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The effects of sprint training regimens and sodium bicarbonate loading on muscle glycolysis, lactate accumulation, acid-base balance, and performance in the racing greyhound

by

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DEDICATION

This dissertation is dedicated to my loving and understanding wife, Barbara, who always encouraged, supported, and consoled me throughout my graduate career. It is also dedicated to my parents, Richard P. Kesl and Adeline F. Kesl, for their love and support throughout my life and educational endeavors.

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GENERAL INTRODUCTION

Historically, the greyhound appears to have its origins in Africa and the Middle East where animals were used for the task of coursing or pursuit of game animals. During their domestication, greyhounds (*Canis domesticus*) were selectively bred to produce an animal with explosive speed, keen eyesight, and the stamina necessary for hunting of game animals (106).

Modern greyhound racing, now an international sport industry, consists of competitive track races which typically range in length from 300 to 800 meters, but may reach approximately 1000 meters in the greyhound equivalent of the "marathon". These running events last from fifteen to sixty seconds in duration and occur at average running speeds in excess of 18 $m \cdot s^{-1}$ (~65 $km \cdot h^{-1}$) over the shorter race distances.

In the United States, 18 states currently sanction competitive greyhound racing. Most states operate on a step grading system in which animals compete in a series of competitive class races, and if successful, progress to higher classes with increased competition. Races are held on tracks with a variety of surfaces (e.g., sand and grass) and shapes (usually oval although straight or U-shaped designs are occasionally used) and typically involve six to eight animals running simultaneously. With circular track designs, animals traditionally run in a counterclockwise direction, begin the

race from a starting box, and pursue a motorized lure ("rabbit").

On the national and international level, the modern greyhound racing industry involves breeding, rearing, training, and the organized competition of a large number of canine athletes. Despite the popularity and growth of the greyhound sport industry, efforts to enhance the performance of the racing greyhound continue to focus primarily on selective breeding practices.

Very few investigations have focused on the physiological responses and adaptations of the greyhound athlete to either acute or chronic exercise stress. Although it may be tempting to generalize across different species assuming that the physiological responses and adaptations to exercise stress follow a similar pattern, interspecies comparisons must be viewed with caution. The greyhound athlete exhibits several unique, cardiorespiratory, and skeletal muscle characteristics which contribute to their superior athletic abilities when compared with their human, equine, or other canine breed counterparts.

Although some greyhound trainers do employ formal regimens of exercise, the primary focus of most training bouts is familiarization with lure pursuit, counterclockwise running, starting boxes, and increased handling by humans. Because of the lack of scientific research applied directly to

training the greyhound athlete, current practices vary widely and, in many cases, have developed from a "trail and error" approach.

The enhancement of human exercise performance has occurred, in part, from an increasing knowledge of how various training strategies impact on physiological adaptation and responses to acute exercise. Human athletes who participate in athletic events requiring short term, maximal intensity exercise commonly engage in training regimens of high intensity, repetitive bouts of exercise that mimic the competitive event itself. Physiological adaptations to sprint training in humans include increased contribution of anaerobic glycolysis to ATP resynthesis (85), activity of glycolytic regulatory enzymes (53,91,103), muscle lactate accumulation (85,103) blood lactate accumulation (47,53,63,101,103,104) and muscle buffer capacity (103). These training adaptations are also typically associated with enhanced exercise performance (12,47,71,83,85,91,103,104).

Although a large body of knowledge currently exists in the scientific literature characterizing the physiological responses to acute exercise stress and adaptations associated with chronic exercise stress in humans, minimal application of scientifically based training principles is being utilized in the greyhound industry (3). Limited information exists concerning the physiological responses of greyhounds to

exercise stress. The investigations which have been reported have focused on an acute exposure to exercise and have been limited primarily to hematological responses (50,65,86,89,107), although a few studies have examined metabolic responses (20,94). Based on a significant decline in muscle glycogen and a concomitant rise in muscle and blood lactate, glycogenolysis, and subsequent anaerobic glycolysis appear to play a major metabolic role in the sprinting greyhound (20,94). These exercise bouts are also associated with a marked disturbance in acid-base balance as illustrated by the significant declines in skeletal muscle and blood pH (20). Training regimens specifically designed to increase anaerobic metabolic capacity may be beneficial. Although training regimens used by other sprinting species such as humans and equine may be useful as initial models, the development of effective training regimens for the greyhound athlete can only be determined by evaluating the effects of various training regimens on the physiology and performance of greyhound athletes. It is generally believed that greyhounds do not require or tolerate the magnitude of exercise performed by elite human athletes. In addition, several syndromes associated with exercise training stress have been identified in the greyhound, including exertional rhabdomyolysis, bald thigh syndrome, chronic dehydration, and chronic musculoskeletal dysfunction (25,111). Investigations

examining the effects of training regimens on racing greyhounds may provide insight leading to more efficient utilization of animals. More efficient utilization of animals may reduce the necessity of large scale breeding practices and the disposal of large numbers of noncompetitive greyhounds. Because of the unique, inherited athletic abilities exhibited by the greyhound, exercise training studies may also provide an interesting model to investigate the upper limits of training-induced adaptations. At present, no investigations have examined whether or not greyhounds will physiologically adapt to formal exercise training and, if adaptations occur, will performance be affected. Therefore, the purpose of the first study presented in this dissertation was to examine the effects of a six-week sprint training program on muscle glycolysis and buffer capacity in racing greyhounds.

The accumulation of lactic acid in both muscle tissue and the vascular system during high intensity exercise is well documented (4,9,29,87). At the pH of the muscle cell, lactic acid (pka = 3.9) readily dissociates, leading to an intracellular accumulation of lactate and hydrogen (H^+) ions. As these metabolites accumulate, a concentration gradient between muscle cells and the interstitium occurs. These metabolites enter the interstitial space and move into the vascular system, altering the acid-base status of blood. Other organic acids such as fatty acids and pyruvic acid also

accumulate, although their relative role in acid-base balance appears to be minor.

The rise in intracellular H^+ ion concentration may contribute to muscular fatigue via several mechanisms, including inhibition of the rate-limiting enzymes such as phosphofructokinase (13,120) and phosphorylase (13,98), inhibition of calcium ion release from sarcoplasmic reticulum (21,23), reduced binding of calcium to troponin (21,23), and alterations in neural impulse propagation (15,113).

During short term, high intensity exercise, performance may be affected by alterations in the acid-base homeostasis of intracellular and extracellular fluids. The collective ability of the body or a specific tissue to resist changes in pH despite changes in H^+ ion activity is referred to as buffer capacity (87). A variety of buffering mechanisms exist to minimize pH disturbance during exercise. These include physico-chemical buffers such as bicarbonate (HCO_3^-), phosphates, and various proteins found within the cell and in the extracellular fluids. Metabolic processes such as the hydrolysis of muscle phosphocreatine functions as a buffer by the "absorption" of a H^+ ion. In addition, the transmembrane movement of H^+ ions in and out of cells also plays a role in maintaining acid-base homeostasis. Respiratory and renal compensatory mechanisms are also involved in the maintenance and/or reestablishment of acid-base homeostasis (48).

Based on Stewart's (114) quantitative approach to determination of acid-base status of biological fluids, several independent variables determine the distribution and concentration of H^+ and HCO_3^- ions. These variables include the concentration and translocation of strong ions (Na^+, K^+, Ca^{2+}, Cl^-, and La^-), the partial pressure of CO_2, and the concentration of weak acids (typically estimated from either total protein or albumin and the total inorganic phosphate concentration).

One factor which may be limiting during short term, high intensity exercise is the capacity to maintain the work rate as metabolites such as lactate and H^+ ions accumulate (100). Studies in humans have documented enhanced skeletal muscle buffer capacity and exercise performance following high intensity training (103). In addition to physical training regimens, the use of various ergogenic aids has become a popular strategy to enhance human athletic performance. Ergogenic aids include a variety of procedures or products that athletes use to improve athletic performance including nutritional, physical, mechanical, psychological, or pharmacologic procedures (70).

Because of the potential role of pH disturbance in muscle fatigue, a number of investigators have attempted to alter buffer capacity before an exercise bout, primarily by ingestion of solutions containing sodium bicarbonate ($NaHCO_3$).

The effects of $NaHCO_3$ ingestion on physiological responses and exercise performance have been investigated in both human and equine athletes with equivocal results. Both a positive effect (5,28,52,56,59,66,72,84,97,116,123) and no effect (7,45,51,58,60,61,64,67,69,81) have been reported for a variety of exercise tasks. The physiological responses and performance of greyhounds during sprint exercise may potentially be influenced by acute alterations in buffer reserve. In addition, racing greyhounds exhibit a high incidence of exertional rhabdomyolysis, a syndrome associated with exercise-induced muscle trauma. It has been suggested that acidosis contributes to this condition, and that a reduction in the acid-base disturbance associated with exercise may prove beneficial (25). Therefore, the purpose of the second study presented in this dissertation was to examine the effects of $NaHCO_3$ ingestion on muscle and blood lactate accumulation, acid-base balance, and exercise performance in greyhounds sprinting over a 603.5 meter distance.

General Research Objective and Rational

Limited information exists concerning the physiological adaptations associated with formal exercise training in the greyhound athlete. Investigations examining the exercising greyhound have focused on the responses to acute bouts of exercise and have been limited primarily to hematological

parameters. Enhancing exercise performance is dependent, in part, on expanding our understanding of how the various physiological systems respond and adapt to the chronic stress imposed by training. Increasing our understanding of how or if greyhounds adapt to formal training regimens could potentially reduce the current mass breeding practices. These breeding practices have led to large numbers of non-competitive greyhounds and the ethical problems associated with their fate.

