

ALTERNATIVE NUTRITIONAL STRATEGIES FOR ENHANCING  
GLYCOGEN RESYNTHESIS IN HORSES

A Thesis

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## ABSTRACT

### **Alternative strategies for enhancing muscle glycogen resynthesis in horses**

Recovery of skeletal muscle glycogen is very slow in horses compared to other mammals studied, and can take 48-72 h, even when a highly soluble carbohydrate meal is fed immediately after exercise. There is a clear need to develop a practical nutritional strategy to enhancing recovery of muscle glycogen in horses. The studies described herein were intended to characterize the effects of supplemental acetate and effective rehydration on plasma and skeletal muscle in horses, in order to determine if the slow rate of muscle glycogen resynthesis after glycogen-depleting exercise is due to a combination of slow recovery of hydration and inadequate shuttling of intracellular glucose towards glycogenesis.

In two separate research studies gluteus medius biopsies and jugular venous blood was sampled from exercise conditioned horses on 2 separate occasions, at rest and for 24 h following a competitive exercise test (CET) designed to simulate the speed and endurance test of a 3-day event and induce significant glycogen and total body water losses. In the first study, following the CETs horses were given either water *ad libitum* and either a hypotonic commercial electrolyte solution via nasogastric tube followed by a typical hay/grain meal, or a hay/grain meal alone. Electrolyte supplementation resulted in an enhanced rate of muscle glycogen resynthesis and faster restoration of hydration, as evidenced by faster recovery of plasma [protein], maintenance of plasma osmolality and greater muscle intracellular fluid volume during the recovery period compared to control.

In the second study, following the CETs horses were allowed water *ad libitum* and either a sodium acetate/acetic acid solution via nasogastric tube followed by a typical

hay/grain meal, or a hay/grain meal alone. Acetate supplementation resulted in a rapid and sustained increase in plasma [acetate], and increases in skeletal muscle [acetyl-CoA] and [acetylcarnitine]. Acetate supplementation also resulted in an enhanced rate of muscle glycogen resynthesis during the initial 4 h of the recovery period compared to control.

It is concluded that both electrolyte and acetate supplementation can be used to enhance glycogen resynthesis in athletic horses.



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# Chapter 1

## 1.0 Introduction

The horse is an extraordinary athlete, the evolutionary product of grazing herbivores whose survival was directly dependent on the speed and endurance required to escape predators and search for food. Subsequent to when horses were first domesticated, humans have continuously attempted to modify and enhance the athletic capabilities of equids by selective breeding. Today equine athletes are involved in a broad range of sporting activities, such as flatracing distances from 400 to 3200m, endurance rides of up to 160km, show jumping, dressage, three-day eventing, heavy draft work, polo, reining, cutting and competitive driving, as well as conventional warm-up exercise and training. Traditionally horse owners have attempted to meet the high energy requirements of equine athletes with diets high in soluble (hydrolyzable) carbohydrate (CHO, i.e. grain). More recently however, it has been recognized that high grain diets can have significant negative consequences such as ulcers, gastrointestinal problems, laminitis, exertional rhabdomyolysis, and the development of stereotypies and 'hot' horses. This in turn has led to increased interest in alternative sources of energy.

Optimum performance of the competitive athlete is highly dependent on the quantity and availability of metabolic fuels that serve muscle contraction and hence locomotion. Both human and equine athletes are highly reliant on muscle glycogen stores to fuel the demands of vigorous exercise characteristic of many forms of sport. Accordingly, vigorous exercise for even brief periods, and/or prolonged submaximal exercise, is associated with depletion of muscle glycogen reserves in both species, contributing to

skeletal muscle fatigue and poor performance. Simply put, CHO is the most important fuel as it is a substrate for aerobic and anaerobic energy production. This appears to be where the similarities in CHO metabolism between horses and humans end however, as it has become increasingly clear that many conclusions drawn from human exercise physiology do not apply to horses. For example, although horses have similar muscle glycogen concentrations to humans, they utilize a substantially higher proportion of CHO compared to fat at the same relative work intensities.

Perhaps one of the most striking differences in CHO metabolism between the two species, and thus one that has received considerable research interest of late, is the difference in skeletal muscle glycogen resynthesis rates after fatiguing exercise. The rate of post-exercise muscle glycogen resynthesis is much slower in horses compared to humans and other mammals studied. The horse evolved as a grazing hindgut fermenting herbivore using primarily fermentable forage CHOs as its main energy source, and may have a limited ability to digest hydrolysable CHOs. Indeed, recent research suggests that the low rate of glycogen resynthesis with oral CHO provision in horses may be due to reduced rate of delivery from the gastrointestinal tract, combined with impairments in skeletal muscle glucose uptake as compared to humans. Consequently, conventional feeding strategies employing diets high in soluble CHOs have not been successful in enhancing glycogen replenishment in horses, and in fact may lead to negative health consequences.

Thus there is a clear need for research into alternate nutritional strategies for optimizing recovery of muscle glycogen in horses. The studies described herein were intended to characterize the effects of supplemental acetate and effective rehydration on plasma and skeletal muscle in horses, in order to determine if the slow rate of muscle glycogen resynthesis after glycogen-depleting exercise is due to a combination of slow recovery of hydration and inadequate shuttling of intracellular glucose towards glycogenesis. The long term goal of this research is to develop a safe and practical nutritional strategy for optimizing the recovery of muscle glycogen in competitive equine athletes so as to improve training and competition performance, particularly with respect to multi-day activities.

## Chapter 2

### 2.0 Literature Review

#### 2.1 Depletion of Muscle Glycogen

Carbohydrate in the form of muscle glycogen is the main energy source for glycolytic and oxidative ATP production during both short term high intensity and prolonged submaximal exercise in horses (Snow et al 1981, Snow and Harris 1991, Lacombe et al 1999, 2001, Hyypa et al 1997). Athletic horses frequently perform endurance exercise and/or multiple bouts of moderate to intense fatiguing exercise, along with conventional warm-up exercises, which results in muscle glycogen utilization. The rate of glycogen use during exercise is related to work intensity. When trotting speed is increased from  $5.0 \text{ m s}^{-1}$  to  $12.5 \text{ m s}^{-1}$ , the rate of glycogenolysis increases from  $1.3$  to  $60.0 \text{ mmol kg}^{-1} \text{ dw}^{-1} \text{ min}$  (Lindholm and Saltin, 1974). In competitive endurance rides of 50-100 km lasting ~4-9 h, muscle glycogenolysis was  $0.6$  to  $1.3 \text{ mmol kg}^{-1} \text{ dw}^{-1} \text{ min}$  (Essen-Gustavsson et al 1984, Snow et al 1981), while high intensity sprinting in Thoroughbreds ( $13.4 \text{ m s}^{-1}$  for 2.5 min) resulted in glycogenolysis rates of  $69 \text{ mmol kg}^{-1} \text{ dw}^{-1} \text{ min}$  (Harris et al 1987a).

High intensity exercise in Thoroughbred or Standardbred racehorses depletes muscle glycogen by 30-40% (Lindholm and Piehl 1974, Harris et al 1987a, Snow and Harris 1991, Hyypa et al 1997). Horses that are exercised frequently or undergo several warm-ups may have depleted glycogen stores that persist at the time of subsequent exercise bouts or performances (Snow and Harris 1991, Hyypa et al 1997). Indeed, persistently low muscle glycogen concentrations have been reported in competitive Standardbreds in

training (Essen-Gustavsson et al 1989). Significantly decreased muscle glycogen has been found in Quarter Horses after repeated high intensity sprints (Wilson et al 1998) and after 15 minutes of trotting on an 11% grade (Miller-Graber et al 1991), and in Thoroughbreds after only 1 hour of moderate intensity (45%  $VO_{2max}$ ) exercise. Prolonged endurance exercise also results in significant depletion of muscle glycogen. Horses competing in 80-160 km endurance races have muscle glycogen content reduced by 50-75% (Snow et al 1981, 1982), while participation in the second day of a 3-day event (the cross-country phase) resulted in a 60% decrease in muscle glycogen concentration in horses (Hodgson et al 1984a).

It is important to note that horses have a higher mass-specific aerobic capacity than humans as a result of greater muscle mitochondrial density, proportion of muscles engaged in exercise and cardiovascular capacity (Jose-Cunilleras and Hinchcliff 2004). While elite human athletes have a  $VO_{2max}$  of  $\sim 70 \text{ mL kg}^{-1} \text{ min}^{-1}$  (Warburton et al 1999), the  $VO_{2max}$  of equine athletes can exceed  $200 \text{ mL kg}^{-1} \text{ min}^{-1}$  (Young et al 2002), therefore in horses exercise intensity above 30%  $VO_{2max}$  is considered moderate. Indeed the contribution from CHO oxidation in horses exercising at 35% and 60%  $VO_{2max}$  is 58% and 75%, respectively (Geor et al 2000). In contrast in well-trained humans exercising at 65%  $VO_{2max}$  the contribution from CHO oxidation is only 40% (Romijn et al 1993)

Muscle glycogen appears to be important for peak performance, as partial depletion of intramuscular glycogen is associated with fatigue during endurance (Snow et al 1981) and high intensity exercise (Lacombe et al 1999, 2001). Indeed, individual muscle fibers

may be devoid of glycogen at the point of exhaustion (Hodgson et al 1984b). Depletion of glycogen by 60-80% before a high speed exercise test was associated with decreased run time to fatigue and decreased anaerobic capacity during exercise (Lacombe et al 1999, 2001). Additionally, low muscle glycogen content is associated with decreased blood lactate concentration at identical work intensities, suggesting a decrease in glycogenolytic ATP production (Valberg et al 1985, Lacombe et al 1999). It is concluded that many forms of exercise result in decreased muscle glycogen in performance horses, and decreased availability of skeletal muscle glycogen results in reduced athletic capability.

## **2.2 Post Exercise Muscle Glycogen Synthesis**

### **2.2.1 Basic biochemical mechanisms**

The first step in skeletal muscle glycogen synthesis is glucose transport across the sarcolemma. Basal entry of glucose into muscle cells occurs by the GLUT-1 transporter (Gaster et al 2000), but with insulin and/or exercise stimulation the GLUT-4 isoform is translocated from intracellular storage vesicles to the sarcolemma and t-tubules (Thorell et al 1999).

Once glucose enters the muscle cell it is rapidly phosphorylated to glucose-6-phosphate (G-6-P) by the enzyme hexokinase, which can then undergo either glycolysis or glycogenesis. During glycogenesis G-6-P is converted to glucose-1-phosphate (G-1-P) and then incorporated into an existing glycogen molecule in a series of reactions, the final of which is catalyzed by the enzyme glycogen synthase (GS). Control of GS activity

occurs by phosphorylation/dephosphorylation, such that GS is active in the dephosphorylated state. GS is activated by insulin binding to its cell surface receptor, and can be allosterically activated by glucose-6-phosphate (G-6-P). GS activity is increased with lower muscle glycogen such that the rate of synthesis is inversely proportional to muscle glycogen content (Bergstrom et al 1972), and this relationship holds true for basal, contraction-, and insulin- stimulated muscle (Nielsen et al 2001).

Following exercise, at least in humans and rats, there are two phases of glycogen synthesis (Price et al 1994, Ivy and Kuo 1998). Initially (first 30-60 min of recovery) there is a rapid insulin independent phase stimulated by glycogen depletion and contraction-stimulated GLUT-4 translocation (Douen et al 1989). This is followed by a slow insulin dependent phase associated with post exercise CHO feeding and marked by increased muscle insulin sensitivity that can persist for >48 h (Cartee et al 1989).

### **2.2.2 Interaction of CHO and fat metabolism**

Glycogen synthesis is ultimately dependent on the demand for glycolytic ATP production, pyruvate oxidation, fat oxidation and provision of exogenous carbohydrate in the form of glucose, lactate and other monocarboxylates. During recovery from exercise there is evidence that exogenous glucose transport into muscle cells is the rate-limiting step in glycogenesis, at least in rats (Fisher et al 2002) and mice (Ren et al 1993).

However the glucose available to a muscle cell for glycogenesis is dependent not only on glucose uptake (as discussed above), but also on glycogenolysis and glycolytic flux.

An important in vivo regulator of fuel oxidation and glycolytic flux in both resting and exercising skeletal muscle is the pyruvate dehydrogenase (PDH) complex. PDH is a multienzyme complex located within the inner mitochondrial membrane that catalyzes the decarboxylation of pyruvate to Ac-CoA, therefore controlling the flux of glycolysis-derived pyruvate into the TCA cycle. PDH is very important in the integration of CHO and fat oxidation in skeletal muscle, such that PDH activity (PDHa) is decreased during periods of increased Ac-CoA accumulation secondary to increased oxidation of fat fuels (Putman et al 1993). Randle et al (1963, 1986) described a classic theory termed the glucose-fatty acid cycle in which, at least in resting humans, decreased PDHa results in the beneficial conservation of intramuscular and whole-body glucose stores when other substrates (fat) are available. Ultimately increases in Ac-CoA lead to decreased PDHa and accumulation of citrate which inhibits phosphofructokinase (PFK), resulting in decreased glycolytic flux and the sparing of G-6-P. Increased G-6-P in turn allosterically activates GS, leading to increased glycogen synthesis.

Resting PDHa decreases with CHO deprivation (Putman et al 1993), starvation (Sugden and Holness 1989) and sodium acetate infusion (Putman et al 1995), but not with acute Intralipid infusion (Dyck et al 1993). There have been no studies to date on either resting or exercising skeletal muscle PDHa in horses.

## **2.3 Nutritional Aspects of Post Exercise Muscle Glycogen Synthesis**

### **2.3.1 Carbohydrate feeding and metabolism**

In rats (Garetto et al 1984) and humans (Costill et al 1981) restoration of muscle glycogen stores after ~50-70% depletion is usually complete within 2.5 h and 24 h, respectively, when a high CHO diet is fed. In contrast in horses, complete restoration of similarly depleted muscle glycogen stores still required 48-72 h, even when a highly soluble CHO meal was fed immediately after exercise (Lacombe et al 2004). It should be noted however, that while trained horses and humans have similar resting skeletal muscle glycogen contents of ~ 550-700 mmol kg<sup>-1</sup> dry wt (Lacombe et al 2004, Costill et al 1981), rats have much lower glycogen contents (~ 130-160 mmol kg<sup>-1</sup> dry wt; Garetto et al 1984).

Previous studies in horses have altered post exercise strategies in attempts to enhance muscle glycogen resynthesis, however conventional feeding strategies using typical grain/hay diets have been ultimately unsuccessful (Snow and Harris 1991, Topliff et al 1983, 1985, Hyypya et al 1997). Feeding a high soluble CHO diet of only grain (corn, oats and barley) (Lacombe et al 2004) or corn (Jose-Cunilleras et al 2005) immediately after exercise resulted in glycogen resynthesis rates of ~12 mmol/kg dw/h, compared with rates of ~8 mmol/kg dw/h for isocaloric low (hay) or mixed (hay and grain) soluble CHO diets. In contrast, in humans initial glycogen resynthesis rates were ~40 mmol kg<sup>-1</sup> dw h<sup>-1</sup> when at least 5 g kg<sup>-1</sup> of oral soluble CHO was provided during the first 4 hours after exercise (Jentjens et al 2001). Similar to humans (Robergs 1991), rates of glycogen resynthesis in horses are highest in the initial 3-6 h post exercise, with sequential decreases occurring until 48 h of recovery (Lacombe et al 2004). While i.v. infusion of large amounts of glucose (~2.7 kg over 12 h) accelerated muscle glycogen replenishment

to within 24 h post exercise (Lacombe et al 2001, 2003, Davie et al 1995, Geor et al 2006), nasogastric administration of a smaller amount of glucose (~0.96 kg) did not enhance glycogen replenishment (Nout et al 2003). I.v. infusion of glucose at ~0.5 to 0.7 g/kg body wt/h (~1.5 kg over 6 h) after exercise resulted in initial (0-6 h) glycogen storage rates of  $19.9 \pm 3.8$  (Davie et al 1995) and  $20.9 \pm 7.3$  (Geor et al 2006)  $\text{mmol kg}^{-1} \text{dw}^{-1} \text{h}$  after ~50% glycogen depletion, while rates up to ~29  $\text{mmol kg}^{-1} \text{dw}^{-1} \text{h}$  have been reported after similar i.v. glucose infusion protocols with ~80% muscle glycogen depletion (Lacombe et al 2001). In summary, rates of glycogen repletion in humans are 2-3 fold higher than the rates seen in horses, for both CHO ingestion (Bergstrom and Hultman 1966b, Jentjens et al 2001) and i.v. glucose infusion (Bergstrom and Hultman 1967, Reed et al 1989).

As hindgut fermenters horses digest soluble CHOs such as monosaccharides, disaccharides and starch in the small intestine by enzymatic hydrolysis, while non-hydrolysable CHOs such as cellulose, hemi-cellulose, lignocellulose, soluble fibres and some oligosaccharides pass to the cecum and large colon where they undergo bacterial fermentation (Hoffman et al 2001). The horse evolved as a grazing hindgut fermenting herbivore using primarily low to moderate quality fermentable forage CHOs as its main energy source. Therefore a reason for the slowed glycogen resynthesis in horses compared to rodents and humans may be the horse's limited ability to digest large amounts of soluble CHOs. It has been suggested that the low rate of glycogen synthesis with oral CHO provision in horses may be due to reduced rate of glucose transport from the gastrointestinal tract (Geor et al 2006), possibly due to a glucose absorption limit or

utilization by the gut tissues. In horses, as in all mammals, glucose and galactose are transported across the intestinal luminal membrane by the Na<sup>+</sup>/glucose cotransporter type 1 (SGLT1) carrier protein. It is known that the major site of glucose absorption in horses is the proximal small intestine, with glucose transport highest in the duodenum, followed by the jejunum and ileum (Dyer et al 2002). However the saturation kinetics of the SGLT1 do not appear to have been studied in horses.

In addition to reduced ability to digest soluble CHO, recent research suggests that the low rate of glycogen synthesis with oral CHO provision in horses may also be a result of a lack of increase in post exercise insulin sensitivity and membrane GLUT-4 translocation (Pratt et al 2007). In humans and rats there is an increase in whole body and skeletal muscle insulin sensitivity after a single bout of glycogen-depleting exercise, concurrent with an increase in skeletal muscle insulin-stimulated GSa and sarcolemmal GLUT-4 translocation (Ivy and Kuo 1998, Nielsen et al 2001, Christ-Roberts and Mandarino 2004). However in horses there is no increase in whole-body insulin sensitivity or muscle membrane GLUT-4 content during 24 h of recovery from glycogen-depleting exercise (Pratt et al 2007).

Finally, an additional challenge to the problem of glycogen resynthesis in equine athletes is that the feeding of large amounts of soluble CHOs is not recommended in horses due to the risk of health issues such as gastric ulcers (Murray 1994), colic (Tinker et al 1997), laminitis (Garner et al 1975) and exertional rhabdomyolysis (McKenzie et al 2003). In humans a daily CHO intake of 7-10 g kg<sup>-1</sup> is required for a substantial increase in

glycogen content (for summary see Jentjens and Jeukendrup, 2003). For a 475 kg horse an equivalent CHO dosage would require the consumption of 6.5 to 9.5 kg of oats per day, however such a high grain intake is neither realistic nor recommended in horses, due to the health issues mentioned above. In summary, consuming large amounts of soluble CHOs immediately after exercise substantially hastens muscle glycogen resynthesis in humans and rats, but not horses. There is a clear need for research into alternate nutritional strategies for optimizing recovery of muscle glycogen in equine athletes.

### **2.3.2 CHO and amino acid supplementation**

There is some evidence in humans that glycogen synthesis may be enhanced further when protein or amino acids are added to the post-exercise CHO supplement. The addition of whey protein (Zawadski et al 1992, Ivy et al 2002) or whey protein plus leucine and phenylalanine (van Loon et al 2000) to a post exercise CHO meal synergistically increased the insulin response and enhanced glycogen synthesis. It was suggested that the higher rates of glycogenesis were a result of increased muscle glucose uptake as a result of higher blood insulin response, however the amount of CHO ingested in these studies was lower (0.8 g/kg/h) than amounts considered optimal for glycogen resynthesis in humans (<1.2 g/kg/h). Indeed when CHO is ingested in sufficient amounts ( $\geq 1.2$  g/kg/h) at frequent intervals (every 30 min) optimal rates of glycogen synthesis are produced (~40 mmol/kg dw/h), and provision of additional protein does not further enhance glycogenesis (van Loon et al 2000, Van Hall et al 2000, Jentjens et al 2001) and may increase gastrointestinal discomfort, though it does appear to stimulate post-exercise net muscle protein anabolism (Ivy et al 2008).

It appears that only one study to date has investigated the effects of protein/amino acid supplementation on glycogen resynthesis in horses. Poso and Hyypa (1999) demonstrated that adding leucine to a post-exercise oral glucose dose increased plasma [insulin] compared to the same glucose dosage alone, however this did not result in enhanced glycogen resynthesis at 22.5 h after exercise. Currently a 10-12% crude protein diet is recommended for mature athletic horses (NRC 2007), and most concentrate rations are formulated to provide this. There is some evidence that feeding higher protein (~15%) could have detrimental effects on exercise performance (as a result of increased exercise-associated acidosis) (Graham-Thiers et al 2001), and respiratory health (due to higher urinary urea load) (Miller-Graber et al 1991).

### **2.3.3 Long and medium chain fatty acid supplementation**

Concern over the potential detrimental effects of feeding large amounts of grain has led to the use of animal and vegetable fat supplemented diets (consisting of medium and long chain fatty acids) as a method of meeting the high energy demands of performance horses. A fat-supplemented diet has been purported to have beneficial effects during low intensity exercise in humans and horses, such as increased capacity for fatty acid uptake and oxidation in muscle with a corresponding decrease in CHO utilization. Horses adapted to a high fat diet (~25% of digestible energy) have a lower respiratory exchange ratio (RER) and decreased glucose utilization during prolonged exercise at 25-35%  $VO_{2max}$  (Pagan et al 2002, Dunnett et al 2002), while humans adapted to an ~70% fat diet have increased fat oxidation and decreased muscle glycogen utilization during prolonged

low intensity exercise (Burke et al 2000, Burke and Hawley 2002). In contrast, horses adapted to a 10% fat diet did not have any difference in RER during submaximal exercise but did have higher resting muscle glycogen contents (Meyers et al 1989). Horses performing high intensity sprint exercise and fed an ~10% fat-supplemented diet have either increased resting muscle glycogen content and glycogen utilization during exercise (Oldham et al 1990), or no change (Eaton et al 1995) compared to a typical high CHO/no fat diet.

There is little research investigating the effect of fat-supplemented diets on muscle glycogen replenishment in horses. Hyypa et al (1999) found that a fat supplemented diet (5% of dry matter) did not affect the rate of post exercise glycogen resynthesis in fat-adapted horses, compared to horses fed a normal hay/grain diet, however glycogen resynthesis was slowed when horses were not adapted to fat feeding.

In diet-adapted rats, a diet high in long-chain triglycerides (~70-75%) had no effect (Conlee et al 1990) on post exercise muscle glycogen resynthesis, as compared to a high (~70%) CHO diet. Alternately, when fat-adapted rats are fed a high CHO diet after exercise they have similar glycogen resynthesis rates as fat-adapted rats fed a high fat diet, however CHO-adapted rats fed a high fat diet after exercise have slowed glycogen synthesis (Conlee et al 1990).

Endurance trained humans adapted to a high (85%) fat diet for 6 weeks had lower resting muscle glycogen contents than those on a high (~66%) CHO diet (Phinney et al 1983).

In non diet adapted humans a high fat (55%) post exercise diet slowed the rate of glycogen synthesis in the first 9 h of recovery, compared to a low fat (15%) high CHO diet (Decombaz et al 2001), however by 30 h of recovery muscle glycogen was not different from pre-exercise with either diet. Thus it appears that in horses, humans and rats increased dietary fat is not associated with slowed muscle glycogen resynthesis, provided time is allowed for diet adaptation. It is important to note however, that a diet considered high in fat for horses (5-20%) is considered normal or low in fat for humans, making comparisons between the species difficult. As well there is considerable variability in the reported effects of fat-supplementation in horses, and inconsistencies in the results between studies could be due to type and amount of fat provided, duration of adaptation and washout periods, the conditioning state of the horses, and the small number (often <6) of subjects per treatment.

#### **2.3.4 Volatile (short chain) fatty acid supplementation**

Horses derive 30-40% of their maintenance energy requirements from volatile fatty acid (VFA, ie: short chain fatty acids) absorbed from the large intestine following hindgut fermentation of primarily forage (Glinsky et al 1976, Hintz et al, 1978, Argenzio et al 1974). Acetate is the VFA produced in the greatest quantity (~70% of all VFAs), and while first pass hepatic clearance of acetate is as high as 75% of portal blood acetate (Remesy et al 1980), it is the only VFA present in measurable amounts in peripheral blood.

Acetate extracted from the portal circulation by the liver can be converted to glucose to be used in cells, converted to hepatic glycogen or fat, or incorporated into a number of compounds, including phospholipids, triglycerides and cholesterol (Anderson and Bridges 1984), however oxidation is the primary fate of exogenous oral (Smith et al 2007) and iv (Akanji et al 1989) acetate in humans. Acetate in peripheral blood is rapidly extracted by muscle, via plasma membrane monocarboxylate transporters (MCTs; Waniewski and Martin 2004, Hosoi et al 2008, Koho et al 2006). Once inside the muscle cell, acetate can enter the mitochondria via MCT's (Benton et al 2004) where it is converted to acetyl-CoA by the enzyme acetyl-CoA synthetase and metabolized via the TCA cycle (Mohme et al 1970), generating ATP within the mitochondria.

Because it is readily taken up and oxidized by muscle, acetate enhances glycogenesis and decreases glycolysis in mammals. Early studies in rat muscle demonstrated that increased acetate oxidation inhibits glycolysis and stimulates glycogenesis *in vitro*, (Williamson 1964, 1965, Randle et al 1970). It was hypothesized that the increase in muscle glycogenesis and decrease in glycolysis in rats was a result of increased acetyl-CoA accumulation inhibiting PDHa and ultimately decreasing glycolytic flux (See Figure 2.1). *In vivo* in rats an ingested acetate and glucose solution containing 2-4% acetate increased muscle glycogen resynthesis after exercise, compared with an isocaloric solution of glucose alone (Fushimi et al 2001). However it was hypothesized that the enhanced glycogen repletion with oral acetate feeding in rats was a result of lowered F-1,6-P<sub>2</sub>/F-6-P, which reflects phosphofructokinase 1 (PFK-1) activity (Wakelam and Pette 1982), suggesting that acetate may directly suppress glycolysis by inhibition of PFK-1, as

there were no changes in PDHa or GSa (Fushimi et al 2001). Indeed, concomitant with the decreased PFK-1 activity, muscle G-6-P was increased in the acetate fed rats and there was a tendency for increased citrate.

Increased acetate availability also decreases glucose oxidation *in vivo* in humans (Richards et al 1982, Chiolero et al 1993), likely resulting from decreased PDHa. Indeed a sodium acetate infusion of 8.3 mmol/kg (~5mmol/kg acetate) increases skeletal muscle acetyl-CoA 2-fold and citrate 1.5 fold (increasing the availability of substrate for the TCA cycle) and decreased PDHa 2.3-fold, consistent with the events of intramuscular glucose restriction (Putman et al 1995). However infusion of 4 mmol/kg sodium acetate (~ 2.4 mmol/kg acetate) has no effect on resting PDHa after acetate infusion, despite large increases in acetyl-CoA and acetylcarnitine (Howlett et al 1999, Evans et al 2001), and other studies in humans have suggested that acetate infusion, while not significantly affecting CHO oxidation, results in decreases in long-chain fatty acid oxidation (Burnier et al 1992, Smith et al 2007). It is clear that increased acetate availability increases muscle acetyl-CoA in humans and that this can lead to decreased flux through PDH, provided sufficient acetate is administered.

Acetate supplementation also enhances glycogen synthesis *in vivo* in pigs. In a dose response study, increasing acetate supplemented to growing pigs at 5 and 10% of energy intake resulted in proportional increases in skeletal muscle glucose extraction and increased glycogen contents of liver, heart and skeletal muscle (Imoto and Namioka 1983).

In horses iv sodium acetate (NaAcetate) clearance was accelerated during exercise, suggesting that acetate is used as a metabolic fuel by contracting skeletal muscle during exercise (Pratt et al 2005). However in contrast to humans in which lipids are the predominant energy source during exercise recovery (Kimber et al 2003, Henderson et al 2007), the availability of triglycerides and nonesterified fatty acids is low during recovery in horses (Poso and Hyypa 1999) therefore circulating glucose appears to be preferentially diverted to energy production (glycolysis and oxidation) instead of to glycogen synthesis (Hyypa et al 1997). Thus it has been speculated that in the horse muscle glycogen replenishment during recovery is so slow because skeletal muscle glucose is needed and used for oxidative ATP synthesis (Poso and Hyypa 1999). At rest, glucose and acetate are the major energy sources for hindlimb muscles in horses (Pethick et al 1993). Therefore during recovery glucose (or acetate) may be diverted to energy production instead of to glycogen synthesis (Hyypa et al 1997).

To date, only Poso and Hyypa (1999) have investigated VFA supplementation on glycogen replenishment in horses. Oral supplementation with either a glucose-electrolyte (GE) solution alone or with 200 ml propionic acid added (GEP) was given after glycogen depleting exercise. Neither treatment increased glycogen content 22.5 h after exercise. However, the amounts of glucose (~38 g) and propionate given were very small, and much lower than the amount of CHO used during the exercise period. Furthermore, neither the GE nor the GEP solutions caused any increase in plasma [glucose], suggesting

that this amount of post-exercise CHO was much less than they would receive from a typical post exercise meal.

In summary, acetate may be a practical alternative energy source for post-exercise muscle glycogen replenishment in horses. In addition, horses fed a high grain diet (typical of performance horses) produce less acetate and have lower cecal VFA concentrations than those fed mainly forage (Hintz et al 1971). Therefore it is certainly possible that for performance horses receiving large amounts of grain in their diet, the provision of supplemental acetate during exercise recovery may “spare” exogenous glucose and thus enhance muscle glycogen resynthesis (due to downregulation of PDH activity and an increase in [citrate] ultimately increasing [G-6-P], see Figure 2.1), however the intestinal absorption and metabolic effects of oral acetate in horses remain largely unknown.

### **2.3.5 Electrolytes and Hydration**

In horses, dehydration from loss of total body water, and alterations in blood/muscle electrolyte and consequently acid-base state, can impair health, well-being, and physical and cognitive performance (Carlson et al 1976, Guthrie and Lund 1998, McKeever 1998, Hoffman et al 2002). There is considerable anecdotal and practical information that dehydrated horses will not eat, and most forms of glycogen-depleting exercise in horses result in dehydration (Lindinger and Waller 2008). Water alone is also not effective in restoring hydration and there is a requirement for electrolytes to osmotically retain water in the appropriate extracellular and intracellular fluid compartments (Maughan et al 1994, Maughan and Lindinger 1995, Jansson and Dahlborn 1999).

In addition, intracellular water and  $K^+$  are required for the resynthesis of muscle glycogen, implying that both hydration status and availability of electrolytes affect muscle glycogen resynthesis. Muscle glycogen is stored in a hydrated form (up to 3 g water/ g glycogen) (Costill 1988) and is tightly associated with  $K^+$ , with 0.5 mmol  $K^+$  deposited for every g of glycogen (Bergstrom and Hultman, 1966a). Thus it seems important to ensure that horses are adequately rehydrated with appropriate electrolyte solutions, during and soon after exercise, in order to facilitate muscle glycogen recovery. Interestingly, i.v. infusion of large amounts of saline (~20 L in 12 h for a total of 107 g NaCl) plus consumption of the hay portion of the diet alone, resulted in glycogen resynthesis rates of ~ 7.6 mmol  $kg^{-1}$  dw  $h^{-1}$  during the first 12 h of exercise recovery in horses (Lacombe et al 2001), which was similar to typical rates of ~8 to ~12 mmol  $kg^{-1}$  dw<sup>-1</sup> h seen with mixed and highly soluble CHO diets (Lacombe et al 2004). These results strongly suggest a role for rehydration in glycogen resynthesis independent of glucose supplementation, and lead to the notion that rehydration with electrolyte solution, with provision of a CHO source, should have an even greater positive effect.

As further evidence that dehydration affects glycogen resynthesis, cell shrinkage is associated with decreased glycogen synthesis in skeletal muscle (Low et al. 1996) and hepatocytes (Haussinger 1996) and increased carbohydrate oxidation and glycogenolysis (Keller et al. 2003). Conversely, glycogen synthesis and other anabolic processes in the liver and muscle are stimulated by cell swelling (Low et al 1996, Haussinger 1996). It has also been demonstrated *in vivo* in humans that cell swelling induced by hypo-

osmolality resulted in decreased carbohydrate oxidation and glycogenolysis and stimulated lipolysis, while cell shrinking resulted in increased glycogenolysis (Berneis et al 1999, Keller et al 2003). These responses to both increased and decreased cell volume appear to involve signal transduction mechanisms similar to those associated with insulin and growth factor signaling, and it has been suggested that the stimulation of glycogen synthesis by cell swelling is likely due to dephosphorylation of glycogen synthase via effects on glycogen synthase kinase-3 (Low et al 1996).

Neufer et al (1991) found no change in glycogen resynthesis in hypo- vs euhydrated human subjects, however CHO ingestion was withheld for 7 h after exercise in that study (approximately half the total recovery time course). In humans the maximal rate of glycogen resynthesis occurs during the first 4 h after exercise, providing that CHOs are given immediately after exercise (Robergs 1991). It is likely that the long delay in CHO provision in the Neufer et al (1991) study had a large effect on the similarity of glycogen resynthesis rates.

It also must be emphasized that horses and humans undergo different types of dehydration with exercise. While humans produce a dilute sweat that results in a hypertonic dehydration (Sawka 1988), horses secrete an electrolyte-rich sweat that results in an isotonic to hypotonic dehydration (McCutcheon et al 1995). Because there is typically no increase in plasma osmolality with exercise-induced dehydration in horses, the osmoreceptors in the hypothalamus are not stimulated to release vasopressin and no thirst response is triggered (Johnson 1998). Thus the importance of replacing the

electrolytes lost in sweat as a means to recovering fluid balance horses is clear. Due to these stark differences in exercise-induced dehydration between horses and humans, and the well-established inherent differences in 'normal' glycogen resynthesis between the two species (discussed above), it is reasonable to postulate that post exercise dehydration may be one reason why muscle glycogen replenishment is so slow in horses. However the concurrent changes in intracellular water,  $K^+$  and glycogen content during exercise and recovery in horses has not been studied.

## **2.4 Acid-Base Status**

### **2.4.1 The Physicochemical Approach to Acid-Base Status**

Measurement and interpretation of acid-base status is important in equine clinical practice and in the racing community to determine if horses have been administered alkalinizing substances for the purpose of performance enhancement. Because both electrolyte mixtures and sodium acetate contain strong ions and can affect hydration status, it is expected that their supplementation should have direct effects on plasma acid-base balance. Traditionally, acid-base status has been described in terms of pH,  $pCO_2$  and  $[HCO_3^-]$  (Hasselbalch 1912). However it is increasingly recognized that this definition does not adequately describe the physical and chemical origins of the acid-base disturbance (Carlson 2000, Lindinger and Waller 2008). Stewart (1983) proposed a comprehensive physicochemical approach to acid-base status that uses both independent and dependent variables to describe acid-base state. The physicochemical approach recognizes that pH (or  $[H^+]$ ) and  $[HCO_3^-]$  are dependent acid-base variables because they cannot be altered without initially (and concurrently) altering the concentrations of

independent acid-base variables. The independent variables that determine acid-base status are the partial pressure of carbon dioxide ( $PCO_2$ ), the concentrations of strong ions in solution – defined as the strong ion difference ( $[SID]$ ), and the concentrations of weak ions (such as plasma proteins: PP) in solution – defined as the total weak acid concentration ( $[A_{tot}]$ ).

Strong ions are electrolytes that, based on their dissociation constant ( $K_A$ ), are completely dissociated in solution. The net strong ion charge was termed the strong ion difference ( $[SID]$ ) by Stewart, and is calculated as:

$$[SID] (\text{meq L}^{-1}) = \sum [\text{strong cations}] - \sum [\text{strong anions}]$$

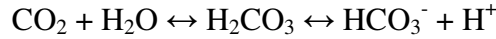
The principal strong ions in equine plasma are  $Na^+$ ,  $K^+$ ,  $Cl^-$  and Lactate $^-$ . The concentrations of the divalent cations and anions ( $Ca^{2+}$ ,  $Mg^{2+}$ ,  $PO_4^{2-}$  and  $SO_4^{2-}$ ) are low, and their charges are approximately equal, thus they can be ignored (Lindinger and Waller 2008). Increases in plasma [cation], and thus  $[SID]$ , contribute to plasma alkalization, while increases in plasma [anion] contribute to plasma acidification.

