  
FT - fast twitch fibres
Table 3.8 Percentage fibre types (mean±SEM), range and coefficient of variation (CV) for ST, FTH and FT fibres for 5 sites in the left m. gluteus medius of Standardbred geldings, as determined from the combination of myosin ATPase and NADH-TR stains (N=5).

<table>
<thead>
<tr>
<th></th>
<th>Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>1</td>
<td>24.3±4.4</td>
<td>14.3 - 38.5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.4±3.6</td>
<td>11.8 - 31.7</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.2±5.3</td>
<td>7.9 - 37.9</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>26.3±3.3</td>
<td>7.6 - 34.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32.8±2.5</td>
<td>24.6 - 41.9</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FTH</th>
<th>Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.0±3.0</td>
<td>49.8 - 64.9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>63.0±2.2</td>
<td>59.1 - 70.1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>63.2±3.9</td>
<td>54.4 - 76.1</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>60.0±2.0</td>
<td>27.5 - 61.6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>58.4±1.5</td>
<td>53.1 - 62.0</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FT</th>
<th>Site</th>
<th>Mean±(SEM)</th>
<th>Range</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.7±1.1</td>
<td>11.7 - 22.0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13.6±2.4</td>
<td>6.1 - 29.0</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14.6±2.2</td>
<td>6.3 - 19.9</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13.6±2.3</td>
<td>6.1 - 16.6</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8.7±2.1</td>
<td>5.0 - 13.3</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

Note: ST - slow twitch fibres       FTH - Fast twitch high oxidative

FT - fast twitch low oxidative
Table 3.9 Percentage fibre types (mean±SEM) and coefficient of variation (CV) for the 8 sites in the right m. gluteus medius for the 6 Standardbred geldings, as determined from the staining for myofibrillar ATPase following preincubation at pH 10.3 (N=6).

<table>
<thead>
<tr>
<th>ST</th>
<th>Horse</th>
<th>Mean±(SEM)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.2±1.71</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.9±1.62</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>26.8±2.02</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15.8±1.92</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18.9±2.14</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18.4±1.34</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FT</th>
<th>Horse</th>
<th>Mean±(SEM)</th>
<th>CV%</th>
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<td>6</td>
<td>81.6±0.64</td>
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</table>

Note: ST - slow twitch fibres
      FT - fast twitch fibres
Table 3.91 Muscle glycogen concentrations (mean±(SEM) (mmol.kg⁻¹.dwt) for the 8
and 5 sites in the m. gluteus medius of Standardbred geldings (N=6).

### 8 Sites

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### 5 sites

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<td>42.9</td>
<td>22.2</td>
<td>26.5</td>
<td>25.3</td>
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3.5 Discussion

Due to its easy accessibility and its major involvement in propulsion, the m. gluteus medius has been widely used in research. However, based on the results of this study, the taking of a single biopsy sample from a muscle may not necessarily be representative of the fibre type of the whole muscle. This confirms results from other anatomical studies which have shown that the percentage of individual muscle fibre types, and the fibre type ratio, can change with both age (Essén et al., 1980; Kline and Bechtel, 1988) and sample depth (Bruce and Turek, 1985; van den Hoven et al., 1985; Lopez-Rivero et al., 1992b).

The results of this study demonstrate that for the Standardbreds tested, there was no significant difference in glycogen concentration within the "8-site" and "5-site" in the m. gluteus medius. However, there was a variation in the proportion of ST and FT fibres between some sample locations but no difference between any sites for FTH fibres. The large difference in the CV between sites for the same fibre type and between the ST and FT fibres indicate that the distribution of fibres within the m. gluteus medius is not uniform. The variation between sites may partly be due to sampling and analysis techniques, however it is large enough to pose a problem if biopsies are taken from different locations for comparative analysis.

Van den Hoven et al. (1985) reported that in samples originating from different zones of the muscle, there was a maximal significant intra-muscle difference in Type I fibres of 36%. In samples originating from the same zone there was a maximal significant difference of 30% at the 8 cm depth of the m. gluteus medius. In this study the highest coefficient of variation was 49% for within horse variability for the 8 sites ST fibres. For
between horse variability the CV was as high as 68% in one of the 8 sites, and up to 55% for one of the 5 sites. This variation is higher than that reported by van den Hoven et al. (1985).

The findings of this study indicate that structural change in fibre type may occur in the m. gluteus medius when moving from the proximal to superior aspects of the muscle. This was largely attributable to the low percentage of ST fibres for sites 2, 5 and 8. It is possible that the 8 cm depth of collection of the muscle biopsy at these sites may have been relatively deeper than in the other sites. However, our findings of low %ST fibres at the 8 cm depths for sites 2, 5 and 8 is contrary to the literature which indicates increasing percentages of ST fibres with increasing depth. Bruce and Turek (1985) reported a general trend of increasing percentages of ST fibres when moving along a line drawn from the tuber coxae of the ilium to the head of the femur. In this study the percentage ST fibres for sites 1, 3 and 6, which were lying closest to the marks utilized by Bruce and Turek (1985), also showed increasing percentages of ST fibres when moving from the tuber coxae to the greater trochanter of the femur. This pattern was not evident for the other sites (7, 4 and 2) which were located parallel to sites 1, 3 and 6, illustrating the variability that may exist between similar sites.

The purpose of the "5-site" biopsy samples were in the main to test how repeatable samples are when taken within a small area. The lack of a significant difference between the muscle samples taken from around an area 4 cm² gives confidence in sampling where slight variations in location of the sample may occur. However, considerable variations were still evident between the sites, even though differences were not statistically different.
The range in glycogen concentrations for the means of the eight sites was 577 to 662 mmol.kg$^{-1}$(dwt), with the range for the eight sites being 399 - 794 mmol.kg$^{-1}$(dwt), which is higher than the ranges of 380-589 mmol.kg$^{-1}$(dwt) reported for Standardbreds (Lindholm et al., 1974; Lindholm and Pichl, 1974; Essén-Gustavsson et al., 1989). The range in muscle glycogen concentration between the sites within a single horse was from 161- 239 mmol.kg$^{-1}$(dwt). Therefore, although glycogen distribution would appear to be more uniform within the m. gluteus medius, the range indicates that caution should be taken when interpreting concentrations from different sites. In addition, because different muscle fibre types are recruited at different exercise intensities (Essén-Gustavsson et al., 1984) care should be exercised when interpreting results of muscle glycogen concentrations, from areas where large differences in muscle fibre type occur.

In conclusion, this study has shown that for the sample sites chosen, the fibre composition of the m. gluteus medius does vary. However, with the "8-site" biopsy sampling area, fibre type variability was not found to occur for the sites 1, 3, 4, 6, 7 and 8 and consequently these sites were used in assessing muscle glycogen resynthesis rates. Biopsies of the m. gluteus medius give repeatable results when the samples differ in location by only 2-4 cm.
Chapter 4

THE EFFECTS OF NASOGASTRIC ADMINISTRATION OF

GLUCOSE OR A GLUCOSE POLYMER

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4.5.1 Experiment 1
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4.6 Discussion
4.1 Introduction

This study was a preliminary one into the effects of nasogastric administration of glucose and a glucose polymer (Polyjoule) on the horse. The purpose was to determine a dose regimen that produced no discomfort since one of the difficulties with the administration of glucose is the possibility of laminitis. The blood glucose response to an oral dose of glucose follows a repeatable characteristic pattern which is dose dependent and possesses two phases (Roberts and Hill, 1973; June et al., 1992). The first phase represents continuous absorption of glucose from the small intestine, and lasts for approximately 120 min. The second phase is dependent on the insulin response to the hyperglycaemia induced by the glucose dose and lasts for approximately 4 hours with a return to resting insulin values by 6 hours.

Glucose absorption, blood glucose concentrations and tests for the functional capacity of the small intestine of the horse have been examined by a number of researchers (Alexander, 1955; Jacobs et al., 1982; Frake, 1986; Mair et al., 1991). However, few studies have examined the plasma glucose concentration and physiological responses to varying glucose concentrations administered by nasogastric tube.

The following experiments were conducted with the aim of determining the maximum glucose dose which could be administered to the horse without causing intestinal dysfunction, and to investigate differences between plasma glucose and insulin responses, to the administration of glucose and a glucose polymer. Glucose polymer studies in humans have shown that its capacity to empty from the stomach is more rapid than glucose (Decombaz et al., 1985; Neufeld et al., 1986; Fielding et al., 1987), has a lower insulin response and is able to be utilized for energy as effectively as glucose.
(Decombaz et al., 1985). This preliminary work examining the physiological, glucose and insulin responses to the administration of glucose or a polyjoule, provided the basis for the later studies investigating muscle glycogen resynthesis after exercise.

4.2 Aims

The aims of the experiments were:

a. To investigate the effects of varying glucose doses on plasma glucose and osmolality:

b. To investigate the responses of plasma glucose and osmolality to glucose compared with a glucose polymer (Polyjoule, Sharpe Laboratories, Sydney):

c. To examine the effects of administering a glucose polymer (4 g.kg\(^{-1}\)) on plasma glucose and osmolality:

d. To examine the physiological and haematological responses following the administration of a glucose polymer (3 g.kg\(^{-1}\)), with a second equivalent dose administered 3 hours later:

e. To examine the physiological and haematological responses following the administration of a glucose polymer (2 g.kg\(^{-1}\)), with a second equivalent dose administered 3 hours later:

f. To examine the hormonal, glucose and osmolality responses to a single versus a repeated dose of a glucose polymer (3 g.kg\(^{-1}\)).
In the first stage of this preliminary work, 3 horses were given a glucose absorption test in accordance with the procedures of Roberts and Hill (1973), in order to eliminate any horse which did not display a normal response curve. All horses had a normal glucose tolerance.

4.3 Materials and Methods

Horses were weighed prior to treatments. Feed was withheld for at least 10 hours before each experiment, but free access to water was permitted during this time. Water was withheld for the two hours after treatment, with free access then permitted.

Blood samples for glucose assays were collected into chilled 5 mL evacuated tubes containing potassium oxalate and sodium fluoride, and those for osmolality and insulin were collected into chilled 5 mL evacuated tubes containing lithium heparin. Blood samples for WBC, PCV and TP were collected into chilled 5 mL evacuated tubes containing EDTA.

Blood was centrifuged for 15 min at 3000 rpm and plasma was decanted into 5 mL plain disposable containers and stored at -20°C until assays were conducted. All samples were measured in duplicate for each time period and the mean of the two readings recorded.

Plasma glucose concentration was measured using an automatic analyser, results reported in mmol.L⁻¹, with plasma insulin concentration measured by radioimmunoassay
with units reported in $\mu U.mL^{-1}$. Osmolality of plasma was measured with an Osmometer and reported in mOsm.kg H$_2$O$^{-1}$.

White blood cell counts were carried out according to the procedure of Dacie and Lewis (1975). TP were measured using a refractometer.

Heart rate was determined over a 30 sec period using a stethoscope.

Rectal temperature was measured ($^\circ$C) by the use of a glass mercury thermometer.

All horses were confined to their stables during the experimental period and feed was kept constant for the 3 weeks before and throughout the experiment.

Areas under the glucose and insulin curves were calculated with time as the X-axis and glucose (mmol.L$^{-1}$) or insulin (μM.ml$^{-1}$) on the Y-axis. For glucose a base Y-axis value of 3.0 mmol.L$^{-1}$ and for insulin a base Y-axis value of zero were used for all calculations. The 3.0 mmol.L$^{-1}$ base line value was subjectively chosen based on the scale of the Y-axis and the area under the curve between the pre-treatment concentration and the zero base line. Area was calculated in square millimeters (sq.mm).

When the same horses were used in consecutive experiments, a minimum of 2 weeks was allowed between experiments.

4.3.1 Experiment 1

Three Thoroughbred geldings (Horses 1, 2 and 3) aged 7.0±0.6 years (mean±SEM) were used in a 3x3 latin square design. Mean body weight was 489±12 kg (mean±SEM).
Treatments:

All horses were administered glucose by nasogastric tube, at concentrations of either:

a. 0.5 g.kg\(^{-1}\) (bwt) as a 20% solution (Treatment A):

b. 1.0 g.kg\(^{-1}\) (bwt) as a 20% solution (Treatment B):

c. 2.0 g.kg\(^{-1}\) (bwt) as a 20% solution (Treatment C).

Horses were allowed free access to water 2 hours after drenching. Blood samples for glucose and osmolality were collected immediately before treatment and then every 30 min for 8 hours after treatment.

4.3.2 Experiment 2

Two Standardbred geldings (Horses A and B) of body weights 561 kg and 494 kg respectively were used in the study.

Treatments:

Each horse was administered a single dose by nasogastric tube at a concentration of either:

a. Glucose, 4 g.kg\(^{-1}\) (bwt) as a 20% solution (Treatment D).

b. Glucose polymer (Polyjoule) 4 g.kg\(^{-1}\) (bwt) as a 20% solution (Treatment E).

Horse A was administered Treatment D and horse B Treatment E.

Blood samples were collected immediately before treatment and every 30 min for the first 3 hours, then at hourly intervals up to 9 hours after treatment with a sample taken at 24 hours for plasma glucose and osmolality analysis.
4.3.3 Experiment 3

Two Standardbred geldings (Horses A & B) of body weights 578 kg and 494 kg respectively were used in the experiment.

*Treatments:*

Both horses were administered the same treatment.

Glucose polymer 4 g kg\(^{-1}\) (bwt) as a 20\% solution, with a second equivalent dose administered 3 hours later (Treatment F).

Blood samples were collected immediately before treatment and every 30 min for the first 6 hours after treatment. Samples then were taken hourly up to 9 hours after treatment, with a final sample taken at 24 hours after commencement of treatment, for plasma glucose and osmolality measurements. Blood samples for measurement of PCV, TP and recordings of heart rate (HR) and rectal temperature (Temp) were taken at 24 hours after first drenching. Horses were allowed free access to water following the 2 hour blood sample. Water was removed prior to the second drenching and replaced 2 hours after the second drenching. Horses were fed following the 9 hour blood sample.

4.3.4 Experiment 4

Two Standardbred geldings (Horses A & B) of body weights of 584 kg and 506 kg respectively were used in the experiment.

*Treatments:*

Each horse was administered the same treatment:
Glucose polymer 3 g kg\(^{-1}\) (bwt) as a 20% solution, with a second equivalent dose administered 3 hours later (Treatment G).

Water was provided 2 hours following the first administration of glucose polymer. Water was then removed prior to the 3 hour blood sampling and replaced 2 hours after the second administration. Blood samples for WBC, PCV, TP and the measurement of Temp were taken immediately before and at 3, 6, 9, 12, 24 and 48 hours after first drenching. Horses were fed following the 9 hour blood sampling.

4.3.5 Experiment 5

Two Standardbred geldings (Horses C & D) aged 4 and 5 years and body weights of 424 kg and 406 kg respectively were used in this experiment.

*Treatments:*

Each horse was administered the same treatment.

Glucose polymer 2 g kg\(^{-1}\) (bwt) as a 20% solution, with a second equivalent dose administered three hours later (Treatment H).

Horses were given free access to water 2 hours following the first administration of glucose polymer. Water was removed prior to the second administration and replaced 2 hours after. Blood samples for measurement of WBC, PCV, TP and the measurement of Temp were taken before treatment and at 3, 6, 9, 12, 24 and 48 hours after first administration. Horses were fed following the 9 hour blood sample.
4.3.6 Experiment 6

Three Standardbred geldings (Horses A, B & C) of mean body weight of 515±46 kg (mean±SEM), were randomly allocated to treatments in a 3x3 latin square design. Feed was withheld for a minimum of 10 hours prior to the experiment, and until after the 9 hour blood sample was collected.

_Treatments:_

Each horse was given one of three treatments by nasogastric tube:

a. Glucose polymer at 3 g.kg⁻¹(bwt) by nasogastric tube, as a 20% solution (Treatment I):

b. Glucose polymer at 1.5 g.kg⁻¹(bwt) by nasogastric tube, as a 20% solution, with a repeated dose 3 hours after the first drenching (Treatment J):

c. Water in a dose volume equivalent to the 3 g.kg⁻¹(bwt) (Treatment K Control).