Although the mechanisms limiting performance in high intensity exercise tasks have not been clearly defined, it is apparent that muscle energy metabolism and the resulting disturbance to intracellular and extracellular acid-base homeostasis are important considerations. The research presented in this dissertation will examine select aspects of muscle metabolism and acid-base balance in greyhounds exposed to chronic training and in an acute exercise model involving a potential ergogenic agent. It is believed that the information gained from this research will be useful in the development of exercise training strategies and will contribute to the overall welfare of the greyhound athlete.

Explanation Of The Dissertation Organization

The following dissertation contains two papers which are based on investigations which focused on the physiological

responses of racing greyhounds subjected to acute and chronic exercise stress. Following this general introduction and contained in a separate chapter is a general review of literature which will include literature relative to the papers included in this dissertation. The papers will follow the general review of literature and each manuscript will include an abstract, materials and methods section, results section, discussion section, and a list of references cited within that paper. Following the papers will be a section including general conclusions, and a list of literature references cited in the general introduction, review of literature, and general conclusion sections. Additional data, not included within the manuscripts, appears in the appendices.

Approval Of The Committee On Animal Care

The Iowa State University Committee on Animal Care in Research reviewed and approved the protocols and procedures used to complete the research described within this dissertation.

LITERATURE REVIEW

The first section of the following review of literature will provide an overview of the physiology of the greyhound as it pertains to responses during acute exercise and exercise performance. Included will be a brief, general description of current training practices utilized within the racing greyhound industry. The first paper presented in this dissertation focuses on examination of selected metabolic adaptations in muscle glycolysis which may occur in the racing greyhound exposed to a chronic exercise regimen. Because of the lack of literature concerning greyhound responses to exercise training programs, the second section of this review of literature will focus on human investigations which have examined the physiological responses to exercise training programs designed to enhance physiologic function and performance in short duration, high intensity exercise tasks. The second study included in this dissertation examines the effect of acute alteration in blood buffer reserve by $NaHCO_3$ ingestion on select aspects of muscle metabolism, acid-base balance, and performance during a 603.5 meter sprint task. A review of the literature, again limited primarily to human studies, concerning the metabolic and performance effects of induced alkalosis on short duration, high intensity will be presented.

Review of Literature - Greyhound Physiology

The athletic ability of the greyhound is the cumulative effect of generations of selective breeding. A number of cardiovascular, skeletal muscle, and body morphology adaptations contribute to the inherent running ability demonstrated by racing greyhounds. It is possible that some of the observed physiological characteristics and subsequent performance potential could be a direct result of current training practices. Unfortunately, scientifically designed investigations examining the effects of various training regimens on greyhound physiology and performance are virtually non-existent in the literature.

Cardiorespiratory characteristics

The heart mass to body mass ratio is higher in the greyhound, averaging 1.2% of total body weight compared to 0.8% in other canine breeds and 0.5% in humans. Stroke volume measurements of 2.2 ml·kg body weight^{-1} (60 ml for the average 27.2 kg dog) at rest and 3.0 ml·kg body weight^{-1} (80 ml for the average 27.2 kg dog) during near maximal exercise have been reported (111). Coupled with maximal heart rates of approximately 300 beats·min^{-1}, impressive cardiac output values may be attained during exercise. Thermal dilution has been used to determine cardiac output values from 200 ml·kg body weight^{-1}·min^{-1} (5.5 l·min^{-1} for the average 60 lb dog) at

rest to 1000 ml·kg body weight^{-1}·min^{-1} (approximately 27 l·min^{-1} for the average 27.2 kg dog) during maximal exercise (111). Other circulatory adaptations are apparent when comparing blood parameters such as blood volume, hemoglobin concentration, and packed cell volume. Greyhounds have a proportionally higher blood volume (11.4% vs 7.2% of body weight) when compared to other canine breeds (111). Resting hemoglobin concentrations from 18 to 22 g·dl^{-1} have been reported (50,65,94,107,117) exceeding typical means for other canines (15.0 g·dl^{-1}), equine (15.6 g·dl^{-1}), and humans (14.8 g·dl^{-1}) (117). Resting packed cell volume percentage, ranging from 55 to 63 (50,65,94,107,117) is also elevated by comparison to other canine breeds (45%), equine (40%) and humans (45%). The total erythrocyte concentrations of 7.2 to 9.5 million·mm$^{3-1}$ (50,65,94,107,117) are also proportionally higher in the greyhound when compared to other canine breeds (6.8 million·mm$^{3-1}$) and humans (5.0 million·mm$^{3-1}$), although equine total erythrocyte counts are typically higher (10.3 million·mm$^{3-1}$) (117). Collectively, these circulatory system characteristics in the greyhound suggest a potential for a high aerobic capacity.

Catalytic activities of oxidative enzymes across a variety of mammalian species suggests that oxidative potential appears to decrease as body mass increases (44). Canines (including greyhounds) exhibit a greater maximal oxygen uptake

than predicted based on their body mass. Staaden (111) has reported average peak oxygen uptake in greyhounds running on high speed treadmills of 150 ml·kg body weight^{-1}·min^{-1}, with one animal exceeding 200 ml·kg body weight^{-1}·min^{-1}. Maximal respiratory rates of 188 breaths·min^{-1} and ventilation volumes of 4 to 6 l·kg^{-1}·min^{-1} (100 to 150 l·min^{-1} for the average 27.2 kg dog) were also observed. There are differences in ventilatory mechanics when comparing bipedal and quadruped species. Respiratory rates are coupled to stride patterns in most running quadruped animals, with inspiration occurring as the pelvic limbs are extended and expiration occurring during their forward movement. In addition, respiratory mechanisms (panting) play a major role in temperature regulation in the greyhound and may affect acid-base status through alterations in respiratory loss of carbon dioxide.

Skeletal muscle characteristics

In addition to the cardiovascular adaptations, several skeletal muscle characteristics are important to note. Muscle tissue constitutes a greater percentage of the total body mass in greyhounds (57%) in comparison to humans (~40%) and other canine breeds (44%) (106). Within canine breeds, greyhounds have a proportionally greater pelvic limb muscle mass. These are characteristics which have been suggested to favor a high stride frequency, thus contributing to higher running speeds

(106). The increased muscle mass appears to be related to an increased number of total fibers as opposed to similar numbers of larger fibers. Gunn (33) reported that transverse sections of the semitendinosus muscle in greyhounds contained approximately 25% more fibers when compared to equal area sections of other breeds (Collie, Afghan, Labrador, and Great Dane). This difference was observed in both young and adult dogs.

Increased muscle blood flow during exercise contributes to an increased rate of oxygen and carbon dioxide exchange between muscle and blood, as well as removal of various metabolites from the muscle cell environment. Analysis of capillary to muscle fiber ratio in the semitendinosus revealed a significantly higher ratio in the adult greyhound compared to other breeds. However, when the area of muscle per capillary was determined, there was no difference between dog breeds, including the greyhound (32). The capillary to muscle fiber ratio and area of muscle per capillary measurements only provide a means of estimating the potential for muscle blood flow which may occur during exercise. Muscle blood flow and the diffusion of gases and metabolites between blood and muscle during exercise is regulated by a number of factors including the recruitment of more capillaries, vasoactive substances released in response to exercise, blood pressure and flow rates, capillary permeability, and alterations in red

cell mass.

Metabolic responses to exercise are dependent, in part, on the biochemical profile and contractile characteristics of the individual muscle fibers which compose the skeletal muscle. Species comparisons of metabolic enzyme activities are interesting and may assist in the explanation of species differences in exercise performance. Differences in fiber type distribution and the degree of homogeneity in comparable muscles may pose a problem when attempting to compare humans, other species, or other canine breeds to the greyhound. When comparisons are made based on enzyme activities expressed per unit tissue weight, a bias occurs due to the relative homogeneity of the greyhound fiber type, specially when comparisons are made to species with more heterogenous fiber type distribution such as humans.

Based on a classification of either a high or low myosin ATPase activity, Guy and Snow (34) examined six primary locomotory muscles in greyhounds and reported that 88.6 ± 2.2 to 99.8 ± 0.2 % of the fibers exhibited high myosin ATPase activity, traditionally referred to as type II, white, or fast twitch fibers. These percentages were lower in crossbred canines (range from 61.4 ± 10.2 to 85.3 ± 2.6 %) within the same respective muscles. Attempts to separate the high myosin ATPase fibers into the classical divisions of type IIA and IIB, based on their relative oxidative capacities determined

via succinate dehydrogenase activities, were unsuccessful. Other investigators (1,6,105) have suggested that classical type IIB fibers, which in humans are characterized as having high glycolytic and low oxidative capacity, do not appear to exist in greyhound (or any canine breed) skeletal muscle. However, all canine high myosin ATPase fibers exhibit moderate to high oxidative capacity based on histochemical assay for nicotinamide adenine dinucleotide tetrazolium reductase or biochemical assay of succinate dehydrogenase activities (1,6,105).

The ability to generate and sustain high locomotory speeds would require a metabolic strategy which could support a high muscle power output. The ability to generate muscle power is dependent, in part, on a continual supply of adenosine triphosphate (ATP) for ATP-consuming reactions associated with the muscle contraction-relaxation cycle processes such as actomyosin cycling, Ca^{2+} transport, and Na^{+}-K^{+} transport. The supply of stored muscle ATP is limited, approximately 4 to 6 $mmole \cdot kg^{-1}$ in human muscle (9,29,43,71,85,109), equine (35,106), and greyhound (20,94,106), thus requiring continued resynthesis of muscle ATP if contraction processes are to continue for more than a few seconds.