The plasma concentration of weak ions is represented by  $[A_{tot}]$ . Weak ions are not fully dissociated in solution, and the extent of their dissociation is determined by their pK (Stewart 1983). An increase in  $[A_{tot}]$  contributes to plasma acidification, as the pK of weak ions is less than 7. Plasma albumin concentration provides the major contribution to plasma  $[A_{tot}]$  (Constable 1997). In equine plasma,  $[A_{tot}]$  is calculated as:

$$[A_{tot}] (\text{meq L}^{-1}) = 2.24 \times [PP] (\text{g dL}^{-1}) \text{ (Constable 1997)}$$

This equation assumes a relatively small and unchanging plasma phosphate and acetate concentrations, and normal albumin-to-globulin ratio.

The third independent variable in the physicochemical model is the pCO<sub>2</sub>. The dissociation of CO<sub>2</sub> in an aqueous solution is described by:



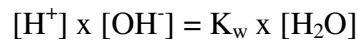
Thus increases in pCO<sub>2</sub> contribute to acidification. The amount of dissolved CO<sub>2</sub> can be derived from its solubility constant and the pCO<sub>2</sub> by the equations:

$$K_c \text{ (eq L}^{-1}\text{)} = ([\text{H}^+] [\text{HCO}_3^-]) / [\text{pCO}_2]$$

$$K_3 \text{ (eq L}^{-1}\text{)} = ([\text{H}^+] [\text{CO}_3^{2-}]) / [\text{HCO}_3^-]$$

where K<sub>3</sub> is the equilibrium dissociation constant for HCO<sub>3</sub><sup>-</sup>.

The dissociation of water is a key physical premise underlying the physicochemical approach:



Since water dissociation occurs rapidly, it has a negligible effect on the water concentration, thus K<sub>w</sub> × [H<sub>2</sub>O] can be considered a constant:

$$K_w \text{ (eq L}^{-1}\text{)} = [\text{H}^+] [\text{OH}^-]$$

Stewart (1983) formulated a 4<sup>th</sup> order polynomial equation to determine [H<sup>+</sup>] in terms of the independent variables, such that:

$$[\text{H}^+]^4 = (K_A + [\text{SID}]) [\text{H}^+]^3 + \{([\text{SID}] - [\text{A}_{\text{tot}}] * K_A) - (K_C * \text{pCO}_2 + K_w)\} [\text{H}^+]^2 - \{K_A (K_C * \text{pCO}_2 + K_w) + (K_3 * K_C * \text{pCO}_2)\} [\text{H}^+] - K_A * K_3 * K_C * \text{pCO}_2 = 0$$

where  $K_w$ ,  $K_A$ ,  $K_3$  and  $K_C$  are the equilibrium constants for dissociations of water, weak acids, carbonic acid and bicarbonate, respectively.

This equation allows the contributions of each of the independent variables ( $[SID]$ ,  $[A_{tot}]$  and  $pCO_2$ ), to the dependent variables ( $[H^+]$  and  $[TCO_2]$ ), to be determined by holding two of the three independent variables constant while calculating  $[H^+]$  in response to changes in the third independent variable (Lindinger et al 1992, Lindinger and Waller 2008, Miller et al 2005). This procedure allows the time course of response for  $[H^+]$  or  $[TCO_2]$  to be determined for changes in each of the independent variables, and thus allows for determination of the origins of disturbances in acid-base state.

The physicochemical approach therefore has advantages over the traditional method in that it allows for identification and quantification of the origins of the acid-base disturbance, and thus is increasingly used in the evaluation of acid-base status in humans (see Miller et al 2005 for references) and horses (see Lindinger and Waller 2008) at rest and during exercise and recovery.

#### **2.4.2 Alkalinizing agents**

The administration of bicarbonate salts as alkalinizing agents has been extensively studied in both horses (Schott and Hinchcliff, 1993) and humans (Heigenhauser and Jones 1991), and is of particular interest to the horseracing community where the testing of horses for illegal performance enhancing alkalinizing agents occurs. NaAcetate has received some attention as an alkalinizing agent and possible alternative to sodium

bicarbonate ( $\text{NaHCO}_3$ ) (Lloyd and Rose 1996). In contrast to  $\text{NaHCO}_3$ , the ergogenic effects of NaAcetate do not appear to have been studied in horses, however a few studies have demonstrated its alkalinizing effect orally (Lloyd and Rose 1996) and intravenously (Kline et al 2005). Of the limited studies of NaAcetate administration in horses, none have measured and reported the major plasma constituents describing acid-base status, therefore the magnitude and time course of variables that contribute to the origins of the metabolic alkalosis have been incompletely characterized; indeed the picture may be different from that obtained with  $\text{NaHCO}_3$  and other alkalinizing compounds.

### **2.4.3 Electrolyte Supplementation**

In order to enhance recovery, horses are commonly supplemented with electrolyte solutions both during and after exercise (Schott and Hinchcliff, 1993). Because electrolyte mixtures contain strong ions and affect hydration status (Marlin et al 1998), it is expected that electrolyte supplementation should have direct effects on plasma acid-base balance. There are few studies of acid-base effects of electrolyte supplementation during recovery from exercise in horses. Szucsik et al (2006) determined that administration of 7 different commercial electrolyte supplements, as hypertonic pastes prior to exercise, had no effect on plasma concentrations of electrolytes,  $\text{TCO}_2$ , or plasma protein concentration ([PP]). The amounts of electrolytes given were relatively small however (1.1-6.5g Na, 0.9-3.7g K, 2.6-13g Cl), corresponding to only an approximate 2 L sweat loss (McCutcheon et al 1995), and the time course was limited to only two samples post-exercise. When a greater amount of total electrolytes (20.2g Na, 9.0g K, 31.1g Cl) was given as an isotonic electrolyte/water solution, increases in plasma  $[\text{Cl}^-]$

and  $[\text{Na}^+]$  (as compared to control), and no changes in plasma  $[\text{K}^+]$  or  $[\text{PP}]$  were seen (Hyypä et al 1996), however the effects on acid-base state were not studied. In contrast, when horses were administered a large amount of electrolytes (51g Na, 24g K, 99g Cl) as a hypertonic paste after a furosemide-induced dehydration (mean losses of water, Na, K and Cl were 18 L, 40g, 20g and 75g, respectively), plasma  $[\text{H}^+]$  and  $[\text{HCO}_3^-]$  had returned to pre-furosemide levels by 2 h post administration, while a plasma alkalosis persisted in the water treated control horses (Sosa Leon et al 1998). No previous studies have measured and reported all the major plasma constituents describing acid-base status after electrolyte supplementation in horses, therefore the acute time course of changes in acid-base state in response to electrolyte supplementation has not been characterized.

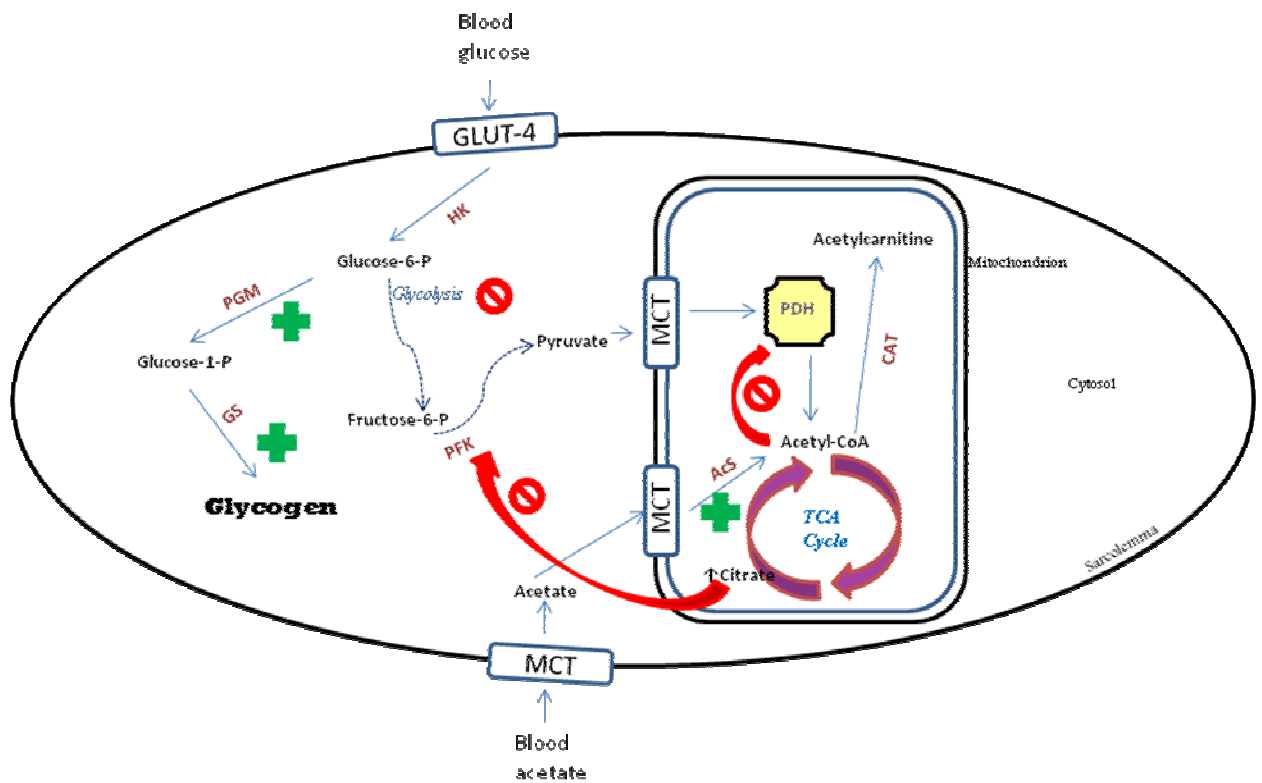
## **2.5 Summary**

Optimum performance of the competitive, athletic horse is highly dependent on the quantity and availability of metabolic fuels that serve muscle contraction and hence locomotion. The high performance athlete is highly reliant on muscle glycogen stores to fuel the demands of vigorous exercise characteristic of many forms of equitation. Accordingly, vigorous exercise for even brief periods, or with prolonged submaximal exercise, is associated with depletion of muscle glycogen reserves and dehydration due to sweating – excessive depletion of glycogen and water/electrolytes contributes to skeletal muscle fatigue and poor performance. Depressed muscle glycogen in days after training or competition will contribute to poor performance.

Unfortunately, compared to other mammals studied, recovery of skeletal muscle glycogen is very slow in horses, and can take 48-72 h, even when a highly soluble carbohydrate meal is fed immediately after exercise. It appears there are limitations to CHO digestion and skeletal muscle glucose uptake in horses as compared to humans and rats. In addition, the availability of triglycerides and nonesterified fatty acids is low during recovery in horses thus circulating glucose appears to be preferentially diverted to energy production instead of to glycogen synthesis. There is a clear need to develop a practical nutritional strategy to enhancing recovery of muscle glycogen in horses.

Acetate is the primary volatile fatty acid produced in horses, and acetate and glucose are the two major energy sources for muscle in horses at rest. Acetate supplementation enhances muscle glycogen resynthesis in other mammals, however there have been no studies investigating the effect of acetate on glycogen resynthesis in horses.

The studies described herein were intended to characterize the effects of supplemental acetate and effective rehydration on plasma and skeletal muscle in horses, in order to determine if the slow rate of muscle glycogen resynthesis after glycogen-depleting exercise is due to a combination of slow recovery of hydration and inadequate shuttling of intracellular glucose towards glycogenesis. The long term goal of this research is to assist in developing a safe and practical nutritional strategy for optimizing the recovery of muscle glycogen in competitive equine athletes so as to improve training and competition performance, particularly with respect to multi-day activities.



**Figure 2.1. Proposed mechanism for acetate supplementation enhancing skeletal muscle glycogen synthesis.** +: denotes hypothetical up-regulated pathways, □ : denotes hypothetical down-regulated pathways; see text for further explanation. GS: glycogen synthase, PGM: phosphoglucomutase, PFK: phosphofructokinase, HK: hexokinase, AcS: acetyl-CoA synthetase, CAT: carnitine acetyltransferase

## Chapter 3

### 3.0 Objectives

#### 3.1 Study 1

*Purpose:* to detail the time course and magnitude of the changes in all key plasma constituents that determine acid-base state in horses, after oral administration of a commercially available electrolyte solution post exercise, and to employ the physicochemical approach to describe the acid-base responses.

*Hypothesis:* oral administration of a hypotonic electrolyte solution after prolonged moderate intensity exercise would result in 1) reduced plasma  $[H^+]$  and  $[TCO_2]$ , and 2) enhanced recovery of hydration status, as compared to a control when no electrolytes are given.

#### 3.2 Study 2

*Purpose:* to determine the effect of administering a hypotonic electrolyte solution immediately after prolonged exercise, and before feed is provided, on rehydration and muscle glycogen and electrolyte recovery in horses.

*Hypothesis:* provision of a balanced electrolyte solution, immediately followed by a typical hay and grain meal, after glycogen-depleting exercise will result in a faster rate of muscle glycogen resynthesis than a grain/hay meal with voluntary access to water alone.

#### 3.3 Study 3

*Purpose:* to detail the time course and magnitude of the changes in key blood constituents that determine plasma acid-base state in horses, after oral administration of a sodium

acetate/acetic acid solution, and to employ the physicochemical approach to describe the resulting acid-base responses.

*Hypothesis:* 1) oral administration of a sodium acetate/acetic acid solution after prolonged submaximal exercise will result in a rapid and sustained increase in plasma [acetate]; and 2) a marked metabolic alkalosis will occur that is characterized by increased plasma volume,  $[\text{Na}^+]$  and [SID].

### **3.4 Study 4**

*Purpose:* to determine the effect of oral acetate administration on plasma acetate appearance, skeletal muscle acetate metabolism and glycogen resynthesis.

*Hypothesis:* provision of a sodium acetate/acetic acid/ potassium chloride solution with a typical hay and grain meal after glycogen-depleting exercise would result in a faster rate of muscle glycogen resynthesis than a grain/hay meal alone. Specifically, the addition of acetate and electrolytes to a typical post exercise meal would result in: 1) rapid appearance of acetate in blood, 2) rapid skeletal muscle uptake of acetate to be converted to acetyl-CoA and acetylcarnitine and 3) decreased pyruvate dehydrogenase activity, ultimately shuttling muscle glucose to glycogenesis.

## Chapter 4

### **Electrolyte supplementation after prolonged moderate intensity exercise results in decreased plasma [TCO<sub>2</sub>] in Standardbreds**

#### **4.1 Introduction**

In horses, both prolonged submaximal exercise and brief vigorous exercise are associated with fluid and electrolyte shifts into and out of the plasma compartment (Lindinger et al 2004, Waller and Lindinger 2005a) and depletion of total body water and electrolytes (Ecker and Lindinger 1995, Hyypa et al 1996, Waller and Lindinger 2005b) that persists well into the recovery period (Waller and Lindinger 2005a, Hyypa et al 1996, Schott et al 1999). Prolonged submaximal exercise is also associated with the development of a mild systemic alkalosis that results from extracellular Cl<sup>-</sup> loss in excess of Na<sup>+</sup> loss as a result of thermoregulatory sweating (McCutcheon et al 1995). In order to enhance recovery, horses are commonly supplemented with electrolyte solutions both during and after exercise (Schott and Hinshcliff 1993). Because electrolyte mixtures contain strong ions and affect hydration status (Marlin et al 1998), it is expected that electrolyte supplementation should have direct effects on plasma acid-base balance. According to the physicochemical approach (Stewart 1983), the independent variables that determine plasma acid-base status are the concentration of strong ions in solution – defined as the strong ion difference ([SID]), the partial pressure of carbon dioxide (PCO<sub>2</sub>), and the concentration of weak acids in solution – defined as the total weak acid concentration ([A<sub>tot</sub>]). Thus the dependent acid-base variables – [H<sup>+</sup>], bicarbonate concentration

( $[\text{HCO}_3^-]$ ) and total carbon dioxide concentration ( $[\text{TCO}_2]$ ) – only change when one or more of the independent variables are altered (Stewart 1983, Lindinger and Waller 2008).

There are few studies of acid-base effects of electrolyte supplementation during recovery from exercise in horses. Szucsik et al (2006) determined that administration of 7 different commercial electrolyte supplements, as hypertonic pastes prior to exercise, had no effect on plasma concentrations of electrolytes,  $\text{TCO}_2$ , or plasma protein concentration ([PP]). The amounts of electrolytes given were relatively small however (1.1-6.5g Na, 0.9-3.7g K, 2.6-13g Cl), corresponding to only an approximate 2 L sweat loss (McCutcheon et al 1995), and the time course was limited to only two samples post-exercise. When a greater amount of total electrolytes (20.2g Na, 9.0g K, 31.1g Cl) was given as an isotonic electrolyte/water solution, increases in plasma  $[\text{Cl}^-]$  and  $[\text{Na}^+]$  (as compared to control), and no changes in plasma  $[\text{K}^+]$  or [PP] were seen (Hyypya et al 1996), however the effects on acid-base state were not studied. Thus the acute time course of changes in acid-base state in response to electrolyte supplementation has not been characterized.

Measurement and interpretation of acid-base status is important in clinical practice and in the racing community to determine if horses have been administered alkalinizing substances for the purpose of performance enhancement. Therefore the purpose of the present study was to detail the time course and magnitude of the changes in all plasma constituents that determine acid-base state in horses, after oral administration of a commercially available electrolyte solution post exercise, and to employ the

physicochemical approach to describe the resulting acid-base disturbances. It was hypothesized that oral administration of a hypotonic electrolyte solution after prolonged moderate intensity exercise would result in 1) reduced plasma  $[H^+]$  and  $[TCO_2]$ , and 2) enhanced recovery of hydration status, as compared to a control when no electrolytes are given.

## 4.2 Methods

**Animals:** 6 Standardbred geldings (body mass  $464 \pm 10$  kg; age 5-12 yrs) from the University of Guelph research herd were used. The study took place in June and July, and horses underwent a 4-6 week diet and exercise acclimation period during which they were housed in individual box stalls with 7 hrs of paddock turnout during the day. Horses were exercise conditioned 5 days/week on a high speed treadmill (SATO, Sweden) and outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada), until able to comfortably perform a ~60 minute competitive exercise test (CET) (Marlin et al 1996, 1999) on a high speed treadmill intended to significantly decrease muscle glycogen content (Poso and Hyypa 1999) and result in total body water losses of 8-10L. The CET is designed to simulate the 2<sup>nd</sup> day (speed and endurance test – classic format) of a one star CCI 3-day event, and includes the following phases: 10 min walk (1.7 m/s), 10 min trot (3.7 m/s), 2 min gallop (10.0 m/s), 20 min trot (3.7 m/s), 10 min walk (1.7 m/s), 8 min canter (8.0 m/s), and 10 min walk (1.7 m/s).

The horses were maintained on a diet consisting of oats, DCAB  $\{Na + K - Cl\} = -54.6$  meq/kg) twice daily and mixed grass hay (DCAB =  $368.8$  meq  $kg^{-1}$ ) three times daily,

with free access to water and a salt block. The amount of feed given was increased over this acclimation period such that during the final two weeks the horses were receiving 4kg sweet feed and 6 kg hay daily (dietary DCAB = 200.4 meq kg<sup>-1</sup>), and there were no significant changes in the body masses of the horses during this time. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

***Experimental Protocol:*** The study consisted of an electrolyte treatment and control, thus each horse performed the CET twice in randomized order, separated by an 8-10 day interval (Hyyppa et al 1996, Marlin et al 1999) during which time exercise conditioning was maintained. The CET was performed in a climate-controlled treadmill room, with conditions intending to approximate those of a typical summer day in a temperate climate (temperature ~25°C, humidity ~70%). On both sampling days beginning at 7 am, the hair coat over the jugular vein, 10-20 cm below the mandible, was clipped short to the skin on both sides of the neck. Each jugular vein catheterization site was aseptically prepared for insertion of catheters. EMLA cream (2.5% lidocaine and 2.5% prilocaine; Astra Pharma, Mississauga, ON, Canada), was applied topically 25-30 min before insertion of catheters to desensitize the skin. Local anaesthetic (2% Xylocaine; Astra Pharma) was injected subcutaneously to complete the anesthesia. Catheters (14-gauge, 5.25 in; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted antegrade into the left and right jugular veins, secured with tape and stitched to the skin. Four-way stopcocks with

50 cm extensions were attached to the catheters for ease of blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2000 IU<sup>-1</sup> NaCl).

A pre-exercise blood sample was taken at 8 am and then the CET was performed.

Immediately upon completion of the final canter an 'end of exercise' blood sample was taken, following which the horse walked for 10 min. 20 minutes after cessation of exercise, the horse either 1) was nasogastically administered a commercially available electrolyte solution (Perform'N Win, Buckeye Nutrition, Dalton, OH) according to manufacturer's direction for an 8 L sweat loss (12 g Na, 24 g Cl, 9 g K), in 8 L of water (Osmolality = 212 mOsm kg<sup>-1</sup>), or 2) stood in stocks for equivalent amount of time.

Within 20 minutes of cessation of exercise, the horses were given 2kg sweet feed and 2kg hay (0 min of recovery), with access to water *ad libitum*. Horses were given 2kg sweet feed and 2kg hay at 6 h of recovery, and 2kg hay at 12 h recovery. Blood samples were taken at 20-60 min intervals up to 8 h of recovery, and again at 24 h of recovery, and horses remained in their stalls for the duration of sampling.

**Sample Analysis:** Each blood sample was collected into 7 ml lithium heparinized vacutainers and immediately analyzed for plasma pH, the partial pressures of carbon dioxide (pCO<sub>2</sub>) and oxygen (pO<sub>2</sub>), and the plasma concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, lactate<sup>-</sup> using a Nova Stat Profile 9<sup>+</sup> (NOVA Biomedical, Waltham, MA). Hematocrit (hct) was measured by conductivity and [HCO<sub>3</sub><sup>-</sup>] and total carbon dioxide (TCO<sub>2</sub>) concentration were calculated using the Henderson-Hasselbach equation by the Nova Stat Profile 9<sup>+</sup>. Blood was then transferred into two 1.5 ml Eppendorf centrifuge tubes and

centrifuged for 5 min at 15 000g to separate the plasma. Plasma protein concentration ([PP]) was determined (coefficient of variation (CV 0.83%) by using refractometry (Atago clinical refractometer model SPR-T2; Atago, Tokyo, Japan).

**Calculations:** Plasma  $[H^+]$  was calculated using the measured pH such that:

$$pH = -\log[H^+]$$

Plasma Strong Ion Difference ([SID]) was calculated as the sum of the plasma concentrations of the strong cations minus the strong anions<sup>10</sup>, such that:

$$[SID] = [Na^+] + [K^+] - [Cl^-] - [lactate^-]$$

In practice, the concentrations of the divalent cations and anions ( $Ca^{2+}$ ,  $Mg^{2+}$ ,  $PO_4^{2-}$  and  $SO_4^{2-}$ ) are small and the sum of their charges close to zero and can be ignored (Lindinger and Waller 2008).

The plasma concentration of weak ions ( $[A_{tot}]$ ) was calculated by multiplying the [PP] (in g dL<sup>-1</sup>) by 2.24 (Constable 1997).

Calculations of dependent acid-base parameters (pH,  $[H^+]$ ,  $[HCO_3^-]$ ,  $TCO_2$ ) were made using AcidBasics II software (©2003, PD Watson), using the equation:

$$[H^+] + (K_A + [SID]) [H^+]^3 + \{K_A ([SID] - [A_{tot}]) - (K_C * pCO_2 + K_W)\} [H^+]^2 - \{K_A (K_C * pCO_2 + K_W) + (K_3 * K_C * pCO_2)\} [H^+] - K_A * K_3 * K_C * pCO_2 = 0$$

where  $K_W$ ,  $K_A$ ,  $K_3$  and  $K_C$  are the equilibrium constants for dissociations of water, weak acids, carbonic acid and bicarbonate, respectively.

$$K_W = 4.4 * 10^{-14} \text{ (eq L}^{-1}\text{)}$$

$$K_A = 2.22 * 10^{-7} \text{ (eq L}^{-1}\text{)}$$

$$K_3 = 5.76 * 10^{-11} \text{ (eq L}^{-1}\text{)}$$

$$K_C = 2.45 * 10^{-11} \text{ (eq L}^{-1}\text{)}^2/\text{mmHg}$$

The contributions of the independent variables  $[A_{\text{tot}}]$  and  $\text{PCO}_2$  to the dependent variable  $[\text{H}^+]$  were determined by holding two of either  $[\text{SID}]$ ,  $\text{pCO}_2$ , or  $[A_{\text{tot}}]$  constant while calculating  $[\text{H}^+]$  in response to changes in the third independent variable (Lindinger et al 1992). The contribution of the  $[\text{SID}]$  to the changes in  $[\text{H}^+]$  was calculated by determining the contributions of  $\text{pCO}_2$  and  $[A_{\text{tot}}]$ , and then subtracting these from the measured change in  $[\text{H}^+]$ .

Total body water loss during the CET was determined as the change in body mass after accounting for fecal losses.

Plasma osmolality was calculated according to the formula of Brownlow and Hutchins (1982) for equine plasma such that:

$$\text{Osmolality (mOsm kg}^{-1}\text{)} = 1.86([\text{Na}^+] + [\text{K}^+]) + [\text{Glucose}] + [\text{Lactate}^-] + 9$$

### *Statistics*

Data are presented as mean  $\pm$  standard error. Changes over time were assessed by one-way repeated measures analysis of variance. Differences between treatments during the recovery period were assessed by two-way repeated measures analysis of variance.

When a significant F-ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when  $P \leq 0.05$  at a power of 0.8.

### 4.3 Results

Ambient temperature and humidity during the CET were  $23.7 \pm 0.2$  °C and  $69.3 \pm 1.9$  %, respectively. Total body water loss during the CET was  $8.4 \pm 0.2$  L.

All horses consumed all the feed offered by the 24 h sample. Every horse finished all of the oats given by 20 min post feeding, and had consumed all the hay given by 3-4 h post feeding.

#### *Independent variables and electrolytes*

The plasma electrolytes are shown in Fig. 4.1. Plasma  $[\text{Na}^+]$  (Fig. 4.1a) was increased from pre-exercise during the initial 20-60 min of the recovery period for both trials, with no difference between treatments ( $P = 0.299$ ). Plasma  $[\text{K}^+]$  (Fig. 4.1b) was increased at the end of exercise, and decreased during the initial 120 min of recovery, with no difference between treatments ( $P = 0.277$ ). Plasma  $[\text{Cl}^-]$  (Fig. 4.1c) was decreased from pre-exercise at 300, 420, and 480 min of recovery in the Control trial, and did not differ from pre-exercise in the Electrolyte trial. There was a trend ( $P = 0.080$ ) towards higher plasma  $[\text{Cl}^-]$  in the Electrolyte trial.

Plasma  $[\text{Ca}^{2+}]$  was decreased at the end of exercise and until 40 min of recovery, with no differences between trials ( $P = 0.361$ ) (Table 4.1). Plasma  $[\text{lactate}^-]$  was increased at the end of exercise to  $3.4 \pm 0.9$  and  $3.4 \pm 1.3$  mmol L<sup>-1</sup> in the Electrolyte and Control trials, respectively, with no differences between trials ( $P = 0.539$ ) (Table 4.1).

The time course of changes in independent acid-base variables is shown in Fig. 4.2. There were no differences from pre-exercise in plasma [SID] (Fig. 4.2a) for either trial, however plasma [SID] in the Electrolyte trial was significantly lower than Control ( $P = 0.015$ ). Plasma  $\text{PCO}_2$  (Fig. 4.2b) was decreased at the end of exercise and increased during the initial 20-60 min of recovery in both trials, with no differences between trials ( $P = 0.108$ ). Plasma  $[\text{A}_{\text{tot}}]$  (Fig. 4.2c) was increased from pre-exercise at the end of exercise until 60 min of recovery in the Electrolyte trial, and until the end of sampling in the Control trial. There was trend ( $P = 0.109$ ) for a lower  $[\text{A}_{\text{tot}}]$  during the recovery phase of the electrolyte trial.

### ***Dependent variables***

Plasma  $[\text{H}^+]$  (Fig. 4.3) was decreased from pre-exercise at the end of exercise and increased during 40-60 min of recovery for both trials. There was a trend ( $P = 0.065$ ) towards higher plasma  $[\text{H}^+]$  in the Electrolyte trial. When the contributions of the independent variables to plasma  $[\text{H}^+]$  were determined (data not shown), the decrease in  $[\text{H}^+]$  at the end of exercise was entirely due to the decreased  $\text{PCO}_2$ , while increases in  $[\text{A}_{\text{tot}}]$  and  $\text{PCO}_2$  were the primary contributors to the increase in  $[\text{H}^+]$  during early recovery in both trials. The primary contributor to the trend towards increased  $[\text{H}^+]$  during 120-480 min of recovery in the Electrolyte trial was the decreased [SID].

Plasma  $[\text{TCO}_2]$  (Fig. 4.4a) at pre-exercise was  $37.3 \pm 0.7$  and  $37.4 \pm 0.5$   $\text{mmol L}^{-1}$  for the Electrolyte and Control trials, respectively. Plasma  $[\text{TCO}_2]$  was decreased at the end of exercise in both trials, and increased at 20 min of recovery in the Control trial only (40.0

$\pm 0.5 \text{ mmol L}^{-1}$ ). There was a significant ( $P = 0.033$ ) treatment effect such that plasma  $[\text{TCO}_2]$  was lower during recovery in the Electrolyte, compared to the Control, trial. The contributions of the independent variables to the change in plasma  $[\text{TCO}_2]$  are shown in Fig. 4.4b and Fig. 4.4c. The main contributor to the decreased  $[\text{TCO}_2]$  at the end of exercise in both trials was the increased  $[\text{A}_{\text{tot}}]$ , with decreased  $\text{PCO}_2$  and  $[\text{SID}]$  contributing to a lesser extent. The increased  $[\text{TCO}_2]$  at 20 min of recovery in the Control trial (Fig. 4.4b) was entirely due to increased  $[\text{SID}]$ , while the overall decreased  $[\text{TCO}_2]$  in the Electrolyte trial (Fig. 4.4c) was primarily due to increased  $[\text{A}_{\text{tot}}]$  during the initial 60 min of recovery, and due to a decreased  $[\text{SID}]$  during the latter 120-480 min of recovery.

Plasma pH and  $[\text{HCO}_3^-]$  (Table 4.2) are shown to provide terms of reference with respect to the majority of acid-base literature. pH was increased at the end of exercise and decreased during the initial 40-60 min of recovery. There was a trend ( $P = 0.061$ ) towards lower pH in the Electrolyte trial. Plasma  $[\text{HCO}_3^-]$  was decreased at the end of exercise, and was significantly lower in the Electrolyte trial ( $P = 0.033$ ).

#### ***Water Consumption, plasma osmolality and other variables***

Calculated plasma osmolality (Fig. 4.5) was increased at the end of exercise, and from 20-60 min and 20-240 min of recovery in the Control and Electrolyte trials, respectively. Plasma osmolality decreased throughout the recovery period in the Control trial such that it was significantly lower than pre-exercise from 360-420 min of recovery, however there was no difference between trials ( $P = 0.104$ ). Total water intake (including the 8 L given

nasogastrically) was significantly greater in the Electrolyte trial, such that at 24 h of recovery (Fig. 4.6) was  $49.5 \pm 4.2$  L and  $34.8 \pm 2.2$  L in the Electrolyte and Control trials, respectively ( $P = 0.018$ ). Total water intake over the entire time course of the trials also showed a treatment effect, with total intake in the Electrolyte trial significantly greater than Control ( $P = 0.014$ ).

[PP] was increased from pre-exercise at the end of exercise until 60 min of recovery in the Electrolyte trial, and until the end of sampling in the Control trial, with no difference between treatments ( $P = 0.169$ ) (Table 4.2). Hematocrit (hct) was increased at the end of exercise and remained increased until 60 min of recovery in both trials, with no difference between trials ( $P = 0.348$ ) (Table 4.2). Plasma  $PO_2$  was increased at the end of exercise in both trials, with no difference between trials ( $P = 0.901$ ) (Table 4.1). Plasma [Glucose] was increased from the end of exercise until 180 min of recovery, with no difference between trials ( $P = 0.399$ ) (Table 4.1).

#### **4.4 Discussion**

This study appears to be the first to detail the time course of acute changes in plasma dependent and independent acid-base variables in response to post exercise electrolyte supplementation in horses. The electrolytes were administered according to manufacturer's direction to replace the approximate sweat losses during exercise, and both the control and electrolyte treatments were designed to imitate recovery protocols typical of the industry and thus included meal feeding with water *ad libitum*. The nasogastric administration of a hypotonic commercial electrolyte solution in 8 L of water,

followed by a typical hay and grain meal, resulted in about a 2 mmol/L decreased plasma [TCO<sub>2</sub>] during the recovery period as compared to Control. The primary contributor to the decreased [TCO<sub>2</sub>] with electrolyte supplementation was a decreased [SID], as a result of the non-significant increase in plasma [Cl<sup>-</sup>].

### ***Independent Variables***

Despite the fact that none of the individual strong ions exhibited a treatment effect of electrolyte administration, plasma [SID] was lower during recovery in the Electrolyte trial. This result illustrates the importance of determining the independent variables as opposed to only looking at individual plasma electrolyte concentrations. In both trials plasma [Na<sup>+</sup>] was increased and plasma [K<sup>+</sup>] was decreased during the initial 60 and 120 min of recovery, respectively, but their concentrations did not differ with electrolyte treatment. This is in agreement with previous studies that found no change in plasma [Na<sup>+</sup>] or [K<sup>+</sup>] with either isotonic (Hyypya et al 1996) or hypertonic (Szucsik et al 2006) electrolyte supplementation when free access to water was provided. This is in large part due to the large distribution volume for supplemented electrolytes, comprising approximately 100 L of extracellular fluid and 200 L of intracellular fluid. The increase in plasma [Na<sup>+</sup>] during early recovery in both trials coincided with increases in [PP] and Hct, and thus is likely a result of a cyclical fluid shift between the plasma compartment and the gastrointestinal tract as a result of feeding (Waller et al 2005). The decrease in plasma [K<sup>+</sup>] during early recovery has been demonstrated previously after moderate (Hyypya et al 1996) and high (Waller and Lindinger 2005a) intensity exercise, and can be explained by high rates of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in previously contracting muscles (Lindinger et al 1995). Plasma [Cl<sup>-</sup>] in the present study did not differ from pre-exercise

throughout the Electrolyte trial, but was significantly decreased from pre-exercise from 300 to 480 min of recovery in the Control trial, indicating that electrolyte supplementation resulted in maintenance of plasma  $\text{Cl}^-$  homeostasis and effectively replaced sweat  $\text{Cl}^-$  losses. Equine sweat contains a high  $[\text{Cl}^-]$  (McCutcheon et al 1995) and decreases in plasma  $[\text{Cl}^-]$  have been demonstrated after endurance (Lindinger and Ecker 1995), moderate intensity (Ecker and Lindinger 1995) and high intensity exercise (Waller and Lindinger 2005a). The maintenance of plasma  $[\text{Cl}^-]$  in the present study resulted in an overall lower  $[\text{SID}]$  with electrolyte treatment, and provides evidence that supplementing with adequate electrolytes diminishes the post exercise alkalosis that results when sweat losses of  $\text{Cl}^-$  exceed those of  $\text{Na}^+$  (McCutcheon and Geor 1998).

In both trials, plasma  $\text{PCO}_2$  was decreased at the end of exercise, likely due to an increased alveolar ventilation as a result of muscular exercise (Parks and Manohar 1984), and increased in the initial 40-60 min of recovery. An increase in glycolytic activity post feeding may account for the increased  $\text{PCO}_2$  during early recovery, as Stutz et al (1992) also found an increase in venous  $\text{PCO}_2$  1 h after the feeding of a mixed hay and grain meal following exercise.

Electrolyte administration resulted in faster restoration of hydration status compared to Control, as evidenced by faster recovery of  $[\text{PP}]$  and  $[\text{A}_{\text{tot}}]$ . Plasma  $[\text{A}_{\text{tot}}]$  and  $[\text{PP}]$  returned to pre-exercise by 120 min of recovery when horses were administered the hypotonic electrolyte solution prior to consuming their typical post exercise meals and water *ad libitum*. In contrast, when the horses were provided with their normal meals and

water *ad libitum* (Control), [PP] and [ $A_{tot}$ ] remained increased from pre-exercise throughout the remainder of the sampling period. Total water intake was significantly greater over the 24 h recovery period when electrolytes were given, even though the supplement was hypotonic. Similarly, Sosa Leon et al (1998) found that horses administered a hypertonic electrolyte paste after furosemide-induced dehydration had higher water consumption and lower [PP] during the 6 h recovery period. In contrast to humans, equine sweat is hypertonic to plasma (McCutcheon et al 1995), resulting in an exercise-induced plasma dehydration that is isotonic to hypotonic. Thus when electrolytes lost in sweat are not replaced, there is no increase in plasma osmolality to stimulate a thirst response. Additionally, if water alone is given after exercise, this reduces plasma tonicity, resulting in greater suppression of thirst and excretion of the water with additional electrolytes in the urine (Schott and Hinchcliff 1993, Maughan and Lindinger 1995). Indeed, in the present study, plasma osmolality was increased up to 4 h after electrolyte administration, while in the Control trial plasma osmolality decreased throughout the recovery period such that it was significantly lower than pre-exercise by 6 h of recovery. In agreement with this, our data demonstrates that electrolyte supplementation after exercise is required for full recovery of hydration status, and that recovery of hydration status is still incomplete 24 h after exercise when no electrolytes are given.