Horses were allowed free access to water following the 2 hour sampling, then the water was removed prior to the 3 hour sampling and replaced 2 hours after the second nasogastric administration. Blood samples for plasma glucose, insulin and osmolality analysis, were collected before treatment and every 30 min for the first 3 hours following treatment, then at hourly intervals up to 9 hours after the first nasogastric administration. A final blood sample was taken 24 hours after the first nasogastric administration. Blood samples for measurement of WBC, PCV, TP and the measurement of Temp were taken prior to treatment and at 3, 6, 9, 12, 24 and 48 hours after the first nasogastric administration.
4.4 Statistics

All results are expressed as mean±(SEM). The major effect of time and treatment were analysed by analysis of variance, with time as a repeated measures (within group) factor. Where F values were significant, a post hoc test of least significant difference was used. The level of statistical significance set was p<0.05.
4.5 Results

4.5.1 Experiment 1

All horses were administered the glucose without showing signs of abdominal discomfort. The mean pre treatment plasma glucose concentrations for treatments A, B and C were 4.1±0.2, 4.3±0.1 and 4.3±0.8 (mean±SEM) mmol.L\(^{-1}\) respectively with mean peak concentrations being 5.6±0.3, 6.9±0.5 and 6.8±1.1 mmol.L\(^{-1}\) respectively (Figure 4.1). There was no significant effect of treatments but there was a significant effect of time on plasma glucose concentration at the p<0.05 level. A Mann-Whitney rank sum test was used to analyse area under the curve. There was a significant difference between treatments A and B and C but not between B and C. The median was 5956.0 (sq.mm) and the 25 to 75 percentiles being 4413.3 and 7021.7 respectively. (Table 4.1).

Plasma glucose concentration peaked by 2 hours in treatments A and B and by 2.5 hours in treatment C (Figure 4.1). Mean plasma glucose concentrations in treatments A and B returned to control levels by 4 hours after treatment, with treatment C remaining elevated for 6 hours after drenching. There was no significant difference between treatments on osmolality. Osmolality showed no relationship between net change and dose (Figure 4.2). Mean osmolality ranged from 277 to 283 mOsm.kg\(^{-1}\) H\(_2\)O, 277 to 283 mOsm.kg\(^{-1}\) H\(_2\)O and 275 to 285 mOsm.kg\(^{-1}\) H\(_2\)O for treatments A, B and C respectively (Figure 4.1).

No horse displayed any signs of inflammatory response to the glucose dosages.
Figure 4.1 Plasma glucose and osmolality values (mean±SEM) in Thoroughbred geldings before and after the administration of glucose at doses of 0.5, 1.0 and 2.0 g.kg⁻¹(bwt) as a 20% solution (N=3).
Table 4.1 Area under the glucose curve after the ingestion of 0.5, 1.0 and 2.0 g.kg\(^{-1}\) (bwt) of glucose as a 20% solution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Area (sq.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=2 0.5 g.kg(^{-1})</td>
<td>3569±60.2 (^a)</td>
</tr>
<tr>
<td>n=3 1.0 g.kg(^{-1})</td>
<td>6346±390.4 (^b)</td>
</tr>
<tr>
<td>n=3 2.0 g.kg(^{-1})</td>
<td>8100±2094.4 (^b)</td>
</tr>
</tbody>
</table>

Note: Area was calculated with time on the X-axis and plasma glucose on the Y-axis (mmol.L\(^{-1}\)).

Mean(SEM) in the same column not sharing the same superscript were significantly different p<0.05
Figure 4.2 Net changes in plasma glucose and osmolality values in Thoroughbred geldings, before and after the administration of glucose at doses of 0.5, 1.0 and 2.0 g.kg\(^{-1}\)(bwt) as a 20% solution (N=3).

Note: Net change is Peak-Rest plasma concentration.
4.5.2 Experiment 2

Both horses received the drench without showing signs of abdominal discomfort.

Peak plasma glucose concentrations were 11.6 and 8.2 mmol.L$^{-1}$ for treatments D and E respectively. Peak plasma glucose concentration occurred within 3 hours of administration of glucose in both treatments, with plasma glucose concentration returning to control levels by 8 hours for treatment D and 5 hours for treatment E (Figure 4.3).

Peak osmolality was 299 and 297 mOsm.kg$^{-1}$ H$_2$O for treatment D and E respectively. Osmolality remained within the normal range of 270-300 mOsm.kg$^{-1}$ H$_2$O (Figure 4.3).

Horse A had reduced appetite at the 8 hour feed, which was still evident 24 hours later. Faeces became more fluid in consistency.

4.5.3 Experiment 3

Peak plasma glucose concentrations were 10.4 and 7.2 mmol.L$^{-1}$ for horses A and B respectively. Peak concentrations were reached within 3 hours of treatment, with plasma glucose concentration still not returning to control levels by 9 hours following the first nasogastric administration (Figure 4.4). Osmolality had peaked by 4 hours for horse B and by 6 hours for horse A. Both horses were within the normal range for osmolality values (Figure 4.4).

Neither horse consumed any water throughout the experimental period. At the 9 hour feeding both horses ate. However, their appetites were low, with feed still remaining
Figure 4.3 Plasma glucose and osmolality values in 2 Standardbred geldings before and after the administration of glucose or a glucose polymer at a dose of 4 g.kg$^{-1}$ (bwt) as a 20% solution.

Note: --- represents a misplaced sample.
Figure 4.4 Plasma glucose and osmolality values in 2 Standardbred geldings before and after the administration of a glucose polymer at a dose of (4 g·kg\(^{-1}\) (bwt)) as a 20% solution, with a second equivalent dose administered 3 hours later.
in their feed bin at the next morning feed, with this reduced appetite persisting for a further 12 hours.

Examination 24 hours after the first administration revealed the following signs: horse A had pain in the front left foot, diarrhoea, a HR of 36 bpm, respiration rate of 20 breaths per min, temp of 37.5\(^0\)C, PCV of 0.30 L.L\(^{-1}\) and TP of 59 g.L\(^{-1}\).

Horse B displayed no lameness but a HR of 50 bpm, temp 39.5\(^0\)C, respiration rate of 48 breaths per min, PCV of 0.34 L.L\(^{-1}\) and TP of 64 g.L\(^{-1}\).

4.5.4 Experiment 4

The effects of 3 g.kg\(^{-1}\) (bwt) of glucose polymer on HR, WBC, TP and PCV are presented in Table 4.2.

Heart rates increased from resting values of 30 bpm to 37 bpm at 24 hours and 35 bpm at 48 hours after administration for horse A, and 31 bpm at rest to 65 bpm and 58 bpm at 24 and 48 hours for horse B. Total proteins and PCV displayed no major changes (see Table 4.2)

WBC for horse A had displayed no change by 24 hours, then had reduced by 48 hours. Horse B, however, had a major change with WBC nearly doubling from 6.7 x 10\(^9\) at rest to 12.8 x 10\(^9\) at 48 hours.

Horse A at the 9 hour feed had poor appetite, increased gut sounds and at 57 hours after first administration was still not eating normally, although general clinical signs were normal.
Table 4.2 HR, WBC, TP and PCV values following the provision of a glucose polymer (Polyjoule) at a dose of 3g.kg\(^{-1}\)(bwt) as a 20% solution, with a second equivalent dose administered 3 hours later in 2 Standardbred geldings.

<table>
<thead>
<tr>
<th>Time Hours</th>
<th>Horse A</th>
<th>Horse B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>WBC (x10^9 \text{L}^{-1})</td>
</tr>
<tr>
<td>PreTreat</td>
<td>30</td>
<td>8.1</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>7.8</td>
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<td>6</td>
<td>28</td>
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<td>37</td>
<td>8.1</td>
</tr>
<tr>
<td>48</td>
<td>35</td>
<td>7.3</td>
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</table>

*Note:* HR at 9 hours was not measured
Horse B at the 9 hour feed was alert. By 24 hours after first administration, the horse was scouring badly, had increased gut sounds, and was reluctant to remain standing. At 48 hours the horse was showing signs of lameness in left front leg, diarrhoea had ceased but appetite remained reduced.

4.5.5 Experiment 5

The influence of the glucose polymer on HR, WBC, TP and PCV is presented in Table 4.3. Results of this study showed that a repeated dose of 2 g.kg\(^{-1}\) (bwt) of the glucose polymer had no major effect on HR, PCV and TP for the horses tested.

WBC counts for horse C increased from a control value of 8.8 \(\times\) 10\(^8\) to 11.3 \(\times\) 10\(^8\) at 24 hours and 11.6 \(\times\) 10\(^8\) at 48 hours. In horse D the WBC decreased over the 24 hour and 48 hour periods from a control value of 10.2 \(\times\) 10\(^9\) to 8.5 \(\times\) 10\(^9\) and 8.0 \(\times\) 10\(^9\) at 24 and 48 hours respectively.

Temperatures at 24 and 48 hours were 38.3 and 37.7\(^0\)C for horse C and 37.6\(^0\)C at both 24 and 48 hours for horse D respectively. These values all lie within the normal range for the horse (Rose and Hodgson, 1994).

Both horses were alert and showed no signs of lameness.

4.5.6 Experiment 6

Results for this experiment are shown in Table 4.4. There was a significant difference between treatment K and treatments I and J for both glucose and insulin, but no
Table 4.3 HR, WBC, TP and PCV values following the administration of a glucose polymer (Polyjoule) at a dose of 2g.kg\(^{-1}\)(bwt) as a 20% solution, with an equivalent second dose administered 3 hours later in 2 Standardbred geldings.

<table>
<thead>
<tr>
<th>Time Hours</th>
<th>Horse C</th>
<th></th>
<th>Horse D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>WBC (x10(^9).L(^{-1}))</td>
<td>TP (g.L(^{-1}))</td>
<td>PCV (L.L(^{-1}))</td>
</tr>
<tr>
<td>Pre-Treat</td>
<td>32</td>
<td>8.8</td>
<td>66</td>
<td>.36</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>8.1</td>
<td>63</td>
<td>.34</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>7.2</td>
<td>61</td>
<td>.33</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>6.2</td>
<td>61</td>
<td>.34</td>
</tr>
<tr>
<td>12</td>
<td>44</td>
<td>9.7</td>
<td>71</td>
<td>.44</td>
</tr>
<tr>
<td>24</td>
<td>40</td>
<td>11.3</td>
<td>67</td>
<td>.38</td>
</tr>
<tr>
<td>48</td>
<td>36</td>
<td>11.6</td>
<td>63</td>
<td>.34</td>
</tr>
</tbody>
</table>
significant difference between treatments I and J at the p<0.05. The same data analysed as
area under the curve similarly showed a significant difference between treatment K and
treatments I and J for both glucose and insulin but no significant difference between
treatments I and J at p<0.05 (Table 4.4).

The plasma glucose concentrations (mean±SEM) prior to nasogastric intubation
were 4.4±0.2, 4.5±0.01, 4.5±0.5 mmol.L⁻¹ for treatments I, J and K respectively. Peak
plasma glucose concentrations (mean±SEM) following treatments were 7.3±0.2 and
7.2±0.5 mmol.L⁻¹ for treatments I and J. For treatment K there was no distinct peak but a
base value of 4.4±0.04 mmol.L⁻¹. Peak plasma glucose concentration for treatments I and
J had been reached by 2 hours after treatment (Figure 4.5), with time to return to pre-
treatment values being longer for treatment J.

The plasma insulin concentrations (mean±SEM) prior to nasogastric intubation
were 6.6±1.6, 6.4±0.8, 5.4±0.8 µU.ml⁻¹ respectively for treatments I, J and K. Peak insulin
concentrations (mean±SEM) following treatments were 47.8±6.9 and 41.2±7.4 µU.ml⁻¹
for treatments I and J respectively. For treatment K there was no distinct peak but a base
value of 5.2±0.3 µU.ml⁻¹ (Figure 4.5). There was no significant difference between
treatments for WBC, HR, TP, PCV and Temp at the p<0.05 level. Ranges for HR, PCV
and Temp, WBC and TP are shown in Figures 4.6 and 4.7 respectively. All were within
the daily variation for horses.

Water consumption varied, with volume consumed in the 3 hour period after
feeding being greater than for the total 9 hour sampling. All horses showed normal
appetite following the 9 hour feed and passed normal faeces during the testing period. At
24 hours there were no signs of lameness and faeces were normal.
Table 4.4 Total area (mean±SEM) under the glucose and insulin curves for the 9 hour period following the administration of 3 g.kg$^{-1}$(bwt) as a single dose, 1.5 g.kg$^{-1}$(bwt) with a second equivalent dose administered 3 hours later or water in a volume equivalent to the 3 g.kg$^{-1}$(bwt) trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose(sq.mm)</th>
<th>Insulin(sq.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=3 Water</td>
<td>3569±60.2$^a$</td>
<td>1721±382.0$^a$</td>
</tr>
<tr>
<td>n=3 1.5 g.kg$^{-1}$</td>
<td>6346±390.4$^b$</td>
<td>9834±2930$^b$</td>
</tr>
<tr>
<td>n=3 3.0 g.kg$^{-1}$</td>
<td>8100±2094.4$^b$</td>
<td>8755±1540.6$^b$</td>
</tr>
</tbody>
</table>

Note: Area was calculated with time on the X-axis and plasma glucose or insulin on the Y-axis (mmol.L$^{-1}$).

Mean(SEM) in the same column not sharing the same superscript were significantly different p<0.05
Figure 4.5 Plasma glucose and insulin values (mean±SEM) in Standardbred geldings before and after the administration of a glucose polymer at either 3 g.kg⁻¹ (bwt) as a single dose, 1.5 g.kg⁻¹ (bwt) with a second equivalent dose administered 3 hours later or water in a dose volume equivalent to the 3 g.kg⁻¹ (bwt) (N=3).
Figure 4.6 Values for HR and PCV (mean±SEM) in Standardbred geldings before and after the administration of a glucose polymer, at a dose of either 3 g.kg\(^{-1}\)(bwt) as a single dose, 1.5 g.kg\(^{-1}\)(bwt) with a second equivalent dose administered 3 hours later, or water in a dose volume equivalent to the 3 g.kg\(^{-1}\) (bwt) (N=3).
Figure 4.7 Values for temp, WBC and TP (mean±SEM) in Standardbred geldings before and after the administration of a glucose polymer at a dose of either 3 g.kg⁻¹(bwt) as a single dose, 1.5 g.kg⁻¹(bwt) with a second equivalent dose administered 3 hours later, or water in a dose volume equivalent to the 3 g.kg⁻¹(bwt) (N=3).
4.6 Discussion

The results of these studies demonstrate that a single dose of glucose in the vicinity of 3 g.kg\(^{-1}\)(bwt), is the maximum safe dose that can be administered to the horse without causing gastrointestinal dysfunction and early signs of laminitis.

A number of studies, (Alexander, 1955; Loeb et al., 1972; Roberts and Hill, 1973), have shown that peak blood glucose concentrations are reached within 2 hours of the administration of glucose at a dose of 1 g.kg\(^{-1}\)(bwt), with returns to pre-treatment concentrations by 4 to 6 hours. Studies by Roberts and Norman (1979), in evaluating the D-(+)-Xylose absorption test, and Roberts and Hill (1973) on glucose absorption tests, found that the time to peak plasma glucose or Xylose concentrations was dose dependent. This characteristic dose dependent pattern of time to peak glucose concentration was seen in these experiments for the lower doses. The fact that the high dose administered was three times as great as the normal glucose tolerance test dose, would offer an explanation for the longer time to peak concentration. Further, that when the dose was repeated, this would delay the return to pre-treatment concentrations. The peak plasma glucose concentration following a second dose was found to be less than that following the first dose. This lower second peak could be due to the higher insulin concentration following the second dose, as insulin stimulates glucose uptake into tissues (Frape, 1986).

In experiments 1 and 2 a hypoglycaemic response occurred, which was dose dependent and varying from 3.5 to 6.5 hours after treatment for the 0.5 g.kg\(^{-1}\) and 2.0 g.kg\(^{-1}\) glucose doses respectively (Figures 4.1 and 4.3). The hypoglycaemic response following the administration of a glucose supplement has been reported previously (Loeb
et al., 1972 and Frape, 1986) and is thought to contribute to a delay in the termination of insulin action.