In short duration, high intensity exercise, several metabolic strategies exist for maintaining adequate

availability of ATP including the myokinase reaction (ADP + ADP → ATP + P_i), phosphocreatine metabolism, and anaerobic glycolysis. The relative importance of the myokinase reaction in energy production has not been characterized in the greyhound. Greyhound muscle does exhibit a high potential for phosphocreatine utilization, as evident by a higher maximal activity of creatinphosphokinase (CPK) in comparison to other species such as humans, equine, or other canine breeds (106). This high CPK activity probably contributes to the rapid acceleration exhibited by greyhounds at the beginning of a race. However, the concentration of stored phosphocreatine in skeletal muscle is limited and is similar in humans, equine, and canine with pre-exercise concentrations of approximately 15 to 20 umol·g wet wt^{-1} reported for the greyhound (20,94,106).

Due to the limited availability of stored ATP and phosphocreatine, continued ATP resynthesis requires anaerobic glycolysis during short duration, high intensity exercise tasks. Disagreement exists concerning the magnitude and time course of glycogen utilization during maximal sprint exercise. It appears, based on determinations of muscle glycogen and muscle lactate accumulation, that muscle glycolysis in humans occurs within seconds after the onset of high intensity exercise (4,54). In contrast to oxidative enzymes activities, muscle anaerobic enzyme activities are in direct proportion to

body mass (44). Glycolytic potential in greyhounds appears to deviate from this relationship based on the two- to three-fold higher activity of glycogen phosphorylase, phosphofructokinase, and lactic acid dehydrogenase when compared to that in humans (106).

Chemical reaction rates are controlled by a variety of mechanisms including feedback inhibition by end product accumulation. The formation of lactic acid is well documented during intense exercise and quantitatively represents an important metabolic fate of pyruvic acid, especially during high intensity exercise. In the presence of NADH, the enzyme lactic acid dehydrogenase catalyzes the reduction of pyruvic acid to lactic acid. This reaction is essential for the maintenance of glycolysis because it regenerates the coenzyme NAD^+ and prevents the accumulation of pyruvic acid. If NAD^+ is not replenished, the cellular supply of this coenzyme is rapidly depleted, the glyceraldehyde-3-phosphate dehydrogenase reaction is inhibited, and glycolysis will cease. Analysis of human muscle (118) has revealed a positive correlation between the percentage of fast twitch muscle fibers and the total and muscle specific activity for lactic acid dehydrogenase. Despite a higher proportion of high myosin ATPase fibers (fast twitch) in the greyhound, the lactate dehydrogenase activity is similar to other canine breeds (106). In addition, the quarterhorse, which is the greyhound equivalent within the

equine breeds, exhibits almost a three-fold higher lactic acid dehydrogenase activity per unit body weight (106). The pyruvic acid produced during glycolysis has several potential fates in addition to the production of lactic acid. Pyruvate may be transaminated to form alanine. The alanine-glucose pathway has been shown to play an important role in the disposal of pyruvate from skeletal muscle as the liver utilizes muscle-generated alanine as a gluconeogenic substrate. It has been suggested that an increased activity of alanine aminotransferase, the enzyme which catalyzes the conversion of pyruvate to alanine, in the greyhound may provide an alternative fate for pyruvate (34,106) and thus a metabolic strategy to decrease the accumulation of lactic acid. The increased alanine aminotransferase activity may also explain the relatively low LDH activity in the greyhound.

In addition, pyruvic acid disposal via conversion to acetyl CoA may occur, resulting in ATP regeneration via oxidative metabolism. A high activity of citrate synthase in greyhounds (in comparison to that of crossbred canines) suggests that oxidative metabolism may also play an important role in energy metabolism (34,106).

Muscle glycogen concentrations in greyhounds are not proportionally higher as might be expected with an elevated glycolytic enzymatic potential. Resting greyhound muscle

glycogen stores of 72.43 ± 1.68, 71.80 ± 8.52, and 63.10 ± 2.81 umol·g wet wt^{-1} have been reported (20) in the vastus lateralis, biceps femoris, and gastrocnemius muscles, respectively. Muscle glycogen concentrations may vary depending on the sample site, training status, and diet of the individual; however, the reported values for greyhound muscle are slightly below the typical reported range for human muscle (80 to 100 umol·g wet wt^{-1}). The relatively low muscle glycogen storage may be related to the traditional high raw meat diets that many greyhound trainers advocate. In addition, it has also been suggested that lower glycogen stores in animals with high glycolytic enzyme activities may act as a "final safety valve" for limiting glycolytic end product accumulation and the associated consequences that may occur such as tissue acidification and osmotic-induced disturbances (44). However, the racing thoroughbred, which exhibits a similar glycolytic potential based on the skeletal muscle enzymatic profile, has substantially higher muscle glycogen storage (~140 umol·g wet wt^{-1}) than does the greyhound. Finally, 3-hydroxyacyl CoA dehydrogenase, an enzyme marker of fatty acid oxidation, is lower in the greyhound when compared to that in other canine breeds (34,106), suggesting a reduced dependence on fatty acid metabolism. The extremely low adipose stores found in the greyhound would also suggest that fat metabolism may play a

lesser role.

A high capacity to minimize exercise-induced intracellular muscle pH disturbances represents one strategy of enhancing the ability to perform high intensity exercise tasks. Measures of muscle buffer capacity are typically obtained by two techniques. Tissue homogenates from the muscle of non-exercising animals may be titrated over a select pH range. The buffer capacity is typically expressed as the micromoles of base or acid required to alter the homogenate pH over the pH range selected. An alternative technique involves the calculation of the ratio of change in muscle lactate from pre-exercise to post-exercise to the change in muscle pH. Buffer capacity is typically expressed as $mmol \cdot kg^{-1} \cdot pH^{-1}$ in units referred to as Slykes (121). Harris et al. (36) evaluated the muscle buffer capacity of the greyhound. Several different muscles were sampled in the greyhound and buffer capacity was determined by hydrochloric acid titration. Muscle buffer capacity was expressed as the umol of H^+ ions required to change the pH of one gram of freeze-dried muscle from a pH of 7.1 to 6.5. Muscle buffer capacity was also determined from the middle gluteal muscle of thoroughbred racehorses and vastus lateralis of humans. Buffer capacities were 117.7 ± 1.9, 105.2 ± 2.0, and 79.5 ± 1.8 $umol\ H^+ \cdot g\ dry\ wt^{-1}$ for the racehorses, greyhounds, and humans, respectively. These investigators also determined the carnosine and anserine

content (muscle dipeptides) in these tissues and calculated the combined contribution of these peptides to the overall muscle buffer capacity. This contribution, expressed as the percentage of the total capacity, was 31%, 25%, and 7% in the horse, greyhound, and human, respectively. Investigations (85,103) in which human muscle buffer capacities have been measured have found buffering capacities ranging from 45 to 71 Slykes. Dobson et al. (20) obtained similar results by homogenate titration in three hindlimb muscles (51.32 ± 1.94, 49.97 ± 3.21, and 55.34 ± 3.05 Slykes for the gastrocnemius, biceps femoris, and vastus lateralis muscles, respectively) of the greyhound. Interspecies comparison of muscle buffer capacity is difficult due to variations in methods of determining buffer capacity, training state of the subjects, muscles sampled, and fiber type distribution. It does seem, however, that skeletal muscle buffer capacities are similar in the greyhound when compared to both human and equine athletes, suggesting that superior buffering capacity does not explain the marked differences in sprinting abilities in these different species.

Physiological responses to acute bouts of exercise

It seems that many of the inherent physiological characteristics of the greyhound athlete explains their sprinting abilities. The majority of investigations reporting

on the physiological responses of greyhounds to exercise are based on acute exposure to exercise and have been limited primarily to hematological responses (50,65,86,89,107), although a few studies have examined metabolic responses including measurement of skeletal muscle metabolites (20,94).

The reliance of the sprinting greyhound on anaerobic metabolism is supported by reductions in muscle phosphocreatine of 40 to 50% and in muscle glycogen of approximately 70% during a run of 800 meters (20). The rate of glycogen utilization (53.48 umol·g wet $wt^{-1}\cdot min^{-1}$) was 25 to 50% higher than rates reported in human or equine athletes sprinting over similar distances. In contrast, Rose and Bloomberg (94) reported no significant pre- to post-exercise changes in muscle phosphocreatine or glycogen in greyhounds sprinting over 400 meters.

The formation of lactic acid is well documented during high intensity exercise, and the accumulation of muscle and blood lactate is frequently used as a general index of glycolytic activity. As expected, based on the nature of the exercise task and the muscle metabolic characteristics, greyhounds exhibit significant post-exercise accumulation of muscle and blood lactate. Studies have documented peak post-exercise blood lactate concentrations between 23 and 33 $mmol\cdot l^{-1}$ over race distances of 400 to 800 meters (20,50,86,89,94,107). The peak post-exercise blood lactate

accumulation is influenced by the timing of the post-exercise sample. It is well recognized that the peak blood lactate accumulation occurs after the exercise bout ceases and is believed to occur at approximately five minutes post-exercise in humans. By using an automated blood sampling system, Pieschl et al. (89) have been able to obtain arterial blood samples at eight second intervals from greyhounds during and after a 402-meter sprint. Lactate accumulation reached a post-exercise peak of 29.3 ± 1.9 $mmol \cdot l^{-1}$ at 3.5 minutes after exercise. Very few studies have examined muscle lactate accumulation in greyhounds. After a 400-meter sprint, a post-exercise muscle lactate accumulation of ~18 $umol \cdot g$ wet wt^{-1} in the vastus lateralis has been reported (94). Dobson et al. (20) reported a post-exercise (30 s) muscle lactate accumulation in the gastrocnemius, biceps femoris, and vastus lateralis muscles of 18.96 ± 2.60, 25.42 ± 3.54, and 30.09 ± 1.94 $umol \cdot g$ wet wt^{-1}, respectively, after an 800 meter sprint.