The presence of dextrose in the electrolyte supplement serves as a direct source of glucose to provide cellular energy to subserve increased rates of epithelial transport of  $Na^+$  and water across the small intestine (Gisolfi et al 1992, Dyer et al 2002). The

amount of dextrose administered, even with 8 L of supplement, was insufficient to result in a glycemic effect compared to Control, indicating an appropriate dextrose concentration (31 mmol/L) of the Perform'N Win supplement.

### ***Dependent Variables***

This appears to be the first study to show that supplementation with a hypotonic electrolyte solution post exercise, in addition to a typical hay/grain meal and water *ad libitum*, resulted in decreased plasma [TCO<sub>2</sub>] during the recovery period as compared to water *ad libitum* and a typical hay/grain meal alone. When the physicochemical determinants of the dependent variables were quantified (Fig. 4b & 4c), the primary contributor to the decreased plasma [TCO<sub>2</sub>] with electrolyte administration was a decreased plasma [SID] from 2-8 h of recovery. The overall lower plasma [SID] in the Electrolyte trial can be entirely attributed to a tendency towards increased plasma [Cl<sup>-</sup>] during recovery compared to Control. An increased plasma [A<sub>tot</sub>] during early recovery also contributed to decreased [TCO<sub>2</sub>] in the Electrolyte trial, however [A<sub>tot</sub>] returned to pre-exercise by 120 min of recovery. In contrast, an increased [A<sub>tot</sub>] throughout the Control trial actually contributed a slight acidifying effect throughout recovery. Finally, despite an overall decreased PCO<sub>2</sub> with electrolyte supplementation, PCO<sub>2</sub> had little effect on plasma [TCO<sub>2</sub>] during either the Electrolyte or Control trial.

The literature is limited with respect to previous studies on the effects of electrolyte supplementation on equine acid-base balance. Szucsik et al (2006) reported that administration of 7 different commercial electrolyte supplements as hypertonic pastes

prior to a simulated race test had no effect on plasma  $[\text{TCO}_2]$ , however the amounts of electrolytes given corresponded to only an approximate 2 L sweat loss, and the time course was limited to only two post exercise samples at 60 and 90 min of recovery. In contrast, when horses were administered a greater amount of electrolytes (51g Na, 24g K, 99g Cl) as a hypertonic paste after a furosemide-induced dehydration (mean losses of water, Na, K and Cl were 18 L, 40g, 20g and 75g, respectively), plasma  $[\text{H}^+]$  and  $[\text{HCO}_3^-]$  had returned to pre-furosemide levels by 2 h post administration, while a plasma alkalosis persisted in the water treated control horses (Sosa Leon et al 1998). In the present study, coinciding with the decreased  $[\text{TCO}_2]$ , plasma  $[\text{H}^+]$  also showed a tendency to be increased in the electrolyte horses as compared with a more alkalotic control, and this was entirely due to decreases in  $[\text{SID}]$ .

An improved understanding of the effects of post exercise electrolyte supplementation on acid-base status is of practical interest to the racing community. A plasma  $[\text{TCO}_2]$  testing threshold of greater than  $37 \text{ mmol L}^{-1}$  is used by many racing jurisdictions to determine whether a horse has been administered an alkalinizing agent for the purpose of performance enhancement (see Lindinger and Waller 2008). Interestingly, pre-exercise plasma  $[\text{TCO}_2]$  for the Electrolyte and Control trials in the present study was  $37.3 \pm 0.7$  and  $37.4 \pm 0.5 \text{ mmol L}^{-1}$ , respectively, with these horses fed a typical racehorse diet. In the Control trial, which was intended to imitate a typical recovery protocol in the Standardbred racing industry, plasma  $[\text{TCO}_2]$  was above  $39 \text{ mmol L}^{-1}$  for the first hour of recovery, and remained above  $37 \text{ mmol L}^{-1}$  for the entire duration of sampling. Accordingly, the results of this study suggest that some horses may naturally demonstrate

[TCO<sub>2</sub>] in excess of the testing threshold, even when no alkalinizing substances have been given. Indeed a previous study by this laboratory also found that Standardbreds exhibited increased plasma [TCO<sub>2</sub>] 90-120 min after short duration high intensity exercise, due to decreases in plasma [Cl<sup>-</sup>] (Waller and Lindinger 2005b). It is concluded that sweat-induced losses of Cl<sup>-</sup> can significantly increase [TCO<sub>2</sub>] by as much as 2 mmol/L with an 8L dehydration. This is also an important concern with dehydration due to equine transport (Friend 2000), excitement (McConaghy et al 1995), and/or high ambient temperatures (Kerr and Snow 1983). Additionally, supplementation with electrolytes according to estimated sweat losses attenuates decreases in plasma [Cl<sup>-</sup>] and results in decreased [TCO<sub>2</sub>] compared to when no electrolytes are given.

#### **4.5 Conclusions**

The present study quantified the magnitude and time course of the main physicochemical determinants of acid-base status in Standardbreds after post exercise electrolyte administration, and compared them to a control recovery protocol. The nasogastric administration of a hypotonic commercial electrolyte solution, followed by a typical hay and grain meal, resulted in decreased plasma [TCO<sub>2</sub>] during the recovery period as compared to control. The primary contributor to the decreased [TCO<sub>2</sub>] with electrolyte supplementation was a decreased [SID], as a result of non-significant increases in plasma [Cl<sup>-</sup>]. It is concluded that oral administration of a hypotonic electrolyte solution after prolonged moderate intensity exercise diminishes the post exercise alkalosis, and that recovery of hydration status is still incomplete 24 h after exercise when no electrolytes are given.

**Table 4.1. Jugular vein plasma ion and metabolite concentrations and PO<sub>2</sub> at rest and during recovery from a Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by a typical feeding protocol (Electrolyte trial), or 2) a typical feeding protocol alone (Control trial).**

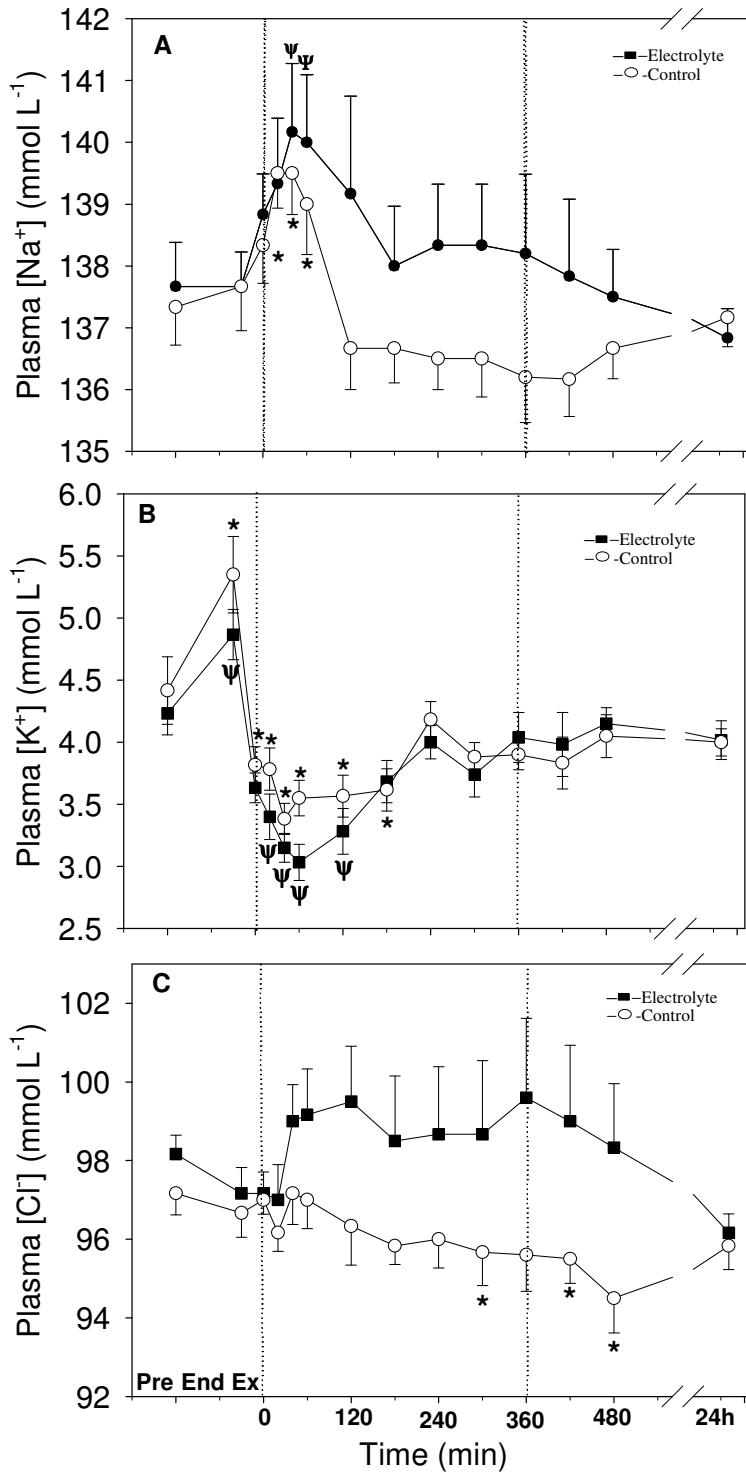
Time (min)	[Ca <sup>2+</sup> ]		[Glucose]		[Lactate]		PO <sub>2</sub>	
	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control
Pre-Ex	1.42 ±	1.42 ±	0.5 ±	0.5 ±	5.5 ±	5.3 ±	81.6 ±	73.3 ±
	0.01	0.01	0.1	0.1	0.2	0.2	20.0	11.0
End Ex	1.26 ±	1.27 ±	3.4 ±	3.4 ±	7.0 ±	6.7 ±	125.1 ±	128.4 ±
	0.01*	0.02*	0.9*	1.3*	0.3*	0.3*	16.4*	11.5*
0'	1.34 ±	1.36 ±	1.3 ±	1.4 ±	6.3 ±	6.4 ±	72.0 ±	119.1 ±
	0.01*	0.03*	0.2	0.3*	0.3*	0.3*	17.1	14.4*
20'	1.38 ±	1.33 ±	1.1 ±	1.0 ±	7.5 ±	6.4 ±	53.6 ±	40.9 ±
	0.03*	0.02*	0.2	0.3	0.5*	0.2*	18.8	4.6
40'	1.37 ±	1.35 ±	0.9 ±	0.8 ±	8.0 ±	6.8 ±	46.1 ±	48.0 ±
	0.01*	0.02*	0.2	0.1	0.6*	0.3*	5.3	5.4
60'	1.40 ±	1.43 ±	0.8 ±	1.0 ±	8.2 ±	7.5 ±	47.1 ±	43.4 ±
	0.01	0.02	0.2	0.1	0.6*	0.3*	4.6	3.7
120'	1.45 ±	1.49 ±	1.0 ±	0.9 ±	8.1 ±	7.9 ±	70.2 ±	70.2 ±
	0.02	0.01*	0.1	0.1	0.5*	0.4*	10.5	14.2
180'	1.48 ±	1.49 ±	1.0 ±	1.0 ±	7.1 ±	7.1 ±	78.5 ±	63.8 ±
	0.01*	0.01*	0.1	0.1	0.4	0.3*	14.1	11.5
240'	1.48 ±	1.43 ±	0.9 ±	0.9 ±	6.1 ±	6.6 ±	86.2 ±	80.4 ±
	0.01*	0.02	0.1	0.1	0.3	0.2*	16.6	16.8
300'	1.48 ±	1.48 ±	0.9 ±	0.8 ±	5.6 ±	5.7 ±	80.1 ±	51.8 ±
	0.01*	0.01*	0.1	0.1	0.3	0.1	24.9	7.4
360'	1.50 ±	1.48 ±	0.9 ±	0.6 ±	5.6 ±	5.1 ±	61.4 ±	78.1 ±
	0.01*	0.02*	0.1	0.2	0.3	0.1	10.2	14.2
420'	1.46 ±	1.47 ±	0.7 ±	0.6 ±	5.7 ±	5.8 ±	70.6 ±	77.6 ±
	0.01	0.01*	0.1	0.2	0.3	0.1	24.8	24.7
480'	1.48 ±	1.46 ±	0.6 ±	0.6 ±	6.0 ±	5.6 ±	49.3 ±	36.7 ±
	0.01*	0.01	0.1	0.1	0.2	0.2	10.6	2.9*
24h	1.47 ±	1.45 ±	0.7 ±	0.7 ±	5.7 ±	5.6 ±	76.8 ±	91.1 ±
	0.01*	0.01	0.1	0.1	0.3	0.3	13.5	20.2

Values are mean ± SE in mmol L<sup>-1</sup> except pO<sub>2</sub> in mmHg. \* Significantly different (P<0.05) from pre-exercise time point.

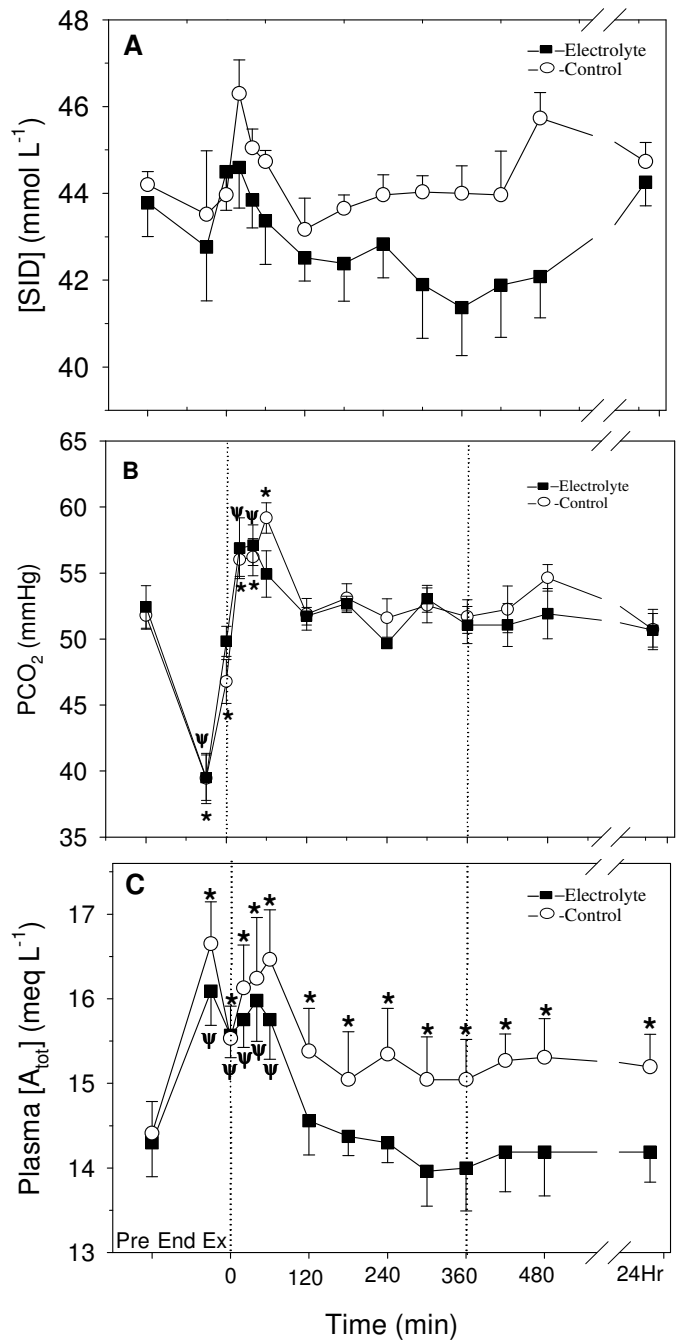
**Table 4.2. Jugular vein plasma pH, hematocrit, and bicarbonate and protein concentrations at rest and during recovery from a Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by a typical feeding protocol (Electrolyte trial), or 2) a typical feeding protocol alone (Control trial).**

Time (min)	pH		[HCO <sub>3</sub> <sup>-</sup> ]		Hct		[PP]	
	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control
Pre-Ex	7.44 ± 0.01	7.45 ± 0.01	35.7 ± 0.7	35.8 ± 0.6	34.5 ± 1.2	35.2 ± 0.9	6.3 ± 0.1	6.4 ± 0.2
End Ex	7.50 ± 0.01*	7.51 ± 0.01*	31.0 ± 0.9*	31.4 ± 1.2*	49.0 ± 0.9*	48.5 ± 1.4*	7.3 ± 0.2*	7.4 ± 0.2*
0'	7.46 ± 0.01*	7.48 ± 0.01*	35.5 ± 0.8	34.6 ± 0.8	37.7 ± 0.9*	37.8 ± 1.3*	7.0 ± 0.1*	6.9 ± 0.2*
20'	7.42 ± 0.02	7.44 ± 0.01	36.7 ± 1.0	38.2 ± 0.5*	38.0 ± 0.6*	37.5 ± 1.0*	7.1 ± 0.2*	7.2 ± 0.2*
40'	7.41 ± 0.01*	7.43 ± 0.01	36.0 ± 0.6	37.5 ± 0.7	38.8 ± 1.0*	38.2 ± 0.7*	7.2 ± 0.2*	7.3 ± 0.3*
60'	7.41 ± 0.01*	7.41 ± 0.01*	34.9 ± 0.8	37.2 ± 0.4	38.8 ± 0.7*	38.2 ± ±0.8*	7.1 ± 0.2*	7.4 ± 0.3*
120'	7.43 ± 0.01	7.44 ± 0.01	34.5 ± 0.4	35.7 ± 1.0	37.4 ± 1.2	37.5 ± 1.3	6.6 ± 0.2	6.9 ± 0.2*
180'	7.43 ± 0.01	7.45 ± 0.01	35.1 ± 1.0	36.9 ± 1.0	36.5 ± 1.0	37.8 ± 0.9	6.5 ± 0.1	6.7 ± 0.3*
240'	7.44 ± 0.01	7.45 ± 0.01	33.9 ± 0.7	36.4 ± 1.3	39.8 ± 1.4*	41.0 ± 1.2*	6.4 ± 0.1	6.9 ± 0.2*
300'	7.43 ± 0.01	7.45 ± 0.01	35.2 ± 1.2	36.5 ± 0.8	35.3 ± 1.6	36.2 ± 0.7	6.5 ± 0.2	6.7 ± 0.2*
360'	7.43 ± 0.01	7.45 ± 0.01	34.2 ± 1.3	36.0 ± 0.4	36.0 ± 1.1	36.8 ± 0.4	6.5 ± 0.2	6.7 ± 0.2*
420'	7.44 ± 0.02	7.45 ± 0.01	34.6 ± 1.2	36.4 ± 0.5	36.0 ± 0.8	37.5 ± 0.4*	6.5 ± 0.2	6.8 ± 0.2*
480'	7.43 ± 0.01	7.44 ± 0.01	34.8 ± 1.1	37.6 ± 0.4	37.6 ± 0.5	37.2 ± 0.5	6.6 ± 0.2	6.8 ± 0.2*
24h	7.45 ± 0.01	7.45 ± 0.01	35.0 ± 0.5	35.7 ± 0.4	40.8 ± 0.9	40.8 ± 0.9*	6.6 ± 0.2	6.8 ± 0.2*

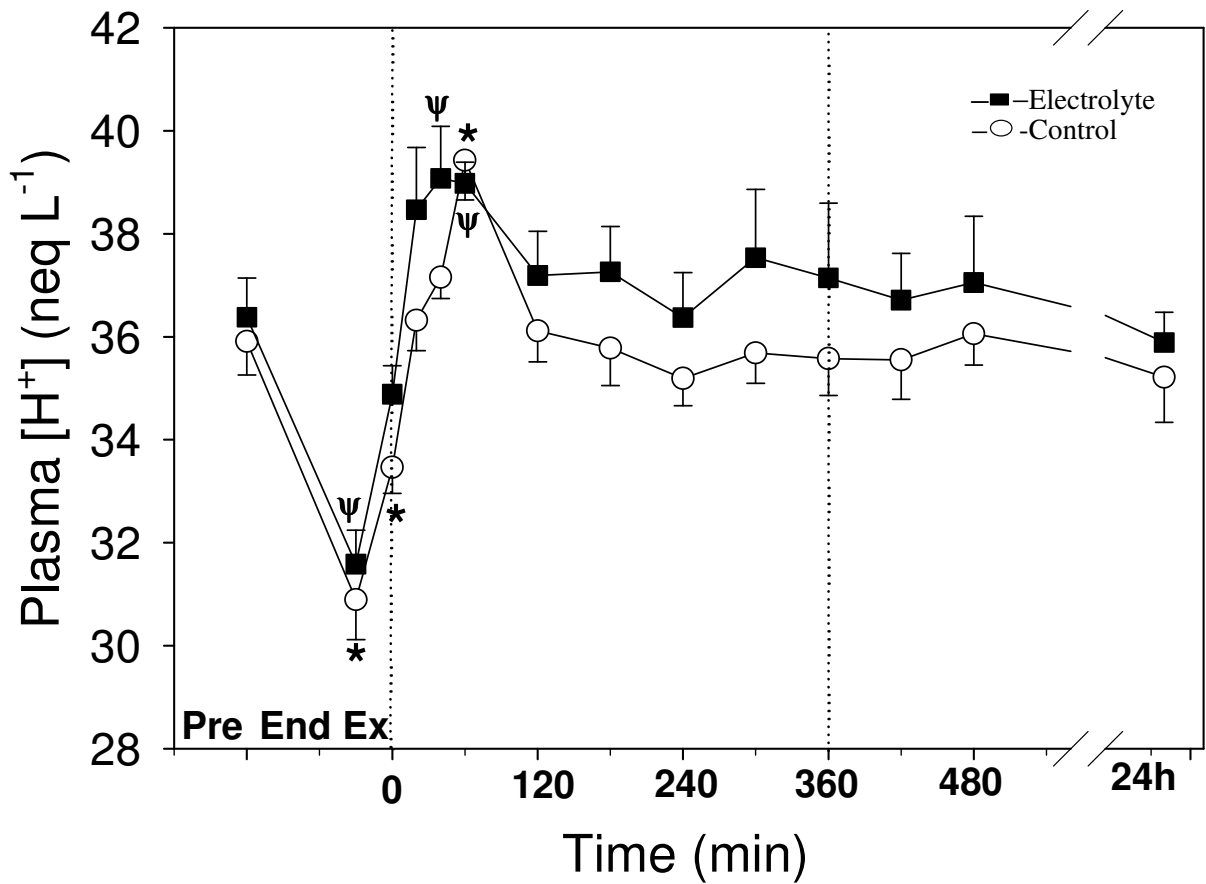
Hct = hematocrit, [PP] = [plasma protein]. Values are mean ± SE. [HCO<sub>3</sub><sup>-</sup>] in mmol L<sup>-1</sup>, Hct in % and [PP] in g dL<sup>-1</sup>. \* Significantly different (P<0.05) from pre-exercise time point.



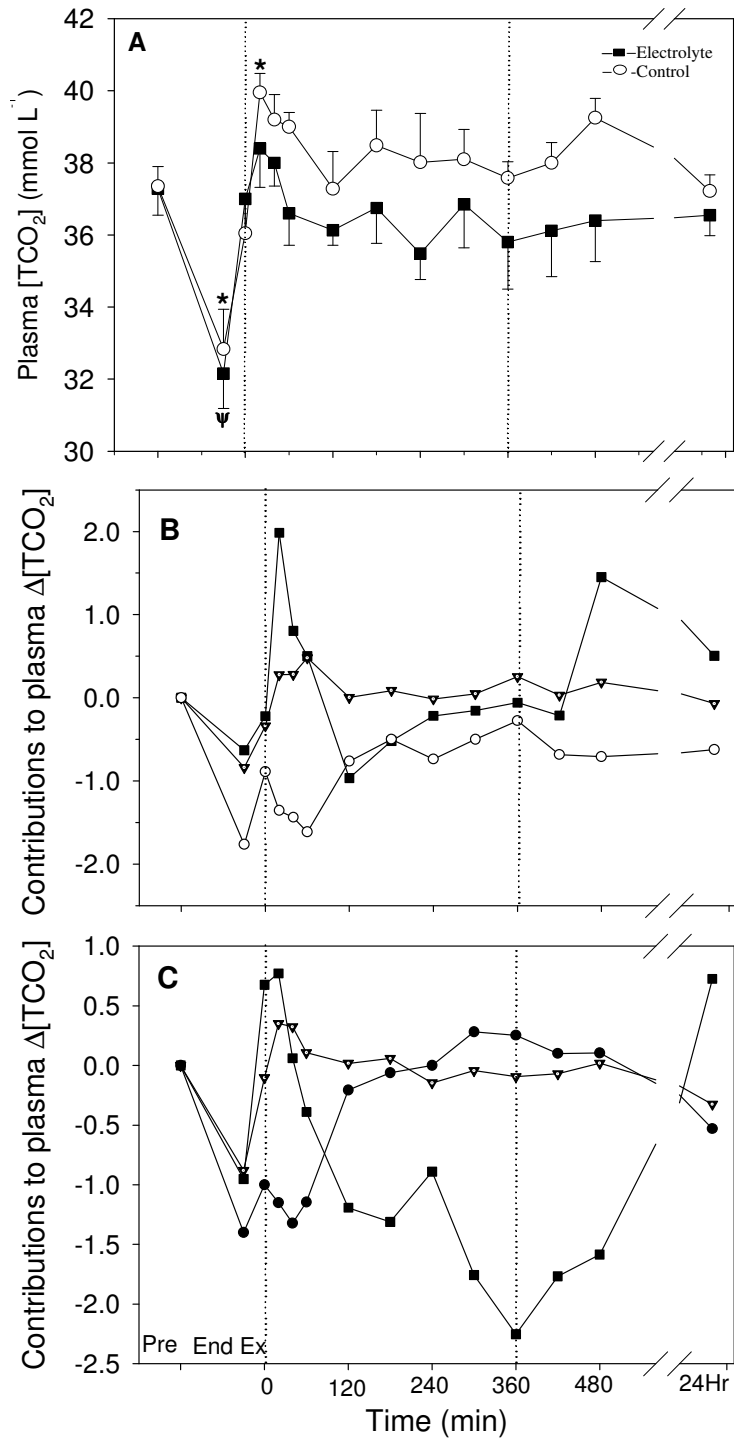
**Figure 4.1. The time course of the plasma electrolytes: (A) sodium ( $\text{Na}^+$ ), (B) potassium ( $\text{K}^+$ ), and (C) chloride ( $\text{Cl}^-$ ), after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line).  $\circ$  denotes Control trial;  $\blacksquare$  denotes Electrolyte trial. Values are mean  $\pm$  SE for 6 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and Electrolyte trials, respectively.



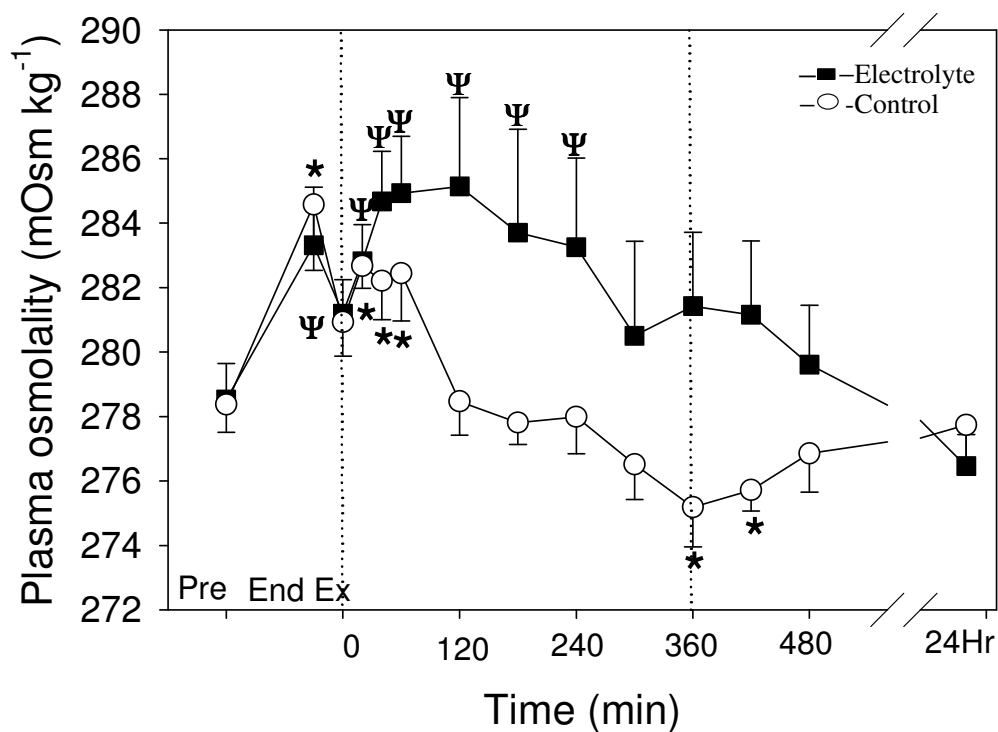
**Figure 4.2. The time course of the independent acid-base variables in plasma: (A) strong ion difference ([SID]), (B) partial pressure of carbon dioxide (PCO<sub>2</sub>), and (C) total weak acid concentration ([A<sub>tot</sub>]), after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control trial; ■ denotes Electrolyte trial. Values are mean ± SE for 6 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and Electrolyte trials, respectively.



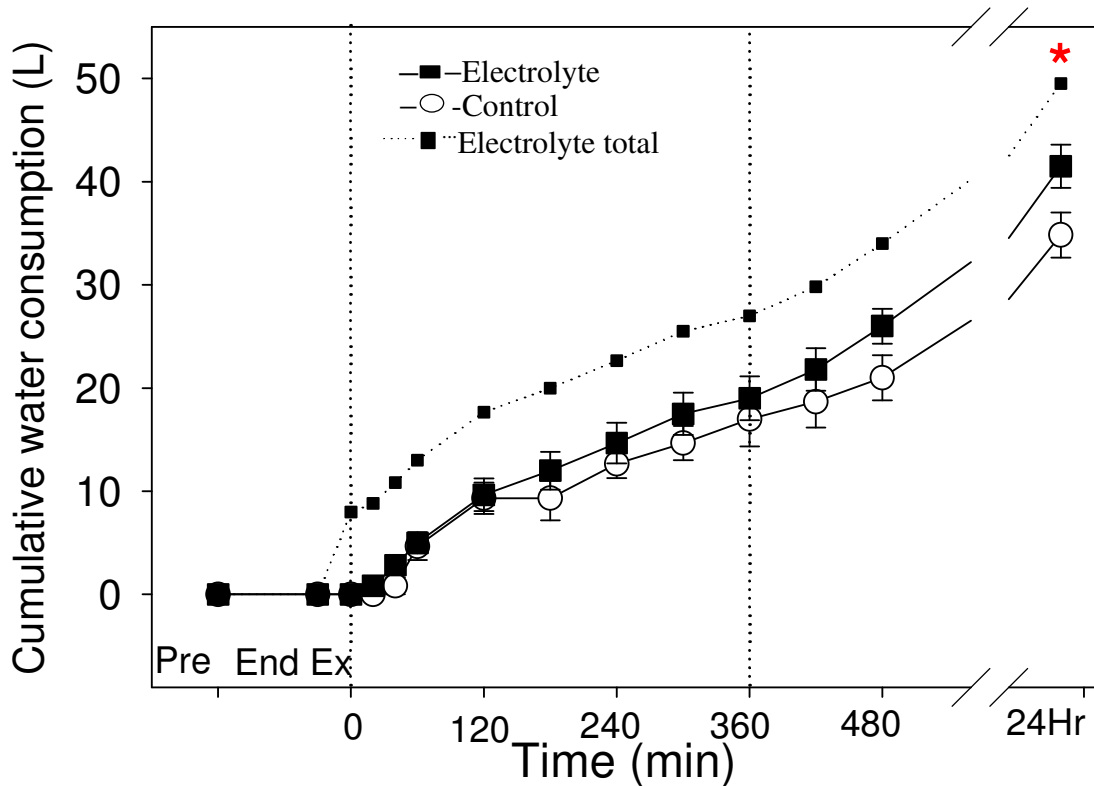
**Figure 4.3.** The time course of the dependent acid-base variable plasma hydrogen ion concentration ( $[H^+]$ ), after a Competition Exercise Test. The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line).  $\circ$  denotes Control trial;  $\blacksquare$  denotes Electrolyte trial. Values are mean  $\pm$  SE for 6 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and Electrolyte trials, respectively.



**Figure 4.4. (A) The time course of plasma total carbon dioxide concentration ([TCO<sub>2</sub>]), after a Competition Exercise Test, and the contributions of the independent variables strong ion difference ([SID], ■), total weak acid concentration ([A<sub>tot</sub>], ○) or partial pressure of carbon dioxide (PCO<sub>2</sub>, ▼) to the change in plasma [TCO<sub>2</sub>], for (B) the Control trial and (C) the Electrolyte trial.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control trial; ■ denotes Electrolyte trial. Values are mean ± SE for 6 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and Electrolyte trials, respectively.



**Figure 4.5. The time course of the changes in plasma osmolality, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control trial; ■ denotes Electrolyte trial. Values are mean ± SE for 6 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and Electrolyte trials, respectively.



**Figure 4.6. The time course of cumulative water consumption, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte trial), or stood in stocks (Control trial). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control trial; ■ denotes Electrolyte trial; ■.....■ denotes total water intake for the Electrolyte trial. Values are mean ± SE for 6 horses. \*: significantly different from Control.

## Chapter 5

### **Fluid and electrolyte supplementation after prolonged moderate intensity exercise enhances muscle glycogen resynthesis in Standardbred horses**

#### **5.1 Introduction**

In the horse, both short term high intensity and prolonged submaximal exercise result in dehydration, loss of water and electrolytes and depletion of skeletal muscle glycogen (Snow et al 1981, Snow and Harris 1991, Lacombe et al 1999, 2001, Hyypa et al 1997, Waller and Lindinger 2005). Resynthesis of muscle glycogen stores requires 48-72 hours in horses, compared to 2.5 h in rats (Garetto et al 1984) and less than 24 h humans (Costill et al 1981). Previous studies in horses have altered post exercise nutrition strategies in attempts to enhance muscle glycogen resynthesis. Despite demonstrations that i.v. infusion of large amounts of glucose (3-6 g kg<sup>-1</sup>) modestly accelerated muscle glycogen replenishment (Davie et al 1995, Geor et al 2006), conventional feeding strategies have not been successful (Snow and Harris 1991, Topliff et al 1983, Topliff et al 1985, Hyypa et al 1997). Initial glycogen resynthesis rates in horses after ingestion of meals with varying soluble CHO contents ranged from ~8 to ~12 mmol kg<sup>-1</sup> dw h<sup>-1</sup> for mixed and high soluble CHO diets, respectively. In contrast, in humans initial glycogen storage rates are ~40 mmol kg<sup>-1</sup> dw h<sup>-1</sup> when at least 5 g kg<sup>-1</sup> of soluble CHO is provided during the first 4 hours after alternating high and low intensity interval exercise (Jentjens et al 2001).

There is considerable anecdotal and practical information that dehydrated horses will not eat, while most forms of glycogen-depleting exercise in horses result in dehydration (Lindinger et al 2004, Waller and Lindinger 2005a). Water alone is not effective in restoring hydration, as there is also a requirement for electrolytes to osmotically retain water in the appropriate extracellular and intracellular fluid compartments (Maughan et al 1994, Jansson and Dahlborn 1999). Interestingly, i.v. infusion of large amounts of saline alone (~20 L in 12 h for a total of 107 g NaCl) resulted in glycogen replenishment rates of ~ 7.6 mmol kg<sup>-1</sup> dw h<sup>-1</sup> during the first 12 h of exercise recovery in horses (Lacombe et al 2001), as compared to typical rates of ~8 to ~12 mmol kg<sup>-1</sup> dw<sup>-1</sup> h for mixed and high soluble CHO diets (Lacombe et al 2004), suggesting a possible role for rehydration in glycogen resynthesis.

The resynthesis of glycogen after exercise requires adequate intracellular water and K<sup>+</sup>. Within muscle and liver, glycogen is stored in a hydrated form (up to 3 g water/ g glycogen) (Costill 1988) tightly associated with 0.5 mmol K<sup>+</sup> / g of glycogen (Bergstrom and Hultman 1966a). Therefore, as muscle contraction occurs and glycogen is utilized, there occurs concurrent release of water and K<sup>+</sup> from the glycogen store. In humans and rodents, prolonged exercise results in net losses of water (Costill et al 1976) and K<sup>+</sup> (Lindinger et al 1995) from contracting muscle, however this may not be the case in horses. It has previously been reported that intracellular water (Lindinger et al 2004) and K<sup>+</sup> (Wilson et al 1998) may be preserved during exercise in horses, despite ongoing glycogen degradation and extracellular dehydration. However the concurrent changes in

intracellular water,  $K^+$  and glycogen content during exercise in horses has not been studied. Furthermore, cell shrinkage (as occurs with dehydration) is associated with decreased glycogen synthesis in skeletal muscle (Low et al 1996) and hepatocytes (Haussinger 1996) and increased carbohydrate oxidation and glycogenolysis (Keller et al 2003). Therefore it is reasonable to postulate that post exercise dehydration may be one reason why muscle glycogen replenishment is so slow in horses.