Time to peak plasma glucose concentration varied between glucose and glucose polymer. In experiment 2, the horse which received the glucose polymer had reached peak plasma glucose concentration by 2 hours, and returned to control values by 5 hours after treatment. In comparison, the glucose dosed horse did not peak until 3 hours, and had not returned to resting levels by 7 hours. However, there was no difference in the initial absorption phase, up to 2 hours, indicating that absorption rates for the two dosages were similar. This concept is supported by the work of Wahlqvist et al. (1978) who reported no difference in absorption rates of mono-penta or polysaccharides consisting exclusively of glucose, and concluded that chain length alone does not appear to influence the rate of glucose absorption across the intestinal wall in humans. However, the data indicates that the glucose polymer may not have released its total glucose content (Figure 4.3). The bioavailability difference could relate to the different chain lengths of the two molecules and offer an explanation for the difference in peak plasma glucose concentration.

The reduced appetite and more fluid faeces of the glucose dosed horse in experiment 2 is indicative of alimentary disturbance, most likely resulting from the high glucose load. It is known that laminitis of alimentary origin is possible (Garner et al., 1975, Moore et al., 1989), which can result in lameness of varying degrees, and increases in HR, TP, PCV and rectal temperature. Although these variables were not measured in this experiment, the loss of appetite and more fluid faeces were indicative of gastrointestinal disturbances which is known to precede the onset of laminitis.
The physiological responses measured in experiment 3 do not support the findings
of Garner et al. (1975). This was to be expected as the dose of carbohydrate administered
in this study was much smaller than that reported by Garner et al. (1975). In addition, the
horses in the study by Garner et al. (1975) all showed signs of laminitis, whereas the two
horses in this study only displayed some early signs. Though the physiological responses
were not severe, an important point that was highlighted was the degree of variability
which existed between the horses in response to the same relative dose rate of a glucose
polymer. Further, the signs such as diarrhoea and mild lameness can occur while the other
responses such as HR, Temp and TP were within the normal ranges. It was also possible
for horses to show no adverse clinical signs with elevations in HR and Temp. In horse B
even though lameness was evident, HR, Temp, and PCV readings of 50 bpm, 39.5°C, and
0.34 L.L⁻¹ at 24 hours after treatment, were comparable to those reported by Garner et al.
(1975) for horses with laminitis. In the fourth experiment the HR, Temp and PCV changes
in horse B were of a similar magnitude to those reported by Garner et al. (1975). These
HR, Temp, and PCV changes were reflected by horse B in the physical signs of severe
diarrhoea, increased gut sounds and lameness, at 24 and 48 hours post administration of
glucose. Increased WBC, HR and Temp were according to Garner et al. (1975) indicators
of an inflammatory response which they indicated was the response from an increased
vascular permeability associated with endotoxaemia. The severity of the inflammatory
response of horses A and B in this experiment, as opposed to the responses in experiment
3, poses the question of whether there is a critical level of glucose intake which triggers
the pathological responses, and above this dose does not magnify the pathological
responses. This critical level concept is supported by the finding that the total dosages for
horse B were 3952 gm versus 3036 gm of glucose for treatments F and G respectively. However, the severity of responses was greater in the lower dose (treatment G) trial. The lack of any major changes in HR in the first 12 hours reported in this study was supported by the work of Garner et al. (1975), in which they did not find a major change in HR until 16 hours after infusion.

The degree of excitement of the horse has been shown to increase hemoglobin, PCV, RBC and WBC readings. Revington (1983) reported a mean WBC count of $10.2 \pm 1.7 \times 10^9 \text{L}^{-1}$, with a range of $5.5 - 21.9 \times 10^9 \text{L}^{-1}$ for Thoroughbreds 1 to 3 hours before racing. In the present study the WBC counts were all within the range reported by Revington (1983).

The changes in TP and PCV in this study are in line with Garner et al. (1975). They indicate the changes in TP and PCV were representative of a compartmental fluid shift, with water moving out of the intravascular compartment. This in turn leads to an increase in blood viscosity and a tendency for cardiovascular insufficiency. The indicator of cardiovascular insufficiency, an increased heart rate, was evident in this study. These signs were most pronounced in horse B in experiment 4 (Table 4.1). The results from experiment 6 illustrate the same pattern of change in PCV and TP. However, the severity of change was not as pronounced, nor were there any physical signs of laminitis. The initial decrease in PCV and TP observed in these experiments may be indicative of a compartmental fluid shift into the vascular system resulting from the large intake of a concentrated fluid.
The administration of a carbohydrate overload to the horse, has been shown to cause a decrease in caecal pH, increase in lactate and free endotoxins, which are indicators of possible acute alimentary laminitis (Moore et al., 1979).

These studies have shown that care needs to be taken when administering large doses of glucose by nasogastric tube. There was no conclusive evidence that a glucose polymer was any less likely to induce gastrointestinal dysfunction. However, a dose rate up to 1.5 g.kg\(^{-1}\) (bwt), repeated at a three hour period or 3 g.kg\(^{-1}\) (bwt) as a single dose, appear safe to be administered to the horse without causing gastrointestinal disturbances, which are known to precede the onset of laminitis.
CHAPTER 5

THE EFFECTS OF AN ORAL GLUCOSE POLYMER ON
MUSCLE GLYCOGEN RESYNTHESIS IN
STANDARDBRED HORSES.

5.1 Introduction

5.2 Materials and Methods

5.2.1 Horses
5.2.2 Glycogen Depletion Run
5.2.3 Muscle Biopsy Procedure
5.2.4 Blood Collection and Analysis
5.2.5 Treatments

5.3 Statistics

5.4 Results

5.5 Discussion
5.1 Introduction

In the horse, endurance competition demands a high level of performance over 1 to 5 days. This has resulted in decreased muscle glycogen concentrations in the range 17% to 87% (Snow et al., 1981; Hodgson et al., 1984b; Essén-Gustavsson et al., 1984). These large decreases in muscle glycogen stores suggest that muscle glycogen levels may be a key factor in the onset of fatigue and the incidence of "exhausted horse syndrome" during endurance events (Snow et al., 1981).

There have been various studies reported investigating muscle adaptations to exercise in horses (Lindholm et al., 1974; Lindholm and Piehl 1974; Snow et al., 1981; Hodgson et al., 1985; Hodgson and Rose, 1987), but the field of muscle glycogen resynthesis has received little attention.

In contrast, studies in humans have shown that the rate of resynthesis is affected by factors such as glucose dose administered post exercise (Blom et al., 1986; Blom et al., 1987), the timing of the administration of the supplement after exercise (Ivy et al., 1988a), the activity state of glycogen synthetase and the initial muscle glycogen concentration (Danforth, 1965; Larner et al., 1967; Bergström et al., 1972; Kochan et al., 1979).

Snow et al. (1987) studied the effects of varying concentrations of carbohydrates in the diets of horses, on muscle glycogen resynthesis following exercise. They compared a low carbohydrate (roughage), normal and high carbohydrate diet, and
reported that the rate of resynthesis during the first 28 hours was lower on a low carbohydrate (roughage) diet, but it was not significantly different from the normal or high carbohydrate diet.

This lack of a significant difference between the high carbohydrate and normal diets in the first 28 hours (Snow et al., 1987) suggests that the restoration of glycogen stores may not entirely depend on the level of carbohydrate in the diet. An alternative is that the level of carbohydrate was not high enough to induce a large enough effect between the normal and high carbohydrate diets utilized by Snow et al. (1987).

Due to the difficulties in administering a large glucose dose in the diet, in this experiment, nasogastric administration of glucose was used to determine if there was a beneficial effect on muscle glycogen resynthesis in the horse.

5.2 Materials and Methods

5.2.1 Horses

Four Standardbred geldings aged 5.5±2.9 years (mean±SEM) and 512±29 kg body weight were randomly allocated to treatments in a cross over design. Testing was carried out on 3 consecutive weeks, with all horses being tested at the same time and on the same day each week.
All horses had been in training for 6 weeks at the commencement of the study. Training was conducted on a treadmill with the slope set at 10%. The training over the final weeks prior to the study consisted of 1000 m at 3 m.s\(^{-1}\) as a warm up, followed by 3000 m at 5 m.s\(^{-1}\) and 840 m at 7 m.s\(^{-1}\) on 5 days per week. On the sixth day horses exercised for 1000 m at 4 m.s\(^{-1}\) and rested the seventh day. All horses were maintained on the same diet for the duration of training and throughout the study.

**5.2.2 Glycogen Depletion Run**

To induce glycogen depletion, horses were exercised at various percentages of their maximal oxygen uptake (\(\dot{V}O_2\text{max}\)) values, based on the results of a preliminary study examining fibre type depletion at different intensities and durations of exercise (Appendix A). The treadmill speeds for the respective percentages of \(\dot{V}O_2\text{max}\) were determined from sub-maximal and maximal treadmill tests conducted the week prior to the experiment.

To deplete muscle glycogen each horse was exercised on the treadmill (inclined at 10% slope) for 45 min at 30% \(\dot{V}O_2\text{max}\), 15 min at 50% \(\dot{V}O_2\text{max}\) after which they were rested for 30 min. This was followed by six one min sprints at 100% \(\dot{V}O_2\text{max}\) with 5 min rest periods between sprints. The aim of this protocol was to induce glycogen
depletion in all muscle fibre types. All glycogen depletion runs were conducted early in the mornings to reduce the thermal stress on the horses. Food was withheld for a minimum of 10 hours prior to the glycogen depletion run, and throughout the 12 hour biopsy sampling period. Horses were allowed free access to water during experiments.

5.2.3 Muscle Biopsy Procedure

Muscle biopsies were taken using the needle biopsy technique of Bergstrom (1962) as modified by Lindholm and Piehl (1974). Biopsies were taken from the m. gluteus medius at a depth of 8 cm using the same location in each horse. Samples were taken before and at the end of exercise, and at 3, 6, 9, 12 and 24 hours following treatments. Muscle glycogen concentrations were determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al. (1987). Muscle sections were preincubated at pH 4.3 and 10.3 to allow identification of slow twitch (ST) and fast twitch (FT) fibre types (Brooke and Kaiser 1970). To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff et al. (1961), with the fast twitch fibres subdivided into high oxidative (FTH) and low oxidative (FT).
5.2.4 Blood Collection and Analysis

Horses were prepared for blood collection in the 30 min between the end of the exercise and administration of the glucose polymer. Blood samples were collected before exercise, immediately before treatment and then every 30 min for 6 hours. Following centrifuging of the blood samples, the plasma was decanted into 5 mL disposable containers and stored at -20°C until analysed for glucose and insulin.

5.2.5 Treatments

Thirty minutes after exercise each horse was given one of three treatments by nasogastric tube;

a. Glucose polymer (Polyjoule) 1.5 g.kg\(^{-1}\) as a 20% solution, with a second equivalent dose administered 3 hours later (Treatment A);

b. Glucose polymer (Polyjoule) 3 g.kg\(^{-1}\) as a single dose in a 20% solution (Treatment B);

c. Water (Control) at a volume equivalent to that administered in treatment B (Treatment C).

The experiment was conducted over 3 weeks with 7 days between treatments. No horse showed any signs of discomfort after treatment.
5.3 Statistics

All results are expressed as mean±(SEM). For glucose and insulin the major effects of time, horse, week and treatment were analysed by MANOVA, with time as a repeated measures (within group) factor. Where F values were significant, a post hoc test of least significant difference was used. The level of statistical significance used was p<0.05.

The rate of glycogen resynthesis in the first 12 hours after exercise was determined by linear regression analysis using the method of least squares. The effects of horse, week and treatment on the slope of the regression line were calculated by MANOVA.

5.4 Results

Mean body weight and $\dot{V}_{O_2}\max$ of the group was 512±29kg and 114±6 ml.kg.$^{-1}$ respectively. Mean speeds at 30%, 50% and 100% $\dot{V}_{O_2}\max$ were 2.3±0.1, 4.1±0.2 and 8.8±0.4 m.s.$^{-1}$ respectively. Mean pre-exercise plasma glucose values were 4.2±0.1, 4.1±0.2 and 4.2±0.2 mmol.L.$^{-1}$ for treatments A, B and C respectively. The changes in plasma glucose following treatments are illustrated in Figure 5.1. Two peaks occurred in
plasma glucose concentration with mean plasma glucose concentrations for the first peaks being 5.7±0.2 and 5.5±0.1 mmol.L⁻¹ for treatments A and B respectively. Peak glucose concentrations occurred 30 min and 1.5 hours for treatments A and B respectively. The second peak plasma glucose concentrations of 5.6±0.2 and 4.8±0.2 mmol.L⁻¹ for treatments A and B occurred at 4.5 hours for treatment A and 5 hours for treatment B. The second peaks represented 99% and 89% of the first peak for treatments A and B respectively. Plasma glucose concentrations were dependent on treatment and time, but there was no significant effect of horse or week at the p<0.05 level.

The mean pre-exercise plasma insulin concentrations were 4.1±0.7, 4.1±0.9 and 6.3±3.0 µU.ml⁻¹ for treatments A, B and C respectively. Plasma insulin response curves to the treatments had two peaks with the first peak concentrations being 26±3.1 and 25.6±3.1 µU.ml⁻¹ respectively for treatments A and B (Figure 5.1). The second peak concentrations were 33.9±5.2 and 11±2.3 µU.ml⁻¹ for treatments A and B. The second peaks represented 130% and 43% of the first peaks for treatments A and B respectively. There was a significant effect of both time and treatment at the p<0.05 level.

Pre-exercise muscle glycogen concentrations ranged from 529 to 800 mmol.kg⁻¹(dwt) with a mean of 652±22 mmol.kg⁻¹(dwt). At the end of exercise and prior to treatment, the muscle glycogen range was 211 to 485 with a mean of 366±25 mmol.kg⁻¹(dwt). The percentage depletion of glycogen ranged from 31% to 65% with a mean of
Figure 5.1 Plasma glucose and insulin values (mean±SEM) in Standardbred geldings before and after the administration of a glucose polymer at either 1.5 g.kg⁻¹(bwt), with a second equivalent dose administered 3 hours later (Treatment A), or 3 g.kg⁻¹(bwt) as a single dose (Treatment B) or control (Treatment C) 30 min after exercise (N=4).
44%. Differences between treatments for the resting and after exercise muscle glycogen concentrations were not significant.

By 12 hours after treatment, muscle glycogen concentrations had increased to 465±33 and 459±17 and 446±45 mmol.kg⁻¹(dwt) for treatments A, B and C respectively. These increases were 69%, 75% and 67% of the mean resting values for each treatment (Figure 5.2). Muscle glycogen concentration was dependent on time but there was no significant effect of treatment, horse or week.

Mean rates of glycogen resynthesis for the 6 to 24 hours after the administration of treatments are presented in Table 5.1. Mean rates of muscle glycogen resynthesis for the three treatments over the 24 hours were not significantly different. Mean rates of resynthesis at 6, 9 and 12 hour sampling periods were significantly different, with the differences being between treatment C and treatments A and B. There was no significant difference between treatments A and B. The mean rate of resynthesis for the three treatments during the 12 hours after treatment was 7.6 mmol.kg⁻¹.h⁻¹. The r² for the individual regression equations for resynthesis rates ranged from 0.5 to 1.0 with a mean of 0.75±0.05. The rate of resynthesis was independent of treatments as there was no significant difference between regression coefficients.

A Spearman rank order correlation was performed to determine if any association existed between muscle glycogen concentration at 24 hours after exercise and both peak insulin and glycogen concentration at the end of exercise. There was no significant
association with insulin, but there was a trend for an association with glycogen concentration at the end of exercise ($R=0.52$, $p=.08$).
Figure 5.2 Muscle glycogen concentrations (mean±SEM) of the m. gluteus medius in Standardbred geldings for the 24 hour period before and after the administration of a glucose polymer at either 1.5 g.kg⁻¹(bwt), with a second equivalent dose administered 3 hours later (Treatment A), or 3 g.kg⁻¹(bwt) as a single dose (Treatment B) or control (Treatment C), 30 min after exercise (N=4).
Table 5.1 Muscle glycogen resynthesis rates (mmol.kg$^{-1}$.h$^{-1}$(dwt)) (mean±SEM) at 3, 6, 9, 12 and 24 hours after the administration of a glucose polymer at either 1.5 g.kg$^{-1}$(bwt), with a second equivalent dose administered 3 hours later (Treatment A), or 3 g.kg$^{-1}$(bwt) as a single dose (Treatment B) or control (Treatment C), 30 min after exercise (N=4).