The accumulation of lactate is associated with marked disturbances in blood and muscle acid-base balance. Resting blood pH in greyhounds seems to be near the typical mammalian value of 7.4, although the "normal" blood pH will be affected by the conditions under which the sample was obtained. Greyhounds in the competitive racing environment are confined in individual kennels along with other animals. These animals are typically very excited which results in excessive panting

and may lead to a respiratory alkalosis and an elevated blood pH (25). This pre-exercise alkalosis has been confirmed (89) by comparing a laboratory "resting" arterial blood sample after 20 to 30 minutes of quiet rest and a pre-exercise sample taken at the running site immediately before exercise. The laboratory sample pH was 7.439 ± 0.03 and PCO_2 was 39.6 ± 3.0 Torr. The pre-exercise sample pH had risen to 7.493 ± 0.03 and PCO_2 decreased to 27.9 ± 2.0 Torr. Post-exercise blood pH nadirs of below 7.0 have been reported in greyhounds sprinting over distances of 722 and 800 meters (20,50). Concomitant declines in blood bicarbonate and an increase in base deficit also occur. Immediately following a 722 meter sprint, arterial blood bicarbonate values of 3.1 ± 0.3 $mmol \cdot l^{-1}$ and a base deficit of -29.1 ± 0.7 $mmol \cdot l^{-1}$ were observed, indicating an acidosis of metabolic origin (50).

Arterial blood gas measurements provide information concerning the acid-base status of the athlete and the capacity of the cardiorespiratory system to maintain adequate gas exchange to active tissues. Ilkiw et al. (50) measured arterial blood gases prior to, immediately after, and three hours after a 722 meter sprint. The greyhounds exhibited a transient elevation in PaO_2 (130.8 ± 3.5 mm Hg) and decline in $PaCO_2$ (13.9 ± 0.6 mm Hg) immediately after exercise, both which had returned to pre-exercise levels by three hours post-exercise. Similar results were obtained during recovery

following a 400 meter sprint (94). At three minutes into recovery, PaO_2 had reached a peak of ~125 mm Hg and $PaCO_2$ a nadir of ~15 mm Hg, both which were no longer statistically different from pre-exercise values by 30 minutes into recovery. These post-exercise changes in arterial blood gases reflect increased respiratory drive related to several factors, including respiratory compensation for the metabolic acidosis incurred during the exercise bout. Sustained hyperventilation leads to an elevation of PaO_2 and reduces $PaCO_2$. Elevations in rectal core temperatures (50,94) and arterial blood temperatures (86) have also been reported following sprint exercise and, in the case of the canine, stimulate respiratory rate as a mechanism of restoring normal body temperature via panting. Arterial blood gases during exercise have also been reported. The $PaCO_2$ of blood samples taken at eight second intervals during a 402-meter run (mean sprint time 29.6 s) were 28.3 ± 2.8 mm Hg (0 s), 29.0 ± 0.9 mm Hg (8 s), 31.7 ± 3.8 mm Hg (16 s), 35.3 ± 4.3 mm Hg (24 s) and 42.8 ± 4.9 mm Hg (32 s) (89). The $PaCO_2$ in these samples indicates a progressive hypercapnia during exercise with respect to the pre-exercise sample (27.9 ± 2.3 mm Hg). However, the samples at 0, 8, and 16 seconds were significantly hypocapnic with respect to a "resting sample" obtained approximately 30 minutes prior to the exercise bout (39.6 ± 3.4 mm Hg) and the 24- and 32-second samples were not

significantly different from the resting sample. Nold et al. (86) reported maximal declines of 17 ± 4.0 mm Hg, 19 ± 1.1 mm Hg, and 19 ± 2.2 mm Hg in PaO_2 during exercise in greyhounds running sprints of 402, 503, and 704 meters, respectively. These data suggest that greyhounds exhibit an arterial hypoxemia during exercise. Measurements of PaO_2 during exercise in equine (2) and human athletes (16) have also indicated that arterial hypoxemia may occur, although the significance of this hypoxemia in limiting performance is uncertain.

Alterations in muscle pH associated with acute exercise have been reported by Dobson et al. (20) in greyhounds following an 800-meter sprint. Pre-exercise muscle biopsies revealed a homogenate pH of 6.92 ± 0.02, 6.88 ± 0.03, and 6.93 ± 0.02 for the gastrocnemius, biceps femoris, and vastus lateralis muscles, respectively. These pre-exercise values are slightly lower than the "resting" muscle pH typically reported for humans (85,103,110), although other investigations in humans have reported similar resting muscle pH (40,97). The lower resting muscle pH in greyhounds may be related to pre-exercise excitability which may produce a sympathetically mediated stimulation of glycolysis via adrenergic stimulation of phosphorylase. Following exercise the muscle pH was reduced to 6.56 ± 0.02, 6.40 ± 0.02, and 6.47 ± 0.01 for the same muscles. Post-exercise muscle pH in

a similar range has been reported for humans following high intensity exercise tasks (11,40,46,85,103). However, these human studies utilized exercise protocols with repeated bouts or single bouts designed to lead to exhaustion. The greyhound post-exercise muscle pH data represents responses to a single sprint bout over a race distance which would not lead to complete exhaustion.

Hematological responses to acute exercise have been reported for the greyhound. A number of investigations have documented exercise-induced increases in packed cell volume, hemoglobin concentration, and erythrocyte concentration (20,50,94,107). An increased hemoglobin concentration increases the blood oxygen carrying capacity and, as mentioned previously, contributes to the high oxygen consumption observed in greyhounds. In contrast to humans, it is generally believed that greyhounds, like equine athletes, mobilize a large number of erythrocytes from the spleen via sympathetic-induced splenic contracture (20). The addition of this cell mass to the vascular system contributes to the increased hemoglobin concentration and packed cell volume observed following exercise. However, exercise-induced increases in other blood components such as total protein concentration (50,89,94,107) and osmolality (94) suggest that significant plasma volume shifts also occur, resulting in hemoconcentration.

Current exercise training practices in the greyhound industry

A general approach to training in greyhounds has been described by Beh (3) and Staaden (111). It should be stressed that training practices vary widely. As mentioned previously, most trainers believe that generations of selective breeding has essentially maximized the physical characteristics of the greyhound athlete. In addition, most training programs are limited due to the availability of training facilities and the time required to train large number of animals.

In the first 12 months, usually referred to as the rearing stage, very little formal training occurs. Animals are allowed to run free in large open areas, and they may be allowed to pursue rabbits to facilitate learning to swerve, avoid obstacles, and change pace. These pursuit skills are important attributes once competitive racing begins, and the athlete is running in close quarters with other animals. In addition, some trainers utilize mechanically or manually driven circular minitracks in which young dogs begin pursuit of a suspended artificial lure traveling in a small circle (20 to 30 meter diameter). Attempts at intensive training during this initial rearing stage are generally thought to be counterproductive.

Organized training sessions mark the beginning of the formal training stage. This stage typically begin with three to four weeks of walking (speeds of approximately five to

eight $km \cdot h^{-1}$) involving road work, treadmills or rotary walking machines. Sessions may range from 15 to 60 minutes in length and involve two workouts per day (morning and evening). This low intensity work is thought to promote general baseline conditioning and help prepare the animal for the stresses of subsequent speed work. During this stage of training animals are placed under greater psychological stress as the transition begins from the freedom of the rearing environment to the strict routine and confinement of the kennel.

Following the walking exercise, animals are gradually exposed to speed work over the next three to four weeks. Many trainers begin this stage by sprinting the animal over short distances (50 to 200 meters) every other day for several weeks. Attempts are made to run the initial sprints at an intensity less than maximal effort (70 to 80%), although it is sometimes difficult to pace greyhounds. Starting boxes are generally not utilized, instead animals are released by handslipping (trainer holds the animal then releases) to other individuals waiting at a distance of several hundred meters and waving a towel or calling to the animal. Gradually the sprint length is increased to between 300 and 500 meters, and animals are run at maximal effort by introducing a lure.

This training phase typically culminates with some exposure to formal track sprints which may occur on race tracks or training tracks. These circular sprints progress

from 200 to 500 meters and are usually performed with seven to ten days between successive sprints. Straight sprints may be interspersed every two days. The length of the circular sprints may be varied dependent on the race event length, but most animals are trained at distances from one third to full race distance. These sprints are thought to be important for several reasons including the conditioning aspects, metacarpal bone remodeling in response to the biomechanics of running in a circular path, right gluteal strengthening, and continued exposure to starting boxes, lure pursuit, kenneling, increased human handling, and running with other animals. In many cases, after four to six circular track gallops, the animals are considered ready to enter competitive racing. Most animals are placed into race competition around the age of 18 months. Once animals begin racing competition, less time is spent with continued training, although this varies with trainers.

Summary - greyhound exercise physiology and training practices

In summary, it appears that selective breeding has resulted in a variety of cardiorespiratory and skeletal muscle adaptations which contribute to the inherent running ability demonstrated by racing greyhounds. Based on glycogenolytic and glycolytic enzyme activities (106) and fiber type distribution (34), greyhounds possess a high anaerobic

metabolic potential. Significant exercise-induced muscle (20,94) and blood lactate accumulation (20,50,86,89,94,107), glycogen utilization (20), and acid-base disturbance (20,50) supports the importance of anaerobic metabolism in the sprinting greyhound. Despite this high potential for anaerobic power, the greyhound seems to possess a relatively moderate ability to sustain acid-base homeostasis based on measures of muscle buffer capacity (20). Reports (3,111) on current training practices include various regimens of repetitive, high intensity exercise although the total work (number of repetitions) and frequency of workouts is minimal, at least by human standards.