The purpose of the present study was to determine the effect of administering a hypotonic electrolyte solution immediately after prolonged exercise, and before feed is provided, on rehydration and muscle glycogen and electrolyte recovery in horses. It was hypothesized that provision of a balanced electrolyte solution, immediately followed by a typical hay and grain meal, after glycogen-depleting exercise will result in a faster rate of muscle glycogen resynthesis than a grain/hay meal with voluntary access to water alone.

## **5.2 Methods**

**Animals:** 6 Standardbred geldings (body mass  $464 \pm 10$  kg; age 5-12 yrs) from the University of Guelph research herd were used. The study took place in June and July, and horses underwent a 4-6 week diet and exercise acclimation period during which they were housed in individual box stalls with 7 h of paddock turnout during the day. Horses were exercise conditioned 5 days/week on a high speed treadmill (SATO, Sweden) and outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada), until able to comfortably perform a ~60 minute competitive exercise test (CET) (Marlin et al 1996, 1999) on a high speed treadmill designed to significantly utilize muscle glycogen stores

(Poso and Hyypa 1999) and result in a total body water loss of 8-10L. The CET is designed to simulate the 2<sup>nd</sup> day (speed and endurance test – classic format) of a one star CCI 3-day event, and includes the following phases: 10 min walk (1.7 m/s), 10 min trot (3.7 m/s), 2 min gallop (10.0 m/s), 20 min trot (3.7 m/s), 10 min walk (1.7 m/s), 8 min canter (8.0 m/s), and 10 min walk (1.7 m/s).

The horses were maintained on a diet consisting of oats twice daily and mixed grass hay three times daily (Table 5.1), with free access to water and a salt block. The amount of feed given was increased over this acclimation period such that during the final two weeks the horses were receiving 4 kg oats and 6 kg hay daily, and there were no significant changes in the body masses of the horses during this time. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

***Experimental Protocol:*** The study consisted of an electrolyte treatment and control, thus each horse performed the CET twice in randomized order, separated by an 8-10 day interval during which time exercise conditioning was maintained (Marlin et al 1999, Hyypa et al 1996). A timeline of the experimental protocol is shown in Figure 5.1. On both sampling days beginning at 7 am, catheters (14-gauge, 5.25 in; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted anterograde into the left and right jugular veins, and four-way stopcocks with 50 cm extensions were attached to the

catheters for ease of blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl.

Pre-exercise blood and muscle samples were taken at 8 am and then the CET was performed. Immediately upon completion of the final canter an 'end of exercise' blood sample was taken, following which the horse walked for 10 min. Ten minutes after cessation of exercise, the horse either 1) received by naso-gastric tube a commercially available electrolyte solution (Perform'N Win, Buckeye Nutrition, Dalton, OH) designed to replace an 8 L sweat loss (12 g Na, 24 g Cl, 9 g K, 1 g Ca & Mg), in 8 L of water (Osmolality = 212 mOsm kg<sup>-1</sup>), or 2) stood in stocks for equivalent amount of time (~10 min). Muscle samples were collected from the gluteus medius by use of the needle biopsy technique (Lindholm and Piehl 1974). Samples were collected by use of aseptic technique after desensitization of the area with 2% mepivacaine (Upjohn, Orangeville, ON). For each trial muscle samples were collected at a uniform depth from 4 standardized sites in the left or right middle gluteal muscles. Biopsies were collected before exercise (baseline), 15 min after cessation of exercise (0 h), and at 4 h and 24 h post treatment. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. In both treatments, within 20 minutes of cessation of exercise the horses were given 2 kg oats and 2 kg hay (0 min of recovery), with access to water *ad libitum*. Horses were given 2 kg oats and 2 kg hay at 6 h of recovery, and 2 kg hay at 12 h recovery. Blood samples were taken at 20-60 min intervals up to 8 h of recovery, and again at 24 h of recovery; the horses remained in their stalls for the duration of sampling.

**Sample Analyses:** Each blood sample was collected into a 7 ml lithium heparinized vacutainer and immediately analyzed for the plasma concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, lactate<sup>-</sup> using a Nova Stat Profile 9<sup>+</sup> (NOVA Biomedical, Waltham, MA). Blood was then transferred into two 1.5 ml Eppendorf centrifuge tubes and centrifuged for 5 min at 15000g to separate the plasma. Plasma protein concentration ([PP]) was determined by refractometry (CV 0.83%) (Atago clinical refractometer model SPR-T2; Atago, Tokyo, Japan).

Total body water loss during the CET was determined as the change in body mass after accounting for fecal losses.

Plasma osmolality was calculated according to the formula of Brownlow and Hutchins (1982) for equine plasma such that:

$$\text{Osmolality (mOsm kg}^{-1}\text{)} = 1.86([\text{Na}^+] + [\text{K}^+]) + [\text{Glucose}] + [\text{Lactate}^-] + 9$$

Muscle biopsies were freeze-dried, dissected of all visible blood, connective tissue and fat, and powdered for subsequent analysis. Muscle H<sub>2</sub>O content (L kg<sup>-1</sup> dry wt) was determined as the difference in sample weight before and after freeze-drying. Skeletal muscle creatine, phosphocreatine (PCr), ATP, lactate and glycerol 3 phosphate (G-3-P) were determined by enzymatic spectrophotometric assays (Bergmeyer 1974, Harris et al 1974). Glycogen content (as glucosyl units) was determined in duplicate after acid

hydrolysis as described by Passonneau and Lauderdale (1974). All muscle measurements were corrected for the highest total creatine measured from each horse.

Muscle ion contents were determined by instrumental neutron activation analysis (INAA) (Lindinger and Heigenhauser 1987). Muscle total tissue water (TTW) ( $\text{L kg}^{-1}$  wet wt) was determined from muscle  $\text{H}_2\text{O}$  contents after correcting for total creatine and muscle wet wt: dry wt. Muscle extracellular fluid volume (ECFV) was calculated using two different versions of the  $\text{Cl}^-$ -space method. One version was calculated as per Lindinger and Heigenhauser (1987), using an intracellular  $[\text{Cl}^-]$  derived from the relationship between membrane potential and plasma  $[\text{Cl}^-]$ . The other version was calculated as per Manery and Bale (1941), using the tissue and plasma  $\text{Cl}^-$  contents and Donnan factors for each ion. The intracellular ion contents ( $\text{ion}_i$ ) were calculated from total muscle ion contents ( $\text{ion}_T$ ) measured by INAA and from the quantity of ion in the muscle extracellular space ( $\text{ion}_{\text{ECF}}$ ) such that

$$\text{ion}_i (\text{meq kg}^{-1} \text{ wet wt}) = \text{ion}_T - \text{ion}_{\text{ECF}}$$

where  $\text{ion}_{\text{ECF}}$  for each individual muscle was determined as

$$\text{ion}_{\text{ECF}} = \text{ion}_{\text{plasma}} (\text{meq L}^{-1}) * \text{ECFV} (\text{L kg}^{-1} \text{ wet wt})$$

The intracellular fluid volume for each muscle was calculated as the difference between the total tissue water (TTW) and the ECFV. The intracellular ion contents ( $\text{ion}_i$ ) calculated using the method of Manery and Bale (1941) were converted to units of concentration by:

$$[\text{ion}]_{\text{ICF}} (\text{meq L}^{-1}) = \text{ion}_i / \text{ICFV} (\text{L kg}^{-1} \text{ wet wt})$$

**Statistics.** Data are presented as mean  $\pm$  standard error. Ion contents and concentrations are expressed in equivalents, a form which takes the ionic valency into consideration. For monovalent ions 1 mmol = 1 meq, and for divalent ions 1 mmol = 2 meq. Statistical analysis was performed with SigmaStat (Systat Software Inc, San Jose, CA). Changes over time within treatments were assessed by one-way repeated measures analysis of variance. Differences between treatments and time points during the recovery period were assessed by two-way repeated measures analysis of variance. When a significant F-ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when  $P \leq 0.05$  at a power of 0.8.

### **5.3 Results**

Ambient temperature, humidity and total body water loss during the Control CET were  $23.9 \pm 0.4$  °C,  $68.4 \pm 2.1$  % and  $8.4 \pm 0.3$  L, respectively. Ambient temperature, humidity and total body water loss during the Electrolyte CET were  $23.6 \pm 0.3$  °C,  $70.2 \pm 2.0$  % and  $8.2 \pm 0.4$  L, respectively.

All horses consumed all the feed offered by the 24 h sample. Every horse finished all of the oats given by 20 min post feeding (~40 minutes post exercise), and had consumed all the hay given by 3-4 h post feeding.

***Muscle metabolites.***

Exercise significantly decreased middle gluteus muscle glycogen content by 21.9 and 22.6% from the initial (Pre-Ex) values the Electrolyte and Control treatments, respectively (Fig. 5.2, Table 5.2). Electrolyte enhanced muscle glycogen replenishment such that at 4 h of recovery muscle glycogen content was not different from Pre-Ex, with a further significant increase at 24 h. In contrast, in the Control treatment glycogen was still significantly decreased from Pre-Ex at 4 h. By 24 h of recovery, muscle glycogen content was not different from Pre-Ex values in either treatment. Muscle glycogen content at 24 h of recovery was 108.9 and 93.4 % of its initial Pre-Ex value in the Electrolyte and Control treatments, respectively. The rate of glycogen resynthesis from 0-4 h of recovery was almost triple in Electrolyte ( $15.0 \pm 9.0 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ) as compared to Control ( $5.7 \pm 9.8 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ) ( $P = 0.440$ ) (Fig. 2). From 4-24 hrs of recovery the rates of glycogen resynthesis were  $5.1 \pm 1.6$  and  $3.4 \pm 2.8 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$  for the Electrolyte and Control trials, respectively ( $P = 0.723$ ). The overall (0-24 h) mean rates of glycogen synthesis were  $6.8 \pm 2.0$  and  $3.8 \pm 1.3 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$  for the Electrolyte and Control treatments, respectively ( $P = 0.343$ ).

There was no effect of time or treatment on muscle ATP, creatine, PCr (Table 5.3), lactate or G-3-P (Table 5.2). in either the Electrolyte or Control treatment

### ***Muscle electrolytes and water content.***

Measured muscle H<sub>2</sub>O contents are shown in Table 5.2. Calculating muscle ECFV and ion contents as per Manery and Bale (1941) appeared to give more accurate values as compared to the method of Lindinger and Heigenhauser (1987), which tended to

overestimate muscle ECFV and therefore resulted in  $\text{Cl}^-$  and  $\text{Na}^+$  contents that appeared to be too low. Therefore from this point onward, only the muscle ion and fluid contents and concentrations as calculated per Manery and Bale (1941) will be discussed, however the values calculated as per Lindinger and Heigenhauser (1987) are shown in Tables 5.4 and 5.5 for comparison.

Muscle electrolyte concentrations are shown in Figure 5.4. There were no changes in  $[\text{Cl}^-]_{\text{ICF}}$  in the Electrolyte treatment, while in Control  $[\text{Cl}^-]_{\text{ICF}}$  was decreased from Pre-Ex at 0 and 4 h of recovery (Fig. 5.4a). There were no changes in  $[\text{Na}^+]_{\text{ICF}}$  (Fig. 5.4b) in the Electrolyte treatment, while in Control  $[\text{Na}^+]_{\text{ICF}}$  at 24 h of recovery was significantly decreased from 0 h.  $[\text{K}^+]_{\text{ICF}}$  (Fig. 5.4c) and  $[\text{Mg}^{2+}]_{\text{ICF}}$  (Fig. 5.4d) did not change throughout sampling in either treatment.

Gluteus medius muscle electrolyte contents are shown in Table 5.4. Muscle  $\text{Ca}^{2+}$  (not shown) was  $<1000$  ppm ( $\sim 6.5$  meq  $\text{kg}^{-1}$  wet wt) for all samples and therefore below the limits of detection with INAA.

Muscle ECFV showed no significant changes over time in either treatment (Fig 5.3a, Table 5.5). In the Electrolyte treatment ICFV (Table 5.5) was increased 18% ( $P= 0.038$ ) from Pre-Ex at 4 h of recovery, compared to only 4% (NS) in the Control treatment.

### ***Plasma glucose, protein and osmolality, and water consumption***

Plasma [glucose] was increased from the end of exercise up to 3 h of recovery in the Electrolyte treatment, reaching a maximum of  $8.18 \pm 0.6 \text{ mmol L}^{-1}$  at 1 h of recovery (Fig. 5.5a). Plasma [glucose] in the Control treatment was increased from the end of exercise until 4 h of recovery, reaching a maximum of  $7.90 \pm 0.4 \text{ mmol L}^{-1}$  at 2 h of recovery. There was no difference between treatments ( $P = 0.377$ ). Plasma [protein] ([PP]) was increased at the end of exercise until 1 h of recovery in the Electrolyte treatment, and until the end of sampling in the Control, with no difference between treatments ( $P = 0.169$ ) (Fig. 5.5b). Calculated plasma osmolality (Fig. 5.5c) was increased at the end of exercise, and from 20 min to 1 h and 20 min to 4 h of recovery in the Control and Electrolyte treatments, respectively. Plasma osmolality decreased throughout the recovery period in the Control treatment such that it was significantly lower than Pre-Ex from 6-7 h of recovery, however there was no difference between treatments ( $P = 0.104$ ).

Total water intake (including the 8 L given via nasogastric intubation) was greater in the Electrolyte treatment, such that at 24 h of recovery was  $49.5 \pm 4.2 \text{ L}$  and  $34.8 \pm 2.2 \text{ L}$  in the Electrolyte and Control treatments, respectively ( $P = 0.018$ ) (Fig. 5.6). Total water intake over the entire 24 h of recovery also showed a treatment effect, with total intake in the Electrolyte treatment significantly greater than Control ( $P = 0.014$ ).

## **5.4 Discussion**

The present study appears to be the first to have determined the effects of administering a hypotonic electrolyte solution immediately after prolonged exercise, and before feed is

provided, on skeletal muscle glycogen and electrolyte contents in horses. The reason for early administration of the electrolyte solution was to enhance the rate of recovery of fluid and electrolyte balance and ensure that there was adequate water and  $K^+$  available for glycogen resynthesis. The volume of water and amount of electrolytes administered approximated the sweat losses during exercise. Both the control and electrolyte treatments were designed to mimic recovery protocols typical of the industry and thus included meal feeding with water *ad libitum*. The naso-gastric administration of a hypotonic commercial electrolyte solution in 8 L of water, followed by a typical hay and oats meal, resulted in an enhanced rate of muscle glycogen resynthesis during the recovery period compared to Control. Additionally, electrolyte supplementation resulted in faster restoration of plasma hydration status compared to Control, as evidenced by faster recovery of plasma [protein] and maintenance of plasma osmolality. There were no differences in muscle electrolyte contents with electrolyte treatment however.

### ***Muscle glycogen.***

The horses in the present study used  $\sim 130$  mmol glucosyl units  $kg^{-1}$  dw in 1 h of exercise, resulting in a mean rate of glycogenolysis of  $\sim 2.2$  mmol  $kg^{-1}$   $dw^{-1}$  min. The CET was adequate to provide a significant and consistent degree of glycogen depletion amongst treatments, however it was not as large as that used in other studies of glycogen depletion / resynthesis. Muscle glycogen content in the present study was decreased by 21.9 and 22.6% from pre-exercise values in the Electrolyte and Control trials, respectively. In contrast, other recent studies of glycogen replenishment in horses utilized an intensive 3-day protocol of repeated sprints and a restrictive diet, resulting in muscle glycogen

reductions of 50-80% (Lacombe et al 2001, 2003, 2004, Jose-Cunilleras et al 2005, 2006). Thus, as a higher degree of glycogen depletion has been shown to result in greater rates of glycogen resynthesis (Price et al 2000, Laurent et al 2000), comparisons between replenishment rates in the present and previous studies must be interpreted with caution. The exercise protocol in the present study (prolonged, moderate intensity), was chosen for its ability to result in significant muscle glycogen depletion after only a single exercise bout, but also for inducing significant water and electrolyte losses, while decreasing the likelihood of musculoskeletal injuries.

The mean rate of glycogen resynthesis from 0-4 h of recovery was almost triple in the Electrolyte treatment compared to Control ( $15.0 \pm 9.0$  vs  $5.7 \pm 9.8$  mmol kg<sup>-1</sup> h<sup>-1</sup> dw, P = 0.440), such that at 4 h of recovery muscle glycogen content was not different from Pre-Ex in the Electrolyte treatment, but was still significantly decreased from Pre-Ex in the Control treatment. Glycogen replenishment was also greater in the Electrolyte treatment from 4-24 h, as evidenced by the significant increase in glycogen content from 4h to 24 h of recovery. The rate of muscle glycogen resynthesis during the first 4 h post exercise in the Electrolyte treatment of the present study was not as high as the maximum reported rates in horses following i.v. infusion of glucose. I.v. infusion of glucose at 6 g kg<sup>-1</sup> bwt after exercise resulted in initial (0-6 h) glycogen storage rates of  $19.9 \pm 3.8$  (Davie et al 1995) and  $20.9 \pm 7.3$  (Geor et al 2006) mmol kg<sup>-1</sup> dw<sup>-1</sup> h after ~50% glycogen depletion, while rates up to ~29 mmol kg<sup>-1</sup> dw<sup>-1</sup> h have been reported with ~80% muscle glycogen depletion (Lacombe et al 2001). Initial glycogen resynthesis rates in horses after ingestion of meals with varying soluble CHO contents range from ~8 to ~12 mmol kg<sup>-1</sup>

$\text{dw}^{-1} \text{ h}$  for mixed and high soluble CHO diets, respectively (Lacombe et al 2004). In contrast, rates of glycogen repletion in humans are 2-3 times higher than the rates seen in horses, for both CHO ingestion (Bergstrom and Hultman 1966b, Jentjens et al 2001) and i.v. glucose infusion (Bergstrom and Hultman 1967, Reed et al 1989).

Glycogen synthesis rate is determined by substrate availability and glycogen synthase activity. Recent research suggests that the low rate of glycogen synthesis with oral CHO provision in horses may be due to reduced rate of delivery from the gastrointestinal tract (Geor et al 2006), combined with a lack of increase in post exercise insulin sensitivity and membrane GLUT-4 translocation (Pratt et al 2007). However, i.v. infusion of large amounts of saline alone (~20 L in 12 h for a total of 107 g NaCl) resulted in rates of replenishment of ~ 7.6  $\text{mmol kg}^{-1} \text{ dw}^{-1} \text{ h}$  during the first 12 h of exercise recovery in horses (Lacombe et al. 2001), suggesting a possible role for rehydration in glycogen resynthesis. Keller et al (2003) demonstrated *in vivo* in humans that cell swelling induced by hypo-osmolality resulted in decreased carbohydrate oxidation and glycogenolysis and stimulated lipolysis, while cell shrinking resulted in increased glycogenolysis. These responses to both increased and decreased cell volume appear to involve signal transduction mechanisms similar to those associated with insulin and growth factor signaling, and it has been suggested that the stimulation of glycogen synthesis by cell swelling is likely due to dephosphorylation of glycogen synthase via effects on glycogen synthase kinase-3 (Low et al 1996). In support of this cell volume hypothesis, in the Electrolyte treatment of the present study calculated ICFV was increased by 18% from Pre-Ex at 4 h of recovery (paired t-test  $P=0.038$ ), as compared to

only 4% in the Control treatment. Electrolyte administration also resulted in a greater total water intake, and faster restoration of hydration status compared to Control, as evidenced by faster recovery of [PP]. However when the horses were provided their normal meals and water *ad libitum* (Control), recovery of hydration status was still incomplete 24 h after exercise. Therefore it is reasonable to postulate that post exercise dehydration affected muscle glycogen replenishment in the present study. In contrast to humans, equine sweat is hypertonic to plasma (McCutcheon et al 1995), resulting in an exercise-induced plasma dehydration that is isotonic to hypotonic. Thus when electrolytes lost in sweat are not replaced, there is no increase in plasma osmolality to stimulate a thirst response. Indeed, in the present study, plasma osmolality was increased up to 4 h after electrolyte administration, while in the Control treatment plasma osmolality decreased throughout the recovery period such that it was significantly lower than pre-exercise by 6 h of recovery. This suggests that a typical feed ration alone is not sufficient and electrolyte supplementation is required for full recovery of hydration status following exercise.

Interestingly, exercise did not result in decreased ICFV or net loss of  $K^+$  from the gluteus medius of the horses in the present study. In contrast, in humans and rodents prolonged exercise results in net losses of water (Costill et al. 1976) and  $K^+$  (Lindinger et al. 1995) from contracting muscle, in seeming contrast to the case in horses. It has previously been reported that intracellular water (Lindinger et al. 2004) and  $K^+$  (Wilson et al. 1998) may be preserved during exercise in horses, despite ongoing glycogen degradation and extracellular dehydration. The reasons for the apparent preservation of muscle water and

$K^+$  during exercise in horses is not clear, but may be related to the fact that, during locomotion, most muscles in the horse are actively recruited to either maintain posture and balance, or to generate propulsive forces, leaving relatively little tissue mass for redistribution of ions lost from contracting muscle.

While the results of the present study support a difference in glycogen replenishment rate based on hydration and electrolyte status, it is possible that some differences in glucose availability occurred due to the presence of dextrose in the electrolyte supplement. The dextrose concentration (31 mmol/L) of the Perform'N Win supplement serves as a direct source of glucose to provide cellular energy to subserve increased rates of epithelial transport of  $Na^+$  and water across the small intestine (Gisolfi et al 1992, Dyer et al 2002). Although the amount of dextrose administered with 8 L of supplement (44g) was insufficient to result in overall differences in plasma [glucose] compared to Control, and significant glycemic responses to feeding occurred in both treatments, plasma [glucose] did increase faster with electrolyte supplementation, peaking at 1 h of recovery in the Electrolyte treatment vs 2 h of recovery in Control (See Fig 5.5). Similar glycemic responses following meals with various soluble CHO content have been demonstrated in horses following the first post-exercise feeding (Brewster-Barnes et al 1995, Lacombe et al 2004). This suggests that exercise attenuates the glycemic response, likely due to an increase in glucose transport into skeletal muscle. Interestingly, although identical hay/grain meals were provided at 0 and 6 h of recovery, and each horse consumed all grain fed by 20 min post feeding, only the first meal resulted in any significant glycemic response. Feeding hay and grain in the same meal has been shown to significantly reduce

glycemic response compared with feeding grain alone (Lacombe et al. 2004, Pagan and Harris 1999), and it is speculated that hay consumption slows the rate of passage of grain through the small intestine, attenuating the glycemic response.

### ***Muscle Electrolytes.***

This study showed no change in gluteus medius electrolyte contents or concentrations with exercise or electrolyte administration, although changes in plasma electrolyte concentrations did occur (see Chapter 4). The ~8.3 L sweat loss corresponded to electrolyte losses of approximately 1.0 % of total body  $K^+$ , 3.7 % of total body  $Na^+$  and 7.6 % of total body  $Cl^-$  (Coenen 1992, McCutcheon et al 1995). As expected, the exercise- and electrolyte administration- induced changes in  $[Na^+]$  and  $[Cl^-]$  were seen in the extracellular (plasma) compartment (Chapter 4), while the estimated 1 % decrease in total body  $K^+$  was not sufficient to alter muscle  $[K^+]$  or content. In contrast, a decreased skeletal muscle  $K^+$  content and an increased  $Na^+$  content occurs during moderate to high intensity exercise in men (Bergstrom et al 1971, Costill and Saltin 1975, Sjogaard 1983). A possible explanation for the maintenance of muscle  $K^+$  at 0 h of recovery (~20 min post exercise) in the present study is that any sweat losses were countered by the high rates of  $Na^+/K^+$ -ATPase activity in previously contracting muscles, helping to restore intracellular  $[K^+]$  during initial recovery (Lindinger et al 1995): the high rate of  $K^+$  uptake and  $Na^+$  removal is facilitated by high concentrations of circulating catecholamines and increases in intramuscular  $[Na^+]$ , known activators of  $Na^+/K^+$ -ATPase activity (Clausen 1992).

Studies on electrolyte contents of equine muscle are rare, however resting values for  $K^+$  and  $Mg^{2+}$  contents in the present study are similar to those found previously in horses using flame photometer or atomic absorption spectrometry (AAS; Lindholm and Piehl, 1974) or x-ray microanalysis (Gottlieb-Vedi et al 1996), whereas  $Na^+$  and  $Cl^-$  contents are somewhat lower than in previous studies ( $Na^+ \sim 10-20 \text{ meq kg}^{-1} \text{ ww}$ ,  $Cl^- \sim 30 \text{ meq kg}^{-1} \text{ ww}$ ) (Lindholm and Piehl 1974, Gottlieb-Vedi et al 1996). The differences in equine muscle electrolyte contents between the present and previous studies may result from the different methods of analysis, method of estimation of the extracellular fluid volumes (or  $Cl^-$  space), genetic differences and differences in trained state. Flame photometry, AAS and x-ray microanalysis require elaborate tissue preparation. In contrast INAA can be performed with a minimum of sample handling and permits the entire spectrum of strong ions to be measured simultaneously in a small sample of tissue (Lindinger and Heigenhauser, 1987). When these methods of ion analysis were compared, similar electrolyte contents were found with INAA and AAS, while the contents of  $Na^+$  and  $Cl^-$  obtained by these techniques were somewhat higher, and that of  $K^+$  lower, than the values obtained by x-ray microanalysis (Wroblewski et al 1989). This can be attributed to the fact that in AAS and INAA the entire muscle biopsy contents are measured, while in x-ray microanalysis only the muscle cell components unaffected by extracellular and nonmuscular tissue are determined. Therefore in order to avoid the error from nonmuscular tissue in the present study, each sample was creatine corrected (to account for blood contamination) and then the  $Cl^-$  method was used to determine muscle ECFV and ICFV. Calculating muscle ECFV using the  $Cl^-$  space has been used extensively (Manery and Bale 1941, Bergstrom and Hultman 1966a, Bergstrom et al 1971, Costill et

al 1976, Lindinger and Heigenhauser 1986), however it appears to consistently overestimate ECFV and underestimate ICFV as measured simultaneously by other techniques (Lindinger and Heigenhauser 1986).

## **5.5 Conclusions**

The nasogastric administration of a hypotonic commercial electrolyte solution, followed by a typical hay and grain meal, resulted in an enhanced rate of muscle glycogen resynthesis during the recovery period as compared to Control. Additionally, electrolyte supplementation resulted in faster restoration of plasma hydration status compared to Control, as evidenced by faster recovery of plasma [protein] and maintenance of plasma osmolality. There were no differences in muscle electrolyte contents between treatments however. Because post exercise dehydration appears to be an important reason for why muscle glycogen replenishment is so slow in horses as compared to other mammals, it is suggested that post-exercise rehydration strategies can be actively used to enhance nutritional status in horses during and after fatiguing exercise.

**Table 5.1. Nutritional analysis of oats and mixed grass hay.**

	Oats	Mixed Hay
Dry Matter (%)	89.6	89.6
Protein (%)	15.1	10.6
ADF (%)	17.9	35.8
NDF (%)	36.6	58.2
Non Fibre Carbohydrate (%)		22.1
Starch (%)	40.3	
Calcium (%)	0.06	0.62
Potassium (%)	0.56	2.11
Magnesium (%)	0.15	0.14
Sodium (%)	0.02	0.02
Chloride (%)	0.09	0.29

Values are on a dry matter basis. ADF: acid detergent fibre, NDF: neutral detergent fibre

**Table 5.2. Gluteus medius metabolite concentrations and water content at rest and during recovery from a prolonged, moderate intensity Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by water *ad libitum* and a typical feeding protocol (Electrolyte trial), or 2) water *ad libitum* and a typical feeding protocol (Control).**

Time (min)	Glycogen		[Lactate]		G-3-P		H <sub>2</sub> O content	
	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control
Pre-Ex	531.3 ± 57.8	571.5 ± 49.5	21.7 ± 8.3	21.7 ± 8.4	0.41 ± 0.18	0.78 ± 0.45	2.75 ± 0.24	2.64 ± 0.13
0 h	414.8 ± 49.7*	442.3 ± 37.1*	17.7 ± 8.3	17.7 ± 8.8	0.50 ± 0.45	0.58 ± 0.38	2.79 ± 0.46	2.77 ± 0.04
4 h	474.8 ± 32.4	465.3 ± 37.4*	19.7 ± 4.1	22.9 ± 7.4	1.44 ± 0.72	0.07 ± 0.04	3.32 ± 0.43	2.75 ± 0.08
24 h	578.6 ± 26.8Ψ	533.9 ± 59.2	15.1 ± 5.0	25.0 ± 7.6	0.14 ± 0.08	0.86 ± 0.39	3.14 ± 0.26	3.01 ± 0.04

G-3-P = glycerol 3 phosphate. Values are mean ± SE for 6 horses. Metabolite units mmol kg<sup>-1</sup> dry wt; H<sub>2</sub>O content in L kg<sup>-1</sup> dry wt. \* = significantly different (P<0.05) from baseline (Pre-Ex) time point. Ψ = significantly different from 4 h time point.

**Table 5.3. Gluteus medius metabolite concentrations at rest and during recovery from a prolonged, moderate intensity Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by water *ad libitum* and a typical feeding protocol (Electrolyte trial), or 2) water *ad libitum* and a typical feeding protocol (Control).**

Time (min)	ATP		PCr		Creatine	
	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control
Pre-Ex	20.7 ± 1.2	19.7 ± 0.3	52.9 ± 4.0	49.7 ± 2.3	12.0 ± 2.1	15.2 ± 1.2
0 h	22.0 ± 1.4	20.9 ± 0.8	51.9 ± 3.3	49.8 ± 4.3	13.0 ± 1.7	15.2 ± 2.0
4 h	21.5 ± 0.7	21.0 ± 1.2	52.0 ± 3.6	51.6 ± 3.6	12.9 ± 1.5	13.3 ± 1.5
24 h	20.1 ± 1.3	20.2 ± 1.1	50.9 ± 3.1	50.4 ± 3.4	14.0 ± 1.5	14.5 ± 1.7

PCr = phosphocreatine. Values are mean ± SE for 6 horses. Metabolite units mmol kg<sup>-1</sup> dry wt; There were no significant differences between time points.

**Table 5.4. Gluteus medius electrolyte contents at rest and during recovery from a prolonged moderate intensity exercise Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by water *ad libitum* and a typical feeding protocol (Electrolyte trial), or 2) water *ad libitum* and a typical feeding protocol alone (Control trial).**

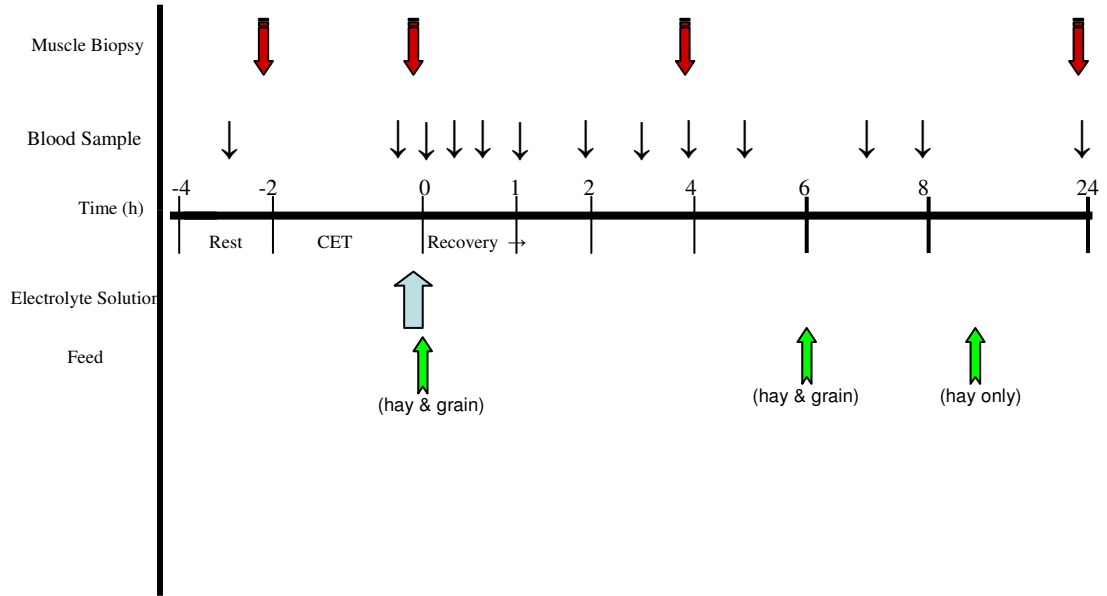
Time (h)	Method of Calc.	Cl <sup>-</sup>		Na <sup>+</sup>		K <sup>+</sup>		Mg <sup>2+</sup>		
		Electrolyte	Control	Electrolyte	Control	Electrolyte	Control	Electrolyte	Control	
Pre-Ex	Manery	4.10 ± 0.3	3.13 ± 0.3	1.90 ± 0.9	1.59 ± 0.4	93.8 ± 5.6	100.2 ± 5.5	26.7 ± 1.9	27.7 ± 2.0	
		Lindinger	2.14 ± 0.1	2.38 ± 0.1	1.36 ± 0.6	2.94 ± 0.2	93.8 ± 5.6	100.1 ± 5.5	26.6 ± 1.9	27.6 ± 2.1
	0'		Manery	2.94 ± 0.2	2.42 ± 0.2	1.96 ± 0.6	3.06 ± 0.4	101.8 ± 11.9	95.8 ± 5.8	26.8 ± 2.3
		Lindinger		2.37 ± 0.1	2.59 ± 0.1	2.84 ± 0.2	4.27 ± 0.6	101.8 ± 11.9	95.6 ± 5.8	26.9 ± 2.2
4'	Manery		3.90 ± 0.8	2.79 ± 0.1	5.42 ± 2.7	2.12 ± 0.3	83.4 ± 5.7	93.5 ± 5.4	25.1 ± 1.2	25.4 ± 1.9
		Lindinger	2.46 ± 0.2	2.44 ± 0.0	1.54 ± 0.6	3.17 ± 0.4	83.4 ± 5.7	93.5 ± 5.4	26.1 ± 1.2	25.4 ± 1.9
	24'		Manery	4.24 ± 0.7	4.44 ± 0.7	9.27 ± 3.1	1.26 ± 0.6	90.7 ± 6.8	98.8 ± 7.3	24.6 ± 1.2
		Lindinger		2.18 ± 0.1	2.28 ± 0.2*	1.01 ± 0.1	1.06 ± 0.7*	90.6 ± 6.8	98.8 ± 7.3	24.7 ± 1.3

Values are mean ± SE in meq kg<sup>-1</sup> dry wt; \* denotes significantly different (P<0.05) from pre-exercise time point.