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=4 3h</td>
<td>12.6±6.6</td>
<td>17.6±4.5</td>
<td>4.5±10.6</td>
</tr>
<tr>
<td>N=4 6h</td>
<td>9.2±3.8$^a$</td>
<td>10.4±3.2$^a$</td>
<td>-6.5±2.9$^b$</td>
</tr>
<tr>
<td>N=4 9h</td>
<td>10.4±2 $^a$</td>
<td>8.7±2.9 $^a$</td>
<td>-0.6±1.0 $^b$</td>
</tr>
<tr>
<td>N=4 12h</td>
<td>9.2±1.9$^a$</td>
<td>10.6±1.3$^a$</td>
<td>2.9±0.8 $^b$</td>
</tr>
<tr>
<td>N=4 24h</td>
<td>8.4±1.1</td>
<td>6.6±1.1</td>
<td>5.4±0.6</td>
</tr>
</tbody>
</table>

Note: Means in the same row not sharing the same superscript were significantly different p<0.05
5.5 Discussion

The results of this study illustrate that the administration of 3.0 g kg\(^{-1}\) of glucose polymer as either a single or split dose, after exercise, does not have a significant effect on the rate of muscle glycogen resynthesis in the 24 hours after exercise. The lack of a significant difference in muscle glycogen resynthesis between control and treatments occurred despite a significant difference in blood glucose and insulin concentration following treatments. This suggests that the blood glucose concentrations resulting from the different treatments were not sufficiently different to affect glycogen resynthesis, or that blood glucose *per se* is not an important factor in glycogen resynthesis in the horse.

The increase in plasma glucose concentration post exercise concurs with results for other studies (Snow and Rose, 1981; Freestone, Wolfsheimer, Kamerling, Church, Hamra and Bagwell, 1991). The glucose polymer doses administered after exercise did not produce a significant differentiation in plasma glucose responses between the two treatments. It is interesting that for the 0-3 hour period, the glucose curves closely follow each other given that the dose administered in treatment A was half that administered in treatment B. The lack of a difference between treatments may reflect a combination of the efficiency of the glucose uptake and the efficiency of hepatic glycogenolysis and increased gluconeogenesis following exercise. Time to peak insulin concentration observed in this study was in agreement with that reported by Stull and Rodiek, 1988. The decreased plasma insulin concentration during exercise is a result of the decreased plasma glucose
concentration and increased sympathetic activity (Snow and Rose, 1981; Church, Evans, Lewis and Rose 1987), with the increase after exercise being the result of a withdrawal of the sympathetic activity on the pancreatic beta cells and an increase in endogenous opioid peptides stimulating insulin release (Freestone et al., 1992). It is known that insulin is both an activator of glycogen synthetase and glucose uptake. The higher insulin response to the second glucose polymer dose in treatment A, would suggest that the glucose polymer would be more effective in the bioavailability of glucose, and may also have higher glycogen synthetase activity and a higher resynthesis rate. However, no association was found between glycogen concentration at 24 hours after exercise and peak insulin values. Further, there was a trend for an association between glycogen concentration at the end of exercise and the 24 hour concentration. As the highest insulin value was coincident with the lowest post exercise glycogen concentration, it could be that the glycogen concentration is a stronger stimulus than insulin in resynthesis. In the control treatment, the increase in insulin concentration post exercise, as described by Church et al. (1987) did not apply, indicating a possible combined action of exercise and feed deprivation on maintaining a low plasma insulin level.

An unexpected finding of this study was the high rate of muscle glycogen resynthesis in the control treatment group. This rate of glycogen resynthesis was in contrast to that reported by Maehlum and Hermansen (1978) and Young et al. (1983). Maehlum and Hermansen (1978) reported that for fasting humans, there was 25%
increase in muscle glycogen concentration after 4 hours, after which there was no further increase over the next 8 hours. Young et al. (1983) found that feeding a carbohydrate free diet to exercised rats did not completely prevent an increase in muscle glycogen concentration, but resulted in muscle glycogen concentrations similar to those found in fasting or fat fed non-exercised rats. The reasons for the high rate of glycogen resynthesis in the control treatment group are unclear. One explanation could be that the contribution of glucose, via hepatic glycogenolysis and gluconeogenesis was adequate to maintain a sufficient level of plasma glucose for glycogen resynthesis.

Also contrasting with the report of Machlum and Hermansen (1978), was the decrease in muscle glycogen concentration between the end of the glycogen depletion run and 6 hours after exercise in the control treatment group. The reasons for this are unclear, as glucose levels either had increased or remained constant. The fact that insulin levels remained low may be a contributing factor because insulin stimulates glycogen synthetase activity. A more likely reason is the variation in glycogen concentration in the different areas of the muscle, in combination with the assay variation.

The major limiting factor in glycogen synthesis according to Fell et al. (1982) is the muscle’s ability to synthesise glycogen, rather than the availability of intracellular substrate. They indicated that a low muscle glycogen content following exercise was a major stimulus for increasing the rate of glucose transported into the muscle in addition to an increased rate of glycogen synthesis. The data from the current study, supports the
concept of the importance of the initial glycogen concentration as a key factor regulating the rate of muscle glycogen resynthesis. There was some indication of an inverse association between the degree of depletion at the end of exercise and the rate of muscle glycogen resynthesis throughout the 24 hour period. However, this resynthesis was not dose dependent, and therefore it would seem that for the horse, the initial concentration of muscle glycogen may be a more significant factor than the blood glucose concentration, in regulating the rate of resynthesis.

An absence of an effect of glucose treatment on muscle glycogen resynthesis concurs with results of Snow et al. (1987). They examined the effects of a low, high or normal carbohydrate diet on muscle glycogen resynthesis following exercise in Thoroughbred horses. Horses receiving the high carbohydrate diet were also given intravenous glucose (0.45 kg) at a constant rate over 6 hours. They reported that the greatest resynthesis of muscle glycogen was in the first 8 hours, with the low carbohydrate group having little further increase after this. During the first 28 hours the repletion rates for the normal and high carbohydrate groups were similar but both were higher than the low carbohydrate diet. Only at 28 hours after depletion was the difference between the low and high/normal diets significant.

The failure of this study to demonstrate a significant difference in the rates of glycogen resynthesis between treatments over the 24 hour period after treatment, supports the findings of Snow et al. (1987), and contrast with results reported for humans (Pichl,
1974; Costill et al., 1981; Blom et al., 1986; Blom et al., 1987; Ivy et al., 1988a; Ivy et al., 1988b; Reed et al., 1989). The difference between the current study and human studies may be the result of the relative doses of glucose administered being insufficient to produce the necessary increase in plasma glucose. However, in the horse, glucose dose rates greater than 3 g kg\(^{-1}\) caused signs of intestinal discomfort and laminitis (See Chapter 4).

For the horse the highest rates of resynthesis have been shown to occur in the first 12 hours of recovery after exercise, with a replenishment rate of 6 mmol kg\(^{-1}\) h\(^{-1}\) and with up to 50% of glycogen replenishment occurring in this period (Hodgson 1984). The rates of glycogen resynthesis in Hodgson's study varied from 7.75 mmol kg\(^{-1}\) h\(^{-1}\) in the first 4 hours to 2.57 mmol kg\(^{-1}\) h\(^{-1}\) between 12 and 24 hours. Maehlum et al. (1977) reported that in humans, the most pronounced increase in glycogen repletion was found in the course of the first 4 hours of recovery from exercise, with the mean rate of glycogen synthesis during this period of 28.8±0.7 mmol kg\(^{-1}\) h\(^{-1}\) (dwt). Others, Ivy et al. 1988a and Ivy et al. 1988b have reported rates of resynthesis up to 30.8 mmol kg\(^{-1}\) h\(^{-1}\) (dwt) in the first two hours following exercise. In the present study, the rate of resynthesis varied from 12.6±6.6 mmol kg\(^{-1}\) h\(^{-1}\) (dwt) in the first 3 hours to 8.4±1.1 mmol kg\(^{-1}\) h\(^{-1}\) (dwt) over the 24 hour period for treatment A, and from 17.6±4.5 mmol kg\(^{-1}\) h\(^{-1}\) to 6.6±1.1 mmol kg\(^{-1}\) h\(^{-1}\) (dwt) for treatment B, and from 4.5±10.6 to 5.4±0.6 mmol kg\(^{-1}\) h\(^{-1}\) (dwt) for treatment C for the
same periods. The mean percentage total glycogen replenishment was 70±2% of mean resting level in the first 12 hours which is much higher than the 50% reported by Hodgson (1984) for the same period.

In studies of glycogen repletion in humans, there is a general consensus that the first 4 hours after exercise appears to be the period of peak glycogen resynthesis (Maehlum et al., 1977; Blom et al., 1987; Ivy et al., 1988a; Ivy et al., 1988b). Our findings tend to support this concept as the highest rate of resynthesis occurred in the first three hours after treatment.

The dose rates of glucose for maximum glycogen resynthesis, as they apply to humans, appear not to apply to the horse. The reasons for this are uncertain but it may be that the glucose concentration per se may not be as crucial for the horse and that the mechanisms of gluconeogenesis are more efficient than those in humans.

The results of this study have demonstrated that the administration of 3 g.kg⁻¹ of glucose polymer, either as a single or split dose after exercise has little influence on the rate of glycogen resynthesis in equine muscle. These results raise the question of whether plasma glucose concentration is a key factor in glycogen resynthesis, and this area is investigated in chapter 6.
CHAPTER 6

EFFECTS OF INTRAVENOUS DEXTROSE INFUSION ON MUSCLE GLYCOGEN RESYNTHESIS AFTER INTENSE EXERCISE

6.1 Introduction

6.2 Materials and Methods
   6.2.1 Horses
   6.2.2 Glycogen Depletion Runs
   6.2.3 Muscle Biopsy Procedure
   6.2.4 Blood Collection and Analysis
   6.2.5 Treatments

6.3 Statistics

6.4 Results

6.5 Discussion
6.1 Introduction

Although the rate of resynthesis of muscle glycogen following exhaustive exercise may be important in events where horses compete on successive days, there is little information concerning factors affecting post-exercise skeletal muscle glycogen resynthesis. Complete glycogen resynthesis may take up to 48 hours following prolonged exercise (Hodgson, 1984). Diet appears to have minimal effect on glycogen resynthesis as Snow et al. (1987) reported that low carbohydrate (roughage), moderate and high carbohydrate diets had similar effects on muscle glycogen resynthesis. In addition, Davie, Evans, Hodgson and Rose (1995), reported that the nasogastric administration of a glucose polymer immediately post exercise did not influence the rate of muscle glycogen resynthesis.

In contrast, studies in humans have shown that the rate of resynthesis is affected by factors such as the glucose dose administered after exercise (Blom et al., 1986; Blom et al., 1987), the timing of the administration of the supplement after exercise (Ivy et al., 1988a), the activity of glycogen synthetase and the initial muscle glycogen concentration (Danforth, 1965; Larner et al., 1967; Bergström et al., 1972; Kochan et al., 1979). The major limiting factor in glycogen synthesis is the muscle's ability to synthesise glycogen, rather than the availability of intracellular substrate (Fell et al., 1982). Fell et al. (1982) reported that a low muscle glycogen content following exercise is a major stimulus for increasing the rate of glucose transport into the muscle, in addition to an increased rate of glycogen synthesis.

If the initial muscle glycogen concentration of the horse is as important for performance as it is for humans (Bergström et al., 1972), the capacity to maximise muscle
glycogen replenishment post-exercise would be an important factor for optimising performance in horses competing on successive days.

The aim of this study was to examine the effect of post-exercise administration of intravenous dextrose (d-(-)-glucose) on the rate of muscle glycogen resynthesis.

6.2 Materials and Methods

6.2.1 Horses

Six Standardbred geldings aged 3.2±0.5 (mean±SEM) years were used in a cross over design in which 3 horses were randomly allocated to the treatment and control groups on the first experimental day. Mean body weight and maximum aerobic capacity ($\bar{V}O_{2\text{max}}$) were 443.5±15.0 kg and 126±8.0 ml.kg$^{-1}$.min$^{-1}$ respectively. Feed was withheld for at least 10 hours before each experiment. Nine hours after exercise 2 kg of lucerne hay was provided. Twelve hours after exercise each horse was given 2 kg pellets (Coprice, Leeton, Australia), 300 g each of oat and lucerne chaff, and 700 g of a "sweet feed" (Stable King Working Horse Mix, Fielders Ltd, Sydney, Australia).

6.2.2 Glycogen Depletion Runs

For the glycogen depletion runs, horses were exercised at various percentages of their maximal oxygen uptake ($\bar{V}O_{2\text{max}}$) values, based on the results of a preliminary study.
examining the degree of fibre type glycogen depletion at different intensities and durations of exercise (Appendix A). The treadmill speeds for the respective percentages of \( \dot{V}O_2 \text{max} \) were determined from sub-maximal and maximal treadmill tests conducted the week prior to the experiment.

To deplete muscle glycogen prior to treatment, horses exercised on a treadmill inclined at 10% slope for 15 min at 50% \( \dot{V}O_2 \text{max} \), 15 min at 70% \( \dot{V}O_2 \text{max} \), 5 min at 90% \( \dot{V}O_2 \text{max} \) and then rested for 30 min. This was followed by six, one min sprints at 100% \( \dot{V}O_2 \text{max} \) with 5 min walking between sprints.

\[ \text{6.2.3 Muscle Biopsy Procedure} \]

Muscle biopsies were taken using the needle biopsy technique of Bergström (1962) as modified by Lindholm and Pehl (1974). Biopsies were taken from the middle gluteal muscle at a depth of 8 cm at the same locations in each horse. Samples were taken before and at the end of exercise, and at 3,6,9,12 and 24 hours after treatment. Muscle samples for biochemical analysis were immediately frozen in liquid nitrogen while samples for histochemistry were mounted on cork blocks and frozen in isopentane chilled at -80°C. Muscle glycogen concentration was determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al. (1987). To determine muscle fibre types, muscle sections (10 μm) were cut on a cryostat microtome and reaction for myosin ATPase performed. The sections were preincubated at
pH 4.3 and 10.3 to allow identification of slow twitch (ST) and fast twitch (FT) fibre types (Brooke and Kaiser, 1970). To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff et al. (1961) and the fast twitch fibres subdivided into high oxidative (FTH) and low oxidative (FT). PAS stains were performed on 20 μm serial sections to enable subjective assessment of muscle fibre glycogen concentrations. Glycogen content of fibres was graded into five categories (0 to ++++) based on the intensity of stain (Hodgson et al., 1983).

Muscle glycogen synthetase was determined on dried muscle according to a modification of the methods of Thomas et al. (1968) and Nuttal and Gannon (1989). The degree of radioactivity turned into glycogen from UDP-glucose C\textsuperscript{14} was measured in the absence and presence of varying concentrations of G-6-P (nmol of glucose into glycogen per min per milligram of tissue (dry) nmol.min\textsuperscript{-1}mg\textsuperscript{-1}) The total glycogen synthetase activity (I+D) was determined in the presence of 10 nmol G-6-P with activity of I being determined in the absence of G-6-P. The fractional velocity as an alternative method of expressing the activation state of glycogen synthetase was proposed by Kochan et al. (1979). Fractional velocity is the velocity of the enzyme at any concentration of G-6-P divided by the velocity at saturation levels of G-6-P. In this study the fractional velocity refers to glycogen synthetase activity at concentrations of G-6-P of zero, 0.1, 0.5, 1.0, 5 and 10 nmol.
6.2.4 Blood Collection and Analysis

Blood samples were collected before exercise, immediately before commencement of infusions (30 min after exercise), and then every 60 min for 8 hours after commencement of treatment. Following centrifuging of blood samples, the plasma was decanted and stored in 5 mL plastic containers until analysed for glucose and insulin. Plasma glucose concentration was measured using an automatic analyser. Plasma insulin concentration was measured by radioimmunoassay.

6.2.5 Treatments

Thirty min after exercise each horse was given either:

a. Dextrose (Parnell Laboratories, Sydney, Australia), 6 g.kg⁻¹(bwt) intravenously as a 20% solution in a commercial isotonic electrolyte mixture (P54 Electrolyte, Parnell Laboratories, Sydney, Australia) at a mean infusion rate of 1.67±0.05 l.h⁻¹ (0.00371 kg⁻¹ h⁻¹) (Treatment) for 8 hours;

or

b. A solution of equivalent volume of polyionic, isotonic fluid (Control) given intravenously at the same infusion rate as treatment.