Review of Literature - Adaptations to High Intensity Training Regimens

Short term, high intensity exercise tasks are heavily dependent on anaerobic metabolic processes for the resynthesis of ATP to support muscle power output (29,85,87,109,110). The inability to generate or maintain a specified or desired power output is commonly used as a definition of fatigue. A number of factors can potentially affect performance in high intensity exercise tasks, including processes related to energy metabolism (e.g., metabolic "machinery" and substrate availability), neural impulse generation and propagation, muscle excitation-contraction coupling processes, acid-base

homeostasis, environmental conditions, psychological factors, and the training status of the athlete.

Exercise training involves the exposure of an athlete to a chronic pattern of exercise stress to induce physiological adaptations which will subsequently enhance physiologic function and performance. Although the specific mechanism(s) of muscle fatigue in high intensity exercise tasks have not been conclusively determined, decreases in muscle power output are related, in part, to the inability of the muscle to maintain or increase the rate of ATP resynthesis. In addition, metabolic processes and physiologic function may be impaired by the inability to maintain cellular homeostasis as metabolites accumulate.

From a biochemical perspective, enhancing metabolic processes related to increasing ATP turnover rate would represent one strategy for enhancing anaerobic power. An alternative or additional strategy would be to maintain the metabolic processes related to the generation of muscle power by maintenance of the muscle cell environmental homeostasis.

A number of studies have focused on physiological adaptations and exercise performance following high intensity training programs. These investigations have employed a variety of training protocols with respect to training mode, intensity, duration, frequency, number of "sprint" repetitions, work to rest ratios, length of training program,

and total work completed. The majority of studies have used either human subjects or rats, making direct comparisons to the greyhound difficult because of species differences in fiber type distribution and biochemical properties.

Metabolic enzyme adaptations following high intensity training regimens

Training-induced metabolic adaptations which could potentially affect high intensity exercise performance include changes in the enzyme systems related to ATP regeneration. Increases in key metabolic enzyme activities could occur by either altering enzyme efficiency (alterations in k_{cat} or turnover number) and/or by increasing the total amount of enzyme present per unit contractile tissue (44). Based on comparisons between different species, it seems that enzyme efficiency is relatively constant and the glycolytic scaling observed across species occurs primarily through alterations in the amount of enzyme present per unit tissue (44). In addition, training adaptations may occur with respect to the concentration and/or utilization of substrates such as stored ATP, phosphocreatine (CP), and muscle glycogen.

Stored muscle CP represents a source for rapid ATP resynthesis via the catalytic action of CPK. Staudt et al. (112) reported an increase in maximal CPK activity (114 ± 3.0 to 128 ± 2.2 $U \cdot g$ wet $wt.^{-1}$) in the primarily slow soleus

muscle of rats subjected to four, 45 second runs each day for three weeks. In contrast, the rectus femoris muscle, representative of a fast twitch muscle, exhibited no change in CPK activity (424 ± 9.8 vs 414 ± 15.2 U·g wet wt.$^{-1}$). Gillespie et al. (27) reported no training effect on CPK activity in the soleus, vastus lateralis profundus, or vastus lateralis superficialis (representing predominantly slow oxidative, fast oxidative glycolytic, and fast glycolytic muscles respectively) muscles in rats following a 12 week interval running program. Contrasting effects have also been reported in human sprint training studies. A six-week, progressive sprint interval cycling program did not alter CPK activity (383 ± 22.7 vs 351 ± 17.3 ukat·g dry wt.$^{-1}$) in the medial vastus lateralis muscle (53). A 36% increase in CPK activity (0.99 ± 0.150 vs 1.35 ± 0.151 mol·g^{-1}·min.$^{-1}$) was found in the medial vastus lateralis after an eight-week program in which subjects progressed from 20, 5-second sprints on a treadmill (19 km·h^{-1} at a 9% grade) to 40, 5-second sprints (24 km·h^{-1} at a 10% grade) (119). This increase in CPK activity following training was not associated with changes in pre-exercise CP or ATP concentration. Other investigators have reported no changes in either pre-exercise CP concentrations (47,85,103) or the magnitude of CP decline during a progressive cycling test to exhaustion (103), 30 second maximal treadmill sprint (85), or a two minute

treadmill run at 110% of VO_2 max (85) following training. Conversely, McDougall et al. (71) reported increases in resting muscle CP and ATP concentrations of 22% and 18% respectively in the triceps brachii muscle following a five month heavy resistance, weight training program. These increases, however, represent the mean change in two different subject groups. One group, which accounts for the majority of the post-training changes in CP and ATP, had their arm immobilized in a cast for five weeks prior to the training program.

Sustained ATP resynthesis during high intensity exercise requires activation of glycolysis, resulting in decreases in muscle glycogen and subsequent accumulation of lactate in blood and muscle. The effects of sprint training regimens on muscle glycolysis have been characterized by measurements of a variety of glycolytic enzyme activities, changes in muscle glycogen concentration, and accumulation of both blood and muscle lactate. Glycogen phosphorylase catalyzes the sequential removal of glycosyl residues from the glycogen molecule and thus regulates the entry of substrate into the glycolytic pathway (assuming negligible contribution from blood-borne glucose). Following high intensity training in the rat, no significant changes in phosphorylase activity have been observed in the rectus femoris muscle (112), red gastrocnemius, white gastrocnemius, or red vastus lateralis

(102). Conflicting results have been reported for the soleus muscle, classified biochemically as a slow oxidative tissue. No training effect on phosphorylase activity was observed in rats exercised daily for three weeks with four, 45-second runs (112). An eleven-week progressive sprint interval program, consisting of 30 second sprints (1:1 work rest ratio) progressing up to 18 repetitions and treadmill speeds of 80.5 $m \cdot min^{-1}$ produced an increase in phosphorylase activity of approximately 70% (12.20 ± 0.96 vs 20.84 ± 2.31 $umol \cdot g$ dry wt^{-1}) in the soleus muscle (102). Sprint training studies in humans have documented increases in phosphorylase activity (~18 vs ~28 $umol \cdot g^{-1} \cdot min^{-1}$) following a five-week program of repetitive 800 meter sprints (91) or no change after an eight-week cycling program consisting of repetitive 30-second sprint bouts (103).

Phosphofructokinase (PFK), considered a key regulatory enzyme in glycolysis, catalyzes the phosphorylation of fructose-6-phosphate. The activity of PFK has been used as a general index of glycolytic potential and to document a training effect on glycolysis. Increases in PFK were observed in the vastus lateralis profundus (+78%) and vastus lateralis superficialis (+51%) muscles of sprint trained rats with no change in the soleus muscle (27). Increases in maximal PFK activity have also been documented in human subjects. Eight-week (103) and six-week (53) high intensity cycling programs

have produced increases in PFK of 46% (19.92 ± 0.95 vs 29.16 ± 2.09 umol·g^{-1}·min^{-1}) and 16% (1.10 ± 0.05 vs 1.28 ± 0.06 ukat·g^{-1}) respectively. Investigations employing running interval sprint regimens have reported increases in PFK of 21% (28.1 ± 3.0 vs 33.9 ± 2.7 umol·g^{-1}·min^{-1}) after three months of training (24) and approximately 100% (~22 vs ~44 umol·g^{-1}·min^{-1}) after only five weeks (91).

Single fiber determinations produced no change in PFK activity in either type I or type II fibers from the vastus lateralis of humans following an eight-week training program (39). The training protocol used in the latter investigation, however, which consisted of five, four-minute work bouts at 101% of VO_2 max, would involve a significant contribution by oxidative metabolism and may explain the lack of change in PFK activity.

The pyruvic acid formed during glycolysis has several potential fates including the formation of lactic acid as lactic dehydrogenase (LDH) catalyzes the reduction of pyruvic acid by NADH. An increased disposal of pyruvic acid via conversion to lactic acid may occur if LDH activity is affected by training. The majority of sprint training studies do not support an enhanced maximal LDH activity. No changes in LDH activity have been reported following high intensity training in rats involving muscles with varying fiber type including the soleus (27,41,112), vastus lateralis profundus

(27), vastus lateralis superficialis (27), or rectus femoris (112). Sprint training in humans has been reported to have no effect on LDH activity (47,104,119) in the vastus lateralis muscle, although the majority of these investigations determined total LDH activity. Roberts et al. (91) reported an increase in LDH activity (~175 vs ~260 $umol \cdot g^{-1} \cdot min^{-1}$) in the gastrocnemius muscle following a five-week training program consisting of 8, 800-meter runs (16 total sessions) separated by a two minute rest period. Changes in total LDH activity may not reflect changes in the various LDH isozymes. It is possible that the heart specific (LDH-1 and LDH-2) isozymes and muscle specific (LDH-4 and LDH-5) isozyme activities could change with minimal changes in total LDH activity. However, Sjodin et al. (119) found no change in total LDH activity or the LDH isozyme pattern following sprint training in humans. At least one investigation has reported an increased LDH activity following a sprint training program.