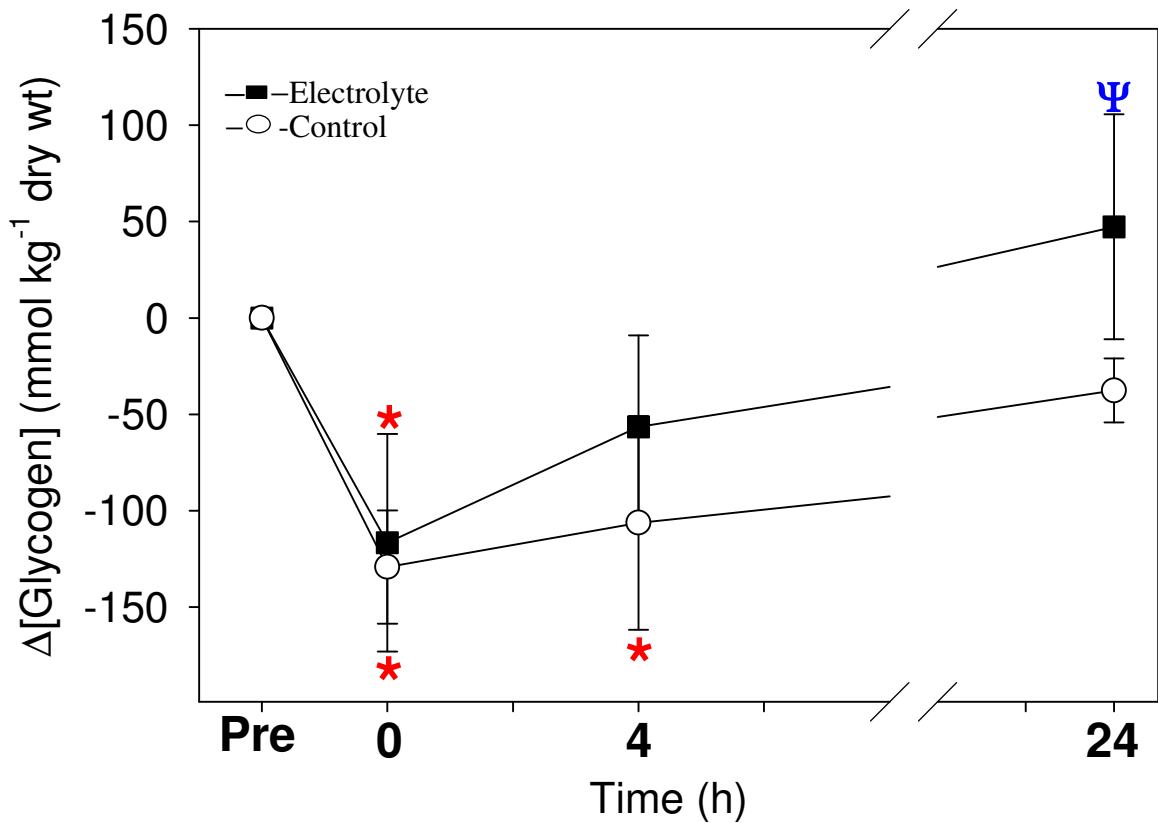
**Table 5.5. Gluteus medius extra- and intra-cellular fluid volume at rest and during recovery from a prolonged moderate intensity exercise Competition Exercise Test, after horses were either given 1) a hypotonic electrolyte solution followed by water *ad libitum* and a typical feeding protocol (Electrolyte trial), or 2) water *ad libitum* and a typical feeding protocol alone (Control trial).**

Time (h)	Method of Calc.	ECFV		ICFV	
		Electrolyte	Control	Electrolyte	Control
Pre-Ex	Manery	0.157 ± 0.02	0.129 ± 0.01	0.535 ± 0.04	0.577 ± 0.03
	Lindinger	0.156 ± 0.02	0.121 ± 0.01	0.536 ± 0.04	0.584 ± 0.03
0'	Manery	0.122 ± 0.01	0.100 ± 0.01	0.578 ± 0.02	0.625 ± 0.01
	Lindinger	0.114 ± 0.01	0.087 ± 0.01	0.586 ± 0.03	0.639 ± 0.01
4'	Manery	0.109 ± 0.01	0.117 ± 0.00	0.632 ± 0.01*	0.599 ± 0.01
	Lindinger	0.097 ± 0.01	0.107 ± 0.00	0.645 ± 0.01*	0.609 ± 0.01
24'	Manery	0.118 ± 0.01	0.139 ± 0.02	0.610 ± 0.01	0.577 ± 0.04
	Lindinger	0.107 ± 0.02	0.133 ± 0.02	0.621 ± 0.02	0.583 ± 0.04

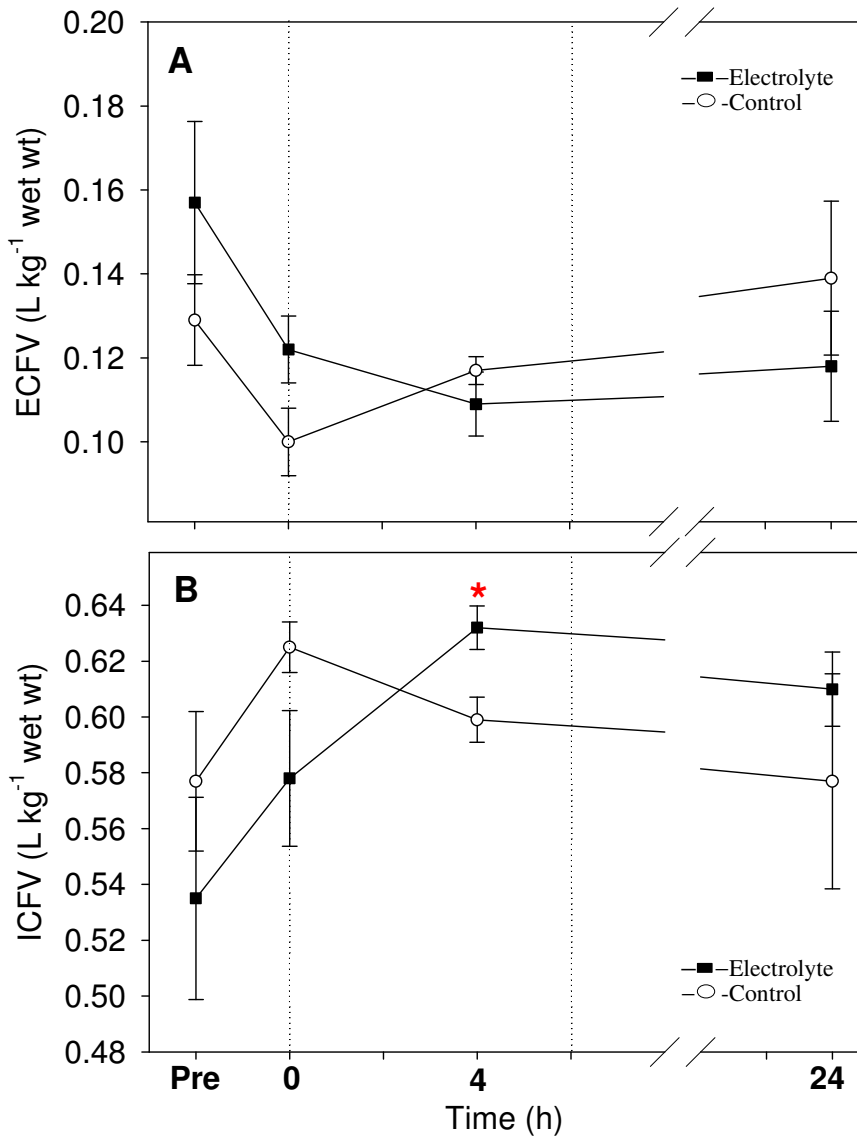
ECFV= muscle extracellular fluid volume, ICFV= intracellular fluid volume. Values are mean ± SE in L kg<sup>-1</sup> wet wt; \* denotes significantly different from Pre-Ex time point



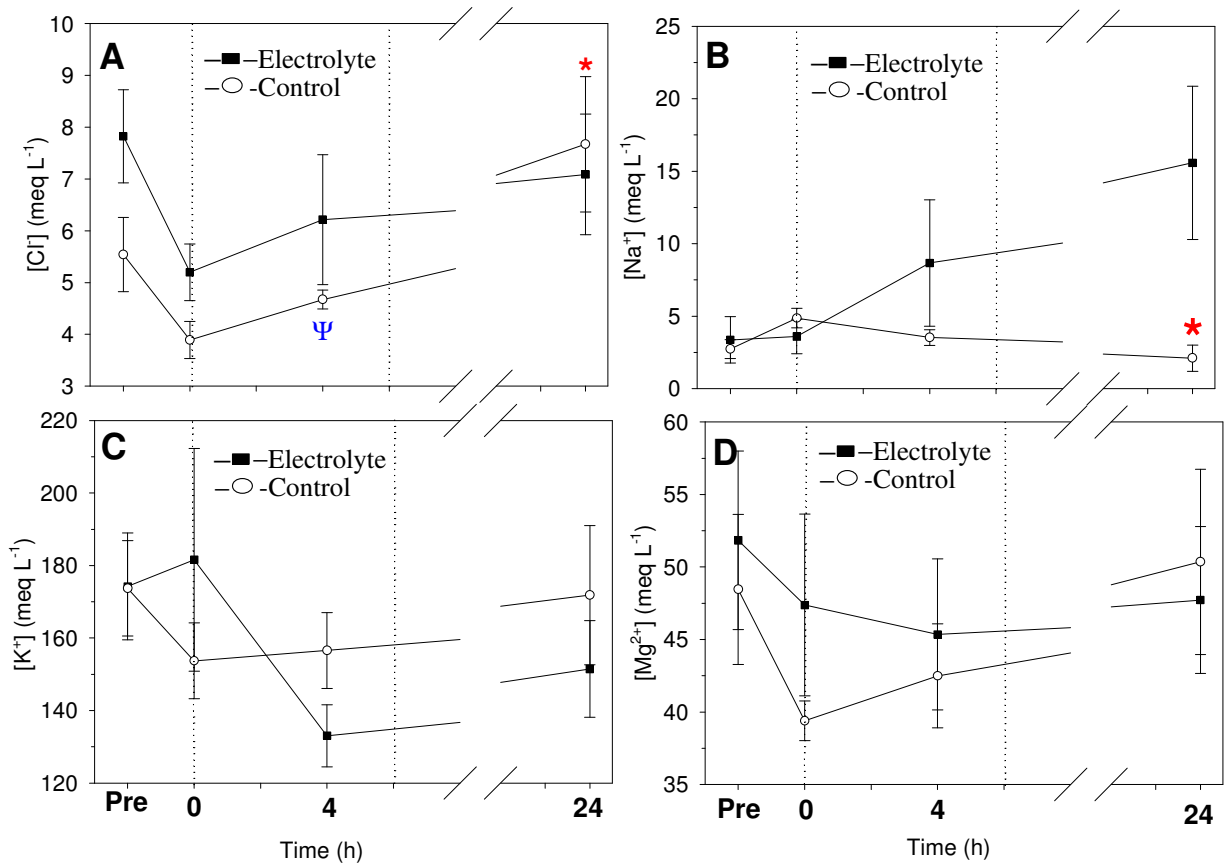
**Figure 5.1. Time line of the experimental protocol. CET = competitive exercise test.**



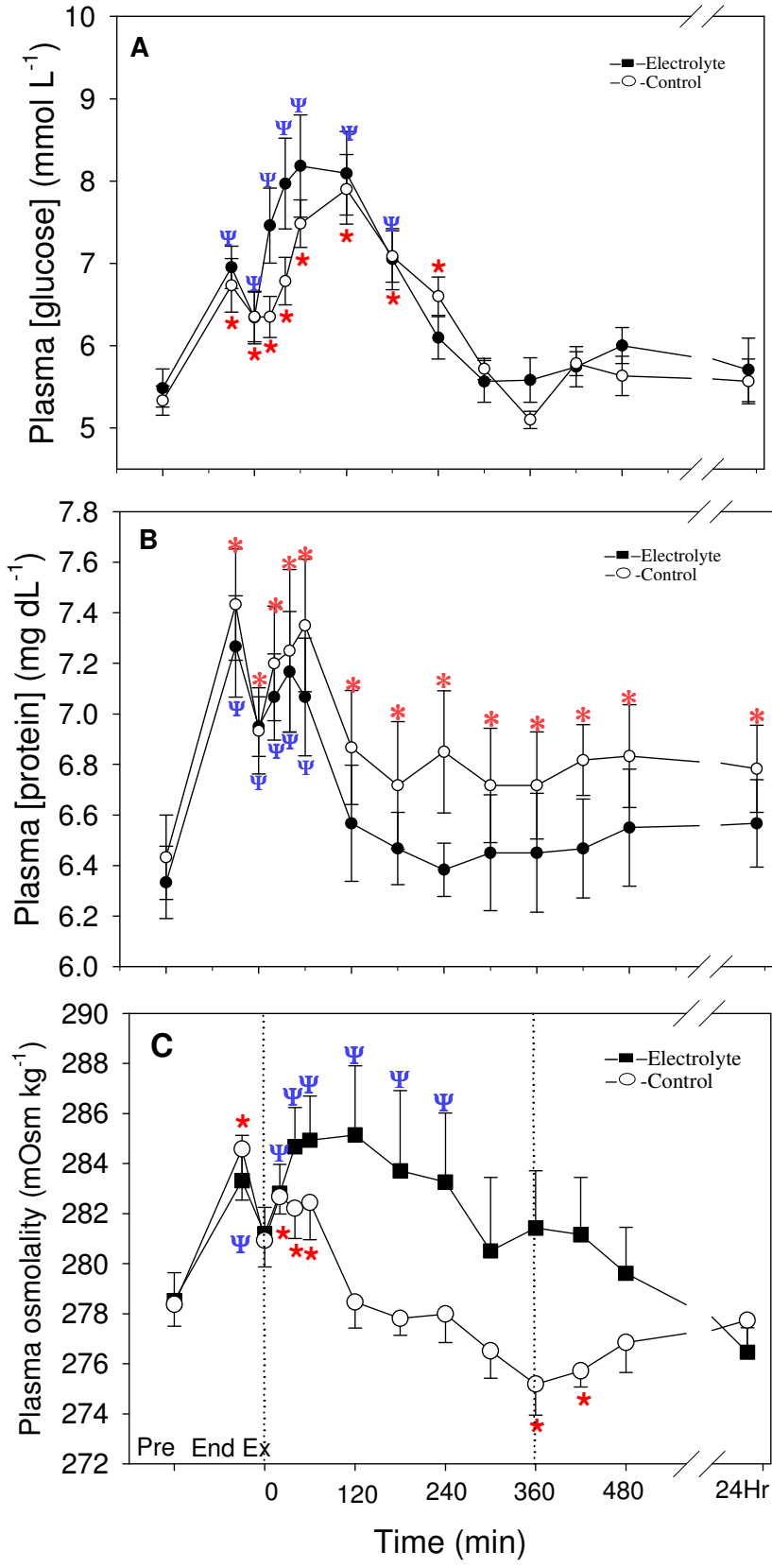
**Figure 5.2. The time course of change in gluteus medius glycogen concentration, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Electrolyte treatment. Values are mean  $\pm$  SE for 6 horses. \*: significantly different from Pre-Ex time point,  $\Psi$ : significantly different from 4 h time point.



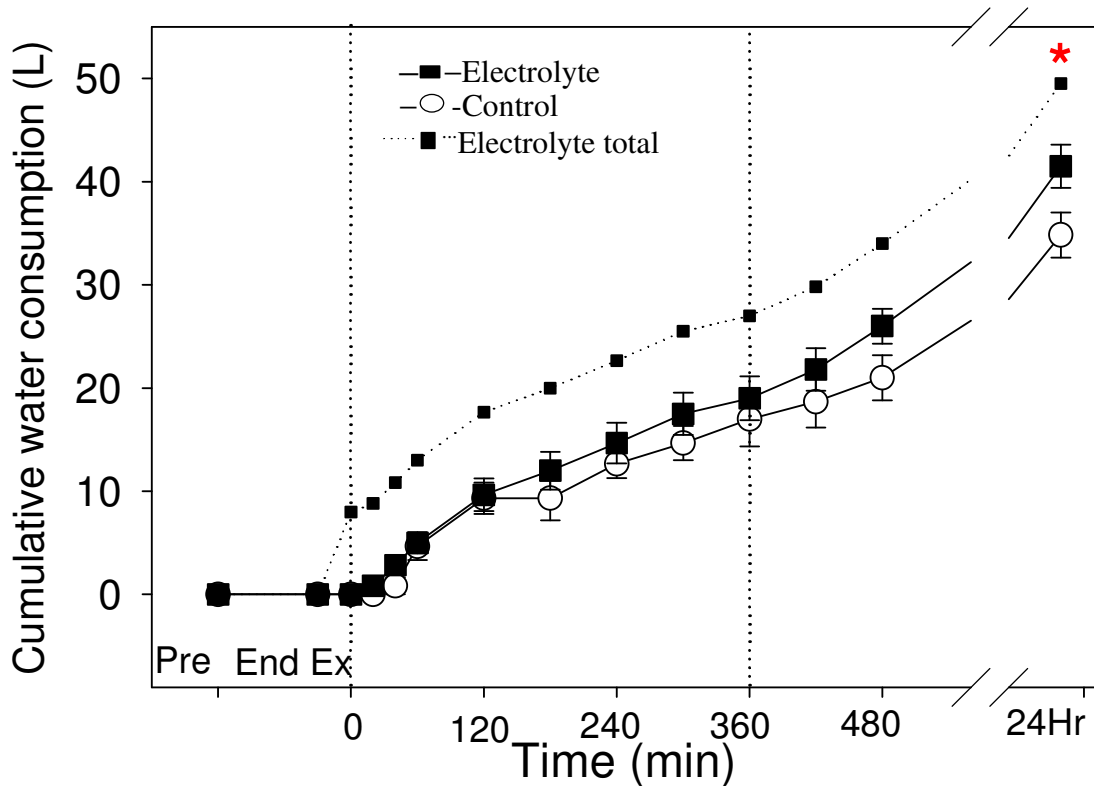
**Figure 5.3. The time course of gluteus medius (A) extracellular fluid volume, and (B) intracellular fluid volume, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control treatment; ■ denotes Electrolyte treatment. Values are mean ± SE for 6 horses. \*; significantly different from Pre-Ex time point.



**Figure 5.4. The time course of gluteus medius electrolyte concentrations: (A) chloride (Cl<sup>-</sup>), (B) sodium (Na<sup>+</sup>), (C) potassium (K<sup>+</sup>), and (D) magnesium (Mg<sup>2+</sup>), after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control treatment; ■ denotes Electrolyte treatment. Values are mean ± SE for 6 horses. \*, Ψ: significantly different from 0 h time point and significantly different from 24 h time point, respectively.



**Figure 5.5. The time course of the changes in plasma a) [glucose], b) protein and c) osmolality after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control treatment; ■ denotes Electrolyte treatment. Values are mean  $\pm$  SE for 6 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and Electrolyte treatments, respectively.



**Figure 5.6. The time course of cumulative water consumption, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypotonic electrolyte solution (Electrolyte), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples (dashed line). ○ denotes Control treatment; ■ denotes Electrolyte treatment; ■-----■ denotes total water intake for the Electrolyte treatment. Values are mean  $\pm$  SE for 6 horses. \*: significantly different from Control.

## Chapter 6

### **The effect of oral sodium acetate administration on plasma acetate concentration and acid-base state in horses**

#### **6.1 Introduction**

The administration of bicarbonate salts as alkalinizing agents has been extensively studied in both horses (Schott and Hinchcliff 1993) and humans (Heigenhauser and Jones 1991), and is of particular interest to the horseracing community where the testing of horses for illegal performance enhancing alkalinizing agents occurs. Sodium acetate (NaAcetate) has received some attention as an alkalinizing agent and possible alternative to sodium bicarbonate ( $\text{NaHCO}_3$ ) (Lloyd and Rose 1996). In contrast to  $\text{NaHCO}_3$ , the ergogenic effects of NaAcetate do not appear to have been studied in horses, however a few studies have demonstrated its alkalinizing effect orally (Lloyd and Rose 1996) and intravenously (Kline et al 2005). Of the limited studies of NaAcetate administration in horses, none have measured and reported the major plasma constituents describing acid-base status, therefore the magnitude and time course of variables that contribute to the origins of the metabolic alkalosis have been incompletely characterized; indeed the picture may be different from that obtained with  $\text{NaHCO}_3$  and other alkalinizing compounds. Identification of the origins of acid-base disturbances is facilitated by use of the physicochemical approach to acid-base status, which uses both dependent and independent variables to describe acid-base state. According to the physicochemical approach, the independent variables that determine plasma acid-base status are the

concentration of strong ions in solution – defined as the strong ion difference ([SID]), the partial pressure of carbon dioxide ( $\text{PCO}_2$ ), and the concentration of weak acids in solution – defined as the total weak acid concentration ( $[\text{A}_{\text{tot}}]$ ). Thus the dependent acid-base variables –  $[\text{H}^+]$ , bicarbonate concentration ( $[\text{HCO}_3^-]$ ) and total carbon dioxide concentration ( $[\text{TCO}_2]$ ) – only change when one or more of the independent variables are altered (Stewart 1983, Lindinger and Waller 2008).

Acetate is produced naturally in the horse by hindgut fermentation, and of all the volatile fatty acids, acetate is produced in the greatest quantity (Hintz et al 1971). Acetate is a metabolic precursor to acetyl-CoA, and tracer studies have shown that it is metabolized mainly to  $\text{CO}_2$  and water via the TCA cycle (Mohme et al 1970), generating ATP within the mitochondria. Indeed acetate appears to be an important energy source for the horse. Pethick et al. (1993) found that acetate contributed up to 32% of total substrate oxidation in hindlimb muscles of horses at rest, while Pratt et al. (2005) found that NaAcetate clearance was accelerated by exercise, suggesting that acetate may be used as an energy source during exercise as well. Thus supplemental acetate may be a practical alternative energy source for horses, however the intestinal absorption and plasma electrolyte and acid-base effects of oral sodium acetate remain largely unknown. Therefore the purpose of the present study was to detail the time course and magnitude of the changes in key blood constituents that determine plasma acid-base state in horses, after oral administration of a sodium acetate/acetic acid solution, and to employ the physicochemical approach to describe the resulting acid-base disturbances. It was hypothesized that: 1) oral administration of a sodium acetate/acetic acid solution after

prolonged submaximal exercise will result in a rapid and sustained increase in plasma [acetate]; and 2) a marked metabolic alkalosis will occur that is characterized by increased plasma  $[\text{Na}^+]$  and elevated plasma [SID].

## 6.2 Methods

**Animals:** 8 Standardbreds and 1 Thoroughbred (7 geldings, 2 mares; body weight  $470 \pm 11$  kg; age 5-12 yrs) from the University of Guelph research herd were used. The study took place between November and February, and the horses underwent a 4-6 week diet and exercise acclimation / conditioning period during which they were housed in individual box stalls with 7 hrs of daily turnout in a half acre paddock with minimal forage available. Horses were exercise trained 4 days/week on a high speed treadmill (SATO, Sweden) and 2 days/week on an outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada), until able to comfortably perform a competitive exercise test (CET) (Marlin et al 1996, 1999) on a high speed treadmill designed to significantly decrease muscle glycogen content (Poso and Hyyppa 1999) and result in total body water losses of 8-10 L [12]. The CET is designed to simulate the 2<sup>nd</sup> day (speed and endurance test – classic format) of a one star CCI 3-day event, and includes the following phases: 10 min walk (1.7 m/s), 10 min trot (3.7 m/s), 2 min gallop (10.0 m/s), 20 min trot (3.7 m/s), 10 min walk (1.7 m/s), 8 min canter (8.0 m/s), and 10 min walk (1.7 m/s).

The horses were maintained on a diet consisting of sweet feed (Purina Check-R-Mix 12%, DCAB  $\{\text{Na}^+ + \text{K}^+ - \text{Cl}^-\} = -68.6$  meq  $\text{kg}^{-1}$ ) twice daily and mixed grass hay (DCAB = 303.2 meq  $\text{kg}^{-1}$ ) three times daily, with free access to water and a salt block. The

amount of feed given was increased over this acclimation period such that during the final two weeks the horses were receiving 4kg sweet feed and 6 kg hay daily (dietary DCAB = 154.5 meq kg<sup>-1</sup>), and there were no significant changes in the body masses of the horses during this time. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

***Experimental Protocol:*** The study consisted of a treatment and control, thus each horse performed the CET twice in randomized order, separated by an 8-10 day interval (Hyypa et al 1996, Marlin et al 1999) during which time exercise conditioning was maintained. On both sampling days beginning at 7 am, the hair coat over the jugular vein, 10-20 cm below the mandible, was clipped short to the skin on both sides of the neck. Each jugular vein catheterization site was aseptically prepared for insertion of catheters. A topical anesthetic, EMLA cream (2.5% lidocaine and 2.5% prilocaine; Astra Pharma, Mississauga, ON, Canada), was applied 25-30 min before insertion of catheters to desensitize the skin. Local anaesthetic (2% Xylocaine; Astra Pharma) was injected subcutaneously to complete the anesthesia. Catheters (14-gauge, 5.25 in; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted antegrade into the left and right jugular veins, secured with tape and stitched to the skin. Four-way stopcocks with 50 cm extensions were attached to the catheters for ease of blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2000 IU l<sup>-1</sup> NaCl).

A pre-exercise blood sample was taken at 8 am and then the CET was performed. Immediately upon completion of the final canter an 'end of exercise' blood sample was taken, following which the horse walked for 10 min. Upon cessation of exercise, the horse either 1) was nasogastically administered a NaAcetate-electrolyte solution consisting of 500g NaAcetate (134g Na & 366 g acetate), 250 ml acetic acid (250 g acetate), 32 g KCl, and 300 g glucose in 8 L of water (Osmolality = 2944 mOsm kg<sup>-1</sup>) (NAA trial), or 2) stood in stocks for equivalent amount of time (Control trial). The acetate + glucose dosage was calculated to be able to replace all the skeletal muscle glycogen glucosyl units degraded during the period of exercise, assuming a ~40% decrease in muscle glycogen content (Poso and Hyyppa 1999). The amount of NaAcetate and acetic acid given was established by a series of 4 pilot studies to determine the maximum amounts tolerated without gastrointestinal upset, and then the amount of glucose needed to replace the remaining glycosyl units was calculated.

Within 20 minutes of cessation of exercise, the horses were given 2kg sweet feed and 3 kg hay (0 min of recovery), with access to water *ad libitum*. Horses were given 2kg sweet feed and 3 kg hay at 6 hrs of recovery, and 2 kg hay at 12 hrs recovery. Blood samples were taken at 20-60 min intervals up to 8 hrs of recovery, and again at 24 hrs of recovery, and horses remained in their stalls for the duration of sampling.

**Sample Analysis:** Each blood sample was collected into 7 ml heparinized vacutainers and immediately analyzed for plasma pH, the partial pressures of carbon dioxide (pCO<sub>2</sub>) and oxygen (pO<sub>2</sub>), and the plasma concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, lactate<sup>-</sup> using a Nova Stat Profile 9<sup>+</sup> (NOVA Biomedical, Waltham, MA). Hematocrit (hct) was measured by

conductivity and  $[\text{HCO}_3^-]$  and total carbon dioxide ( $\text{TCO}_2$ ) concentration were calculated using the Henderson-Hasselbach equation by the Nova Stat Profile 9<sup>+</sup>. It is important to note that the Nova may yield  $[\text{HCO}_3^-]$  and  $\text{TCO}_2$  values 2-3  $\text{mmoles L}^{-1}$  higher than the instruments used (typically a Beckman model analyzer) by horse racing jurisdictions (Lolekha et al 2001). Remaining blood was transferred into two 1.5 ml conical centrifuge tubes and centrifuged for 5 min at 15 000g to separate the plasma. Plasma protein concentration ([PP]) was determined (coefficient of variation (CV 0.83%) by using refractometry (Atago clinical refractometer model SPR-T2; Atago, Tokyo, Japan). Plasma [acetate] was measured in duplicate spectrophotometrically using a commercially available kit (R-Biopharm, Marshall MI) (CV 1.6%).

**Calculations:** Plasma  $[\text{H}^+]$  was calculated using the measured pH such that:

$$\text{pH} = -\log[\text{H}^+]$$

Plasma Strong Ion Difference ([SID]) was calculated as the sum of the plasma concentrations of the strong cations minus the strong anions [5], such that:

$$[\text{SID}] = [\text{Na}^+] + [\text{K}^+] - [\text{Cl}^-] - [\text{lactate}^-]$$

In practice, the concentrations of the divalent cations and anions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{PO}_4^{2-}$  and  $\text{SO}_4^{2-}$ ) are small and the sum of their charges close to zero and can be ignored (Lindinger and Waller 2008).

The plasma concentration of weak ions ( $[\text{A}_{\text{tot}}]$ ) was calculated as:

$$[\text{A}_{\text{tot}}] = 2.04 * [\text{PP}] (\text{g dL}^{-1}) + \text{Ac} (\text{mmol L}^{-1})$$

where  $2.04 * [\text{PP}]$  was taken from Constable (1997) based on formulas for estimating  $[\text{A}_{\text{tot}}]$  in equine plasma with known albumin and globulin concentrations. Ac is the

plasma acetate contribution to  $[A_{tot}]$ , calculated using AcidBasics II software (©2003, PD Watson) for each horse at each time point using measured values for  $[SID]$ ,  $pCO_2$  and  $[PP]$ , and a  $K_A$  for acetate of  $1.74 \times 10^{-5}$  (eq L<sup>-1</sup>).

Calculations of dependent acid-base parameters (pH,  $[H^+]$ ,  $[HCO_3^-]$ ,  $TCO_2$ ) were made using AcidBasics II software using the equation:

$$[H^+] + (K_A + [SID]) [H^+]^3 + \{K_A ([SID] - [A_{tot}]) - (K_C * pCO_2 + K_W)\} [H^+]^2 - \{K_A (K_C * pCO_2 + K_W) + (K_3 * K_C * pCO_2)\} [H^+] - K_A * K_3 * K_C * pCO_2 = 0$$

where  $K_W$ ,  $K_A$ ,  $K_3$  and  $K_C$  are the equilibrium constants for dissociations of water, weak acids, carbonic acid and bicarbonate, respectively ( $K_W = 4.4 * 10^{-14}$  (eq L<sup>-1</sup>),  $K_A = 3.9 * 10^{-8}$  (eq L<sup>-1</sup>),  $K_3 = 5.76 * 10^{-11}$  (eq L<sup>-1</sup>),  $K_C = 2.45 * 10^{-11}$  (eq L<sup>-1</sup>)<sup>2</sup> mmHg<sup>-1</sup>.  $K_A$  is the equilibrium constant for dissociation of weak acids in equine plasma in the present study, determined by linear regression analysis of measured vs calculated  $[H^+]$ . (See Fig. 6.1;  $r^2 = 0.365$ ,  $SEE = 2.32$ , slope = 0.622)

The contributions of the independent variables  $[A_{tot}]$  and  $PCO_2$  to the dependent variable  $[H^+]$  were determined by holding two of either  $[SID]$ ,  $pCO_2$ , or  $[A_{tot}]$  constant while calculating  $[H^+]$  in response to changes in the third independent variable (Lindinger et al 1992). The contribution of the  $[SID]$  to the changes in  $[H^+]$  was calculated by determining the contributions of  $pCO_2$  and  $[A_{tot}]$ , and then subtracting these from the measured change in  $[H^+]$ .

Total body water loss during the CET was determined as the change in body mass after accounting for fecal losses. Plasma osmolality was calculated according to the formula of Brownlow and Hutchins (1982) for equine plasma modified to include plasma [acetate] such that:

$$\text{Osmolality (mOsm kg}^{-1}\text{)} = 1.86([\text{Na}^+] + [\text{K}^+]) + [\text{Glucose}] + [\text{Lactate}^-] + [\text{acetate}] + 9$$

*Statistics.* Data are presented as mean  $\pm$  standard error. Changes over time were assessed by one-way repeated measures analysis of variance. Differences between treatments were assessed by two-way repeated measures analysis of variance. When a significant F-ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when  $P < 0.05$  at a power of 0.8.

### **6.3 Results**

Ambient temperature and humidity during the CET were  $20.5 \pm 0.2$  °C and  $40.5 \pm 3.0$  %, respectively. Total body water loss during the CET was  $8.3 \pm 0.3$  L.

#### *Independent variables and electrolytes*

Plasma  $[\text{Na}^+]$  (Fig. 6.2a) was increased from pre-exercise from 20-480 min and 20-60 min of recovery in the NAA and Control trials, respectively, with a significant difference between treatments ( $P < 0.001$ ). Plasma  $[\text{K}^+]$  (Fig. 6.2b) was decreased from 0-240 min of recovery in the Control trial, and from 0 min to the end of sampling in the NAA trial,

with no difference between treatments ( $P = 0.292$ ). Plasma  $[Cl^-]$  (Fig. 6.2c) was decreased from pre-exercise from 300 min to the end of sampling in the NAA trial, and did not differ from pre-exercise in the Control trial, with a significant difference between treatments ( $P < 0.001$ ). Plasma  $[Ca^{2+}]$  (Fig. 6.2d) was decreased from pre-exercise from the end of exercise to 20 min of recovery, and increased from pre-exercise 60-480 min of recovery in the Control trial. Plasma  $[Ca^{2+}]$  in the NAA trial was decreased from pre-exercise throughout the recovery period from the end of exercise to the end of sampling. There was a significant difference between trials ( $P < 0.001$ ).

Plasma [lactate] was increased from the end of exercise to 0 min of recovery and 100 min of recovery in the Control and NAA trials, respectively, with no differences between trials ( $P = 0.858$ ) (Table 6.1).

Plasma [acetate] in the NAA trial was increased from pre-exercise ( $0.56 \pm 0.07 \text{ mmol L}^{-1}$ ) from 40-300 min of recovery and reached a maximum of  $3.56 \pm 0.58 \text{ mmol L}^{-1}$  at 100 min of recovery (Fig. 6.3). Plasma [acetate] was not different from pre-exercise in the Control trial, and there was a significant difference between trials ( $P = 0.002$ ).

The time course of changes in independent acid-base variables are shown in Fig. 6.4.

Plasma [SID] (Fig. 6.4a) was increased from pre-exercise at 20 min of recovery in the Control trial, and from 60 min to the end of sampling in the NAA trial, with a significant difference between trials ( $P = 0.005$ ). Plasma  $PCO_2$  (Fig. 6.4b) was decreased at the end of exercise in both trials, and increased from 20-60 min and 210-480 in the Control and

NAA trials, respectively, with a trend towards an increased  $\text{PCO}_2$  in the NAA trial ( $P = 0.071$ ). Plasma  $[\text{A}_{\text{tot}}]$  (Fig. 6.4c) was increased from the end of exercise to 120 min of recovery and 300 min of recovery in the Control and NAA trials, respectively, with a significant difference between trials ( $P = 0.009$ ).

#### *Dependent variables*

Plasma  $[\text{H}^+]$  (Fig. 6.5a) in the NAA trial was increased from 40-180 min of recovery, and decreased from 300-480 min of recovery. Plasma  $[\text{H}^+]$  in the Control trial was increased from pre-exercise from 20 min of recovery to the end of sampling. There was a significant difference between trials ( $P = 0.006$ ). The contributions of the independent variables to the change in plasma  $[\text{H}^+]$  are shown in Fig. 6.5b and Fig. 6.5c. The main contributor to the decreased  $[\text{H}^+]$  at the end of exercise in both trials was the decreased  $\text{PCO}_2$ . The decreased  $[\text{H}^+]$  during the latter recovery period of the NAA trial (Fig. 6.5b) was entirely due to increased  $[\text{SID}]$ , while increases in  $\text{PCO}_2$  and  $[\text{A}_{\text{tot}}]$  contributed an acidifying effect.

Plasma  $[\text{TCO}_2]$  (Fig. 6.6a) in the NAA trial was decreased at the end of exercise and 20-60 min of recovery, and increased from 210-480 of recovery. Plasma  $[\text{TCO}_2]$  in the Control trial was increased at 20 min of recovery and decreased at 120, 180 and 300-480 min of recovery. There was a significant difference between trials ( $P < 0.001$ ). The contributions of the independent variables to the change in plasma  $[\text{TCO}_2]$  are shown in Fig. 6.6b and Fig. 6.6c. The increased  $[\text{TCO}_2]$  in the latter recovery period of the NAA

trial (Fig. 6.6b) was entirely due to increases in [SID], while an increased  $[A_{\text{tot}}]$  contributed an acidifying effect.

Plasma pH and  $[\text{HCO}_3^-]$  (Table 6.1) are shown to provide terms of reference with respect to the majority of the acid-base literature. Plasma pH in the NAA trial was decreased from 40-180 min of recovery, and increased from 300-480 min of recovery. Plasma pH in the Control trial was decreased from pre-exercise from 20 min of recovery to the end of sampling, and there was a significant difference between trials ( $P = 0.004$ ). Plasma  $[\text{HCO}_3^-]$  in the NAA trial was decreased at the end of exercise and 20-60 min of recovery, and increased from 210-480 of recovery. Plasma  $[\text{HCO}_3^-]$  in the Control trial was increased at 20 min of recovery and decreased at 120, 180 and 300-480 min of recovery. There was a significant difference between trials ( $P < 0.001$ ).

#### *Plasma osmolality, protein and water consumption*

Calculated plasma osmolality (Fig. 6.7) was increased at the end of exercise in both trials, and from 20-480 min and 20-60 min of recovery in the Acetate and Control trials, respectively. There was a significant difference between trials ( $P < 0.001$ ). Horses consumed significantly more water in the NAA trial such that total water consumption (including the 8 L given nasogastrically) at 24 h of recovery (Fig. 6.8) was  $46.2 \pm 2.3$  L and  $38.1 \pm 2.0$  L in the Acetate and Control trials, respectively ( $P = 0.043$ ).

[PP] was increased from the end of exercise to 120 min and 240 min of recovery in the Control and NAA trials, respectively, with no difference between trials ( $P = 0.428$ )

(Table 6.2). Hematocrit (hct) was increased at the end of exercise in both trials and from 0-20 min and 40-140 min of recovery in the Control and NAA trials, respectively, with no difference between trials ( $P = 0.175$ ) (Table 6.2). Plasma  $PO_2$  was increased at the end of exercise in both trials, with no difference between trials ( $P = 0.301$ ) (Table 6.2). Plasma [glucose] was increased from the end of exercise until 360 min of recovery in the Control trial and until the end of sampling in the NAA trial, with no difference between trials ( $P = 0.386$ ) (Table 6.2).

## **6.4 Discussion**

This study appears to be the first to detail the time course of acute changes in plasma dependent and independent acid-base variables after NaAcetate supplementation in horses. The nasogastric administration of a NaAcetate/acetic acid solution in 8 L of water, followed by a typical hay and grain meal, resulted in a profound plasma alkalosis marked by decreased plasma  $[H^+]$  and increased plasma  $[TCO_2]$  and  $[HCO_3^-]$  as compared to Control. The primary contributor to the plasma alkalosis was an increased [SID], as a result of increased plasma  $[Na^+]$  and decreased plasma  $[Cl^-]$ . An increased  $[A_{tot}]$ , due to increases in [PP] and a sustained increase in plasma [acetate], contributed a minor acidifying effect.