Solutions were infused through a 14 gauge catheter inserted into the left jugular vein, and the right jugular vein was used for blood collection. Infusion pumps were used
to ensure precise control of infusion rate. Testing was carried out over three consecutive weeks, and treatment and control experiments were conducted at the same time of the morning for individual horses.

6.3 Statistics

All results are expressed as mean±(SEM). The effects of horse and treatment were analysed by analysis of covariance, with time as a repeated measure (within group) factor, and values after exercise as the covariate. Where F values were significant, a post hoc test of least significant difference was used. Rates of resynthesis were compared by MANOVA, examining effect of horse, treatment and week. The level of statistical significance used was p<0.05. For fractional velocity the effects of horse treatment and G-6-P concentration were analysed by analysis of variance with time as a repeated measure factor.

6.4 Results

Muscle glycogen concentrations (mmol.kg⁻¹(dwt)) decreased significantly from a mean of 588±35 before to 328±45 after exercise for treatment, and 624±18 before, to 304±42 after exercise for control. Glycogen concentrations were not significantly different between treatment and control, before and after exercise. By 12 hours after
treatment, muscle glycogen concentrations had increased to 503±16 and 333±34 mmol.kg⁻¹ (dwt) for treatment and control respectively, representing 85% and 53% of the mean pre-exercise values for treatment and control groups respectively (Table 6.1). There were significant effects of treatment on glycogen concentrations at 9, 12 and 24 hours after commencement of infusion. At 24 hours after infusion, glycogen concentrations had increased by 51% in the treated horses and only 30% for the control, above the immediate post exercise concentration.

Mean rates of resynthesis for the first 6 hours after the commencement of infusion were 19.8±3.8 mmol.kg⁻¹ h⁻¹ and 9.0±6.2 mmol.kg⁻¹ h⁻¹ (dwt) respectively for treatment and control. During the first 12 hours these rates had decreased to 14.6±2.6 mmol.kg⁻¹ h⁻¹ and 2.6±3.1 mmol.kg⁻¹ h⁻¹ (dwt) respectively for treatment and control, and to 7.1±1.6 mmol.kg⁻¹ h⁻¹ and 3.8±1.2 mmol.kg⁻¹ h⁻¹ (dwt) for the 24 hour period after commencement of infusion. Resynthesis rates were significantly greater in the treatment group over the 12 hour period, but were not significantly different for the 6 hour (p<0.25) or 24 hour periods (p<0.21).

The PAS stains revealed that the exercise protocol resulted in some glycogen depletion in all fibre types, but was most evident in the ST and FTH fibres. The dextrose infusion resulted in an increase in the staining intensity of the ST and FTH fibres at 3 hours and 6 hours after exercise (Figures 6.1, 6.2 and 6.3).
Table 6.1 Glycogen concentrations (mmol.kg⁻¹(dwt)) (mean±SEM) before and after exercise, and 3, 6, 9, 12 and 24 hours after commencement of intravenous treatment with a hypertonic glucose and electrolyte solution, or an isotonic saline solution (Control). Significant differences due to treatment * p <0.05, ** p <0.01

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before exercise</td>
<td>588 ± 35</td>
<td>624 ± 18</td>
</tr>
<tr>
<td>After exercise</td>
<td>328 ± 45</td>
<td>304± 42</td>
</tr>
<tr>
<td>3 hours</td>
<td>391 ± 39</td>
<td>333 ± 39</td>
</tr>
<tr>
<td>6 hours</td>
<td>447 ± 43</td>
<td>356 ± 48</td>
</tr>
<tr>
<td>9 hours</td>
<td>464 ± 31</td>
<td>315 ± 41 **</td>
</tr>
<tr>
<td>12 hours</td>
<td>503 ± 16</td>
<td>333 ± 34 **</td>
</tr>
<tr>
<td>24 hours</td>
<td>497 ± 30</td>
<td>393 ± 32 *</td>
</tr>
</tbody>
</table>
Figure 6.1 Comparison of PAS staining pattern in slow twitch muscle fibres before and immediately after exercise and at 3 and 6 hours after treatment with dextrose or control solutions (N=6).
Figure 6.2 Comparison of PAS staining pattern in fast twitch high oxidative muscle fibres before and immediately after exercise and at 3 and 6 hours after treatment with dextrose or control solutions (N=6).
Figure 6.3 Comparison of PAS staining pattern in fast twitch muscle fibres before and immediately after exercise and at 3 and 6 hours after treatment with dextrose or control solutions (N=6).

CONTROL

DEXTROSE INFUSION
Glycogen synthetase fractional velocity increased after exercise and was still elevated by 6 hours after exercise. Figure 6.4 shows a comparison of glycogen synthetase fractional velocities for the control and dextrose treatments at varying concentrations of G-6-P. The effect of treatment on fractional velocity was not significant. For the dextrose treatment these data show that the activation level of glycogen synthetase immediately after exercise and at 3 and 6 hours following the commencement of infusion was significantly different than at rest.

The G-6-P concentrations are reported for only three of the six horses, due to available muscle volumes. The mean pre-exercise concentrations of G-6-P in muscle were 8.0±1.8 and 10.4±0.2 μg.ml⁻¹ for treatment and control respectively. At the end of exercise and at 3 and 6 hours after exercise they were 13.1±3.1, 6.2±1.2 and 6.1±1.5 μg.ml⁻¹ and 13.4±2.0, 12.9±3.5 and 9.3±0.5 μg.ml⁻¹ respectively for treatment and control. There was no significant difference between treatment and control.

Mean pre-exercise and mean peak plasma glucose concentrations during the infusion were 4.9±0.1 and 28.6±2.8 mmol.L⁻¹ and 4.7±0.2 and 6.0±0.5 mmol.L⁻¹ for treatment and control respectively. Peak plasma glucose concentrations occurred 2 hours after commencement of infusion (Figure 6.5). Plasma glucose concentrations were dependent on treatment and time.

Mean pre-exercise and mean peak insulin concentrations during infusion were 7.0±1.5 and 122.9±22.1 μU.ml⁻¹, and 6.0±1.4 and 12.2±1.8 μU.ml⁻¹ for treatment and control respectively. Mean peak insulin concentration had been reached by 3 hours (Figure 6.6). There were significant effects of both time and treatment.
Figure 6.4 Plot of Fractional Velocity (FV) (mean±SEM) of muscle glycogen synthetase from exercised muscle at various concentrations of G-6-P, before and after exercise, and at 3 and 6 hours following commencement of dextrose or control infusion (N=6).
Figure 6.5 Plasma glucose concentrations (mean±SEM) before exercise, before commencement of infusion (Treatment) and at hourly intervals after infusion of glucose (•) and control (○) solutions (N=6).
Figure 6.6 Plasma insulin concentrations (mean±SEM) before exercise, before commencement of infusion (Treatment) and at hourly intervals after infusion of glucose (---) and control (- - -) solutions (N=6).
6.5 Discussion

The results of this study illustrate that the administration of dextrose intravenously at a rate of 6 g·kg\(^{-1}\) post-exercise does influence the rate of muscle glycogen resynthesis in horses. The rates of muscle glycogen resynthesis for the treatment group were much higher than those reported previously after a routine hay/grain diet in the post-exercise period (Hodgson 1984). Further, Snow et al. (1987) reported that the additional infusion of 0.45 kg glucose over 6 hours in the post-exercise period had little effect on blood glucose or the rate of glycogen resynthesis. However, the glucose infusion rate and dose used were much lower than those in the present study.

Glycogen resynthesis rates varying from 3.4 to 7.2±0.7 mmol·kg\(^{-1}\)·h\(^{-1}\) (dwt) were reported by Snow et al. (1987) and Hodgson (1984). Hodgson (1984) reported that the highest rates of resynthesis occurred in the first 12 hours after exercise, with a mean repletion rate of 6.0 mmol·kg\(^{-1}\)·h\(^{-1}\), with up to 50% of glycogen repletion occurring in this period. The reported rates of glycogen resynthesis in Hodgson’s study varied from 7.8 mmol·kg\(^{-1}\)·h\(^{-1}\) (dwt) in the first 4 hours to 2.6 mmol·kg\(^{-1}\)·h\(^{-1}\) (dwt) between 12 to 24 hours. Machlum et al. (1977) reported that in humans, the most pronounced increase in glycogen resynthesis occurred during the first 4 hours of recovery from exercise, with a mean rate of glycogen synthesis during this period of 28.8±0.7 mmol·kg\(^{-1}\)·h\(^{-1}\) (dwt).

The mean rates of muscle glycogen resynthesis in this study were higher than previously reported for horses and over the first 6 hours were about twice as high with the dextrose infusion when compared to control. This difference was more pronounced by 12 hours, with the resynthesis being about 5 times faster in the treatment compared to the
control group. The mean relative glycogen concentrations 12 hours after infusion were 85% and 53% of mean concentrations before exercise for treatment and control respectively. The mean muscle glycogen resynthesis rates during the first 6 hours were about twice that reported in the glucose polymer study in Chapter 5. By 12 hours the rate had decreased to be about one third greater than for the same period in the Chapter 5, with the rates over the 24 hours being about the same in both studies.

The finding reported by Blom et al. (1987) for humans, that the increase in muscle glycogen resynthesis corresponds with the amount of carbohydrate ingested, but there being an upper limit to the glucose dose on muscle glycogen resynthesis, may also apply to the horse. However, for the horse there may be a lower dose limit, as up to a dose of 3 g.kg⁻¹(bwt), there was no difference between treatment and control. If an upper dose limit exists for the horse it is above 6 g.kg⁻¹(bwt).

Based on the similar rates of resynthesis between the control group in this study and those reported by Hodgson (1984), it is suggested that even when feed is withheld, as occurred with the control group in this study, the capacity for glycogen resynthesis is similar to that found during normal feeding in the first 6 hours after exercise. For this to occur, gluconeogenesis must be adequate to maintain a plasma glucose concentration sufficient for glycogen resynthesis.

The rate of resynthesis for the control group in this study is much higher than that reported in studies involving humans where exercise was followed by a period of fasting. Maehlum and Hermansen (1978), examined the effects of 12 hours of fasting after prolonged exercise on muscle glycogen concentration in humans. Muscle glycogen
concentration decreased by 69% in response to exercise. After 4 hours of recovery, muscle glycogen had increased by 25%. During the next 8 hours of recovery no further increase in glycogen concentration was observed. They concluded that muscle glycogen resynthesis may occur during recovery even under fasting conditions when plasma concentrations of glucose and insulin are relatively low, findings similar to those occurring in the horses in this study.

For glycogen synthetase, the present results are supported by several previous findings in other species, that glycogen synthetase is activated by a decrease in muscle glycogen concentration (Bergstrom et al., 1972; Piehl et al., 1974; Kochan et al., 1979; Bak and Pederson, 1990). Bergstrom et al. (1972) found that the percentage of synthetase I activity in the exercised muscle increased concomitant with the fall in glycogen content. In addition, the percentage of glycogen synthetase in the I form decreased as muscle glycogen content returned to normal. They concluded that an inverse relationship existed between glycogen content and synthetase I activity at normal and low glycogen levels. The relationship between muscle glycogen concentration and synthetase I activity is according to Larner et al. (1967), the result of both synthetase and phosphatase being bound to the glycogen molecule. A decrease in glycogen stores results in the release of both the synthetase and phosphatase molecules, resulting in an increase in the I form of the synthetase. Also, following exercise glycogen synthetase is activated as it becomes more sensitive to the allosteric activator G-6-P and thus becomes stimulated in favour of glycogen synthesis (Bak and Pedersen, 1990). The increased fractional velocity at the low concentrations of G-6-P following exercise show an increased sensitivity of the enzyme.
indicating a more active state. This increased activity state following exercise has been reported previously in other species (Piehl et al., 1974; Maehlum et al., 1977; Kochan, Lamb, Reimann and Schlender, 1981), however, it is the first reported synthetase activity changes with exercise in the horse. The greater increase in activity state for the dextrose treatment (Figure 6.4) may be a result of the large insulin response (Lerner et al., 1967).

Stull and Rodiek (1988) reported mean fasting glucose and insulin concentrations of 5.4±0.3 mmolL\(^{-1}\) and 4.3±0.2 \(\mu\)U.ml\(^{-1}\) respectively in four 2 year old quarter horses. After a meal of 50% alfalfa and corn they reported peak concentrations for insulin and glucose were 7.9 mmolL\(^{-1}\) and 49.9 \(\mu\)U.ml\(^{-1}\) respectively. Peak concentrations of glucose were observed approximately 3.5 hours after ingestion of a meal. Peak insulin concentrations were observed 75 to 195 min following the meal. The results of the present study for time to peak glucose concentration during infusion are in agreement with results obtained by Alexander (1955) and Stull and Rodiek (1988). The higher peak glucose concentrations reported in this study compared to those reported elsewhere in the literature are probably related to the higher glucose doses used and the different route of administration.

This study has indicated that the rate of muscle glycogen resynthesis in the first twelve hours after exercise is greatly enhanced by provision of large intravenous doses of glucose. This finding may have potential practical implications for horses required to compete on successive days in endurance events. The provision of glucose intravenously overcomes the potential problems of gastrointestinal disturbances and laminitis which can be associated with oral glucose administration. The following research was to establish whether
relationships exist between glycogen concentrations in skeletal muscle before exercise and exercise capacity.
CHAPTER 7

EFFECTS OF GLYCOGEN DEPLETION ON SOME
METABOLIC AND PHYSIOLOGICAL RESPONSES TO
SUBMAXIMAL EXERCISE

7.1 Introduction

7.2 Materials and Method

7.2.1 Horses
7.2.2 Training
7.2.3 Determination of Exercise Intensities
7.2.4 Glycogen Depletion Protocol
7.2.5 Endurance Run
7.2.6 Muscle Biopsy Procedure
7.2.7 Blood Collection and Analysis
7.2.8 Temperature Measurement

7.3 Statistics

7.4 Results

7.5 Discussion
7.1 Introduction

The onset of fatigue in human endurance events has been related to muscle glycogen depletion (Bergstrom et al., 1967a; Hargreaves et al., 1984; Coyle, 1991). Bergstrom et al. (1967a) found a strong correlation between the initial muscle glycogen concentration and work time during endurance exercise. At exercise intensities of between 70-80% \( \dot{V}O_2 \text{max} \), exhaustion coincided with the muscle's glycogen stores being depleted (Saltin and Karlsson, 1971).

Topliff et al. (1983) reported that in horses, the onset of fatigue during a run at 3.0 m.s\(^{-1}\) on a treadmill, was shorter when the muscle glycogen concentration was reduced prior to the run. In endurance rides of 160 km, muscle glycogen depletion of more than 70% of ST fibres and substantial depletion of FT have been reported in horses (Hodgson et al., 1983). In the roads and tracks component of three day event competitions, mean decreases in muscle glycogen concentration of 306 mmol.kg\(^{-1}\)(dwt), with a mean rate of utilization of 4.1 mmol.kg\(^{-1}\)(dwt), have been reported (Hodgson et al., 1984b).

In humans, a high initial muscle glycogen concentration, has been shown to attenuate the increases in heart rate and \( \dot{V}O_2 \) during exercise, compared to a low initial glycogen concentration (Spencer et al., 1992). In addition, blood lactate concentration was approximately four times higher after exercise in the high glycogen group compared to the low glycogen concentration. This is in contrast to the earlier reports (Bergstrom et al., 1967a) in which the oxygen uptake during exercise was similar when a fat/protein diet was compared to a carbohydrate diet during an exhaustive exercise bout.
In horses, Topliff et al. (1983) has been the only study to examine the effect of decreased muscle glycogen concentration on endurance performance, and the associated metabolic responses. Their results, however, were based on three horses, therefore this study was to further investigate the role of muscle glycogen in endurance performance in the horse.

An understanding of the metabolic responses may be important in events where horses compete over several days, as muscle glycogen concentrations have been reported to be decreased by 70% following an endurance ride (Hodgson et al., 1983). Further, an understanding of the role of muscle glycogen in fatigue of horses during endurance exercise, may be of assistance in the development of nutritional strategies to be used before such exercise.

7.2 Materials and Methods

Six Thoroughbred geldings were randomly allocated to two treatments or control in a 3x3 latin square repeated design. Testing was carried out over consecutive weeks with all horses being tested at the same time and on the same day each week.