Other enzymatic adaptations to sprint training regimens have been reported. Increases in hexokinase and citrate synthetase activity in both the soleus and rectus femoris muscles of rats were observed following high intensity training (112). In addition, pyruvate kinase activity was significantly increased in the rat soleus muscle (102). Human subjects have demonstrated increases in other enzymes involved in non-oxidative metabolism including glyceraldehyde phosphate

dehydrogenase (91) and myokinase (119). Oxidative enzyme activities have also been reported to increase in response to high intensity training in humans. Roberts et al. (91) reported an increase in malate dehydrogenase, a citric acid cycle enzyme which catalyzes the conversion of malate to oxaloacetate. Increases have also been reported for citrate synthetase (53) and succinate dehydrogenase (39), although in the latter investigation the increase in succinate dehydrogenase was found only in type II fibers with no change in the type I fibers. Other human training studies (24,103) have not found significant changes in succinate dehydrogenase activity following sprint training.

Adaptations in lactate accumulation and acid-base balance following high intensity training regimens

In addition to enzymatic adaptations, sprint training may affect glycogen storage and utilization, blood and muscle lactate accumulation, and acid-base disturbances during standard exercise bouts. Increased maximal activities of glycolytic enzymes suggest a potential for a greater rate of glycolysis. A higher lactate accumulation in either muscle or blood following training would also support an accelerated glycolytic rate, assuming that an increase in lactate accumulation is the result of increased production and not the result of impaired clearance from these compartments.

A number of investigations in humans have examined the effects of sprint training on peak post-exercise blood and muscle lactate accumulation following a standard exercise task. Following high intensity training, blood lactate accumulation has been reported to increase after a 45-second maximal cycling task (103), a 30-second Wingate test (53), a 60-second and 90-second run for distance (47), and a treadmill run (~45 seconds) to exhaustion (104). The increased blood lactate accumulation was associated with enhancement (47,103) or no effect (53) on exercise task performance. Other investigators have found no significant changes in post-training blood lactate accumulation following a 30-second treadmill sprint (85), two-minute run at 110% of VO_2 max (85), treadmill run (16 $km \cdot h^{-1}$ at 15% grade) to exhaustion (91), a two- to three-minute treadmill run (83), or a 30-second treadmill run (83). However, despite the absence of an increase in blood lactate accumulation in these studies, performance was enhanced (85,91) and a significantly higher anaerobic capacity (83) was reported. Fewer studies have examined changes in muscle lactate accumulation following training. Post-exercise muscle lactate accumulation was higher following high intensity cycling (103) and running (85) training regimens and was associated with enhanced exercise performance.

During high intensity exercise tasks, the accumulation of

blood and muscle lactate is associated with an increase in H^+ ion concentration, which may be reflected in a declining blood and/or muscle pH. Following an eight-week sprint training program, Nevill et al. (85) reported a greater decline in blood pH, but no further change in muscle pH immediately after a 30-second maximal treadmill sprint. Sharp et al. (103) found no significant difference in post-exercise muscle pH after eight weeks of high intensity cycling training.

The change in muscle pH associated with exercise is determined by the production of H^+ ions and the buffer capacity of these tissues. The ability to resist changes in pH may be a significant factor limiting anaerobic performance, thus an increased muscle buffer capacity may enhance performance (87). McKenzie et al. (82) examined the skeletal muscle buffer capacity in elite 800-meter runners, marathon runners, and untrained controls. Subjects ran to exhaustion on a treadmill at 3 $m \cdot s^{-1}$ and a 20% grade and muscle biopsies from the vastus lateralis were obtained to determine buffer capacity via HCl titration. The 800-meter runners exhibited a greater time to exhaustion, higher muscle buffer capacity, and higher percentage of fast twitch muscle fibers in comparison to both marathon runners and controls. A significant, positive relationship ($p=0.026$, $r=0.57$) was reported between muscle buffer capacity and anaerobic performance. There was also a tendency ($p=0.09$, $r=0.45$) for a higher muscle buffer

capacity as the percentage of fast twitch muscle fibers increased. Higher muscle buffer capacities have been reported in subjects whom participate in highly anaerobic sport activities when compared to sedentary controls (99). The higher skeletal muscle buffer capacities exhibited by athletes participating in highly anaerobic sport activities may represent an adaptation to training. Conversely, it is possible that differences in muscle buffer capacities simply reflect inherent characteristics which have predisposed athletes to excel in activities in which significant acid-base disturbances occur. Sharp et al. (103) examined the effects of an eight-week, high intensity cycling regimen on muscle buffer capacity. Buffer capacity was calculated by the ratio of the change in muscle lactate to change in muscle pH over an incremental cycling task to exhaustion. Post-training buffer capacity (61.04 ± 4.11 Slykes) was higher than either pre-training (44.68 ± 3.03 Slykes) values or values from a control group of endurance trained subjects (47.21 ± 7.26 Slykes), suggesting that muscle buffer capacity may be influenced by high intensity training regimens. Conversely, muscle buffer capacity was reported unchanged after eight weeks of treadmill sprint training (85). Titration of muscle homogenates revealed no change in buffer capacity (67.6 ± 2.7 vs. 71.2 ± 1.8 slykes), however, when the buffer capacity was determined by changes in muscle lactate and pH, a "tendency" (no

statistics reported) towards an increased buffer capacity was reported.

Effects of high intensity training regimens on exercise performance

A number of investigators have reported enhanced exercise performance (12,47,83,85,91,103,104,119) following high intensity training regimens. Houston and Thomson (47) reported an increase in the mean distance that subjects ran over a 60-second (+13.7%) and a 90-second (+13.4%) period following six weeks (four sessions per week) of intermittent, high intensity hill running. Endurance time for a high-speed treadmill run (215 $m \cdot min^{-1}$ and 20% grade) was also increased and was associated with an increase (+14%) in venous blood lactate accumulation. Following an eight-week (three to four sessions per week) program consisting of repetitive (progressing from 20 to 40 repetitions) five second maximal treadmill sprints, increases in maximal voluntary isometric leg contraction (MVC), endurance time at 50% of MVC, distance in a vertical jump test, and power output as measured by a stair climbing test protocol have been reported (119). In addition, significant reductions in a 25-meter sprint times were observed. Nevill et al. (85) trained subjects for eight weeks (three to four sessions per week) using a high intensity, treadmill sprint protocol including 30-second,

repetitive six second, and two-minute runs. After training, subjects exhibited reductions in both 50-meter and 200-meter track sprint times. Peak power output during a 30-second maximal treadmill sprint was increased by 12%, while mean power output was reported to increase by 6% (non-significant). Endurance treadmill time to exhaustion (at 16 $km \cdot h^{-1}$ and 15% grade) was reported to increase following a five-week (three to four sessions per week) program consisting of eight, 200-meter track runs at approximately 90% of maximal speed (91). Sjodin et al. (104) trained subjects for an eight-week period (three sessions per week) using a mixed protocol, consisting of exhaustive treadmill sprints (~one minute), 300- to 600-meter track sprints, and a 250-meter uphill running protocol. Endurance time for a treadmill sprint test (at a predetermined speed to result in exhaustion in approximately 45 seconds) was increased following training. Medbo and Burgers (83) trained subjects for six weeks (three sessions per week) using two different protocols. One group ran three, two-minute treadmill runs (with eight minute rest intervals) at a speed designed to cause exhaustion in approximately three minutes. A second group ran eight, 20-second sprints (with five minute rest intervals) at a speed designed to cause exhaustion in approximately 40 seconds. Using the accumulated maximal O_2 deficit during a 30-second maximal treadmill sprint as an index of anaerobic capacity, training in both groups resulted

in significant and similar increases in anaerobic capacity. A six-week (five sessions per week) program consisting of 220 yard interval runs and 2.5 mile distance runs increased (+23%) time to exhaustion for a treadmill test performed at a 20% grade and a speed which caused pre-training exhaustion in 30- to 60-seconds (12). Sharp et al. (103) trained subjects using an eight-week, high intensity cycling program (four sessions per week) consisting of eight, 30-second maximal bouts (four minute passive recovery between bouts). Following training, subjects exhibited an increase in peak torque and total work measured during a 45-second maximal cycling bout.

Not all high intensity training programs have resulted in significant post-training effects on performance. Jacobs et al. (53) trained subjects for six weeks (average of 2.5 sessions per week) using a progressive, interval sprint cycling program. Subjects progressed from two, 15-second and two, 30-second maximal sprints to six, 15-second and six, 30-second maximal sprints. No differences were detected for peak power output, mean power output, or percentage power output decline in a 30-second maximal cycling task.

Summary - adaptations to high intensity training regimens

Conflicting results exist concerning the physiological adaptations associated with high intensity training programs. Differences in training regimens (mode, intensity, duration,

frequency, total work, and work/rest intervals) and exercise performance tasks may explain some of these contradictions, as well as species and/or individual subject variation in characteristics such as muscle fiber type distribution. In general, it seems that high intensity training is associated with an enhanced glycogenolytic and glycolytic capacity based on increased activities of various glycolytic enzymes including phosphorylase (91,102), PFK (24,27,53,91,103), GDH (91), pyruvate kinase (102) and LDH (91), although other investigations have found no significant alterations in enzyme profiles. Oxidative capacity may also be enhanced based on increases in malate dehydrogenase (91), citrate synthetase (53,112), and succinate dehydrogenase (39). Endurance trained individuals exhibit higher activities of oxidative enzymes (citrate synthetase, malate dehydrogenase, and 3-OH-acylCoA dehydrogenase) in type I, IIA, and IIB fiber types, as well as higher PFK activity in type I fibers (22), suggesting that all three fiber types possess the potential to increase the oxidative capacity and that endurance training may increase glycolytic capacity in type I fibers. Based on the literature cited within this review, high intensity training regimens may affect both the oxidative (39,53,91,112) and glycolytic (24,27,53,91,102,103) capacity of muscle, although the responses within specific muscle fiber types has not been reported.