### ***Electrolytes and Independent Variables***

Plasma [SID] in the NAA trial was increased from 60 min post exercise until the end of sampling, with a peak increase of  $8 \text{ mmol L}^{-1}$  above the pre-exercise time point. The increase in plasma [SID] was primarily due to a large and sustained increase in plasma

[Na<sup>+</sup>], secondary to intestinal absorption of the large sodium load given (~11.4% of total extracellular sodium) (Schott and Hinchcliff 1993). Based on the amount of Na<sup>+</sup> given in the solution, if complete and simultaneous distribution of the Na<sup>+</sup> in the entire ECFV (~100 L) occurred, the peak increase in plasma [Na<sup>+</sup>] could have been up to 58 mmol L<sup>-1</sup>. Thus the time course of plasma [Na<sup>+</sup>] in the present study represents the interactions between intestinal rate of Na<sup>+</sup> absorption, tissue uptake and renal Na<sup>+</sup> excretion. To date there appear to be no previous studies reporting the effects of oral NaAcetate on plasma electrolytes in horses, however similar increases in plasma [Na<sup>+</sup>] have been seen with comparable doses of NaHCO<sub>3</sub> (500 to 750g given nasogastrically in 3 L of water) (Rivas et al 1997). In contrast, when a smaller amount of NaAcetate (110g) was given intravenously (i.v.) the increase in plasma [Na<sup>+</sup>] was more rapid and less prolonged, occurring from 20-60 min post infusion (Kline et al 2005).

Also contributing to the increased [SID] in the NAA trial of the present study was a decreased plasma [Cl<sup>-</sup>], which has been demonstrated previously with oral NaHCO<sub>3</sub> administration in horses (Freestone et al 1989, Hanson et al 1993) and humans (Heigenhauser and Jones 1991). This decreased plasma [Cl<sup>-</sup>] is not likely a result of increased renal excretion of Cl<sup>-</sup>, as NaHCO<sub>3</sub> ingestion in humans slightly increased renal tubular Cl<sup>-</sup> absorption (Lindinger et al 2000), as would be expected with increased HCO<sub>3</sub><sup>-</sup> delivery to the tubules. A dilutional effect of increased plasma volume as a result of increased plasma osmolality also does not appear to be the cause because [PP] and hct did not decrease with NaAcetate administration. However, increased extracellular osmolality results in rapid activation of volume regulatory mechanisms in both

erythrocytes (Lindinger and Grudzien 2003) and skeletal muscle (Lindinger et al 2002, Gosmanov 2003). Within these cells, shrinkage elicits a regulatory volume increase driven by an inwardly directed  $\text{Na}^+\text{-K}^+\text{-2Cl}^-$  cotransporter (NKCC) with coinciding increase in  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity (Lindinger et al 2002, Gosmanov 2003). The decreased plasma  $[\text{Cl}^-]$  and  $[\text{K}^+]$  in the NAA trial of the present study is consistent with increased activities of the NKCC and  $\text{Na}^+\text{-K}^+\text{-ATPase}$ . Short-term regulators of  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity include intracellular  $[\text{Na}^+]$ , extracellular  $[\text{K}^+]$ , plasma [insulin] and plasma catecholamines (Clausen 1992). It has also been suggested that a  $\text{NaHCO}_3$ -induced alkalosis increases intracellular  $[\text{Na}^+]$  through exchange of intracellular  $\text{H}^+$  via the  $\text{Na}^+\text{-H}^+$  antiporter, which would in turn activate the  $\text{Na}^+\text{-K}^+\text{-ATPase}$  (Adroque and Madias 1981) and result in decreased extracellular  $[\text{K}^+]$ . Indeed  $\text{NaHCO}_3$  therapy is used clinically in treating horses with hyperkalemic periodic paralysis (Naylor 1997). There was likely also a renal contribution to the decreased plasma  $[\text{K}^+]$  in the present study, as alkalosis has been shown to stimulate  $\text{Na}^+\text{-K}^+\text{-ATPase}$ -mediated uptake of  $\text{K}^+$  into the principal cells of the kidney, resulting in enhanced  $\text{K}^+$  secretion (Silva et al 1973), and  $\text{NaHCO}_3$  loading has been shown to result in increased renal excretion of  $\text{K}^+$  in humans (Gennari and Cohen 1975) and horses (Freestone et al 1989).

A rapid and prolonged decrease in plasma  $[\text{Ca}^{2+}]$  also occurred in the present study, which is consistent with the results of previous equine studies using NaAcetate (Kline et al 2005) or  $\text{NaHCO}_3$  (Freestone et al 1989, Beard and Hinchcliff 2002), and likely caused by an increased binding of plasma proteins to ionized calcium secondary to the reduced plasma  $[\text{H}^+]$  associated with the induced alkalosis (Oberleithner et al 1982). Kline et al.

(2005) found that iv infusion of NaAcetate decreased serum  $[Ca^{2+}]$  and  $[K^+]$  to a greater extent than  $NaHCO_3$ , suggesting that the neuromuscular problems that may occur in horses given large doses of  $NaHCO_3$  (Freestone et al 1989, Frey et al 1995), may also arise with NaAcetate administration. Indeed several horses in the present study briefly exhibited mild muscle fasciculations.

Several studies have demonstrated that venous  $PCO_2$  increases after oral  $NaHCO_3$  administration in resting horses (Rivas et al 1997, Freestone et al 1989, Hanson et al 1993), with larger doses  $\geq 500$  g inducing hypercapnia for at least 12 hours (Rivas et al 1997). I.v. infusion of NaAcetate led to an increased  $PCO_2$  in humans (Burnier et al 1992), but not in horses (Kline et al 2005). In the present study, however,  $PCO_2$  was increased from 210-480 min of recovery in the NAA trial, likely due to the marked metabolic alkalosis initiating a hypoventilary response (Aquino and Luke 1973).

NaAcetate administration resulted in a larger and more prolonged increase in plasma  $[A_{tot}]$ , compared to Control, due to greater increases in [PP] and plasma [acetate]. [PP] was increased during the initial recovery period for both trials, which is expected to occur as a result of exercise-induced fluid shifts (Waller and Lindinger 2005a) and bi-directional flow of water and electrolytes between the blood and gastrointestinal tract during feeding (Kerr and Snow 1982). However, the very hypertonic NaAcetate solution ( $2944 \text{ mOsm kg}^{-1}$ ) would have resulted in a greater flux of low [ion] fluid into the gastrointestinal tract, resulting in the 8% greater increase in [PP] in the early recovery period of the NAA trial as compared to Control. As mentioned above, although plasma

volume expansion could be expected with NaAcetate administration due to the large and prolonged increase in plasma osmolality (Lloyd and Rose 1996, Lindinger et al 1999), this does not appear to have occurred as there was no decrease in [PP]. It is likely that increased plasma osmolality resulted in intracellular fluid loss, but no net gain of extracellular fluid volume occurred due to a large intraluminal fluid shift. Additionally, horses in the NAA trial consumed significantly more water over the 24 h sampling period, however they generally did not begin consuming water until at least 120 min of recovery, which would have contributed to the plasma dehydration and increased [PP] up to 240 min of recovery.

To our knowledge this study appears to be the first study to measure plasma [acetate] after oral acetate administration. Plasma [acetate] was increased from 40-300 min post NaAcetate administration and reached a maximum of  $3.56 \pm 0.58 \text{ mmol L}^{-1}$  at 100 min of recovery in the NAA trial, contributing to the increased  $[A_{\text{tot}}]$ . When 112 g NaAcetate was given intravenously (Pratt et al 2005), plasma [acetate] increased to  $\sim 16 \text{ mmol L}^{-1}$  by 2 min post infusion and remained increased until 40 min post infusion. The more sustained and smaller increase in plasma [acetate] with oral administration likely represents a steady state between intestinal absorption and tissue (primarily skeletal muscle) extraction. Acetate transport across lipid bilayer membrane is facilitated by plasma membrane monocarboxylate transporters (Waniewski and Martin 2004), thus cellular uptake occurs rapidly. Indeed, based on the total amount of acetate given in the present study, if complete and simultaneous distribution in the ECFV ( $\sim 100 \text{ L}$ ) occurred, the peak increase in plasma [acetate] could have been up to  $102 \text{ mmol L}^{-1}$ . Thus the time

course of plasma [acetate] in the present study implies that tissue extraction of absorbed acetate occurs rapidly, suggesting that oral acetate could be a potential alternate energy source for horses. This supports the findings of Pratt et al. (2005) who found that when NaAcetate was given intravenously prior to exercise the acetate was cleared from the plasma more rapidly than at rest, suggesting that the acetate may have been used as an energy source for the working muscle. Clearly the supplementation of even small amounts of NaAcetate to racehorses prior to racing would be ill-advised due to the risk of exceeding the testing threshold for an alkalinizing agent, however acetate could still potentially be an effective post race or post training supplemental energy source. In the present study the effect of prior exercise on NaAcetate uptake and metabolism is unknown, and a potential future direction of study would be to compare the effects of post-exercise acetate administration vs resting acetate administration.

### ***Dependent Variables***

Nasogastric administration of a hypertonic NaAcetate/acetic acid solution in 8 L of water, followed by a typical hay and grain meal, resulted in a profound plasma alkalosis marked by decreased plasma  $[H^+]$  and increased plasma  $[HCO_3^-]$  and  $[TCO_2]$ . When the physicochemical determinants of the dependent variables were quantified (Figs. 6.5b/c & 6.6b/c), the primary contributor to the plasma alkalosis was an increased [SID] from 60 min post administration until the end of sampling, as a result of increases in plasma  $[Na^+]$  and decreases in plasma  $[Cl^-]$ . In contrast, an increased  $[A_{tot}]$ , due to increases in [PP] and a sustained increase in plasma [acetate], contributed a minor acidifying effect.

Finally, despite a tendency towards increased  $\text{PCO}_2$  with NaAcetate supplementation,  $\text{PCO}_2$  had little effect on plasma  $[\text{H}^+]$  or  $[\text{TCO}_2]$ .

This appears to be the first study to detail the effects of NaAcetate administration on plasma  $[\text{TCO}_2]$  in horses and knowledge of the effects of NaAcetate supplementation on acid-base status is of practical interest to the racing community. A plasma  $[\text{TCO}_2]$  testing threshold of greater than 37 mmol/l is used by many racing jurisdictions to determine whether a horse has been administered an alkalinizing agent for the purpose of performance enhancement (see Lindinger and Waller 2008). Lloyd and Rose (1996) compared the effects of nasogastric administration of ~250 g NaAcetate or  $\text{NaHCO}_3$  in 2 L of water and found that plasma  $[\text{HCO}_3^-]$  (which is ~95% of  $[\text{TCO}_2]$ ) peaked at 8 h post NaAcetate administration with an average increase of 11.3 mmol/l from baseline, while the increase in  $[\text{HCO}_3^-]$  and time to peak for  $\text{NaHCO}_3$  administration was 7.6 mmol  $\text{L}^{-1}$  and 5.5 h, respectively. The authors concluded that NaAcetate produced a greater metabolic alkalosis with a later peak alkalinizing effect than  $\text{NaHCO}_3$ . In the present study, plasma  $[\text{TCO}_2]$  was not increased until 210 min post NaAcetate administration, reaching a peak of 46.3 mmol  $\text{L}^{-1}$  at 420 min (7 h) post administration (an increase of 8.3 mmol  $\text{L}^{-1}$  above pre-exercise). Thus the results of the present and previous studies suggest that NaAcetate could be used as an alkalinizing compound for performance enhancement, and in fact the delay in peak metabolic alkalosis that appears to occur with NaAcetate administration -- this may make detection of the alkalinizing agent more difficult when pre-race  $\text{TCO}_2$  testing is used. Interestingly, pre-exercise plasma  $[\text{TCO}_2]$  for the NAA and Control trials in the present study was  $38.0 \pm 0.6$  and  $37.5 \pm 0.7$  mmol

L<sup>-1</sup>, respectively, with these horses fed a typical racehorse diet. Accordingly, the results of this study show that some horses naturally demonstrate [TCO<sub>2</sub>] that may approach or exceed the testing threshold, even when no alkalinizing substances have been given.

### ***Usefulness and limitations of the physicochemical approach***

The main advantage of the physicochemical approach is the ability to identify and quantify the origins of an acid-base disturbance. The knowledge of why changes in [H<sup>+</sup>] and [TCO<sub>2</sub>] occur enhances the understanding of acid-base physiology, and may be useful in developing alternative testing strategies to determine whether illegal alkalinizing agents have been given.

The calculation of [A<sub>tot</sub>] proposed for use in horses previously (Constable 1997) assumes normal and constant plasma acetate and phosphate concentrations. Because acetate is a weak acid and plasma [acetate] was altered in the present study, it was necessary to include an acetate component in the calculation of [A<sub>tot</sub>]. The plasma acetate contribution to [A<sub>tot</sub>] in the present study was calculated using AcidBasics II software (©2003, PD Watson) for each horse at each time point using measured values for [SID], pCO<sub>2</sub> and [PP], and a K<sub>A</sub> for acetate of 1.74X10<sup>-5</sup> (eq L<sup>-1</sup>). The contributions of the independent variables to changes in the dependent variables (pH, [H<sup>+</sup>], [HCO<sub>3</sub><sup>-</sup>], TCO<sub>2</sub>) were also determined using AcidBasics II software, using an equilibrium constant for dissociation of weak acids (K<sub>A</sub>) determined by linear regression analysis of measured vs calculated [H<sup>+</sup>]. Some of the difference between measured and calculated [H<sup>+</sup>] in the present study is likely due to inherent inaccuracy in the new dissociation constant for [A<sub>tot</sub>] and the

conversion factor used to produce  $[A_{\text{tot}}]$  from plasma [protein] and plasma [acetate]. It appears that additional research using many more horses may be required to obtain accurate values for  $[A_{\text{tot}}]$  when large changes in plasma [acetate] occur.

## 6.5 Conclusions

The present study quantified the magnitude and time course of the main physicochemical determinants of acid-base status in horses after post exercise oral NaAcetate. The nasogastric administration of a NaAcetate/acetic acid solution in 8 L of water, followed by a typical hay and grain meal, resulted in a profound plasma alkalosis marked by decreased plasma  $[H^+]$  and increased plasma  $[TCO_2]$  and  $[HCO_3^-]$  as compared to Control. The primary contributor to the plasma alkalosis was an increased [SID], as a result of increased plasma  $[Na^+]$  and decreased plasma  $[Cl^-]$ , while an increased  $[A_{\text{tot}}]$ , due to increases in [PP] and a sustained increase in plasma [acetate], contributed a minor acidifying effect. It is concluded that oral NaAcetate could potentially be used as both an alkalinizing agent and an alternative energy source in the horse.

**Table 6.1. Jugular vein plasma pH, bicarbonate and lactate concentrations, at rest and during recovery from a Competition Exercise Test, after horses were either given 1) an oral sodium acetate/acetic acid solution followed by a typical feeding protocol (NAA trial), or 2) a typical feeding protocol alone (Control trial).**

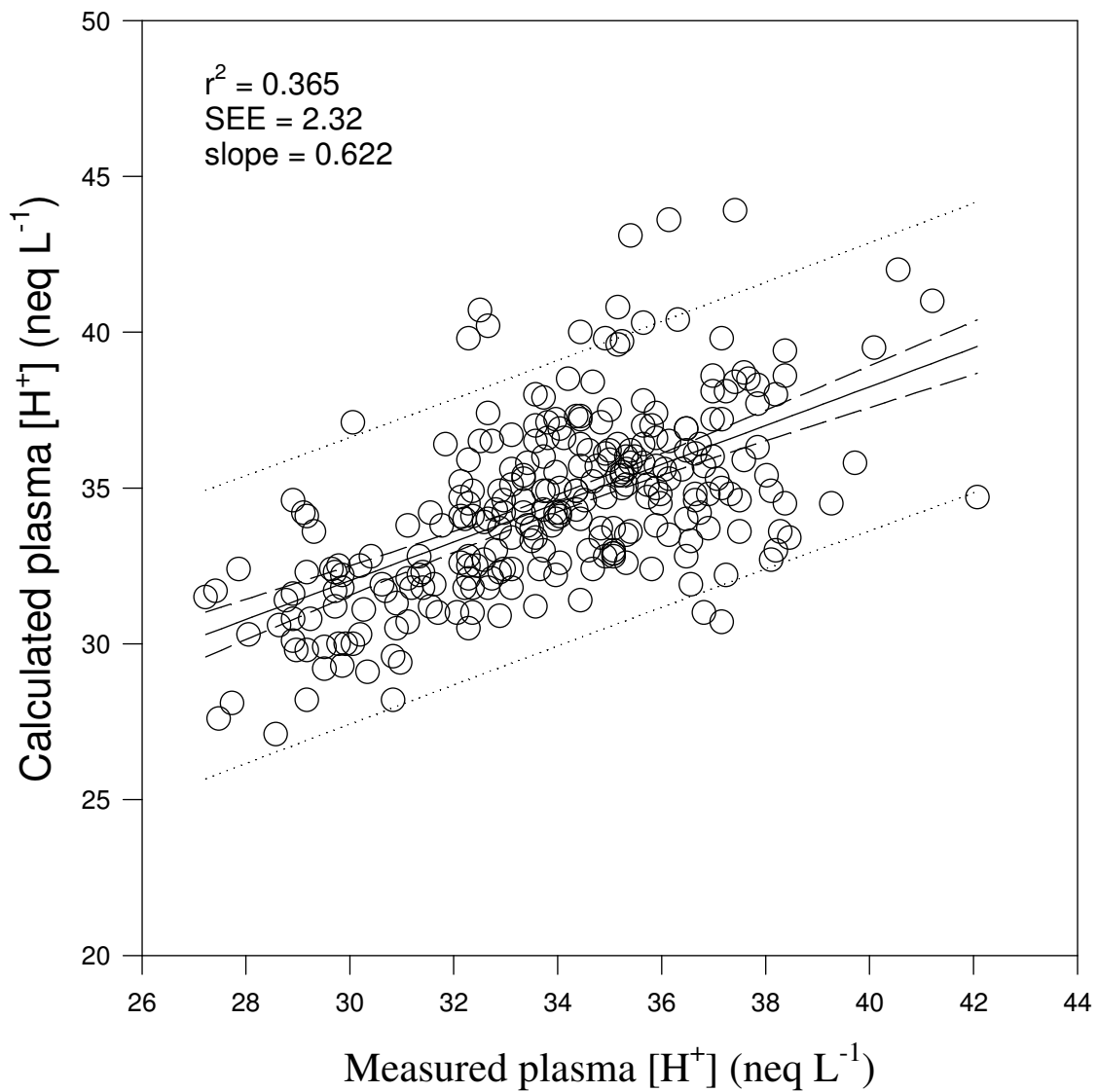
Time (min)	pH		[HCO <sub>3</sub> ]		[Lactate]	
	NAA	Control	NAA	Control	NAA	Control
Pre-Ex	7.48 ± 0.01	7.48 ± 0.01	36.5 ± 0.6	36.4 ± 0.9	0.7 ± 0.2	0.8 ± 0.2
End Ex	7.52 ± 0.01*	7.53 ± 0.01*	33.56 ± 0.4*	34.7 ± 0.8	2.8 ± 0.6*	2.6 ± 0.6*
0'	7.47 ± 0.01	7.48 ± 0.01	36.4 ± 0.8	35.9 ± 0.9	1.6 ± 0.1*	1.7 ± 0.3*
20'	7.47 ± 0.02	7.48 ± 0.01*	33.7 ± 1.4*	38.0 ± 0.9*	1.5 ± 0.2*	1.1 ± 0.3
40'	7.44 ± 0.01*	7.45 ± 0.01*	33.16 ± 1.2*	36.2 ± 1.1	1.5 ± 0.2*	1.2 ± 0.3
60'	7.43 ± 0.01*	7.44 ± 0.01*	33.8 ± 1.0*	34.4 ± 0.5	1.5 ± 0.2*	1.1 ± 0.3
80'	7.44 ± 0.01*		34.5 ± 1.2		1.4 ± 0.2*	
100'	7.44 ± 0.01*		34.8 ± 0.8		1.4 ± 0.2*	
120'	7.45 ± 0.01*	7.44 ± 0.1*	36.8 ± 0.8	34.3 ± 0.6*	1.2 ± 0.2	1.1 ± 0.2
140'	7.45 ± 0.01*		38.0 ± 0.8		1.2 ± 0.2	
180'	7.46 ± 0.01	7.46 ± 0.01*	37.8 ± 1.1	33.7 ± 0.4*	1.1 ± 0.2	1.3 ± 0.2*
210'	7.47 ± 0.01		39.7 ± 1.0*		1.1 ± 0.2	
240'	7.49 ± 0.01	7.46 ± 0.01*	40.46 ± 0.9*	34.9 ± 0.6	1.1 ± 0.2	1.1 ± 0.2
300'	7.51 ± 0.01*	7.46 ± 0.01*	42.01 ± 1.0*	34.2 ± 0.6*	1.2 ± 0.1*	1.0 ± 0.3
360'	7.51 ± 0.01*	7.45 ± 0.01*	44.1 ± 0.8*	33.8 ± 0.5*	1.1 ± 0.1	1.2 ± 0.3
420'	7.53 ± 0.01*	7.45 ± 0.01*	44.8 ± 0.9*	34.4 ± 0.4*	1.0 ± 0.2	0.8 ± 0.2
480'	7.53 ± 0.01*	7.46 ± 0.01*	44.5 ± 0.5*	34.0 ± 0.2*	0.9 ± 0.1	0.9 ± 0.2
24h	7.49 ± 0.01	7.46 ± 0.01*	39.3 ± 1.5*	34.2 ± 0.7	1.2 ± 0.2	0.9 ± 0.2

Values are mean ± SE in mmol/l. \* Significantly different (P<0.05) from pre-exercise time point.

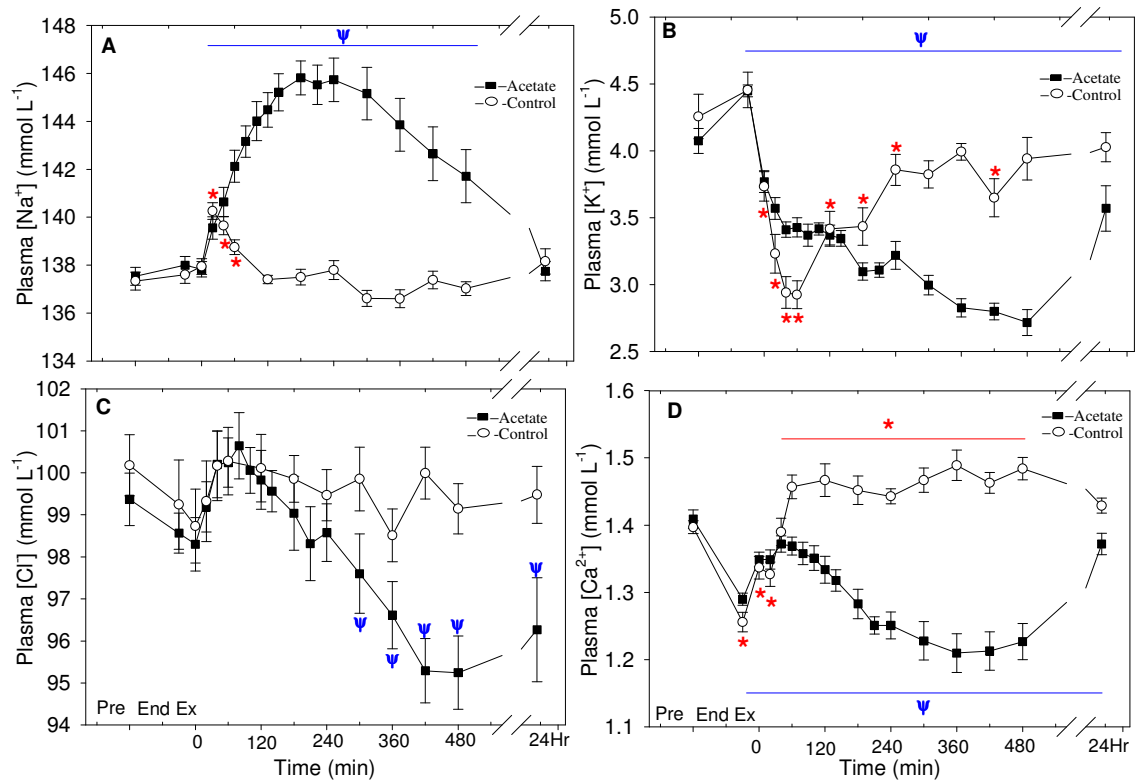
**Table 6.2. Jugular vein hematocrit, plasma glucose and protein concentrations, and PO<sub>2</sub> at rest and during recovery from a Competition Exercise Test, after horses were either given 1) an oral sodium acetate/acetic acid solution followed by a typical feeding protocol (NAA trial), or 2) a typical feeding protocol alone (Control trial).**

Time (min)	Hct		[Glucose]		[PP]		PO <sub>2</sub>	
	NAA	Control	NAA	Control	NAA	Control	NAA	Control
Pre-Ex	37.1 ± 0.6	37.3 ± 0.7	4.7 ± 0.1	4.7 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	59.4 ± 7.9	69.6 ± 13.9
End Ex	49.4 ± 0.5*	50.2 ± 0.7*	5.6 ± 0.2*	5.2 ± 0.3*	7.2 ± 0.1*	7.2 ± 0.2*	128.8 ± 7.6*	150.2 ± 11.5*
0'	39.7 ± 0.6	39.2 ± 0.6*	5.2 ± 0.2*	5.2 ± 0.1*	6.8 ± 0.1*	6.7 ± 0.2*	75.4 ± 11.6	77.9 ± 9.2
20'	40.3 ± 0.8*	38.7 ± 0.6*	5.5 ± 0.2*	5.5 ± 0.1*	7.0 ± 0.2*	6.9 ± 0.2*	93.9 ± 21.*	38.3 ± 2.1*
40'	41.1 ± 1.1*	38.2 ± 0.7	5.9 ± 0.1*	6.6 ± 0.1*	7.2 ± 0.2*	6.9 ± 0.2*	71.5 ± 14.3	45.2 ± 6.7*
60'	41.9 ± 0.7*	38.2 ± 0.8	6.1 ± 0.2*	7.1 ± 0.2*	7.2 ± 0.2*	6.8 ± 0.2*	41.8 ± 3.4	47.6 ± 11.0*
80'	41.3 ± 0.6*		6.5 ± 0.3*		7.1 ± 0.1*		43.5 ± 3.2	
100'	41.1 ± 0.7*		6.8 ± 0.3*		7.0 ± 0.2*		51.4 ± 11.9	
120'	40.9 ± 0.8*	37.5 ± 1.3	6.8 ± 0.4*	6.9 ± 0.4*	7.0 ± 0.2*	6.5 ± 0.2*	38.6 ± 1.6	75.2 ± 19.9
140'	40.7 ± 0.8*		6.6 ± 0.4*		6.9 ± 0.2*		44.2 ± 3.6	
180'	39.3 ± 0.5	37.8 ± 0.9	6.2 ± 0.3*	6.0 ± 0.3	6.7 ± 0.1*	6.4 ± 0.2	50.6 ± 10.5	56.1 ± 2.5
210'	39.0 ± 0.3		5.9 ± 0.2*		6.5 ± 0.1*		44.1 ± 5.6	
240'	40.1 ± 0.8*	41.0 ± 1.2*	6.2 ± 0.3*	5.3 ± 0.1*	6.4 ± 0.1*	6.4 ± 0.1*	60.5 ± 8.6	48.8 ± 4.0*
300'	37.2 ± 0.8	36.2 ± 0.7	6.0 ± 0.3*	5.3 ± 0.1*	6.2 ± 0.2	6.2 ± 0.2	68.2 ± 14.8	50.4 ± 7.3
360'	36.8 ± 0.9	36.8 ± 0.4	5.8 ± 0.2*	5.3 ± 0.2*	5.9 ± 0.2	6.2 ± 0.2	75.0 ± 12.3	65.5 ± 10.2
420'	36.0 ± 1.0	37.5 ± 0.4	5.5 ± 0.2*	5.0 ± 0.2	5.9 ± 0.2	6.3 ± 0.2	39.6 ± 3.2	46.7 ± 7.1
480'	36.8 ± 1.0	38.2 ± 0.2	5.6 ± 0.2*	5.0 ± 0.1	5.8 ± 0.2	6.5 ± 0.1*	46.3 ± 3.8	49.6 ± 5.6
24h	40.7 ± 1.1*	41.0 ± 0.4*	5.5 ± 0.2*	4.9 ± 0.1	5.9 ± 0.2	6.4 ± 0.1	80.2 ± 18.9	124.2 ± 34.1*

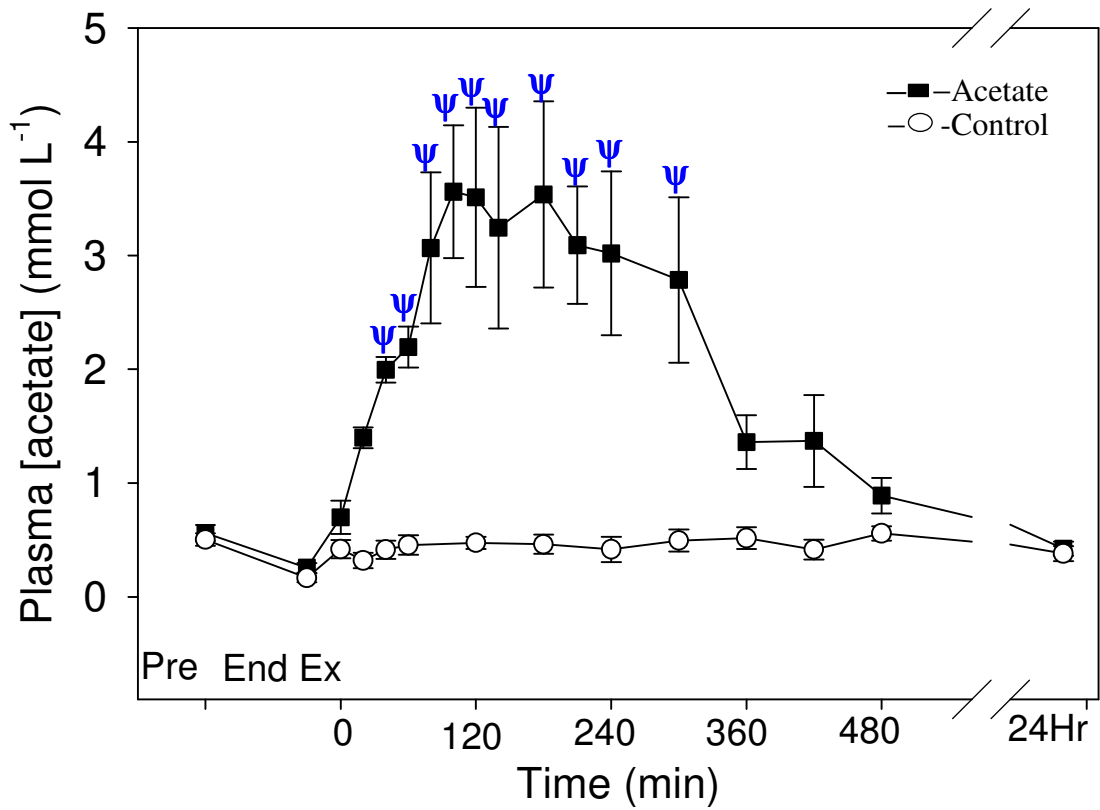
Hct = hematocrit, [PP] = [plasma protein]. Values are mean ± SE, with [Glucose] in mmol/l, Hct in %, [PP] in g/dl and pO<sub>2</sub> in mmHg. \* Significantly different (P<0.05) from pre-exercise time point.



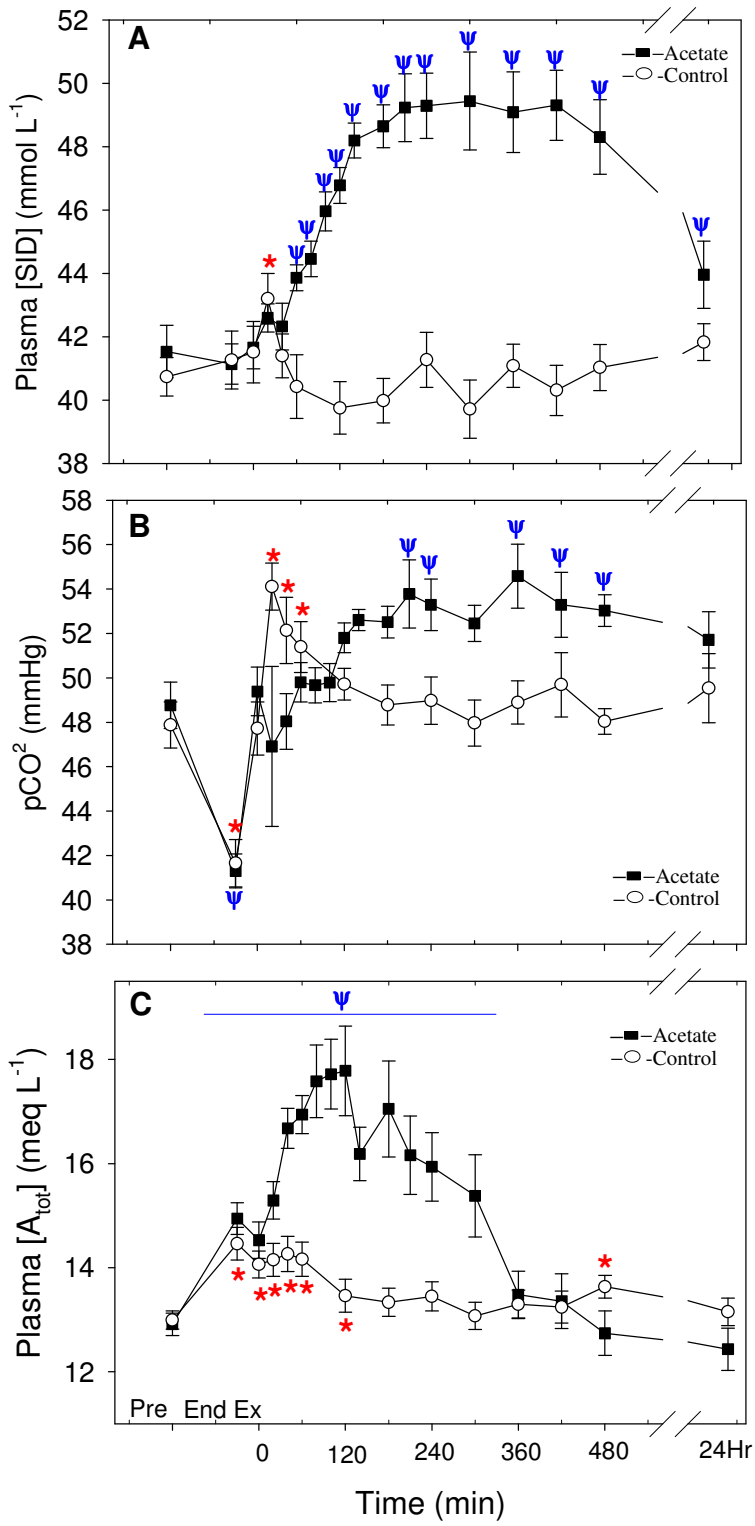
**Figure 6.1. Linear regression relationship (solid lines) and 95% confidence interval (dashed lines) between measured and calculated plasma  $[H^+]$  in 9 horses before and after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). SEE = standard error of the estimate.