The experiment involved horses completing one of two glycogen depletion runs. Five hours after the glycogen depletion run, each horse exercised at 60% $\dot{V}O_2$ max for 30 min on a treadmill inclined at 10% slope.
7.2.1 Horses

Six Thoroughbred geldings of age 5.8±0.54 years (mean±SEM) and body weight of 487±12.0 kg (mean±SEM) were used. All horses had been maintained on a constant diet, 2 kg pellets (Coprice, Leeton, Australia), 200 g lucerne and 800 g oaten chaff as morning and night feeds, with 2.5 kg lucerne hay at lunch, for the 10 weeks of training prior to, and throughout the study.

7.2.2 Training

All horses had been in training for 10 weeks at the time of the study. All training was conducted on a treadmill (Mustang 2000, Kagra AG, Switzerland) with the slope set at 10%.

Each horse’s \( \dot{V}O_2 \max \) was determined prior to and at the completion of the study to determine if there was a training effect.

7.2.3 Determination of Exercise Intensities

Glycogen depletion and endurance run intensities for each horse were determined from sub-maximal and maximal treadmill tests conducted the week prior to the experiment. For all testing the treadmill was set at 10% slope as it was for the training.
7.2.4 Glycogen Depletion Protocol

To deplete muscle glycogen concentration prior to the endurance run, two protocols were used. All glycogen depletion runs were conducted between 7am and 10am in the mornings to reduce the thermal stresses on the horse. Feed was withheld for at least 10 hours before each experiment and during the 5 hours leading up to the endurance run. Horses were given free access to water during the experiment.

Protocol A: Horses ran on the treadmill (10% slope) for 45 min at 30% \( \dot{V}O_2 \text{max} \) then for 15 min at 50% \( \dot{V}O_2 \text{max} \). The aim of this protocol was to deplete mainly type I fibres.

Protocol B: Horses trotted at 3.2 m.s\(^{-1}\) for 1000 m followed by six one min sprints at 115% \( \dot{V}O_2 \text{max} \) with 5 min walking at 1.5 m.s\(^{-1}\) between sprints. The aim of this protocol was to deplete mainly type II fibres.

Control: No exercise prior to endurance run.

7.2.5 Endurance Run

Horses were run on a treadmill, (Mustang 2000, Kagra, AG Switzerland) with the slope set at 10% for 30 min at a speed equivalent to 60% \( \dot{V}O_2 \text{max} \). All endurance runs were conducted 5 hours after the glycogen depletion runs. Mean temperature and humidity throughout the testing were 23°C and 60% respectively. Throughout the endurance run,
expired gas samples were collected every min for the first 5 min, then at 5 min intervals for the remainder of the exercise. Heart rate was recorded every 5 min throughout the endurance run. A core temperature of greater than 42⁰C or horses unable to maintain speed of the treadmill were criteria for stopping the exercise.

7.2.6 Muscle Biopsy Procedure

Muscle biopsies were taken using the needle biopsy technique of Bergstrom (1962) as modified by Lindholm and Piehl (1974). Biopsies were taken from the m. gluteus medius at a depth of 8 cm at the same locations in each horse. Samples were taken immediately before the glycogen depletion and endurance runs and within 20 sec of the completion of the endurance run. Muscle samples for biochemical analysis were immediately frozen in liquid nitrogen while samples for histochemistry were mounted on cork blocks and frozen in isopentane chilled at -80⁰C. Muscle glycogen concentrations were determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al. (1987). Muscle sections were preincubated at pH 4.3 and 10.3 to allow identification of slow twitch (ST) and fast twitch (FT) fibre types (Brooke and Kaiser, 1970). To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff et al. (1961), with the fast twitch fibres subdivided into high oxidative (FTH) and low oxidative (FT). PAS stains were performed on serial sections to enable subjective
assessment of muscle fibre glycogen concentration. Glycogen content of fibres was graded into five categories (0 to ++++) based on the intensity of stain (Hodgson et al., 1983).

7.2.7 Blood Collection and Analysis

Horses were prepared for blood collection prior to the exercise. Blood was collected before the endurance run then at 5 min intervals throughout the exercise for glucose, lactate and insulin assays. Plasma glucose and lactate concentrations were measured using an automatic analyser and plasma insulin was measured by radioimmunoassay.

7.2.8 Temperature Measurement

Skin over the opposite jugular vein to that used for blood collection was prepared as described for the procedures for blood collection. A 14 gauge catheter was placed into the jugular vein and a sterile thermistor was introduced through the catheter into the vein to the level of the right atrium. Temperatures were recorded every 5 min.

7.3 Statistics

All results are expressed as mean±SEM. The effects of time, horse, week and treatment were analysed by MANOVA, with time as a repeated measures (within group)
factor. Where F values were significant, a post hoc test of least significant difference was used. The level of statistical significance used was p<0.05.

7.4 Results

Due to horses in only 11 of the 18 runs being able to complete the 30 min of exercise, only the first 20 min of data has been reported.

Mean glucose, insulin and lactate responses to the 20 min of exercise are presented in Figures 7.1-2.

Mean±(SEM) pre and post exercise plasma glucose concentrations for the three trials were 5.2±0.2 and 7.4±0.3 for control, 5.7±0.3 and 6.2±0.4 for protocol A and 4.5±0.2 and 7.0±0.4 for protocol B respectively. There were no significant effects of treatment on plasma glucose, insulin or lactate responses to the exercise at p<0.05 level.

Mean±(SEM) pre and post exercise plasma lactate concentrations for the three trials were 0.5±0.04 and 7.3±1.0 mmolL⁻¹ for control, 0.8±0.1 and 5.6±1.1 mmolL⁻¹ for protocol A and 1.0±0.1 and 8.0±1.0 for protocol B.

Mean±(SEM) muscle glycogen concentrations for pre-exercise, prior to, and immediately after the endurance run for the three treatments presented in Table 7.1. There was no significant difference between the three treatments for glycogen concentrations prior to the depletion runs and between protocol A and B for pre-endurance run muscle glycogen concentrations. There was a significant difference between the three treatments for
Figure 7.1 Plasma glucose and insulin concentrations (mean±SEM) in Thoroughbred geldings, immediately before and during 20 min of exercise at 60% $\dot{V}O_2$ max on a treadmill (N=6).
Figure 7.2 Plasma lactate concentrations (mean±SEM) in Thoroughbred geldings immediately before and during 20 min of exercise at 60% \( \dot{V}O_2 \) max on a treadmill (N=6).
Table 7.1 Muscle glycogen concentrations (mean±SEM) for protocols A, B and C before glycogen depletion runs, then before and after 20 min exercise at 60% $\bar{V}O_2$ max on a treadmill (N=6).

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Before Depletion Run</th>
<th>Before Exercise</th>
<th>After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>641±18.2</td>
<td>507±41.0</td>
<td>319±55.8a</td>
</tr>
<tr>
<td>B</td>
<td>664±39.9</td>
<td>471±30.5</td>
<td>279±44.3b</td>
</tr>
<tr>
<td>Control</td>
<td>668±35.7</td>
<td>668±35.7</td>
<td>497±39.6a</td>
</tr>
</tbody>
</table>

Note: Means in the same column not sharing the same superscript were significantly different.
post endurance run glycogen concentration. There was a significant difference between pre-
glycogen depletion protocols and post endurance run glycogen concentrations. A significant
difference was found between the glycogen concentrations in the control protocol compared
to protocol B. PAS stains revealed that depletion protocols resulted in some depletion in all
fibres. For protocols A and B the decrease in intensity of stain was most evident in ST and FT
fibres respectively.

Mean percentage of glycogen depletion between rest and pre-endurance run was
21% and 29% for protocol A and B respectively. Mean percentage of glycogen depletion
between pre- and post endurance were 37% and 41% for protocols A and B respectively.
Mean percentage decreases in glycogen concentration between pre-glycogen depletion runs
and post endurance runs were 50%, 58% and 25.4% for protocols A and B and control
respectively (Figure 7.3).

\( \dot{V}O_2 \), HR and Temp were not significantly different between treatments during the
endurance run. There was a significant effect with respect to time for \( \dot{V}CO_2 \), and treatment
for RER at \( p<0.05 \) level.

\( \dot{V}CO_2 \), \( \dot{V}O_2 \), HR, RER and Temp responses to treatments are presented in Figures 7.3-4.
Figure 7.3 $\dot{V}O_2$, $\dot{V}CO_2$ and RER values (mean±SEM) in Thoroughbred geldings during 20 min of exercise at 60% $\dot{V}O_2$ max on a treadmill (N=6).
Figure 7.4 Temperature and HR values (mean±SEM) in Thoroughbred geldings during 20 min of exercise at 60% \( \dot{V}O_2 \) max on a treadmill (N=6).
7.5 Discussion

The results of this study have shown that for the Thoroughbred horse, muscle glycogen concentrations before performance can be reduced by 29% without having major effects on the physiological responses during exercise at 60% $\dot{V}O_2$max.

The total reduction in muscle glycogen concentrations for protocols A and B respectively were 323 and 385 mmol.kg$^{-1}$, which is greater than that reported following an endurance ride and a 4 hour slow trot (Lindholm et al., 1974; Hodgson et al., 1983).

In endurance rides of 160 km, muscle glycogen depletion of more than 70% of ST fibres and substantial depletion of type IIB has been reported (Hodgson et al., 1983). In the same event, after 40 km and 110 km rides, IIB fibres still contained large glycogen concentrations (Hodgson et al., 1983). In the roads and tracks component of a three day event competition, in which horses average approximately 3.6 m.s$^{-1}$, decreases in muscle glycogen concentration of 306 mmol.kg$^{-1}$(dwt) with a mean rate of utilization of 4.08 mmol.kg$^{-1}$.min$^{-1}$(dwt) have been reported (Hodgson et al., 1984b). Lindholm et al. (1974), reported that during a 4 hour slow trot (5 m.s$^{-1}$) muscle glycogen concentration decreased from a resting level of 476 mmol.kg$^{-1}$ to 192 mmol.kg$^{-1}$. At a faster trot of 8.3 m.s$^{-1}$ glycogen concentration decreased from 412 to 172 mmol.kg$^{-1}$.

In the present study the highest mean decrease in muscle glycogen was 58% following protocol B. The fact that in 7 of the 18 endurance runs, horses were unable to complete the 30 min of exercise, but substantial quantities of muscle glycogen were still available, would
indicate that muscle glycogen concentration was not the key contributing factor to fatigue. It is interesting to note that in humans, similar results have been reported. Costill et al. (1971), Gollnick et al. (1973) and Symons and Jacobs (1989) reported that for both prolonged and short exhaustive runs, glycogen depletion was the unlikely cause of fatigue. However, Gollnick et al. (1973) state that even though the total muscle glycogen concentration had decreased by 63%, the loss of glycogen from the FT fibres may have been sufficient to result in the inability of these fibres to function adequately. In contrast, Bergstrom et al. (1967a) found a good correlation between the initial muscle glycogen concentration and exercise time.

In the Bergstrom et al. (1967a) study, the work intensity was 75% $\dot{V}O_2\text{max}$, 77-80% $\dot{V}O_2\text{max}$ in the endurance run of Costill et al., (1971) and 150% $\dot{V}O_2\text{max}$ in Gollnick et al., (1973). The intensities in two of the studies compare with the 60% $\dot{V}O_2\text{max}$ in this study. For glycogen concentration to be a key factor in fatigue in the horse, the work intensity may have to be lowered substantially to enable the exercise period to be long enough for substantial depletion of muscle glycogen concentrations.

In humans, metabolic responses to exercise have been found to differ as a result of differences in muscle glycogen concentrations. Spencer et al. (1992) reported that increases in heart rate and $\dot{V}O_2$ during exercise were attenuated when muscle glycogen concentrations were high compared to when they were low. Lactate concentration was approximately four times higher after exercise with a high muscle glycogen concentration. This higher glycolytic rate and lactate concentration in muscle could indicate that glycolysis was
higher during exercise when muscle glycogen levels were high. Both before and after exercise the lactate concentration in plasma was greater with the high carbohydrate treatment. This is in contrast to the earlier work of Bergstrom et al. (1967a) who reported that oxygen uptake during exercise was similar for a fat, a protein and a carbohydrate diet during an exhaustive exercise bout. Differences in intensities of exercise between the two studies could account for this.

The present study does not indicate that in the horse there is an increased rate of glycolysis, when the initial muscle glycogen concentrations are high, as has been reported for humans (Spencer et al., 1992). The finding of no significant difference between the three treatments for HR, \( \dot{V}O_2 \) and temperature gives support to the findings of Topliff et al. (1983). There was a difference for R and \( \dot{V}CO_2 \), but the \( \dot{V}CO_2 \) was only significant with respect to time. The higher R values for the control group could indicate an increased reliance on carbohydrate metabolism, or an increased reliance on FFA utilization by the glycogen depleted horses. However, even though by the 10 min of exercise \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were nearly constant, lactate progressively increased. As other factors can contribute to changes in R values, (lactate buffering), changes in R that occur when lactate concentrations are increasing make it difficult to utilize R as an indicator of substrate metabolism. This lack of a significant difference was despite the initial glycogen concentrations between the control and two treatments at the commencement of the endurance run, being 197 mmol.kg\(^{-1}\) for glycogen depletion protocol B and 162 mmol.kg\(^{-1}\) for glycogen depletion protocol A.
Throughout the endurance run, glucose concentrations increased slowly with each protocol. The effects of exercise on plasma glucose seem to be variable depending on the intensity and duration of the exercise (Church et al., 1987). Anderson (1975), reported a slight increase in plasma glucose during 10 km exercise which included a 4 km trot, a 2 km canter and a 4 km gallop.

Insulin concentrations have been shown to decrease in both humans and horses throughout exercise, followed by a sharp increase after exercise (Church et al., 1987; O'Brien, Viguie, Mazzeo and Brooks, 1993). The characteristic decrease in insulin concentration with exercise was evident in this study.

In the present study fatigue, as expressed by the horses' inability to complete the treadmill run, did not appear to be the result of muscle glycogen depletion or high lactate accumulation. Other factors that may have contributed to the fatigue could be accumulation of ammonia or increased temperature (Greenhaff, Leiper, Ball and Maughan, 1991). Miller and Lawrence (1986) exercised horses to fatigue and observed glycogen depletion levels of only 25% and lactate concentrations of 10.5 mmol.L⁻¹ at fatigue. They suggest that the accumulation of ammonia and its effects on metabolic and physiological systems may have been the cause of fatigue. Ammonia can have a negative effect on pyruvate carboxylation and on isocitrate dehydrogenase.

It is likely that in this experiment, thermal stress was a major factor that induced the onset of fatigue. Central blood temperatures after the 20 min of exercise were 42.6, 42.1 and 42.3°C for the control, endurance and sprint treatments respectively. As exercise intensity
increases the major mechanism for the loss of heat is sweating. When sweating, the horse is not only losing water but a valuable supply of electrolytes, both of which may have detrimental effect on performance. With prolonged and profuse sweating, the loss of water and electrolytes may be associated with exertional rhabdomyolysis (Carlson, 1983).

In conclusion, the results have shown that several physiological and metabolic responses to moderate intensity exercise are not dependent on the muscle's initial glycogen concentration. It could be however, that because of the large glycogen pool the horse possesses compared to humans (Lindholm et al., 1974), the initial glycogen concentration does not impose the same limitations on performance in the horse, as it does in humans. Or that the degree of muscle glycogen depletion was not severe enough to pose a problem for the horse.
CHAPTER 8

EFFECTS OF GLYCOGEN DEPLETION ON SOME METABOLIC
AND PHYSIOLOGICAL RESPONSES TO HIGH
INTENSITY EXERCISE

8.1 Introduction
8.2 Materials and Method

8.2.1 Horses
8.2.2 Training
8.2.3 Determination of Exercise Intensities
8.2.4 Glycogen Depletion Runs
8.2.5 Run to Exhaustion (RE)
8.2.6 Muscle Biopsy Procedure
8.2.7 Blood Collection and Analysis

8.3 Statistics
8.4 Results
8.5 Discussion
8.1 Introduction

The horse, in comparison to humans, has a high skeletal muscle glycogen concentration (Lindholm et al., 1974). The importance of muscle glycogen and blood glucose for energy supply during prolonged exercise in humans has been highlighted by the work of Bergstrom et al. (1967); Ahlborg et al. (1967) and Ivy et al. (1983). However, the muscle's initial glycogen concentration appears to be unimportant for performance of high intensity exercise (Costill et al., 1981; Symons and Jacobs, 1989). Further, the provision of a carbohydrate supplement prior to high intensity exercise, also has been shown not to be beneficial (Snyder, Moorhead, Luedtke and Small, 1993), whereas the provision of a carbohydrate supplement during 4 hours of cycling, followed by a sprint ride to exhaustion at 100% $\dot{V}$O$_{2\text{max}}$, resulted in enhanced high intensity performance capacity (Hargreaves et al., 1984). Glycogen supercompensation, which is used for performance enhancement in prolonged exercise, also has been shown not to enhance high intensity exercise performance in humans (Housh, deVries, Johnson, Evans, Tharp, Housh and Hughes, 1990; Madsen, Pedersen, Rose and Richter, 1990).