Increased blood (47,53,63,101,103,104) and muscle (85,103) lactate accumulation following high intensity training supports the concept that glycolytic capacity has been enhanced, although other investigators have reported no change in blood lactate accumulation (83,85,91). Lactate accumulation, however, is determined by alterations in rates of production, oxidation (in the case of muscle accumulation), and clearance, making interpretation of blood lactate accumulation tenuous. Based on the higher skeletal muscle buffer capacity exhibited by athletes participating in highly anaerobic sport activities (82,99), and the increase in buffer capacity exhibited following high intensity training (103), another adaptation strategy which seems to occur is the improved maintenance of intracellular acid-base homeostasis during high glycolytic rates.

The investigator's choice of the pre-training and post-training exercise performance task may also affect the interpretation of results. A number of investigations have shown enhanced exercise performance (12,47,83,85,91,103,104,119) following high intensity training regimens. Tasks which require an effort over a fixed distance or time may be interpreted as testing the changes in the rate of anaerobic energy production or anaerobic power. An increase in the distance run in 60- and 90-seconds (47), O_2 deficit incurred in a 30-second maximal treadmill run (83),

peak torque and total work during a 45-second maximal cycling task (103), peak power output during a 30-second treadmill sprint (85), and a significant reduction in 50- and 200-meter sprint time (85) all suggest an increased rate of anaerobic energy production. Tasks in which subjects exercise to exhaustion at the same absolute intensity before and after training are testing the ability to sustain a fixed rate of anaerobic energy production over an extended period of time. Increases in time to exhaustion (91,104) and accumulated O_2 deficit (83) in treadmill running tasks at the same absolute speed and grade have been reported. Improved performance in these tasks may reflect a change in anaerobic capacity and not necessarily a change in maximal anaerobic power. It is likely that both maximal anaerobic power and anaerobic capacity are enhanced following high intensity training regimens, although there may be differences with respect to the optimal training regimen to maximize these two characteristics.

Review of Literature - Effects of $NaHCO_3$ on Responses to Exercise

Fatigue during short duration, high intensity exercise tasks may be related, in part, to disturbances in intracellular and extracellular acid-base homeostasis. The capacity to maintain acid-base homeostasis despite changes in H^+ ion activity is referred to as buffer capacity.

Comparisons of anaerobically trained athletes to either endurance athletes or sedentary controls suggests that a higher muscle buffer capacity exists in the anaerobically trained athlete (82,99). Enhanced muscle buffer capacity has also been demonstrated following high intensity sprint training (103).

A number of innate buffering strategies exist to assist in the maintenance of acid-base homeostasis which develops during high intensity exercise. The capacity to buffer the metabolic acids (primarily lactic acid) formed during high intensity exercise may be limiting performance in these exercise tasks. The use of HCO_3^- containing solutions to acutely alter blood buffer capacity is based on the premise that these solutions will significantly increase blood HCO_3^- concentrations and/or blood pH, and that blood HCO_3^- ions constitute an important buffer system in the maintenance of blood pH during exercise. If these assumptions are valid, then an increased blood HCO_3^- concentration, induced via $NaHCO_3$ ingestion, may increase the blood buffer capacity or reserve, and minimize the blood pH disturbances associated with intense exercise. The actual mechanism(s) by which $NaHCO_3$ actually alters acid-base status is debated. According to Stewart's (114) quantitative approach to the determination of acid-base status of biological fluids, both blood HCO_3^- and H^+ ion concentrations (pH) are dependent on other variables which

include the partial pressure of CO_2, the concentrations of strong ions (Na^+, K^+, Ca^{2+}, Cl^-, and La^-), and the concentration of weak acids (typically estimated from either total protein or albumin and the total inorganic phosphate concentration). Changes in these variables determine the distribution and concentration of HCO_3^- and H^+ ions, suggesting that the changes in blood HCO_3^- and pH observed with $NaHCO_3$ ingestion are not directly related to the HCO_3^- component of $NaHCO_3$ solutions.

To effect muscle fatigue, an extracellular alkalosis must alter some aspect of intracellular acid-base balance, assuming that changes in pH are important in the development of fatigue. If alterations in intracellular pH can be minimized, a reduction in the inhibition of metabolic or contractile processes related to these pH disturbances will occur, thus muscle function will be maintained to either sustain power output or reduce the rate of decline in power output.

Intracellular pH disturbances will be minimized by a reduced accumulation of H^+ ions within the muscle cell. Decreases in H^+ ion accumulation may occur by three mechanisms; a decreased production of H^+ ions, an increased intracellular capacity to buffer, and/or an increased efflux of H^+ ions (clearance) to other compartments such as interstitial or extracellular fluids.

It is unlikely that a decreased production of lactic acid (thus a decrease in H^+ ion accumulation) would be coupled with

the ability to sustain or increase power output during high intensity exercise. If performance is to be enhanced in high intensity exercise tasks, it is likely that the glycolytic rate would be increased or maintained at a higher rate, which could potentially lead to an increase in the accumulation of intracellular metabolites such as lactate and H^+ ions, not a decline. An increase in maximal muscle lactate accumulation (85,103) and blood lactate accumulation (47,53,63,101,103,104) has been demonstrated following high intensity sprint training programs. An increase in the maximal activities of various glycogenolytic and glycolytic enzymes following high intensity training also supports the notion that enhanced performance in high intensity exercise tasks is associated with an acceleration rather than a decline in the rate of glycolysis. These adaptations to chronic training suggest that an increased glycolytic rate is one strategy to enhance performance in high intensity exercise tasks. It is unlikely that enhancing performance via $NaHCO_3$ ingestion occurs with a decline in glycolytic rate.

Increased intracellular buffering capacity may occur if the administration of $NaHCO_3$ increases intracellular HCO_3^- content and/or increases muscle pH. Measurements of intracellular muscle HCO_3^- content during manipulation of extracellular $NaHCO_3$ concentrations, however, support the concept that the sarcolemma is relatively impermeable to HCO_3^-

(8,10,92). Muscle pH seems to be unaffected by ingestion of $NaHCO_3$. Costill et al. (11) found no change in muscle pH 60 minutes after a 200 mg·kg body wt^{-1} dose of $NaHCO_3$, and Rupp et al. (97) reported no difference between control trial muscle pH and the pH after ingestion of a 300 mg·kg body wt^{-1} dose, although no pre-ingestion values were reported. Forearm muscle pH, determined by ^{31}P nuclear magnetic resonance, was similar after ingestion of either ammonium chloride, $NaHCO_3$, or a placebo (46). The administration of a 600 mg·kg body wt^{-1} dose of $NaHCO_3$ did not alter either pre-exercise muscle pH or muscle buffer capacity (determined by acid titration) in racing thoroughbreds (30). These results suggest that any intracellular buffering effect of $NaHCO_3$ is the result of an extracellular effect of $NaHCO_3$. Conversely, increasing the perfusate $NaHCO_3$ concentration in isolated tissues has been reported to increase the intracellular HCO_3^- content in rabbit hearts (115) and the intracellular pH in rat diaphragm muscle (37).

Isolated tissue studies

Early investigations (17,19) failed to demonstrate a beneficial effect of a pre-exercise induced alkalosis on subsequent exercise tasks. A renewed interest in the potential ergogenic effects of altering blood buffer reserve prior to exercise occurred after several investigations

utilizing isolated tissue systems demonstrated that increasing extracellular buffer reserve may alter intracellular events by affecting lactate or H^+ efflux rates. Mainwood, Worsley-Brown, and Paterson (76) examined the effects of fatiguing isolated, perfused frog sartorius muscle in either a 1 or 25 $meq \cdot l^{-1}$ HCO_3^- solution. Muscle lactate increased during the stimulation period in both trials with the 1 $meq \cdot l^{-1}$ trial exhibiting a greater muscle lactate concentration following the stimulation period (25.9 ± 1.95 vs 20.41 ± 1.34 $umol \cdot g$ wet $weight^{-1}$). Tissue preparations were also observed during a 70 minute recovery period after being fatigued. Recovery muscle lactate half-times (time to reach a concentration which is one half of the peak level) were shorter in the high HCO_3^- trial (~25 vs ~50 minutes). Measurements of lactate accumulation in samples of effluent collected during recovery were made to differentiate between lactate efflux and other metabolic fates of lactate (oxidation or interconversion to other metabolites). Peak lactate efflux (which occurred between 10 and 20 minutes post-fatigue) was greater in the high HCO_3^- trial (0.28 ± 0.04 vs 0.12 ± 0.01 $umol \cdot g^{-1} \cdot min^{-1}$) and declined in a pattern suggesting dependence on a concentration gradient. In the low HCO_3^- trial, not only was efflux lower, but a relatively constant efflux was noted. According to the authors, this constant efflux suggests that lactate efflux was dependent on some factor other than concentration gradient.

When the final muscle lactate concentration (70 minutes post-stimulation) was compared to the immediate post-fatigue concentration, the high HCO_3^- trial was associated with a 96% decline in muscle lactate with 56% of the loss attributed to efflux compared to a 50.5% decline and 33% loss attributed to efflux in the low HCO_3^- trial. The balance of the decline in both trials was attributed to oxidation and/or other metabolic interconversions which were greater in the high HCO_3^- trial (5.2 vs 1.4 $umol \cdot g^{-1}$).