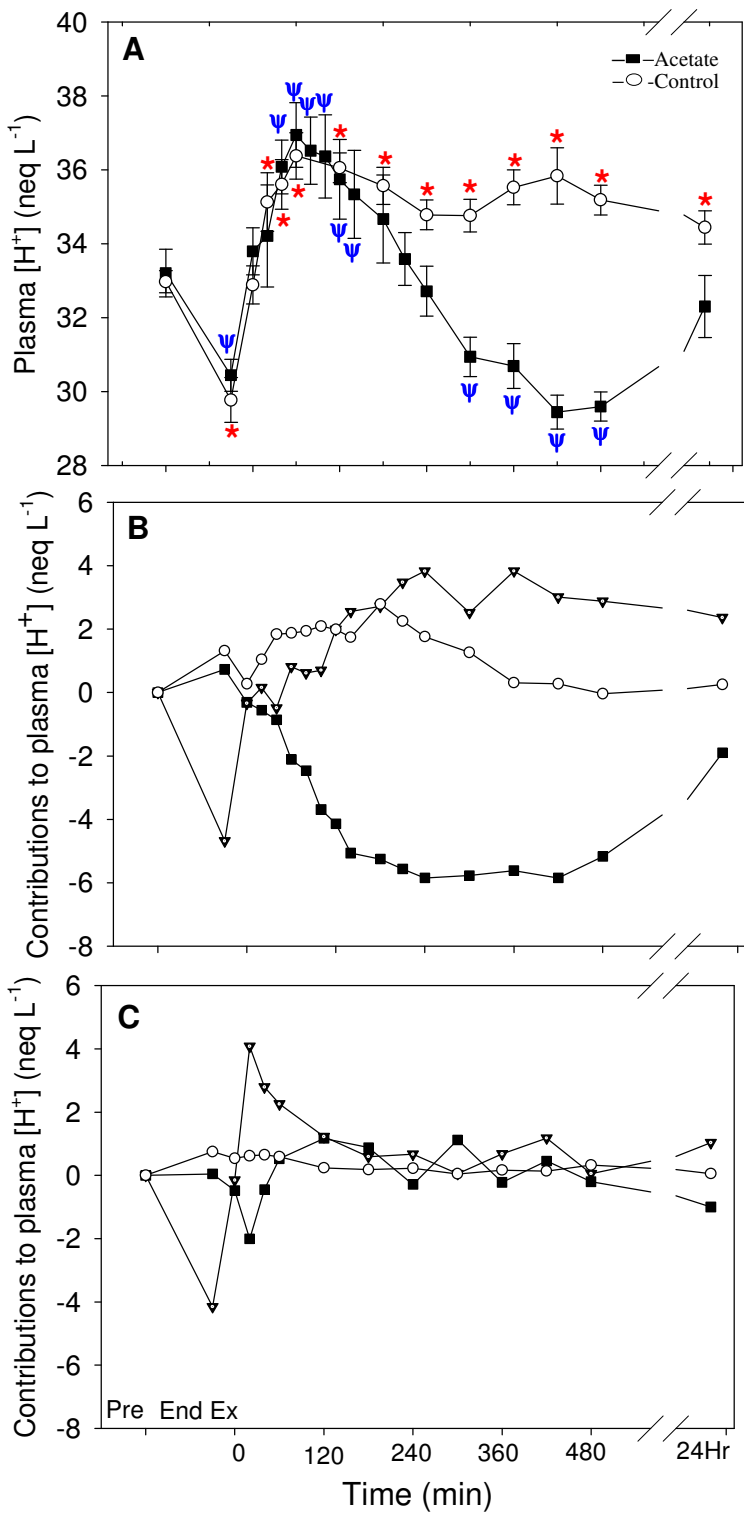
**Figure 6.2. The time course of plasma electrolyte concentrations: (A) sodium (Na<sup>+</sup>), (B) potassium (K<sup>+</sup>), (C) chloride (Cl<sup>-</sup>) and (D) calcium (Ca<sup>2+</sup>) after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean  $\pm$  SE for 9 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.



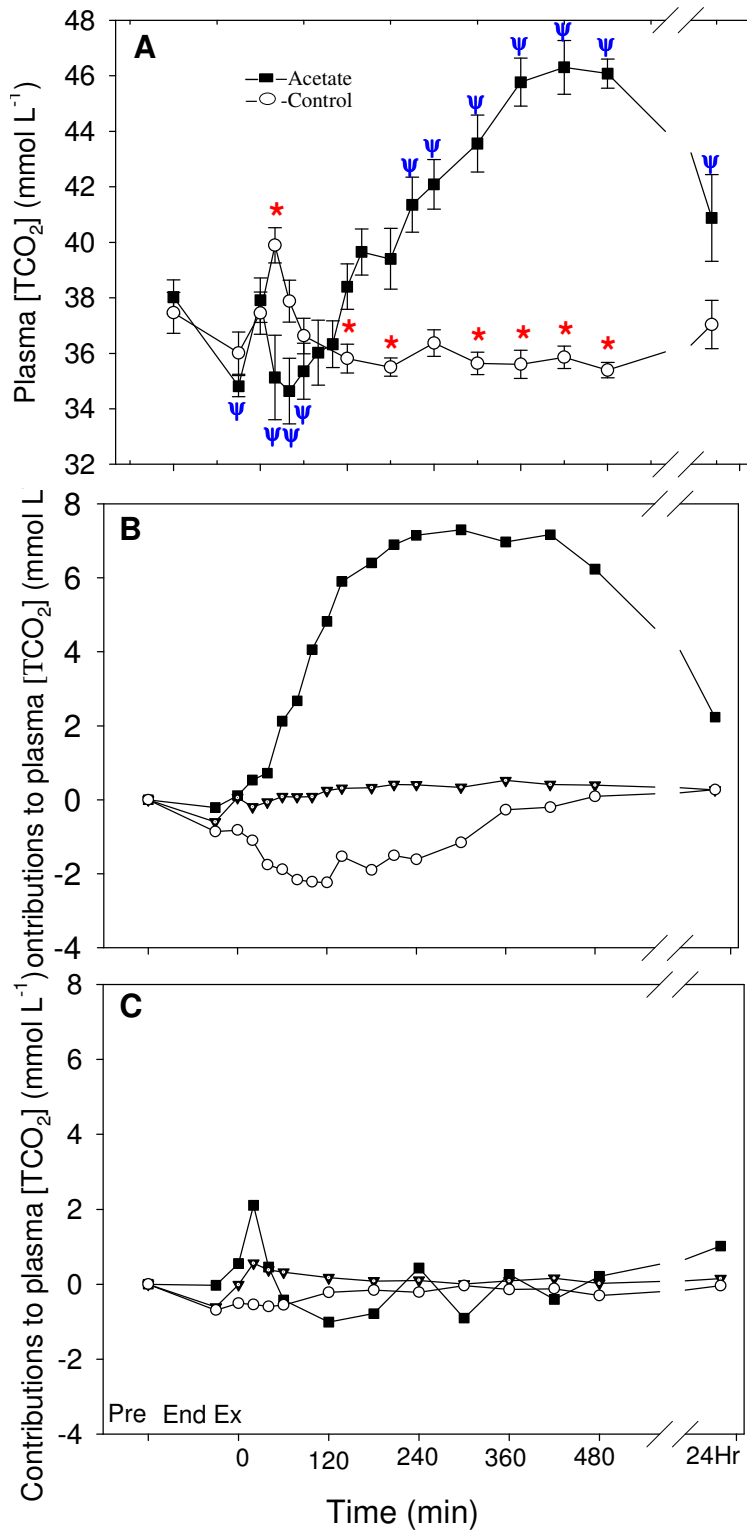
**Figure 6.3. The time course of plasma acetate concentration after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean  $\pm$  SE for 9 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.



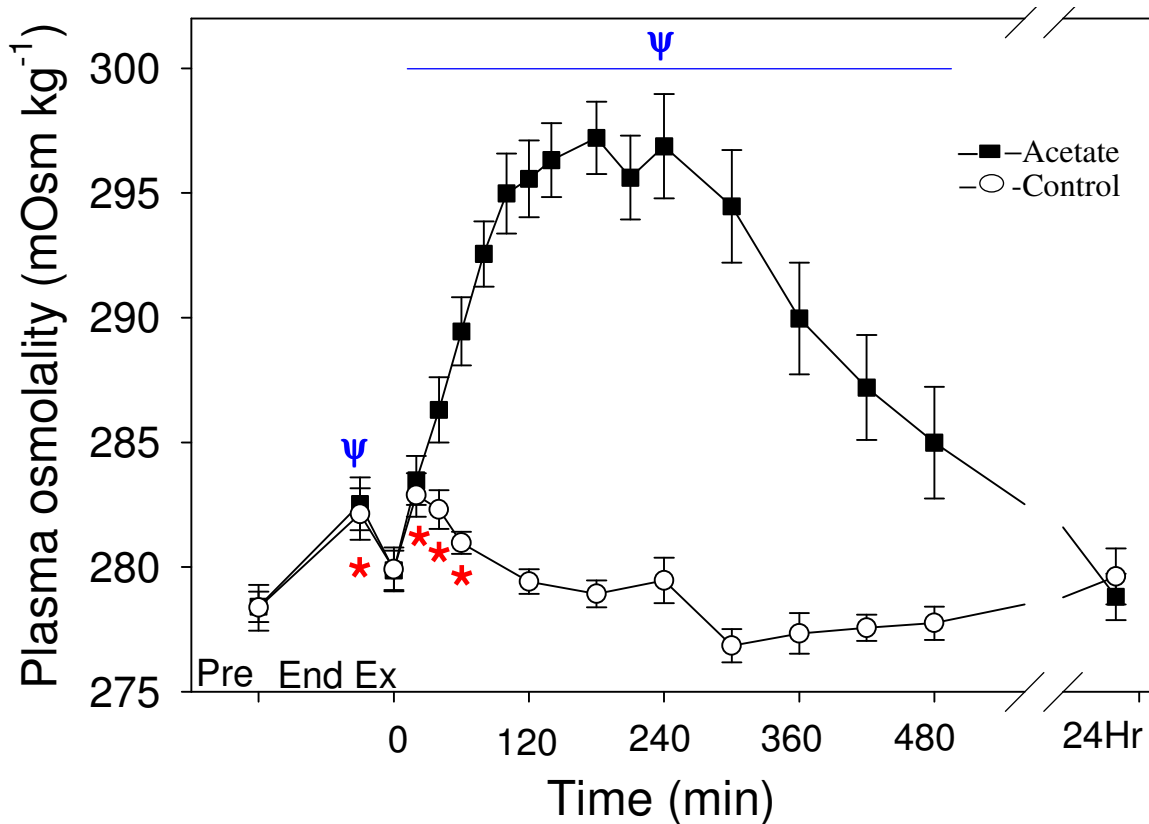
**Figure 6.4. The time course of the independent acid-base variables in plasma: (A) strong ion difference ([SID]), (B) partial pressure of carbon dioxide (PCO<sub>2</sub>), and (C) total weak acid concentration ([A<sub>tot</sub>]), after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean ± SE for 9 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.



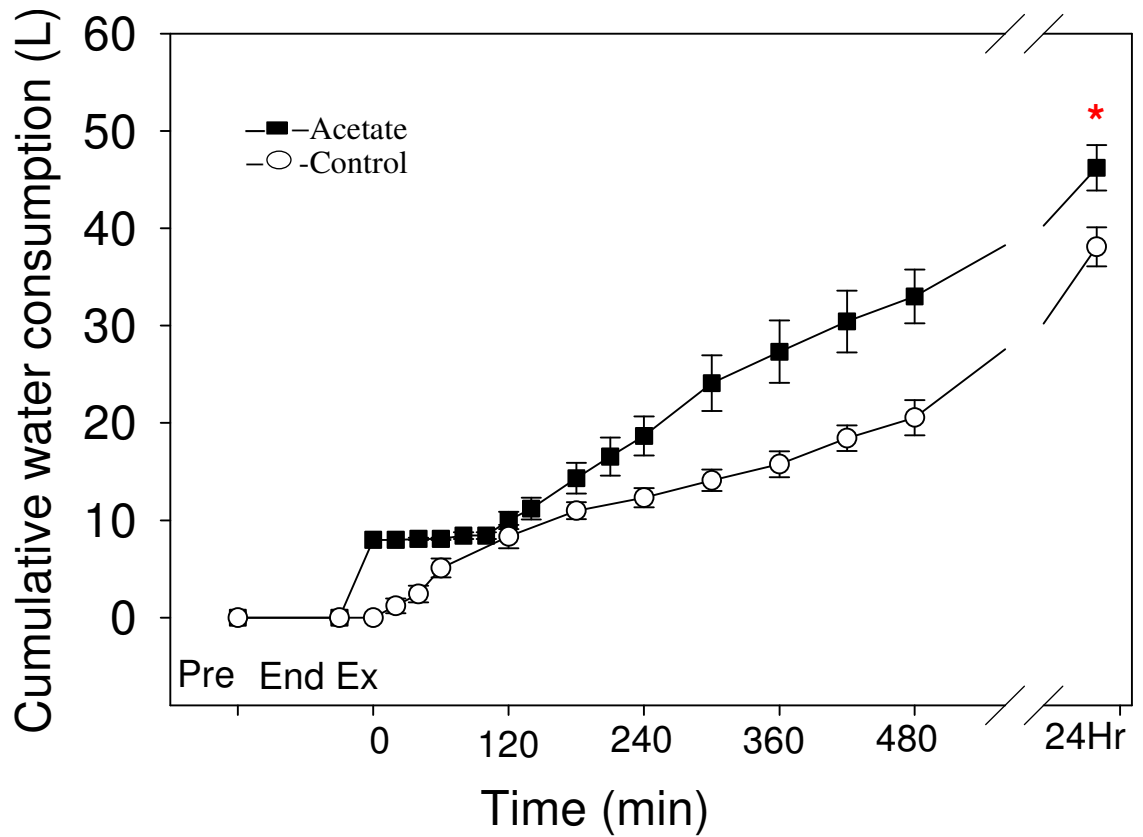
**Figure 6.5. The time course of the dependent acid-base variable plasma hydrogen ion concentration ( $[H^+]$ ) after a Competition Exercise Test, and the contributions of the independent variables strong ion difference ( $[SID]$ , solid square), total weak acid concentration ( $[A_{tot}]$ , hollow circle) or partial pressure of carbon dioxide ( $PCO_2$ , solid triangle) to the change in plasma  $[H^+]$ , for (B) the NAA trial and (C) the Control trial.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean  $\pm$  SE for 9 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.



**Figure 6.6. The time course of the plasma total carbon dioxide concentration ([TCO<sub>2</sub>]) after a Competition Exercise Test, and the contributions of the independent variables strong ion difference ([SID], solid square), total weak acid concentration ([A<sub>tot</sub>], hollow circle) or partial pressure of carbon dioxide (PCO<sub>2</sub>, solid triangle) to the change in [TCO<sub>2</sub>], for (B) the NAA trial and (C) the Control trial. The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean ± SE for 9 horses. \*, Ψ: significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.**



**Figure 6.7. The time course of the changes in plasma osmolality, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean  $\pm$  SE for 9 horses. \*,  $\Psi$ : significantly different from baseline (pre-exercise) time point for Control and NAA trials, respectively.



**Figure 6.8. The time course of cumulative water consumption, after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (NAA trial; solid square), or stood in stocks (Control trial; hollow circle). Horses were fed immediately after the 0 and 360 min samples. Values are mean  $\pm$  SE for 9 horses. \* = significantly different from the Control trial.

## Chapter 7

### **Oral Acetate Supplementation After Prolonged Moderate Intensity Exercise Enhances Early Muscle Glycogen Resynthesis in Horses**

#### **7.1 Introduction**

Carbohydrate in the form of muscle glycogen is the main energy source for glycolytic and oxidative ATP production during both short term high intensity and prolonged submaximal exercise in horses (Snow et al 1981, Snow and Harris 1991, Lacombe et al 1999, 2001, Hyypa et al 1997). Muscle glycogen appears to be important for peak performance, as depletion of intramuscular glycogen stores is associated with fatigue during both endurance (Snow et al 1981) and high intensity exercise (Lacombe et al 1999, Lacombe et al 2001). In addition, horses that are exercised frequently or undergo several warm-ups may have depleted glycogen stores that persist at the time of subsequent exercise bouts or performances (Snow and Harris 1991, Hyypa et al 1997). Indeed, persistently low muscle glycogen concentrations have been reported in Standardbreds during training (Essen-Gustavsson et al 1989).

In rats (Garetto et al 1984) and humans (Costill et al 1981) post-exercise restoration of muscle glycogen stores is usually complete within 2.5 h and 24 h, respectively. In contrast, in horses complete restoration of muscle glycogen stores requires 48-72 h, even when a high soluble CHO meal is fed immediately after exercise (Lacombe et al 2004). Previous studies in horses have provided supplemental glucose as a strategy to enhance

muscle glycogen resynthesis. I.v. infusion of large amounts of glucose (3-6 g/kg) modestly accelerated muscle glycogen replenishment (Davie et al 1995, Geor et al 2006), however conventional feeding strategies have not been successful (Snow and Harris 1991, Topliff et al 1983, Topliff et al 1985, Hyypa et al 1997, Jose-Cunilleras et al 2006). Additionally, the feeding of large amounts of soluble CHOs is not recommended in horses, due to the risk of gastrointestinal disorders (Murray 1994, Tinker et al 1997) and laminitis (Garner et al. 1975). There is a clear need for research into alternate nutritional strategies for optimizing recovery of muscle glycogen in horses.

It has been speculated that muscle glycogen replenishment during recovery is so slow in horses because skeletal muscle glucose is used as a substrate for pyruvate production and oxidation, because the availability of other substrates, such as triglycerides and nonesterified fatty acids is low (Poso and Hyypa 1999). At rest, glucose and acetate, a volatile fatty acid (VFA) absorbed from the large intestine following hindgut fermentation (Glinsky et al 1976, Hintz et al 1978, Argenzio et al 1974), are the major energy sources for hindlimb muscles in horses (Pethick et al 1993). Acetate is a metabolic precursor to acetyl-CoA, and tracer studies have shown that it is metabolized mainly to CO<sub>2</sub> and water via the TCA cycle and respiratory chain (Mohme et al 1970), generating ATP within the mitochondria. Based on *in vitro* results (Williamson 1964, 1965, Randle et al 1970), an ingested acetate and glucose solution increased muscle glycogen replenishment and glycogen synthase activity in rats after exercise, compared with glucose alone (Fushimi et al 2001, 2002). In a dose response study, increasing acetate supplemented to growing pigs resulted in proportional increases in skeletal

muscle glucose extraction and increased glycogen contents of liver, heart and skeletal muscle (Imoto and Namioka 1983).

While the upper gastrointestinal tract absorption and tissue metabolic effects of oral acetate remain largely unknown in horses, i.v sodium acetate clearance was accelerated during exercise (Pratt et al 2005), suggesting that acetate is used as an energy source during exercise. These results led us to speculate that acetate may be a practical alternative energy source for enhancing post-exercise muscle glycogen resynthesis in horses. Therefore the purpose of the present study was to determine the effect of oral acetate administration on plasma acetate appearance, skeletal muscle acetate metabolism and glycogen resynthesis. It was hypothesized that the provision of a sodium acetate/acetic acid/ potassium chloride solution with a typical hay and grain meal after glycogen-depleting exercise would result in a faster rate of muscle glycogen resynthesis than a grain/hay meal alone. Specifically, the addition of acetate and electrolytes to a typical post exercise meal would result in: 1) rapid appearance of acetate in blood; and 2) rapid skeletal muscle extraction of acetate and conversion to acetyl-CoA and acetylcarnitine, which will be metabolized via the TCA cycle generating ATP within the mitochondria, ultimately directing extracted muscle glucose to glycogenesis.

## **7.2 Methods**

**Animals:** Eight Standardbred and one Thoroughbred horses (7 geldings, 2 mares; body weight  $470 \pm 11$  kg; age 5-12 yrs) from the University of Guelph research herd were used

between November and February. The horses underwent a 4-6 week diet and exercise acclimation / conditioning period during which they were housed in individual box stalls with 7 h of daily turnout in a half acre paddock with minimal forage available. Horses were exercise trained 4 days/week on a high speed treadmill (SATO, Sweden) and 2 days/week on an outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada), until able to comfortably perform a competitive exercise test (CET) (Marlin et al. 1996, 1999) on a high speed treadmill designed to significantly decrease muscle glycogen content (Poso and Hyypya 1999) and result in total body water losses of 8-10 L (Marlin et al 1999). The CET is designed to simulate the 2<sup>nd</sup> day (speed and endurance test – classic format) of a one star CCI 3-day event, and includes the following phases: 10 min walk (1.7 m/s), 10 min trot (3.7 m/s), 2 min gallop (10.0 m/s), 20 min trot (3.7 m/s), 10 min walk (1.7 m/s), 8 min canter (8.0 m/s), and 10 min walk (1.7 m/s).

The horses were maintained on a diet (see Table 7.1) consisting of sweet feed (Purina Check-R-Mix 12%, DCAB {Na<sup>+</sup> + K<sup>+</sup> – Cl<sup>-</sup>} = -68.6 meq kg<sup>-1</sup>) twice daily and mixed grass hay (DCAB = 303.2 meq kg<sup>-1</sup>) three times daily, with free access to water and a salt block. The amount of feed given was increased over this acclimation period such that during the final two weeks the horses received 4 kg sweet feed and 6 kg hay daily (dietary DCAB = 154.5 meq kg<sup>-1</sup>), and there were no significant changes in the body masses of the horses during this time. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

**Experimental Protocol:** The study consisted of a treatment and control, thus each horse performed the CET twice in randomized order, separated by an 8-10 day interval (Marlin et al 1999, Hyypa et al 1996) during which time exercise conditioning was maintained. A timeline of the experimental protocol is shown in Figure 7.1. On both sampling days beginning at 7 am, catheters (14-gauge, 5.25 in; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted anterograde into the left or right jugular veins, secured with tape and stitched to the skin. Four-way stopcocks with 50 cm extensions were attached to the catheters for ease of blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2000 IU<sup>-1</sup> NaCl).

A pre-exercise blood sample was taken at 8 am and then the CET was performed. Immediately upon completion of the final canter an 'end of exercise' blood sample was taken, following which the horse walked for 10 min. Within 10 min of cessation of exercise, the horse either i) received by nasogastric intubation an acetate-electrolyte solution consisting of 500g sodium acetate (134g Na & 366 g acetate), 250 ml acetic acid (250 g acetate), 32 g KCl, and 300 g glucose in 8 L of water (Osmolality = 2944 mOsm kg<sup>-1</sup>) (Acetate treatment), or ii) stood in stocks for equivalent amount of time (Control treatment). The acetate + glucose dosage was calculated to be able to replace all the skeletal muscle glycogen glucosyl units degraded during the period of exercise, assuming a ~40% decrease in muscle glycogen content (Poso and Hyypa 1999). The amount of NaAcetate and acetic acid given was established by a series of 4 pilot studies to

determine the maximum amounts tolerated without gastrointestinal upset, and then the amount of glucose needed to replace the remaining glycosyl units was calculated.

Within 20 minutes of cessation of exercise, the horses were given 2 kg sweet feed and 3 kg hay (0 min of recovery), with access to water *ad libitum*. Horses were given 2 kg sweet feed and 3 kg hay at 6 hrs of recovery, and 2 kg hay at 12 hrs recovery. Blood samples were taken at 20-60 min intervals up to 8 hrs of recovery, and again at 24 hrs of recovery; horses remained in their stalls for the duration of sampling.

Muscle samples were collected from the gluteus medius by use of the needle biopsy technique (Lindholm and Piehl 1974). Samples were collected by use of aseptic technique after desensitization of the area with 2% mepivacaine (Upjohn, Orangeville, ON). Muscle samples were collected at a uniform depth from four standardized sites in the left or right middle gluteal muscles before exercise (baseline), 15 min after cessation of exercise (0 h), and at 4 h and 24 h post treatment. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

**Sample Analysis:** Each blood sample was collected into 7 ml heparinized vacutainers and immediately analyzed for plasma [glucose] using a Nova Stat Profile 9<sup>+</sup> (NOVA Biomedical, Waltham, MA). Remaining blood was transferred into two 1.5 ml conical centrifuge tubes and centrifuged for 5 min at 15 000g to separate the plasma. Plasma [acetate] was measured spectrophotometrically, in duplicate, using a commercially available kit (CV 1.6%; R-Biopharm, Marshall MI).

A small piece of frozen (liquid N<sub>2</sub>) wet muscle (20-30 mg) was homogenized as described by Putman et al (1993), and analyzed for pyruvate dehydrogenase activity (PDHa), using a commercially available kit (CV <10%; MitoSciences, Eugene OR). PDHa was calculated from the rate of absorbance change of NADH and Beer's Law. The remainder of the biopsy sample was freeze-dried, dissected of all visible blood, connective tissue and fat then powdered for subsequent analysis. Muscle H<sub>2</sub>O content (L kg<sup>-1</sup> dry wt) was determined as the difference in sample weight before and after freeze-drying. Skeletal muscle creatine, phosphocreatine (PCr), ATP, lactate, glycerol 3 phosphate (G-3-P), glucose 6 phosphate (G-6-P), glucose 1 phosphate (G-1-P) and fructose 6 phosphate (F-6-P) were determined by enzymatic spectrophotometric assays (Bergmeyer 1974, Harris et al 1974). Glycogen content (as glucosyl units) was determined in duplicate after acid hydrolysis as described by Passonneau and Lauderdale (1974). Acetyl-CoA (Ac-CoA) and acetylcarnitine (Ac-Carn) were determined by radiometric measures (Cederblad et al 1990). All muscle measurements were corrected for the highest total creatine measured from each horse.

**Statistics.** Data are presented as mean ± standard error. Differences between means with respect to treatment and time were assessed by two-way repeated measures analysis of variance. Within treatment, changes over time were assessed by one-way repeated measures analysis of variance. When a significant F-ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when P < 0.05 at a power of 0.8.

### 7.3 Results

In the Control treatment every horse consumed all of the sweet feed by 20 min post feeding (40 minutes post exercise) and the hay by 4 h post feeding. In the Acetate treatment the horses consumed all the hay by 6 h post feeding, however they typically consumed only 1/4 of the sweet feed offered within 20 min post feeding, and did not eat the remaining grain from both meals until between 12-24 h of recovery.

Plasma [glucose] was increased from the end of exercise until 360 min of recovery in Control and until the end of sampling in the Acetate treatment, with no difference ( $P = 0.386$ ) between trials (Fig 7.2).

Acetate supplementation elevated plasma [acetate] such that in the Acetate treatment plasma [acetate] was increased from pre-exercise ( $0.56 \pm 0.07 \text{ mmol L}^{-1}$ ) from 40-300 min of recovery and reached a maximum of  $3.56 \pm 0.58 \text{ mmol L}^{-1}$  at 100 min of recovery (Fig. 7.3). In the Control treatment there was no change in plasma [acetate] over time. There was an overall treatment effect such that plasma [acetate] was significantly greater than in Acetate vs Control ( $P = 0.002$ ).

Gluteus medius [Ac-CoA] (Fig. 7.4a) and [Ac-Carn] (Fig. 7.4b) were increased with exercise in both treatments. Acetate supplementation increased [Ac-CoA] such that at 4 h of recovery [Ac-CoA] was significantly higher in the Acetate treatment as compared to Control ( $P = 0.038$ ). Acetate supplementation increased [Ac-Carn] such that at 4 h of

recovery [Ac-Carn] was significantly higher in the Acetate treatment as compared to Control ( $P = 0.030$ ).

There were no significant differences in gluteus medius PDHa over time or between treatments.

Exercise significantly decreased middle gluteal muscle glycogen content by 21% and 17% from the initial (Pre-Ex) values in the Acetate and Control treatments, respectively (Fig. 7.6, Table 7.2). Acetate supplementation enhanced muscle glycogen resynthesis during the initial recovery period such that muscle glycogen content was not different from Pre-Ex at 4 h of recovery in the Acetate treatment. In contrast, in the Control treatment muscle glycogen content was still significantly decreased from Pre-Ex at 4 h. The rate of glycogen resynthesis from 0-4 h of recovery was ~triple in Acetate ( $22.1 \pm 11.3 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ) compared to Control ( $6.8 \pm 7.9 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ) ( $P = 0.261$ ) (Fig. 7.6, Table 7.2). From 4-24 h of recovery in Control there was a significant increase in glycogen content, such that by 24 h of recovery, muscle glycogen content was not different from Pre-Ex values with either treatment. From 4-24 h of recovery there was a tendency ( $P = 0.064$ ) for the rate of glycogen resynthesis to be higher in Control ( $7.2 \pm 1.9 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ) compared to Acetate ( $3.7 \pm 1.4 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$ ). The overall (0-24 h) mean rates of glycogen synthesis were similar ( $P = 0.891$ ) at  $5.7 \pm 2.1$  and  $7.1 \pm 1.7 \text{ mmol kg}^{-1} \text{ h}^{-1} \text{ dw}$  for the Acetate and Control treatments, respectively.

There was no effect of time or treatment on the intermediates of glucose metabolism G-6-P, G-1-P, F-6-P, lactate and G-3-P (Table 7.2), or on muscle metabolites ATP, PCr and creatine (Table 7.3), for either the Acetate or Control treatments. At 4 h of recovery there was a trend ( $P = 0.088$ ) towards decreased H<sub>2</sub>O content (Table 7.3) in the Acetate treatment.

## **7.4 Discussion**

The present study appears to be the first to have determined the effects of providing a sodium acetate/acetic acid solution with a typical hay and grain meal after prolonged exercise on muscle carbohydrate metabolism and skeletal muscle glycogen resynthesis in horses. Acetate supplementation resulted in a rapid and sustained increase in plasma [acetate], and increases in skeletal muscle [Ac-CoA] and [Ac-Carn], supporting our hypothesis of substantial tissue extraction of the supplemented acetate. Acetate also enhanced the rate of muscle glycogen resynthesis during the initial 4 h of the recovery period compared to Control, while by 24 h of recovery there was no difference in glycogen replenishment between treatments. It is concluded that oral acetate is an alternative energy source in the horse and that it can be used to enhance the early resynthesis of skeletal muscle glycogen post-exercise.

### ***Glycogen Resynthesis***

Based on the decrease of skeletal muscle glycogen, the horses in the present study used ~132 mmol glucosyl units kg<sup>-1</sup> dw during 1 h of exercise. The rate of glycogen resynthesis from 0-4 h of recovery was approximately triple in the Acetate treatment

compared to Control ( $22.1 \pm 11.3$  vs  $6.8 \pm 7.9$  mmol kg<sup>-1</sup> h<sup>-1</sup> dw), such that muscle glycogen content was not different from Pre-Ex at 4 h of recovery in the Acetate treatment, but was still significantly decreased from Pre-Ex in the Control treatment. From 4-24 h of recovery there was a significant increase in glycogen content in the Control treatment only, such that by 24 h of recovery, muscle glycogen content was not different from Pre-Ex values in either treatment. Interestingly, the rate of muscle glycogen resynthesis during the first 4 h post exercise in the Acetate treatment of the present study was similar to the maximum reported rates in horses following i.v. infusion of glucose. I.v. infusion of glucose at 6 g kg<sup>-1</sup> bwt after exercise resulted in initial (0-6 h) glycogen storage rates of  $19.9 \pm 3.8$  (Davie et al 1995) and  $20.9 \pm 7.3$  (Geor et al 2006) mmol kg<sup>-1</sup> dw<sup>-1</sup> h after ~50% glycogen depletion, while rates up to ~29 mmol kg<sup>-1</sup> dw<sup>-1</sup> h have been reported with ~80% muscle glycogen depletion (Lacombe et al 2001). Initial glycogen resynthesis rates in horses after ingestion of industry-typical meals with varying soluble CHO contents range from ~8 to ~12 mmol kg<sup>-1</sup> dw<sup>-1</sup> h for mixed and high soluble CHO diets, respectively (Lacombe et al 2004). In contrast, rates of glycogen repletion in humans are 2-3 fold higher than the rates seen in horses with CHO ingestion (Bergstrom and Hultman 1966, Jentjens et al 2001) or i.v. glucose infusion (Bergstrom and Hultman 1967, Reed et al 1989).

The exercise protocol in the present study (prolonged, moderate intensity) was chosen for its ability to significantly decrease muscle glycogen with a single exercise bout without musculoskeletal injury, however the glycogen utilization was not as large as with an intensive 3-day protocol of repeated sprints and a restrictive diet that reduced muscle

glycogen by 50-80% (Lacombe et al 2001, 2003, 2004, Jose-Cunilleras et al 2005, 2006). Thus, in contrast to previous reports that glycogen resynthesis requires 48-72 h with a mixed CHO diet (Lacombe et al 2004), the decreased glycogen depletion seen in the present study in the Control trial horses had resynthesized muscle glycogen by 24 h of recovery.

The low rate of glycogen synthesis with oral CHO provision in horses has been suggested to be due to reduced rate of delivery from the gastrointestinal tract (Geor et al 2006), combined with a lack of increase in post exercise insulin sensitivity and membrane GLUT-4 translocation (Pratt et al 2007). Additionally, in contrast to humans in which lipids are the predominant energy source during recovery (Kimber et al 2003, Henderson et al 2007), the availability of triglycerides and nonesterified fatty acids is low during recovery in horses (Poso and Hyypa 1999). Therefore in horses it may be that glucose (or acetate) may be preferentially diverted towards energy production instead of to glycogen synthesis (Hyypa et al 1997). Furthermore, the feeding of large amounts of soluble CHOs is not recommended in horses, due to the risk of gastrointestinal disorders (Murray 1994, Tinker et al 1997) and laminitis (Garner et al 1975). Thus there is a clear need for research into alternate nutritional strategies for optimizing recovery of muscle glycogen in horses. Horses derive 30-40% of their maintenance energy requirements from volatile fatty acid (VFA) absorbed from the large intestine following hindgut fermentation of primarily forage, of which acetate is the VFA produced in the greatest quantity (Glinsky et al 1976, Hintz et al 1978, Argenzio et al 1974). Pethick et al (1993) found that acetate contributed ~32% of the total hindlimb substrate oxidation in horses at

rest, therefore we hypothesized that the provision of supplemental acetate during recovery may “spare” glucose for glycogen replenishment. Indeed during the first 4 h of recovery in the present study, despite similar plasma [glucose] in both treatments, muscle glycogen synthesis, [Ac-CoA] and [Ac-Carn] were all greater in the Acetate treatment, suggesting significant skeletal muscle availability of the supplemental acetate for oxidation in the TCA cycle, potentially shuttling muscle glucose to glycogenesis. There were no changes in [G-6-P] or other measured intermediates of glucose metabolism in the present study, similar to previous studies of glycogen resynthesis during exercise recovery in humans (Richter et al 1982, Medbo et al 2006).

To date, only Poso and Hyypa (1999) have investigated the effect of VFA supplementation on glycogen resynthesis in horses. Horses were orally supplemented with either a glucose-electrolyte (GE) solution alone or with 200 ml propionate added (GEP) after glycogen depleting exercise. Neither treatment increased glycogen content 22.5 h after exercise. However, the amount of glucose (~38 g) and propionate given were very small, given that humans consume at least 2.4 g/kg (Jentjens and Jeukendrup 2003). As well, neither the GE nor the GEP solutions caused any increase in plasma [glucose], suggesting that this amount of post-exercise CHO was much less than they would receive from a typical post exercise meal.

The findings of the present study agree with previous research on the effects of acetate supplementation on glycogenesis in pigs and rats (Imoto and Namioka 1983, Fushimi et al 2001, 2002, Nakao et al 2001). The enhanced glycogen repletion and increased

glycogen synthase activity by acetate feeding in rats was not due to differences in the activities of muscle hexokinase or plasma [insulin] (Fushimi et al 2001), indicating that acetate did not affect the glucose transport or phosphorylation steps. Plasma [insulin] was not measured in the present study, so although acetate does not affect the insulin response in horses (Argenzio and Hintz 1971), differences in the degree of insulinemia between treatments as a result of varied feed intake cannot be discounted.

### ***Acetate metabolism***

In the Acetate treatment the sustained and relatively constant elevation in plasma [acetate] from 40–300 min post acetate administration and ( $3.56 \pm 0.58 \text{ mmol L}^{-1}$  at 100 min of recovery) represented a steady state between intestinal absorption and tissue (primarily skeletal muscle) extraction. Based on the total amount of acetate given in the present study, if complete absorption and simultaneous distribution in the ECFV (~100 L) occurred, the peak increase in plasma [acetate] would be  $102 \text{ mmol L}^{-1}$ . Thus the time course of plasma [acetate] implies that tissue extraction of acetate occurred rapidly, likely facilitated by plasma membrane monocarboxylate transporters (Waniewski and Martin 2004, Hosoi et al 2008, Koho et al 2006).

Acetate extracted by skeletal muscle is readily converted to Ac-CoA by Ac-CoA synthetase. When the rate of Ac-CoA generation from acetate uptake, glycolysis or  $\beta$ -oxidation exceeds the rate at which it combines with oxaloacetate to form citrate, Ac-CoA will accumulate. Excess Ac-CoA binds rapidly with carnitine to form Ac-Carn in a reversible reaction catalyzed by carnitine acetyltransferase. The present study is the first

to demonstrate an increase in muscle [Ac-CoA] and [Ac-Carn] after acetate supplementation in horses, although this has been shown after iv acetate infusion in humans (Putman et al 1995, Evans et al 2001). Resting values for Ac-CoA and Ac-Carn in the present study are similar to those previously found in horses (Foster and Harris 1987, Carlin et al 1990, Harris and Foster 1990). During exercise accumulation of Ac-CoA and Ac-Carn increases with increasing work intensity in both horses (Carlin et al 1990, Harris and Foster 1990) and humans (Howlett et al 1998). Indeed in the present study significant increases in these metabolites were still present at 15 min after cessation of exercise (0 h recovery) in both treatments. In the present study, acetate supplementation increased skeletal muscle [Ac-CoA] and [Ac-Carn] at 4 h of recovery compared to Control, providing further evidence of sustained skeletal muscle extraction of supplemented acetate. The ability of carnitine to act as an acetyl group buffer has been demonstrated in both humans (Constantin-Teodosiu et al 1991, Harris et al 1987b) and horses (Foster and Harris 1987, Carlin et al 1990, Harris and Foster 1990). The formation of Ac-Carn functions to maintain the mitochondrial CoASH pool and to provide a store of readily available 'active acetate' for transacetylation back to Ac-CoA and entry into the TCA cycle (Harris et al 1987b).