In contrast to human studies, Topliff et al. (1985) reported that a decrease in muscle glycogen concentration in the horse, does affect their capacity for anaerobic work. In their study, they utilized the distance the horse could drag a sled, multiplied by the weight of the horse plus sled, as a measure of relative work. However, no studies have examined the effects of glycogen depletion on physiological responses to high intensity exercise.
The aim of this study was to examine the effects of a decreased muscle glycogen concentration on metabolic and physiological responses to high intensity exercise. The results of this investigation will assist in further understanding the role of muscle glycogen concentration in high intensity exercise, as well as further development of nutritional strategies before race day.

8.2 Materials and Methods

Six Thoroughbred geldings were randomly allocated to two treatments or control in a 3x3 latin square repeated design. Testing was carried out over consecutive weeks, with all horses being tested at the same time and on the same day each week.

The experiment involved horses completing one of two different glycogen depletion runs, with a run to exhaustion (RE) at 115% \( \dot{V}O_2\text{max} \) 5 hours after the glycogen depletion run. The run to exhaustion was used as a measure of physical work capacity.

8.2.1 Horses

Six Thoroughbred geldings of age 5.8±0.5 years(mean±SEM) and body weight of 487±12 kg(mean±SEM) were used. All horses had been maintained on a constant diet; 2 kg pellets (Coprice, Leeton, Australia), 200 g lucerne and 800 g oaten chaff as morning and night feeds, with 2.5 kg lucerne hay at lunch, for the 10 weeks of training
prior to and throughout the study. On the day of testing feed was withheld for a minimum of 10 hours before commencement of experiments and withheld during the 5 hours to RE run. Horses were allowed free access to water during the 5 hour period between glycogen depletion run and RE run.

8.2.2 Training

All horses had been in training for 10 weeks at the time of the study. Training was conducted on a treadmill (Mustang 2000, Kagra AG, Switzerland) with the slope set at 10%.

8.2.3 Determination of Exercise Intensities

Glycogen depletion and RE run intensities for each horse were determined from sub-maximal and maximal treadmill tests conducted the week prior to the experiment. For all testing the treadmill was set at 10% slope as for training.

Speeds for the RE run were obtained from a regression equation of speed and oxygen uptake values from the submaximal and max test.

8.2.4 Glycogen Depletion Runs

Two protocols were used to reduce the concentration of glycogen in skeletal
muscle. All glycogen depletion runs were conducted between 7 am and 10 am, with testing carried out on consecutive weeks.

*Protocol A:* Horses ran on the treadmill set at 10% slope for 45 min at 30% $\dot{V}O_2\max$, then for 15 min at 50% $\dot{V}O_2\max$. The aim of this protocol was to deplete mainly type I fibres:

*Protocol B:* Horses trotted at 3.2 m.s$^{-1}$ for 1000 m, followed by six one min sprints at 115% $\dot{V}O_2\max$, with five min of walking at 1.5 m.s$^{-1}$ between each sprint:

*Control:* in which horses only completed the RE run.

### 8.2.5 Run to Exhaustion (RE) Run

- The RE tests were conducted 5 hours after glycogen depletion runs.

*Protocol:*

Horses warmed-up for 5 min at 50% $\dot{V}O_2\max$ then stood on the treadmill for 3 min, followed by 2 min walking at 1.5 m.s$^{-1}$. The treadmill was then accelerated to the speed that would produce 115% $\dot{V}O_2\max$. The acceleration time and total time of the run were recorded. Run time was taken from the time the horses reached the required speed until they could not maintain pace with the speed of the treadmill.

Throughout the RE run expired air samples were collected every 15 sec for analysis and the calculation of oxygen uptake, carbon-dioxide output and R.
8.2.6 Muscle Biopsy Procedure

Muscle biopsies were taken using the needle biopsy technique of Bergstrom (1962) as modified by Lindholm and Pfehl (1974). Biopsies were taken from the m. gluteus medius at a depth of 8 cm at the same locations in each horse. Samples were taken immediately before the glycogen depletion and RE runs and within 20 sec of the completion of the RE run, for protocols A and B. For control, samples were taken immediately before RE runs and within 20 sec of the completion of the RE run. Muscle samples for biochemical analysis were immediately frozen in liquid nitrogen while samples for histochemistry were mounted on cork blocks and frozen in isopentane chilled at -80°C. Muscle glycogen concentration was determined on freeze dried muscle specimens according to the method of Harris and Hultman (1984) as modified by Snow et al. (1987). To determine muscle fibre types, muscle sections were preincubated at pH 4.3 and 10.3 to allow identification of ST and FT fibre types (Brooke and Kaiser, 1970). To determine oxidative capacity of individual fibres, staining for NADH-TR was performed using the method of Novikoff et al. (1961). PAS staining was performed on 20μm serial sections to enable subjective assessment of muscle fibre glycogen concentration. Glycogen concentration of fibres was graded into five categories (0 to ++++) based on the intensity of stain (Hodgson et al., 1983).

8.2.7 Blood Collection and Analysis

Blood was collected immediately before the RE run, then at 5, 10 and 15 min after the run. The blood was centrifuged for 15 min at 3000 rpm (Orbital 400 Clements...
Phoenix Scientific) and the plasma stored at -20°C until assays were conducted. Samples for plasma lactate were collected into chilled 5 mL evacuated tubes containing potassium oxalate and sodium fluoride.

8.3 Statistics

All results are expressed as mean±SEM. For lactate, glycogen and $\dot{V}O_2$ the major effects of time, horse, week and treatment were analysed by MANOVA, with time as a repeated measures (within group) factor. Where F values were significant, a post hoc test of least significant difference was used. The level of statistical significance used was $p<0.05$.

8.4 Results

Muscle glycogen concentrations for before depletion runs and before and after the RE run, for the three treatments are shown in Table 8.1. There was a significant difference between the three treatments at the $p<0.05$ level, with the post hoc test revealing that the difference was between the control and both protocols A and B. There was no significant difference in muscle glycogen concentrations between protocols A and B. Evaluation of the PAS staining intensities revealed that both protocols resulted in some glycogen depletion in all fibres. For protocols A and B the decreases were most evident in ST and FT fibres respectively. Following protocol A, the percentage of fibres
having a PAS staining intensity of one(+) increased from 12.5% to 43% for ST and from 0 to 3.3% for FT fibres. For protocol B the percentage of ST fibres having a PAS stain intensity of one (+) increased from 8 to 24%, and no FT fibres had a staining intensity of one (+). However, the number of FT fibres showing a staining intensity of two (+ +) increased from 3 to 38% (Figure 8.1), indicating depletion of predominately ST fibres in protocol A, with greater recruitment and depletion of FT fibres in protocol B.

Total glycogen utilized during the RE runs were 188±23.2, 204±12.3 and 146±43.6 mmol.kg⁻¹ for protocols A, B and Control respectively. Mean rates of glycogen usage were 1.9±0.34, 2.2±0.25 and 1.7±0.28 mmol.kg⁻¹s⁻¹ for protocols A, B and Control respectively. There were no significant differences between the treatments and control at the p<0.05 level.

Oxygen uptake responses to the three protocols are illustrated in Figure 8.2. The oxygen uptake increased rapidly at the commencement of exercise and then began to plateau after 45 sec. There was no significant difference between protocols and control at p<0.05 level.

Plasma lactate concentrations are illustrated in Figure 8.3. There was no significant effect of treatment at p<0.05 level.

Mean run times for the three protocols were 103±9.2, 101±14.6 and 97±12.0 sec for protocols A, B and Control respectively and there was no significant difference between these values.
Table 8.1 Muscle glycogen concentrations (mean±SEM) of the m. gluteus medius, for glycogen depletion protocols A, B and C, before glycogen depletion runs, and before and after the run to exhaustion (RE) (N=6).

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Before Depletion Run</th>
<th>Before RE Run</th>
<th>After RE Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>591±19.5</td>
<td>517±20.1</td>
<td>329±16.2 a</td>
</tr>
<tr>
<td>B</td>
<td>647±14.6</td>
<td>502±17.0</td>
<td>297±18.0 a</td>
</tr>
<tr>
<td>Control</td>
<td>608±53.8</td>
<td>608±53.8</td>
<td>462±26.2 b</td>
</tr>
</tbody>
</table>

Note: Superscripts in the same column not sharing the same letter were significantly different p<0.05
Figure 8.1 Comparison of PAS staining patterns in ST and FT muscle fibres, before glycogen depletion exercise and run to exhaustion (RE) for protocol A (slow run) and protocol B (fast run) (N=6).

**Slow Twitch**

![Graph showing PAS staining patterns in slow twitch fibres](image)

**Fast Twitch**

![Graph showing PAS staining patterns in fast twitch fibres](image)
Figure 8.2 $\dot{V}O_2$ values (mean±SEM) for protocols A, B and control groups during the run to exhaustion (RE) (N=6).
Figure 8.3 Plasma lactate concentrations (mean±SEM) for protocols A, B and control groups during the run to exhaustion (RE) (N=6).
8.5 Discussion

The results of this study have shown that muscle glycogen concentration in the horse, can be reduced by 22% without having a significant effect on physical work capacity during high intensity exercise. These findings support the work of Miller and Lawrence (1986). Although the intensity of exercise in that study was much lower than in the current study, they reported that the cause of fatigue, in a group of Quarterhorses, was not due to substrate depletion or lactate concentration, as there was only a 25% reduction in muscle glycogen concentration at fatigue. They suggest that the cause may have been an accumulation of ammonia. However, Topliff et al. (1985), reported a decrease in the relative work capacity of the horse when pre-exercise muscle glycogen concentration was reduced by approximately 41%. The relative work capacity, represented the distance the horse was able to drag a sled multiplied by the weight of the horse and sled, with the task of dragging the sled considered to be an anaerobic activity. The relative work performed by each horse following a 28 day training phase, a 5 day glycogen depletion phase and a 3 day glycogen repletion phase were compared. They reported a significant difference in relative work performed between the glycogen depletion phase, the end of the 28 day training phase and the 3 day repletion phase. There was no difference however, between the end of training and the glycogen repletion phase. A concern with this study is whether the task of dragging the sled, was a reliable measure of high intensity activity and because of the nature of the activity, which involves slow muscle contraction, it is difficult to relate the results to a high velocity muscle contraction activity as sprinting. The reported lactate concentrations of approximately 13 mmolL\(^{-1}\) at fatigue, would also indicate, based on reported lactate
concentrations from both treadmill and field testing (Bayly, Grant and Pearson, 1987; Harris, Marin and Snow, 1987) that the intensity of exercise was not maximal.

The pattern of PAS staining for the two glycogen depletion protocols, indicated that the percentage glycogen contribution from the ST and FT fibres was different for the two protocols. In protocol A, there was a greater recruitment of the ST fibres, whereas with protocol B the FT fibres were preferentially recruited. This pattern indicates a greater recruitment of FT fibres with the increase in intensity of exercise. This specific contribution is in agreement with studies in humans (Piehl, 1974; Vøllestad et al., 1992) and horses (Lindholm et al., 1974; Snow et al., 1981; Essén-Gustavsson et al., 1984).

Studies in humans have shown that lactate production is reduced with a decreased muscle glycogen concentration. The basis of a reduced lactate formation with a low initial muscle glycogen concentration is the relation between energy demand and by-product removal. During high intensity exercise, the rate of glycolysis needs to be increased several fold. At such high intensity, oxygen supply is limited and the rate of H⁺ ion production exceeds the capacity for NADH to shuttle the H⁺ off to the electron-transport chain (McArdle et al., 1991). Pyruvate is then forced into becoming an H⁺ ion acceptor to enable glycolysis to continue with a resultant increase in lactate. If the glycogen concentrations are low to begin with, then the available substrate for glycolysis is reduced, leading to a reduced rate of glycolysis and reduced lactate production.

Costill et al. (1971) examined muscle glycogen utilization and lactate production in humans, during a 16.1 km run which was used to reduce muscle glycogen
concentration, and during a $\dot{V}O_2$-max test conducted after the run. Peak lactate concentrations following a $\dot{V}O_2$-max run conducted after the 16 km run were significantly lower than when the $\dot{V}O_2$-max run was conducted prior to the exercise. They suggested that the reduced lactate concentration was caused by the lower glycogen concentration. The concept of reduced lactate production with a reduced glycogen concentration was indicated in earlier works by Saltin and Hermansen (1967). Jacobs, (1981) suggested that in humans a critical muscle glycogen concentration of 160 mmol.kg$^{-1}$ exists for inflicting an effect on lactate production.

The reduced lactate production with low muscle glycogen concentrations has also been shown in horses by Topliff et al. (1985). The reduced lactate concentrations reported by Topliff et al. (1985), however, were only trends and peak lactates were not significantly different. The lack of a significant difference between the two protocols and control for plasma lactate concentrations in this study indicates that a reduced glycogen concentration appears not to affect lactate production during high intensity exercise in the horse. The mean muscle glycogen concentrations of 502 and 517 mmol.kg$^{-1}$(dwt) for protocols A and B at the commencement of the RE run are well above the threshold level of 160 mmol.kg$^{-1}$ reported by Jacobs (1981). Whether a threshold exists for the horse, however, is not known.

The $\dot{V}O_2$ responses to the three protocols are in agreement with responses reported for humans. Bergstrom et al. (1967a) reported similar oxygen uptake responses during exercise regardless of the differing initial glycogen concentrations. Widrick,
Costill, Fink, Hickery, McConnell and Tanaka (1993), investigated subjects performing in time trials of 120 min duration under conditions of varying initial muscle glycogen concentrations and carbohydrate feeding throughout the trials. For the first 71% of each trial the VO₂ was similar for all conditions, but in the low glycogen trial VO₂ had decreased below the other trials by the end of the exercise period.

The rate of change of the VO₂ in this study were not affected by the varying initial glycogen concentrations. There was no significant difference between treatments for time to peak oxygen uptake, with all horses reaching peak oxygen uptake levels between 45 sec to 60 sec. This time to peak VO₂max supports the findings of Bellenger, Davie, Evans, Hodgson and Rose (1994) who reported that horses reached 90% to 95% of mean steady state VO₂ by 45 sec at speeds of both 4 and 8 m.s⁻¹.

The mean rate of glycogen utilization of 1.9±0.55 mmol.kg⁻¹.s⁻¹ in this study is similar to that reported for humans and Thoroughbred horses at maximum intensity (Snow et al., 1985; Vøllested et al., 1992) but higher than that reported for Standardbred horses (Hodgson, 1984). The similar rates of glycogen utilization between this study and those reported for testing, validates use of the treadmill as an ideal method for investigating factors affecting performance of high intensity exercise in race horses.

One of the limitations of this study was the methodology used for muscle glycogen depletion. The intensity of exercise utilized for the two protocols was appropriate to have a selective activation of different fibres and induce a large reduction in individual muscle fibre glycogen concentration. However, the total glycogen reduction
was not large. The difficulty was that in the laboratory environment, humidity was a problem for the horses and thermal stress prevented them from working for longer periods, thereby reducing the amount of glycogen depletion. To alleviate the thermal stress aspect, future research could adopt a combination of diet and exercise over a period of approximately one week. This should incorporate a reduced carbohydrate intake coupled with long low intensity exercise sessions, culminating with the high intensity sprints on the morning of testing.

As certain metabolic changes in muscle, such as increased lactate and creatine phosphate reduction (Sahlin, 1992), and increased ammonia concentrations (Miller and Lawrence, 1986) have been associated with fatigue in high intensity exercise, the measurement of these variables may have provided more insight into the physiological responses to exercise with reduced concentration of glycogen in skeletal muscle.