PDH is a multienzyme complex located within the inner mitochondrial membrane that catalyzes the decarboxylation of pyruvate to Ac-CoA, therefore controlling the flux of glycolysis-derived pyruvate into the TCA cycle. PDH is important in the integration of CHO and fat oxidation in skeletal muscle, such that its activity (PDHa) is decreased during periods of increased Ac-CoA accumulation secondary to increased oxidation of fat

fuels (Putman et al 1993). Because no studies to date have examined skeletal muscle PDH in horses, based on research in humans (Putman et al 1995) we hypothesized that PDHa would decrease with acetate administration as a result of increased Ac-CoA. There were no differences in PDHa however. It remains unknown if there was sufficient accumulation of citrate to effect a downregulation of PFK activity in the Acetate treatment to effect increased incorporation of glucose into glycogen. Alternatively, the enhanced glycogen repletion with acetate feeding in the present study and in rats (Fushimi et al 2001) may have resulted in lowered F-1,6-P<sub>2</sub>/F-6-P, which reflects phosphofructokinase 1 (PFK-1) activity (Wakelam and Pette, 1982), suggesting that acetate may directly suppress glycolysis by inhibition of PFK-1.

### ***Perspectives and Limitations***

The horse evolved as a grazing hindgut fermenting herbivore using primarily fermentable forage CHO as the main energy source. Thus one possible reason for the difference in glycogen resynthesis rates between horses and humans may be the horse's limited ability to digest large amounts of soluble CHO (i.e. large amounts of grain). In humans a daily CHO intake of 7-10 g kg<sup>-1</sup> is required for a substantial increase in glycogen content (Jentjens and Jeukendrup, 2003). For a 475 kg horse an equivalent CHO dose would require the consumption of 6.5 to 9.5 kg of oats per day. Such a high grain intake is neither realistic nor recommended in most horses due to the risk of gastric ulcers (Murray 1994), colic (Tinker et al 1997) and laminitis (Garner et al 1975). Therefore based on the positive results of previous studies in other species that supplemented acetate in addition to glucose or regular meals, the protocol of supplementing acetate immediately prior to a

typical grain/hay meal was utilized in order to investigate a *practical* nutritional strategy for enhancing the recovery of muscle glycogen in horses. However based on pilot research demonstrating reduced feed consumption after acetate administration, 300 g of glucose was added to the acetate solution to replace the remaining glycosyl units degraded during exercise. In contrast the Control horses ingested 0.83 kg of starch from the first post exercise grain meal (Table 7.1). Therefore, assuming a small intestinal grain digestibility of ~80% (likely a conservative estimate for sweet feed; Meyer et al 1995), the Control horses received ~664 g of glucose after the initial grain feeding, while the Acetate horses probably consumed a maximum of 466 g glucose (if 1/4 the grain was consumed). Thus any difference in glucose provision between treatments is not likely have accounted for the increased glycogen resynthesis with acetate supplementation.

Although the acetate solution used in the present study was tolerated by the horses, most exhibited signs of moderate gastrointestinal disturbance (limited interest in eating or drinking, loose stool) during the initial hours after administration. Therefore despite increased glycogen resynthesis with acetate supplementation during the initial (0-4 h) recovery period, from 4-24 h of recovery glycogen synthesis was greater in Control. It is important to note that the horses in the Acetate treatment hardly consumed any grain until at least 12 h of recovery, whereas the Control horses immediately ate all the grain provided at 0 and 6 h of recovery. Thus although the glycosyl units provided with the acetate/glucose solution in the Acetate treatment were more than sufficient to replace all the skeletal muscle glycogen degraded during the CET, it is possible that providing them in a single large bolus slowed the rate of glycogen synthesis in the latter recovery period.

Indeed in humans it has been shown that smaller, more frequent doses of oral CHO (1.0-1.8 g/kg/h for the first 4 h) increase glycogen resynthesis rates compared to one equivalent large dose (Jentjens and Jeukendrup, 2003). Individual differences in the amount and rate of feed consumption in the acetate-supplemented horses led to substantial variation in individual glycogen synthesis rates, contributing to the lack of significance when comparing the rates between treatments. Ultimately a more palatable formulation of acetate is certainly needed before it can be considered a feasible nutritional supplement.

Additionally, the Acetate solution resulted in substantial osmotic and acid-base disturbances due to its large sodium load (see Chapter 6), which likely led to the tendency for decreased muscle H<sub>2</sub>O content at 4 h of recovery in the Acetate treatment. This intracellular dehydration may actually have contributed to decreasing the rate of glycogen resynthesis in the Acetate treatment, as has been suggested previously in horses (Chapter 5), humans (Keller et al 2003) and rats (Low et al 1996). It must also be stressed that ultimately our results reflect only the CET exercise protocol we utilized; employing this particular solution and supplement strategy in horses undergoing more severe or prolonged activity with greater fluid and electrolyte losses could lead to detrimental consequences.

## **7.5 Conclusions**

The present study determined the effects of providing a sodium acetate/acetic acid solution with a typical hay and grain meal after prolonged exercise on skeletal muscle glycogen resynthesis in horses. Acetate supplementation resulted in a rapid and sustained increase in plasma [acetate], and increases in skeletal muscle [Ac-CoA] and [Ac-Carn], suggesting substantial tissue extraction of the supplemented acetate. Acetate also enhanced the rate of muscle glycogen resynthesis during the initial 4 h of the recovery period, compared to Control. However, by 24 h of recovery, there was no differences in glycogen replenishment between treatments. It is concluded that oral acetate supplementation effectively modifies post-exercise glucose utilization by skeletal muscle, serving to preferentially direct extracted glucose towards glycogen resynthesis.

**Table 7.1 Nutritional analysis of sweet feed and mixed grass hay.**

Values are on a dry matter basis except for DCAB. ADF: acid detergent fibre, NDF: neutral detergent fibre, DCAB: dietary cation anion balance (Na + K – Cl).

	Sweet Feed	Mixed Hay
Dry Matter (%)	85.5	83.3
Protein (%)	12.7	12.0
ADF (%)	8.8	42.9
NDF (%)	28.5	58.3
Starch (%)	41.5	----
DCAB (meq/kg)	-68.6	303.2

**Table 7.2. Gluteus medius glucose metabolism intermediates at rest and during recovery from prolonged moderate intensity Competition Exercise Test, after horses were either given 1) an oral sodium acetate/acetic acid solution followed by water *ad libitum* and a typical feeding protocol (Acetate treatment; Ac), or 2) water *ad libitum* and a typical feeding protocol (Control treatment).**

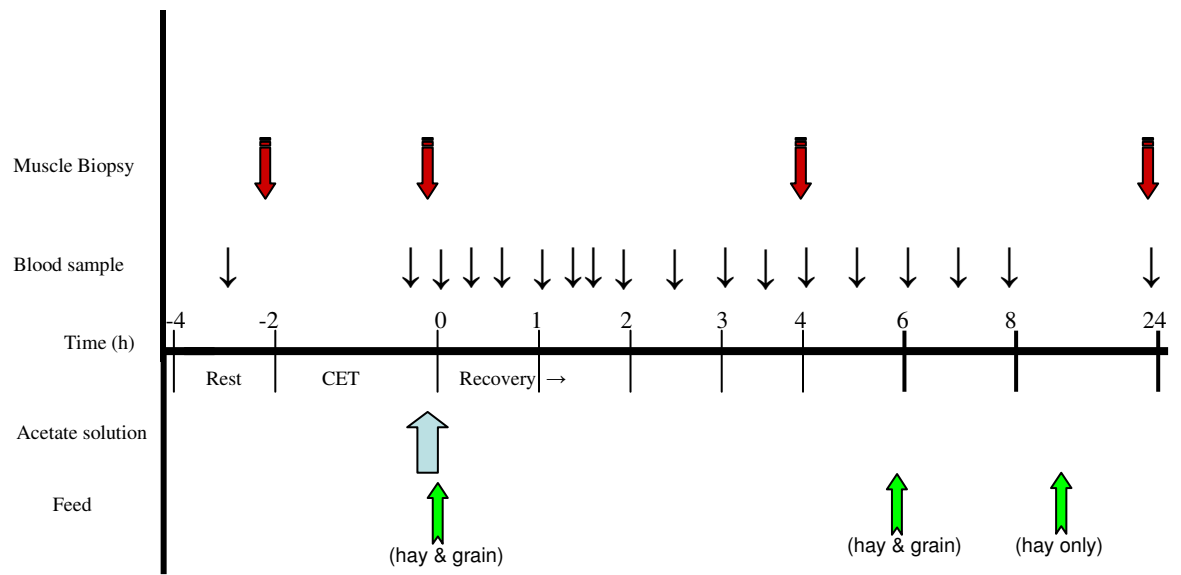
Time (h)	Glycogen		G-1-P		G-6-P		F-6-P		G-3-P		Lactate	
	Ac	Control	Ac	Control	Ac	Control	Ac	Control	Ac	Control	Ac	Control
Pre-Ex	731.7	639.9	0.08	0.08	0.10	0.18	0.12	0.08	2.21	2.52	8.5	7.9
	± 31.2	± 34.3	± 0.03	± 0.03	± 0.06	± 0.06	± 0.04	± 0.03	± 0.3	± 0.4	± 1.3	± 0.7
0	576.0	531.7	0.06	0.03	0.22	0.14	0.10	0.10	1.96	2.04	7.6	9.9
	± 16.6*	± 31.1 *	± 0.03	± 0.02	± 0.07	± 0.07	± 0.03	± 0.03	± 0.3	± 0.1	± 1.0	± 2.3
4	664.3	558.8	0.17	0.12	0.12	0.15	0.05	0.08	1.87	2.30	5.7	7.3
	± 53.3	± 23.4 *	± 0.06	± 0.04	± 0.06	± 0.05	± 0.04	± 0.04	± 0.4	± 0.6	± 1.4	± 1.2
24	737.4	702.0	0.08	0.09	0.16	0.16	0.14	0.13	1.83	2.03	9.5	8.7
	± 61.0	± 41.6	± 0.04	± 0.03	± 0.08	± 0.07	± 0.05	± 0.05	± 0.3	± 0.2	± 1.9	± 1.2

G-1-P = glucose 1 phosphate, G-6-P = glucose 6 phosphate, F-6-P = fructose 6 phosphate, G-3-P = glycerol 3 phosphate. Values are mean ± SE for 9 horses. Metabolite units mmol kg<sup>-1</sup> dry wt. \* = significantly different from Pre-Ex.

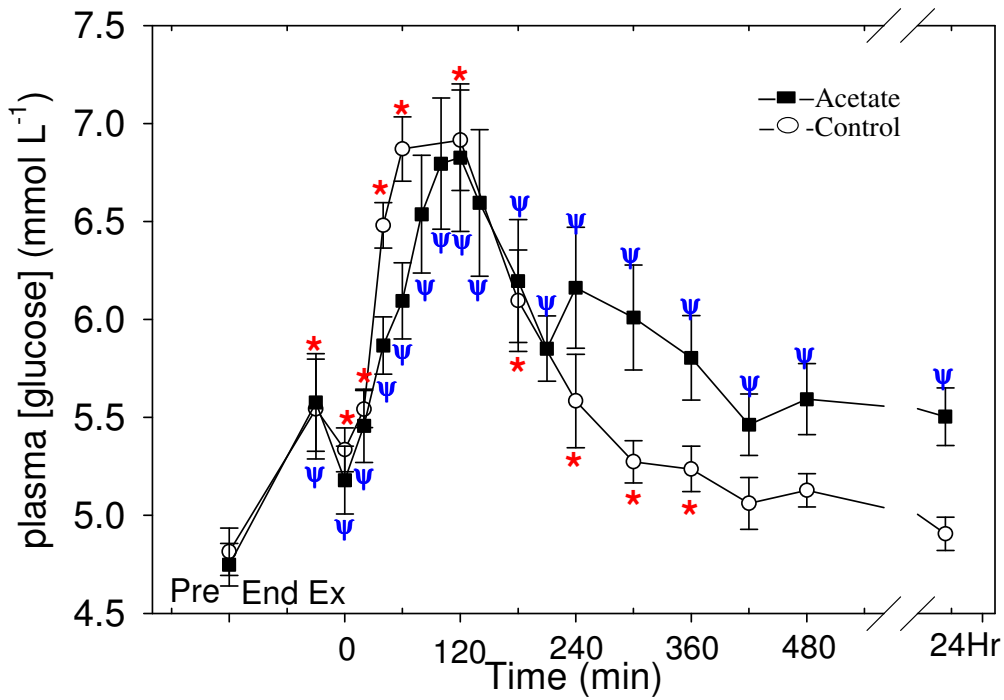
**Table 7.3. Gluteus medius metabolite concentrations and water content at rest and during recovery from a prolonged moderate intensity Competition Exercise Test, after horses were either given 1) an oral sodium acetate/acetic acid solution followed by water *ad libitum* and a typical feeding protocol (Acetate treatment; Ac), or 2) water *ad libitum* and a typical feeding protocol (Control treatment).**

Time (h)	ATP		Creatine		PCr		H <sub>2</sub> O content	
	Ac	Control	Ac	Control	Ac	Control	Ac	Control
Pre-Ex	21.2 ± 0.8	20.1 ± 0.8	14.5 ± 2.1	19.0 ± 1.4	50.6 ± 2.8	46.1 ± 1.7	2.50 ± 0.09	2.49 ± 0.11
	19.1 ± 0.4	20.9 ± 0.8	19.0 ± 0.9	16.5 ± 1.3	46.7 ± 2.1	48.6 ± 1.7	2.48 ± 0.10	2.50 ± 0.09
0	21.1 ± 1.7	21.6 ± 1.0	18.8 ± 2.2	16.9 ± 1.8	45.8 ± 2.0	48.2 ± 2.3	2.34 ± 0.07	2.58 ± 0.08
	20.7 ± 0.8	20.1 ± 1.1	15.3 ± 1.3	18.4 ± 1.8	49.7 ± 2.9	46.6 ± 2.2	2.46 ± 0.13	2.38 ± 0.18

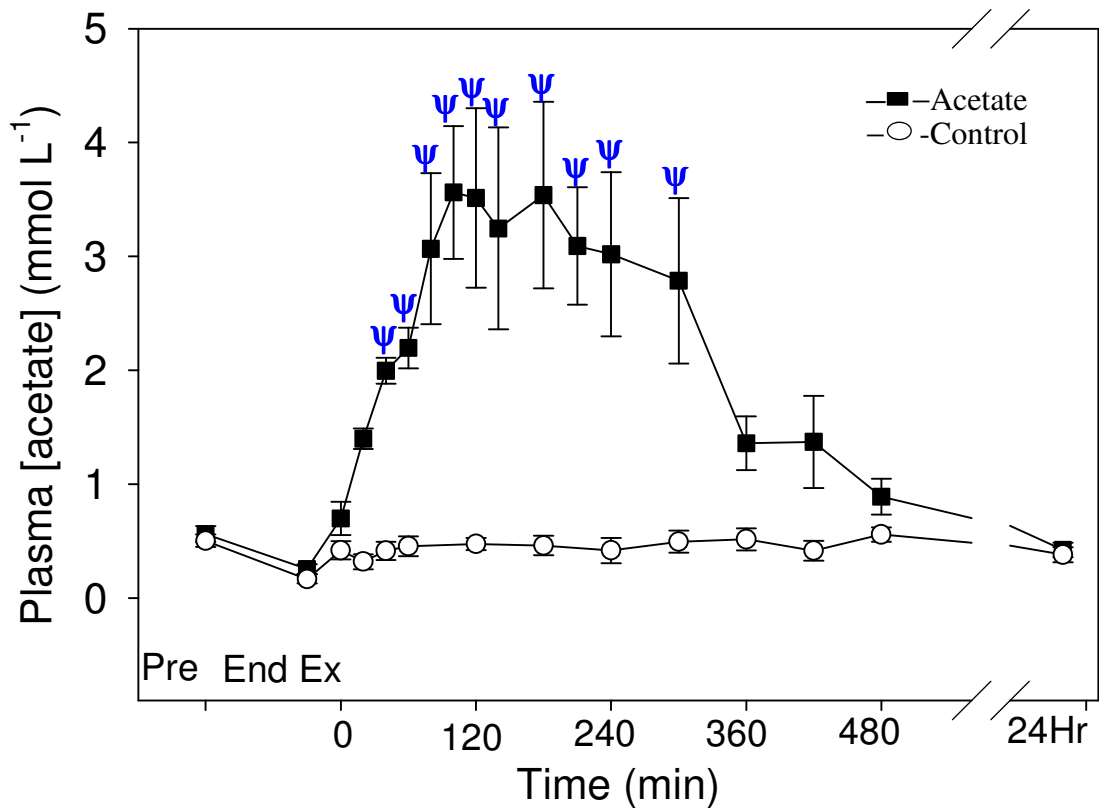
PCr = phosphocreatine. Values are mean ± SE for 9 horses. Metabolite units mmol kg<sup>-1</sup> dry wt; H<sub>2</sub>O content in L kg<sup>-1</sup> dry wt. No significant differences (P<0.05) between treatments and from pre-exercise time point for any of the variables.



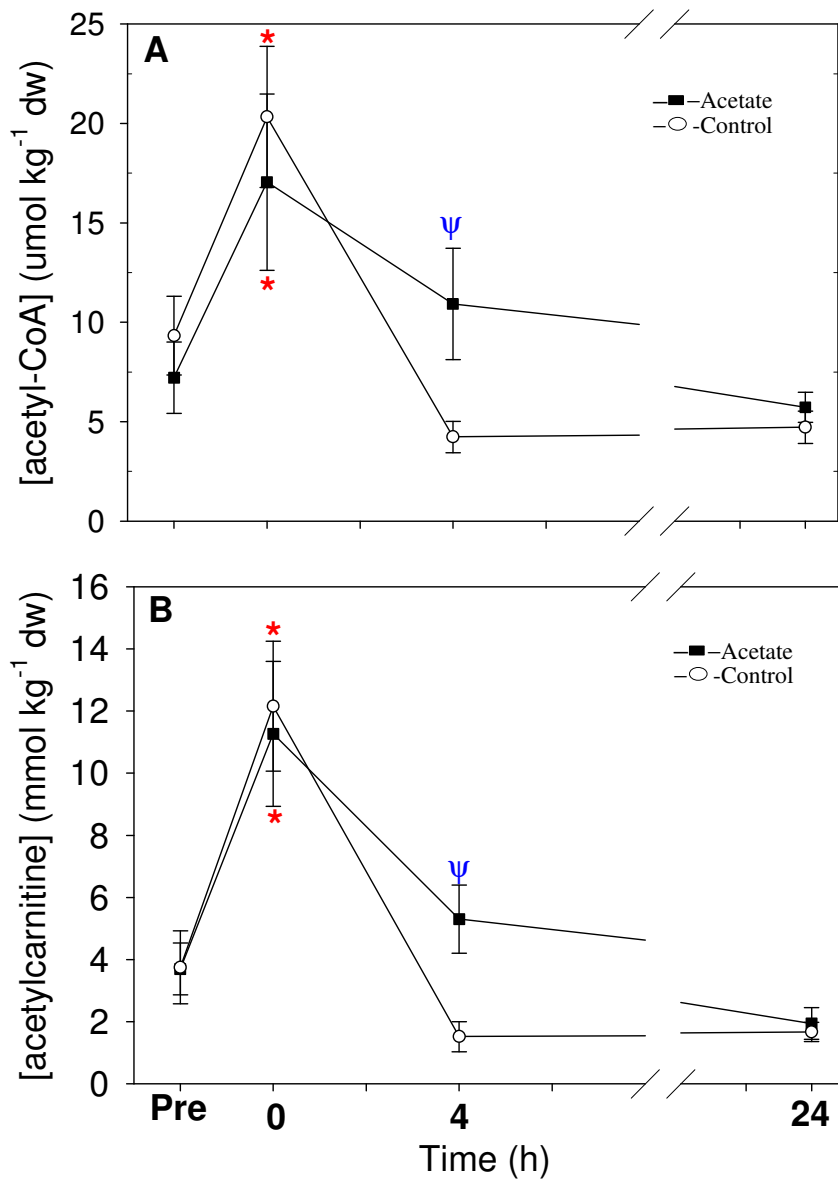
**Figure 7.1. Time line of the experimental protocol. CET = competitive exercise test**



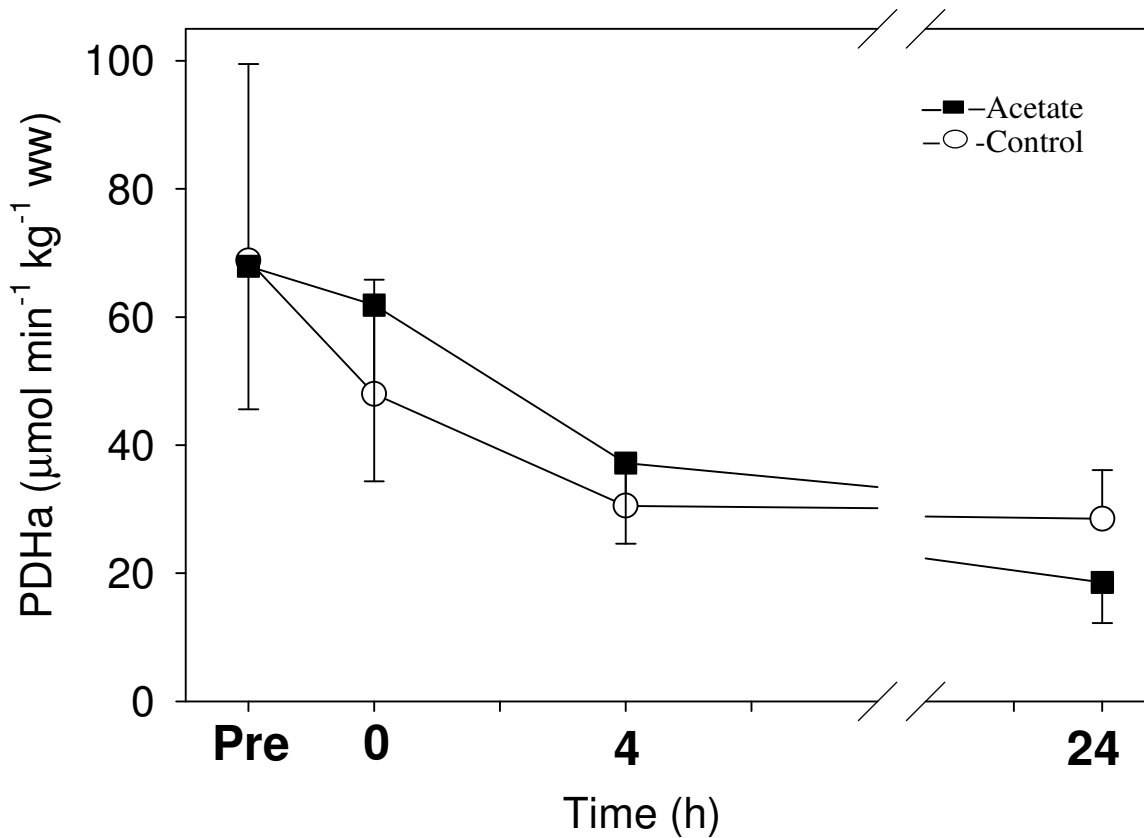
**Figure 7.2. The time course of the changes in plasma [glucose], after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/ acetic acid solution (Acetate), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Acetate treatment. Values are mean  $\pm$  SE for 9 horses. \*,  $\Psi$ : significantly different from Pre-Ex time point for Control and Acetate treatments, respectively.



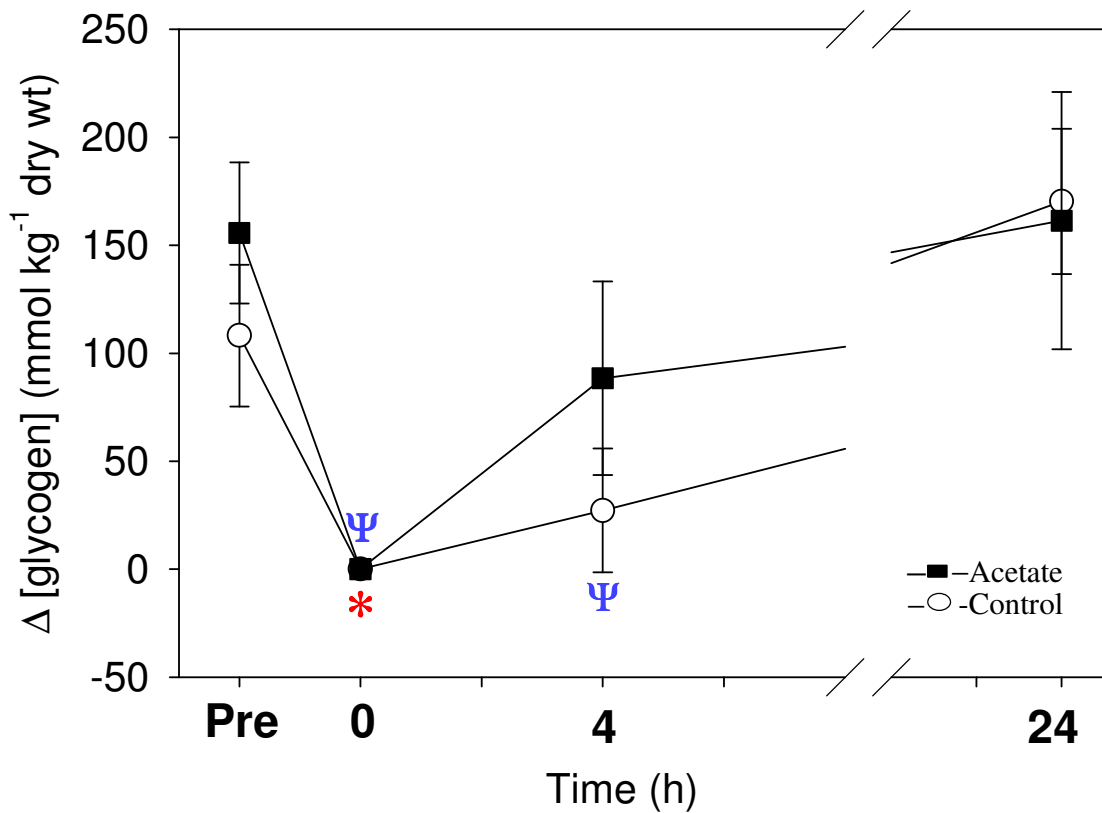
**Figure 7.3. The time course of the changes in plasma [acetate] after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/ acetic acid solution (Acetate), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Acetate treatment. Values are mean  $\pm$  SE for 9 horses.  $\Psi$ : significantly different from Pre-Ex time point.



**Figure 7.4. The time course of gluteus medius (A) [Ac-CoA], and (B) [Ac-Carn] after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/ acetic acid solution (Acetate), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Acetate treatment. Values are mean ± SE for 9 horses. \*: significantly different from Pre-Ex time point, Ψ: significantly different from Control.



**Figure 7.5. The time course of gluteus medius pyruvate dehydrogenase activity (PDHa) after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/ acetic acid solution (Acetate), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Acetate treatment. Values are mean ± SE for 9 horses. There were no significant differences between time points.



**Figure 7.6. The time course of the change in glutemus medius glycogen concentration after a Competition Exercise Test.** The exercise recovery period begins at 0 min, after horses were either given a hypertonic sodium acetate/acetic acid solution (Acetate), or stood in stocks (Control). Horses were fed immediately after the 0 and 360 min samples. ○ denotes Control treatment; ■ denotes Acetate treatment. Values are mean ± SE for 9 horses. Ψ, \*: significantly different from Pre-Ex time point for Control and Acetate, respectively.

## Chapter 8

### 8.0 General Discussion and Future Research

The primary goal of the work undertaken in this thesis was to characterize two nutritional strategies for enhancing recovery of skeletal muscle glycogen in horses after fatiguing exercise. The effects of supplementing either electrolytes or acetate with electrolytes immediately after prolonged exercise on muscle glycogen resynthesis and whole body electrolyte, acid-base and hydration status were investigated.

The first major finding of this research was that nasogastric administration of a balanced hypotonic commercial electrolyte solution post exercise, followed by a typical hay and grain meal, resulted in enhanced muscle glycogen resynthesis during the recovery period, compared to a typical hay/grain meal alone. Our interpretation is that the enhanced glycogen resynthesis was a result of faster recovery of whole body hydration status with electrolyte supplementation, including increased skeletal muscle intracellular fluid volume. Indeed enhanced glycogen synthesis with increased intracellular fluid volume (and conversely decreased glycogen synthesis with intracellular dehydration) has been demonstrated previously in humans (Keller et al 2003) and rats (Low et al 1996), and appears to be due to dephosphorylation of GS via effects on glycogen synthase kinase-3 (Low et al 1996). Thus an interesting possibility for further research would be to investigate the effects of dehydration and rehydration on GS activity in equine skeletal muscle.

Electrolyte supplementation also resulted in significant alterations in plasma acid-base and electrolyte state, such that plasma [TCO<sub>2</sub>] was decreased during the recovery period compared to control, primarily due to increased plasma [Cl<sup>-</sup>]. Also, electrolyte supplemented horses had a faster restoration of whole body hydration status.

This research has practical significance, as it was designed to imitate two recovery protocols (regular meal feeding and water *ad libitum*, with or without a commercially available electrolyte supplement) that are typical in the industry. We hypothesized that post exercise dehydration is an important reason for why muscle glycogen resynthesis is slowed in horses compared to other mammals, and therefore electrolyte supplementation (something simple that all horse owners can do) can be actively used to enhance nutritional status in horses during and after fatiguing exercise. Indeed an important finding of our research was that non-supplemented horses were still dehydrated a full 24 h after exercise, even though water was provided *ad libitum*. This research clearly underlines the importance of providing a balanced electrolyte solution along with *ad libitum* water to equine athletes during exercise recovery since, in addition to its role in slowing glycogen synthesis, it is well-known that dehydration and depletion of electrolytes can result in impairments in health, and cognitive and physical performance (Maughan and Lindinger 1995).

The second major finding of this research was that nasogastric administration of a sodium acetate / acetic acid solution post exercise, followed by a typical hay and grain meal,

resulted in enhanced muscle glycogen resynthesis during the initial 4 h recovery period, as compared to a typical hay/grain meal alone. Acetate administration resulted in a rapid and sustained increase in plasma [acetate], and increased skeletal muscle [Ac-CoA] and [Ac-Carn], suggesting substantial tissue extraction of the supplemented acetate. Our interpretation is that the supplemental acetate was oxidized in the skeletal muscle mitochondria, ultimately sparing glucose extracted by recovering skeletal muscle for glycogen replenishment. Indeed oxidation is the primary fate of exogenous oral (Smith et al 2007) and iv (Akanji et al 1989) acetate in humans. It was thus concluded that oral acetate is an effective, alternative energy source in the horse. Certainly an interesting possibility for further research would be to do a tracer study using labeled glucose and acetate, in order to determine the ultimate metabolic fate of each. For example, oral [<sup>13</sup>C]acetate and [6,6,<sup>2</sup>H<sub>2</sub>]glucose could be administered and this could be compared to a control treatment of identical glycosyl units of [6,6,<sup>2</sup>H<sub>2</sub>]glucose alone. It would also be useful to measure muscle [citrate] and PFK activity, in addition to PDHa and the intermediates of glucose metabolism measured in our study, in order to better understand whether the glucose-fatty acid cycle is operating as classically proposed (Randle et al 1963, 1986).

Another interesting question is whether the exercise protocol affected acetate uptake and metabolism. I.v. sodium acetate clearance was accelerated during exercise in horses (Pratt et al 2005), however it is unknown if acetate clearance is increased during exercise recovery as well. It would be interesting to compare the plasma and muscle kinetics of oral acetate administration in non-exercised vs exercised horses. Indeed after i.v. acetate

infusion in humans, skeletal muscle acetate uptake and oxidation is more than doubled in active vs inactive muscle (van Hall et al 2002).

The objective of this research was to investigate a potential alternative energy source for competitive equine athletes, so as to avoid the health problems associated with feeding diets very high in soluble CHOs. Although the sodium acetate/acetic acid solution administered was tolerated by the horses, most exhibited signs of moderate gastrointestinal disturbance (limited or no interest in eating or drinking, loose stool) during the initial hours after administration. Certainly we advise against horse owners using nasogastric intubation to administer acetate to their horses. Thus a more palatable and easily consumed formulation of acetate is needed before it can be considered a feasible nutritional supplement. During initial pilot research we found that horses could only be trained to voluntarily drink a very small amount of acetate (~50 g sodium acetate in 16 L of water), suggesting this would not be a practical method of supplementation. A formulation of acetate that is sufficiently palatable to be used a top-dressing or additive to grain would be ideal. It is important to note that the single dosage of acetate we administered was intended to replace all the skeletal muscle glycogen degraded during exercise. If acetate could be successfully added to the horses' grain ration, the amount of acetate the horse would need to consume per feeding would be ~1/8 to 1/4 of the dosage we administered.

The sodium acetate/acetic acid solution administered also resulted in substantial osmotic and acid-base disturbances, characterized by a profound plasma alkalosis marked by

increased  $[\text{TCO}_2]$  and  $[\text{Na}^+]$  and decreased  $[\text{Cl}^-]$ . Caution must be taken if administering a similar solution to racehorses, due to the risk of exceeding the testing threshold for an alkalinizing agent, and certainly administration prior to racing would be ill-advised. It must also be stressed that ultimately our results reflect only the CET exercise protocol we utilized; employing this particular solution and supplement strategy in horses undergoing more severe or prolonged activity with greater fluid and electrolyte losses could lead to detrimental consequences.

The effects of plasma alkalosis on glycogen resynthesis in horses does not appear to have been studied, although in humans ingestion of  $0.3 \text{ g kg}^{-1} \text{ NaHCO}_3^-$  does not affect resting muscle [glycogen], [Ac-CoA], [Ac-Carn] or PDHa (Hollidge-Horvat et al 2000).

However the large sodium load of the solution also increased plasma osmolality and resulted in a tendency for intracellular fluid loss, which can decrease glycogen synthesis, as demonstrated by the work in this thesis and in humans (Keller et al 2003) and rats (Low et al 1996). It may seem paradoxical that glycogen resynthesis was enhanced with acetate supplementation despite a tendency towards decreased muscle intracellular fluid volume. It's possible that a significant increase in substrate availability enhanced glycogenesis even in the face of skeletal muscle shrinkage, and that glycogenesis may have been further enhanced with acetate supplementation if a less hypertonic solution was used.

Alternatively, the sodium load of the acetate solution could be reduced by administering a combination of  $\text{Na}^+$ - and  $\text{K}^+$ - acetate.  $\text{K}^+$ , in contrast to  $\text{Na}^+$ , does not remain in the

extracellular compartment but is rapidly taken up by the tissues, reducing the extent of the extent of the extracellular alkalosis presumably at the expense of the intracellular compartment (Lindinger et al 1999). The amount of potassium acetate that can be safely given is much smaller than that of sodium acetate however, due to the risks of hyperkalemia (Lindinger et al 2000).

Clearly the large alterations in fluid, electrolyte and acid-base state resulting from administration of the large dose of sodium acetate/acetic acid solution could have negative consequences in some situations. As mentioned above, ultimately more research into a more feasible formulation of acetate is needed in order to reduce the total dosage of acetate and sodium acetate required. Triacetin is a triester of three acetates on a glycerol backbone that when added to the basal diet of growing pigs at 5 or 10% of intake resulted in proportional increases in skeletal muscle glucose extraction and increased glycogen contents of liver, heart and skeletal muscle (Imoto and Namioka 1983). Triacetin has also been used as a source of dietary fat for rats (Karlstad et al 1992, Lynch et al 1994), however the effects of triacetin administration in horses do not appear to have been studied. A possible benefit of administering acetate as triacetin is that, while hyperosmotic, glycerol is lipid soluble and can rapidly enter all body compartments, resulting in transient fluid retention and hyperhydration of both the extra- and intra-cellular fluid compartments (Lyons and Riedesel 1993). Thus supplementing triacetin to horses may avoid the large alterations in plasma osmolality and acid-base state resulting from administration of the large dose of sodium acetate. Additionally, glycerol can be converted to a glycolytic substrate for subsequent metabolism and could

be a beneficial fuel, in addition to acetate, for sparing muscle glucose oxidation and enhancing glycogen synthesis. Certainly the effects of supplemental triacetin in horses deserves study, however it is likely that palatability would still be a major issue.

In summary, from Thoroughbreds in daily training, to show jumpers warming up and competing multiple times a day for several days in a row, to Standardbreds running in multiple heats in a single day, to endurance horses undergoing multi day rides, to 3-day eventers competing in the ultimate multidisciplinary test, it is clear that many performance horses could benefit from faster glycogen resynthesis. It is presently generally accepted that the best methods to resynthesize glycogen in horses are i.v. glucose administration or feeding very high amounts of soluble CHOs. Unfortunately these options are not practical and have detrimental health consequences. However the research presented in this thesis suggests that horse owners could consider alternative nutritional strategies, although these require additional development. It is concluded that both electrolyte and acetate supplementation can be used to enhance glycogen resynthesis in athletic horses. Hopefully the information gained from these studies will help encourage the development of more effective recovery practices in the horse community, thus enhancing both athletic potential and health and well-being of the horses.

## Chapter 9

### 9.0 References

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