In conclusion the results of this study suggest that the rate of glycogen usage and the physiological and metabolic responses to high intensity exercise, may not be affected by a reduced initial muscle glycogen concentration of up to 22%.
Chapter 9

GENERAL DISCUSSION

In this thesis two methodological issues were addressed. Firstly that for the Standardbred, a number of locations on the m. gluteus medius were examined to validate that biopsies taken contained consistent distribution of fibre types and also similar glycogen concentrations between sites. The results showed that there was no significant difference in muscle glycogen concentration between the muscle biopsy sample locations. However, there was a variation in the proportion of ST and FT fibres between some of the locations, but no differences between any of the sites for FTH fibres. The large difference in the coefficient of variations, both between sites for the same fibre type and between the ST and FT fibres, indicates that the muscle is not uniform in its distribution of fibres, which could present some problems in interpreting data from a single biopsy. These results provided a basis not only for the current studies but have also provided a basis for future studies in which multiple biopsies are required.

Secondly, the issue of a safe glucose dosage regime for the horse was investigated. The result of this work was that a dose of 3 g.kg⁻¹ (bwt) was observed to be the upper limit of the dose range for nasogastric administration of glucose without the risk of producing laminitis. Further, the provision of a glucose polymer, as a substitute for glucose did not increase the tolerable dose range for the horse and there was no difference in the initial absorption phase, up to 2 hours after administration, between the glucose and glucose polymer.
The hypothesis that muscle glycogen resynthesis following exercise could be influenced by the provision of exogenous glucose was supported by the infusion study (Chapter 6) but not by the glucose polymer study (Chapter 5).

The second hypothesis that reduced pre-exercise muscle glycogen concentration does not have a detrimental effect on the metabolic and physiological responses to low and high intensity exercise was supported by the findings reported in Chapters 7 and 8.

Glycogen resynthesis in humans has been shown to be affected by factors such as the glucose dose administered post exercise (Blom et al., 1986; Blom et al., 1987), the timing of the administration of the supplement after exercise (Ivy et al., 1988), the activity of glycogen synthetase and the initial glycogen concentration (Danforth 1965; Larner et al., 1967; Bergström et al., 1972; Kochan et al., 1979). In the infusion study, the intravenous administration of a glucose polymer following exercise was observed to cause a significant increase in the rate of muscle glycogen resynthesis over the first 12 hours. When this dose was halved and administered nasogastrically, the rate of resynthesis over the first 6 hours was half that of the infusion trial and not significantly different from the control treatment.

From a practical viewpoint the situation is made difficult by the fact that the maximum safe dose of a glucose polymer that could be administered to the horse nasogastrically, without causing gastrointestinal disturbances and early signs of laminitis, was 3 g.kg\(^{-1}\) (bwt). However, a significant effect on resynthesis was only evident at a
much higher intravenous dose (6 g.kg$^{-1}$(bwt)). Therefore, unless a large glucose dose can be administered intravenously immediately following exercise, the provision of normal feed may be as effective as any oral glucose supplement.

The practicality of increasing muscle glycogen resynthesis rates, and its importance for the Thoroughbred and Standardbred horse, is questionable. This belief is based on the findings of Chapters 5 and 6, in which muscle glycogen resynthesis over the 24 hour period immediately after exercise, was similar for the control and glucose polymer treatments. Despite being deprived of feed, muscle glycogen concentrations of the control group still reached 67% and 63% of the initial concentration by 24 hours after exercise for the glucose polymer and infusion studies respectively. These results, in combination with the findings of Chapters 7 and 8, which showed that physiological responses to exercise were not affected by decreases in muscle glycogen concentration of up to 29%, indicate that the provision of glucose after exercise, in an endeavour to increase the rate of muscle glycogen resynthesis, may not be necessary for most forms of competitive exercise in horses. However, for the endurance horse, muscle glycogen reductions of up to 70% of ST fibres and substantial depletion of FT fibres have been reported (Hodgson et al., 1983). Based on the findings of Topliff et al. (1983 and 1985) that muscle glycogen reductions of 41% can have a detrimental effect on performance, then maximising the rate of resynthesis may be beneficial when prolonged and repeated bouts of exercise are necessary. It would seem however, based on the findings of Chapters 5 and 6 that the most effective means of doing this would be via infusion of a
glucose polymer, and that the provision of an oral glucose supplement would seem to provide no major benefit over the short time period available.

In humans, some investigators have addressed the glycaemic index of foods to maximise glucose absorption for muscle glycogen resynthesis (Blom et al., 1987). However, it would seem highly unlikely, based on the findings of Chapters 4 and 5, that differences in the glycaemic index of feeds could produce significant effects on muscle glycogen resynthesis in the horse, as glucose given by nasogastric tube and the glucose polymer had similar absorption rates, and even large doses of glucose polymer were unable to produce a significant increase in muscle glycogen resynthesis.

An intriguing factor observed between studies was the variability that existed in glycogen resynthesis rates for feed deprived horses. The reasons for these differences are uncertain but may be addressed by factors such as the training state of the horses, and their hydration status resulting from any thermal stress encountered during the glycogen depletion run.

For the second hypothesis, the results in this thesis show that muscle glycogen concentrations can be reduced by 29% and 22% before moderate and high intensity exercise respectively, without having an apparent detrimental effect on performance, or the physiological responses to exercise. One could argue that because of the large muscle glycogen pool that the horse possesses compared to humans (Lindholm et al., 1974), that the degree of muscle glycogen depletion in both studies was not severe enough to pose a major problem to the horse. Support for this argument is provided by the work of Topliff et al. (1983 and 1985) in which they observed a detrimental effect on
low intensity and relative work performance when muscle glycogen concentration was reduced by 41%. Future experimental designs for the investigation of muscle glycogen resynthesis, or the effects of reduced glycogen concentration on performance, should consider the utilization of a combination of diet and exercise over several days. The 5 hours recovery period between the glycogen depletion run and the exercise tests, based on data from Chapters 5 and 6, was also long enough to allow resynthesis of muscle glycogen, despite the horse being deprived of feed.

The results from Chapters 7 and 8 have a practical application for the racing and endurance horse industries. Based on the reported decreases in muscle glycogen concentration of 19-25% following gallops of 1000 m and 1600 m respectively in fit Thoroughbred horses (Snow and Harris, 1991), and the results from Chapters 7 and 8, it would seem that depletion of muscle glycogen per se is not the main cause of fatigue in Thoroughbred racing.

The issue of glycogen loading, as it applies to humans, would seem to have little practical application for the racing horse based on the degree of depletion that occurs during such events (Snow and Harris 1991) and the findings of Topliff et al. (1983 and 1985), which showed that increases in muscle glycogen concentration of up to 36% above resting concentrations did not have a significant effect on capacity for high or low intensity exercise. However, glycogen loading may have some practical application for the endurance horse. Although glycogen loading has been reported in horses (Topliff et al., 1983, 1985), a major problem in feeding a high carbohydrate diet to horses is the likelihood of causing intestinal disturbances resulting in laminitis.
The results presented in this thesis have not only provided the answers to several questions but at the same time have unearthed further questions. Unfortunately there is a lack of information within the literature to provide concrete answers to these questions. The future direction of research into muscle glycogen resynthesis in the horse may seek to answer some questions such as:

a. What is the time period that the supplement needs to be provided post exercise? This was indicated by the fact that in the first 12 hours, muscle glycogen had returned to 85% of the resting value and by 24 hours this had not shown any further significant increase during the glucose infusion. Further, that for the glucose polymer, the control treatment had also returned to 81% of resting value by 24 hours after exercise.

b. Could the provision of a glucose supplement intravenously for 8 hours post exercise versus the provision of the same dose over 4 hours with normal nutrition being provided for the remainder of the period of investigation, prove just as effective for resynthesis?

c. What are the effects of training status on glycogen resynthesis? Training may have a positive effect on hepatic glycogenolysis and gluconeogenesis. This would require the biopsying of the liver in the immediate pre and post exercise periods at the commencement and conclusion of a training study. Some of the cardiovascular responses to training may be effective in assisting better distribution of glucose and a more efficient uptake following exercise.
d. Is the activity state of glycogen synthetase in the horse affected by age? Determination of whether the sensitivity of glycogen synthetase, for changing from its active to its non-active state, is affected by age.

e. Does the hydration state of the horse have an effect on muscle glycogen resynthesis? Changes in blood volume and redistribution of blood following exercise may affect transport of and distribution of both hepatic and exogenous glucose.

The experimental findings of this thesis have answered specific questions in relation to glycogen resynthesis in the horse. However, the findings whilst enlightening on certain issues of muscle glycogen resynthesis in the horse, have at the same time exposed questions that need further investigation.

In conclusion it has been demonstrated that the rate of muscle glycogen resynthesis in the horse is difficult to influence. Further, that dosages that do have an influence are impractical to administer. However, it has been shown that the rate of resynthesis is highest in the first 6 hours after exercise and that in this period the glycogen synthetase activity state is elevated.

This study has been the first to report glycogen synthetase changes in horse muscle both before and after exercise and during muscle glycogen resynthesis. The activity state changes observed for glycogen synthetase were similar to those reported for other species (Danforth, 1965; Kochan et al., 1979).

From a practical perspective, the importance of trying to influence muscle glycogen concentrations and resynthesis rates is questionable. This is based on the fact that the muscle glycogen concentration in the horse is higher than that reported for other
species, even though the rate of glycogen utilization has been shown to be similar. In addition that muscle glycogen reductions of approximately 22% do not influence physiological responses to exercise or treadmill exercise capacity. These arguments do not imply that glycogen concentration is not important in the horse, but its role in fatigue and muscle glycogen resynthesis does not appear to be as critical as it is in humans.
Bibliography


APPENDIX A
Introduction

The degree of activation and rate of glycogen usage by selective muscle fibres is governed by the intensity, duration and type of exercise. However, the literature is not in total agreement in relation to the fibre recruitment pattern and glycogen usage rates of the selective fibres. These differences, may as suggested by Vøllestad (1984) be due to differences in methodology of glycogen measurement, protocols and mode of exercise utilized for depletion.

Reported muscle glycogen depletion levels in humans following endurance exercise vary from 40% following a 16.1 km run to 86% following a 2 hour run (Ahlborg et al., 1967; Costill et al., 1971; Gollnick et al., 1973; Essén and Henriksson, 1974). Piehl (1974) found that after bouts of both endurance and sprints in which the total glycogen had decreased by approximately 82%, the percentage in fibres showing either negative or light staining was 95% for ST and 65% for FT fibres. This variability in rate of muscle glycogen reduction between fibre types with duration and intensity of exercise was further supported by the work of Vøllestad et al. (1984) and again by Vøllestad et al. (1992).

High intensity exercise periods of between 30 sec to 2-3 min duration has shown the decline in muscle glycogen to be 30 to 35% less in ST fibres compared to FT fibres, with no difference being the subgroups of FT fibres (Vøllestad et al., 1992).

This pattern of glycogen usage and selective fibre depletion with varying intensity of exercise has also been shown for horses. Essén-Gustavsson et al. (1984) found that following a 100 km endurance ride approximately 90% of ST fibres stained low and 69% of FT fibres stained medium for glycogen. In relation to total glycogen used with exercise, Snow et al. (1981) found a decrease of 56% in glycogen content following a 80 km
endurance ride with ST fibres showing the greatest depletion. Increasing trotting speed from approximately 5 m.s\(^{-1}\) to 12.5 m.s\(^{-1}\) the rate of glycogen usage increased from 0.3 mmol.kg\(^{-1}\) to 14 mmol.kg\(^{-1}\) min \((\text{Lindholm, 1974})\).

**Aim of the Study:**

The aim of this study was to evaluate a glycogen depletion protocol to determine the quantity of glycogen depleted and the fibre types from which the glycogen was predominately depleted.

**Materials and Methods:**

Six standardbred geldings of mean age 3.2±0.5(SEM) yrs were used. Mean body weight and maximum aerobic capacity (\(\dot{\text{V}}\text{O}_2\text{max}\)) of the group were 443.5±15.0 kg and 126±8.12 ml.kg\(^{-1}\).min\(^{-1}\), respectively. Feed was withheld for the 8 hour period prior to testing.

**Depletion protocol:**

To deplete muscle glycogen prior to treatments, horses exercised on a treadmill inclined at 10% slope, for 15 min at 50% \(\dot{\text{V}}\text{O}_2\text{max}\), 15 min at 70% \(\dot{\text{V}}\text{O}_2\text{max}\), 5 min at
90% $\dot{V}O_2$max then rested for 30 min. This was followed by six one min sprints at 100% $\dot{V}O_2$max with 5 min rest periods between sprints. This protocol was repeated a week later to determine the variability between depletion runs.

**Muscle biopsies:**

Muscle biopsies were taken using the needle biopsy technique of Bergström (1962) as modified by Lindholm and Piehl (1974). Biopsies were taken from the m. gluteus medius at a depth of 8 cm using the same locations in each horse. Samples were taken before and at the end of exercise. Samples for biochemistry analysis were immediately frozen in liquid nitrogen while samples for histochemistry were examined then mounted on cork blocks and frozen in isopentane. Muscle glycogen was determined according to the method of Harris and Hultman (1984) as modified by Snow et al., (1987). PAS stains were performed on 20 $\mu$m serial sections to enable subjective assessment muscle fibre glycogen concentrations. Glycogen content of fibres was graded into five categories (0 to ++++) based on the intensity of the stain (Hodgson et al., 1983).

**Statistics:**

All results are expressed as mean±(sem). The pre and post glycogen concentrations were compared using a t-test. The level of statistical significance used was $p<0.05$. 
Results and Discussion

The mean PAS stain intensities for ST, FTH and FT at rest and following the depletions runs were compared using a T-test matrix for two dependent variables. As there was no significant difference between fibre types for PAS intensity for the two runs the data was pooled.

Muscle glycogen concentrations decreased from a mean of 588±35.3 before to 328±45.0 mmol.kg⁻¹ (dwt) after exercise for run two and 624±18.0 before to 304±41.8 mmol.kg⁻¹ after exercise for run one. Muscle glycogen concentration decreased by a mean of 43% for the two runs. Glycogen concentrations between the two depletion runs for before and after exercise were not significantly different. The PAS stains revealed that the exercise protocol resulted in some depletion of glycogen for all fibres, but was most evident in the ST and FTH fibres.

The protocol for muscle glycogen depletion utilized in this study resulted in some depletion off all fibres, with the greatest degree of depletion being evident in the ST and FTH fibres. The percentage of FTH fibres having a high PAS stain decreased by 88.4% compared with a decrease of 55% for FT fibres. For the medium PAS stain intensity, decreases of 88.5%, 76% and 39% were witnessed for the ST, FTH and FT fibres respectively. This pattern of glycogen depletion indicates that the ST and FTH fibres made the greatest glycogen contribution to the energy requirements, indicating that the activity was aerobic predominately. The mean speeds for the group at 50%, 70%, 90% and 100%
\( \dot{V}O_2 \text{max} \) were 4.4±0.7, 5.7±1.7, 7.3±3.3 and 8.7±3.1 m.s\(^{-1}\) respectively, which in comparison to maximum trotting speed of around 12 m.s\(^{-1}\) (Hodgson et al., 1984) would indicate that the speed was predominately aerobic in nature.

Fibre type glycogen depletion patterns have been shown to follow a similar pathway for both man and horses (Lindholm, 1974; Piehl, 1974; Vøllestad et al., 1984; Hodgson et al., 1983; Essén-Gustavsson et al., 1984; Vøllestad et al., 1992). Lindholm (1974) reported that at slow trotting speeds (5 m.s\(^{-1}\)) the ST fibres are depleted of glycogen first followed by the FTH. At maximal speeds (12.5 m.s\(^{-1}\)) the FT fibres contribution of glycogen increased dramatically leading to large reductions in muscle glycogen concentration.

In conclusion this study has shown that exercise intensities of 30 min duration at a trotting speed of 4.5 to 7.0 m.s\(^{-1}\) results in approximately 43% depletion with the PAS stains showing that the glycogen depletion occurred predominately from the ST and FTH fibres.
APPENDIX B
Figure B.1 Specificity of the insulin assay was evaluated by serial dilutions of insulin standard (A) and a sample of known insulin concentration (B).