Hirche et al. (42) examined the rate of lactic acid permeation (output) from supramaximally stimulated canine gastrocnemius muscle in an isolated, blood perfused preparation. Lactic acid permeation, calculated via the Fick method, was determined during metabolic acidosis (infusion of 0.20 N HCl) and alkalosis (infusion of 0.33 M $NaHCO_3$). No values for pre-infusion were reported, however pre-stimulation values for arterial blood base excess in the acidosis and alkalosis trials respectively were -20.1 ± 2.9 and +16 $meq \cdot l^{-1}$, standard HCO_3^- was 11.0 ± 1.6 and 38.0 $mmol \cdot l^{-1}$. The pre-stimulation blood pH values were 7.10 and 7.63 for the acidosis and alkalosis trials, respectively. During the alkalosis trial, the average rate of lactic acid output was 3 $umol \cdot g^{-1} \cdot min^{-1}$ vs 1.2 $umol \cdot g^{-1} \cdot min^{-1}$ in the acidosis trial over the 12 minute stimulation period. Muscle lactic acid concentration was similar over the first one to two minutes of

stimulation, however, the concentration decreased more rapidly in the alkalosis trial over the remainder of the stimulation period. There were no significant differences between trials in work per unit time during the first five to six minutes of stimulation. During the remainder of the stimulation period however, the alkalotic trial was associated with higher power output and a reduced muscle lactic acid concentration.

The effects of extracellular buffer concentrations on lactate efflux could be related to either the increased blood buffer reserve and/or the alkaline shift in vascular pH. The effects of altering both extracellular pH and buffer content was examined by Mainwood and Worsley-Brown (75) using isolated, perfused sartorius muscle from frogs. Peak lactate efflux measured during recovery from fatigued muscle (stimulated for 200 seconds) was two- to three-times higher (400 vs 150 $nm \cdot g^{-1} \cdot min^{-1}$) in muscles perfused with a 25 mM HCO_3^- solution (pH 7.9) vs a 1 mM HCO_3^- solution (pH 6.5). Muscles perfused with a series of HCO_3^- concentrations (1, 5, 10, 15, 20, and 25 mM) demonstrated that the lactate efflux rate was enhanced as the concentration increased from one to ten mM, however, increasing the concentration above ten mM had no additional effect on lactate efflux rates. To distinguish between the effects of buffer concentration vs changes in pH, trials were conducted in which PCO_2 was increased to lower extracellular pH from 7.9 to 6.6, while buffer concentration

remained constant at 25 mM. Although efflux was reduced, the rate was still approximately two-times greater than muscles perfused in a low pH (6.6) and low HCO_3^- solution (1 mM). Finally, in a low buffer (10 mM), high pH (8.0) solution, lactate efflux was greater than H^+ ion efflux, indicating an effect of buffer concentration/pH on the degree of coupling between H^+ and lactate ion loss. In addition, trials using buffer systems other than HCO_3^- supported the authors conclusion that the effect on lactate efflux was independent of the type of buffer system used.

To determine if extracellular HCO_3^- concentration influenced lactate efflux in mammalian muscle, Mainwood and Cechetto (73) placed strips of rat diaphragm muscle (considered to be fast fatigue resistance or type IIA) in solutions containing either 2, 10, or 25 mM HCO_3^- and at temperatures of either 30°C or 37°C. The tissues were equilibrated for 30 minutes and then fatigued via electrostimulation by increasing the frequency of pulse train delivery. After tension decreased to less than 30% of the control tension, which was defined as the criterion of fatigue, the stimulation interval was returned to the pre-fatigue regimen for a 30 minute recovery period. The time required to induce fatigue was not significantly different between the HCO_3^- solutions or temperatures. There were, however, differences in tension recovery following fatigue in

the various solutions. Expressed as the time (minutes) to reach a tension equivalent to 50% of the pre-fatigue maximum, the values for 30°C were 7, 3.5, and less than 1 minute for the 2mM, 10 mM, and 25 mM solutions, respectively. A similar pattern was observed at 37°C with 4.5, 1.6, and less than 1 minute for the 2 mM, 10 mM, and 25 mM solutions, respectively. Muscle lactate was higher following fatigue in the 25 mM trial and had decreased to a lower value by 4 minutes into recovery when compared to the 2 mM trial. In an investigation (108) utilizing an electrically stimulated rat hindlimb preparation, peak muscle lactate release was reported to be higher (19.7 ± 1.6 $mmol \cdot min^{-1}$) when compared to control values (15.5 ± 1.1 $umol \cdot min^{-1}$) in tissues perfused with a high HCO_3^- (27.1 ± 0.8 $mmol \cdot l^{-1}$) solution compared to a lower HCO_3^- (20.7 ± 0.5 $mmol \cdot l^{-1}$) solution.

The results of these isolated tissue studies suggest that external buffer concentrations and/or pH affects lactate efflux from muscle. Mainwood, Worsley-Brown, and Paterson (76) suggest four possible models to explain the apparent dependence of lactate efflux on buffer concentrations. The first model is based on the premise that either the HCO_3^- ion or the associated change in external pH may alter muscle membrane permeability to lactate ions. This explanation is supported by several studies (49,74) in which an increase in pH has been shown to increase transmembrane anion conductance.

The authors indicate, however, that this type of passive lactate permeability doesn't explain the low lactate efflux response observed in the low HCO_3^- environment. In addition, the permeability of muscle membranes to large ions is slow (76). A second model involves a transmembrane anion exchange of extracellular HCO_3^- ion and intracellular lactate ion. However, this exchange also seems to be too slow to accommodate the efflux of lactate observed experimentally. Two additional models were based on the premise that the HCO_3^- ion serves as an extracellular buffer. The third model suggests the independent movement of both lactate and H^+ ions across the cell membrane with extracellular HCO_3^- ions buffering the additional H^+ ions entering the extracellular space. The preferred model, according to the authors, is the transmembrane movement of undissociated lactic acid across the cell membrane at which point it dissociates and subsequent H^+ ion buffering occurs. Measurements of H^+ and lactate ion efflux from fatigued isolated rat diaphragm muscle (38) however, have demonstrated a significant difference in equilibration half times for H^+ ions (<1 minute) and lactate ions (6 minutes). This difference in lactate and H^+ ion equilibration times suggest that lactic acid efflux occurs predominantly in the dissociated form and that the rates of efflux may differ for the lactate and H^+ ion.

These studies suggest that altering extracellular buffer

reserve does influence the rate of lactate efflux from the intracellular to extracellular compartments, although it seems that the type of buffer (73) is not critical. During stimulation, tissues perfused in solutions containing high buffer concentrations exhibited higher lactate efflux rates (42). Tissues recovering from stimulation exhibit a greater peak lactate efflux (75,76), faster recovery of pre-exercise lactate concentration (76), and a faster recovery of muscle tension (73). Studies which utilize isolated tissues or in situ muscle preparations, however, should be viewed with caution when extrapolating to whole body responses. The responses in isolated tissue preparations may not represent whole body responses due to the lack of hormonal or neural influence.

The movement of metabolites from the intracellular space to the vascular system can potentially be affected by the properties of the cell membrane and the capillary wall. Evidence for a H^+ linked lactate-monocarboxylate plasma membrane carrier for lactate has been reported in several tissues including mouse diaphragm muscle (62), mouse cardiac cells (77), human red blood cells (18), and frog muscle (79), suggesting that a family of H^+ linked lactate-monocarboxylate carriers exist in the plasma membranes of different cell types (90). Detailed characterization of lactate and H^+ ion transmembrane flux has been made possible by utilization of

various compounds which selectively inhibit cellular membrane transport mechanisms. Juel (57) reported that the primary mechanism for lactate transport in mouse soleus muscle preparations was a monocarboxylate carrier which was involved in the co-transport of lactate and H^+ ions. When inorganic anion exchange (HCO_3^- and lactate) was selectively inhibited, lactate efflux rates were not significantly altered, suggesting that inorganic anion exchange plays a minor role in overall lactate efflux. In addition, under experimental conditions which inhibited both the monocarboxylate carrier and inorganic anion exchange mechanisms, some lactate and H^+ ion efflux continued, suggesting that non-ionic diffusion did take place. The differences observed in lactate and H^+ ion efflux were attributed to a Na^+/H^+ ion exchange mechanism which could selectively facilitate H^+ ion extrusion.

To further isolate and examine sarcolemmal transfer mechanisms, investigators have studied lactate transport utilizing preparations of sarcolemmal membrane vesicles. Vesicle preparations have several advantages over isolated cells, including absence of organelles, minimal metabolic activity, and the potential to alter both the intravesicular and extravesicular composition (95). Studies utilizing vesicle preparations have confirmed that at the physiological pH of the active muscle cell, passive diffusion plays a minor role in lactate transport. The transport process seems to be

stimulated by a proton gradient across the sarcolemmal membrane, resulting in the acceleration of lactate and H^+ ion efflux from muscle as intracellular pH decreases during high intensity exercise (95). Whether training alters the sarcolemmal lactate transport rate or capacity remains unclear. Roth and Brooks (96) prepared sarcolemmal vesicles from sprint trained (ST), endurance trained (ET), and control rats (C). The time course of lactate uptake (1 mM solution) was similar in all three groups. Group comparisons made under a variety of lactate concentrations revealed similar values for lactate transport K_m (40 ± 5, 37 ± 6, and 46 ± 6 mM for C, ST, and ET, respectively) and V_{max} values (139 ± 5, 140 ± 7, and 146 ± 6 $mmol \cdot mg^{-1} \cdot min^{-1}$ for C, ST, and ET, respectively). The similar K_m and V_{max} values, according to the authors, suggest that training did not affect lactate transport rate or capacity. The sarcolemmal preparations, however, were made from muscle tissue with a heterogenous fiber type, potentially masking adaptations in specific fiber types.

Investigations which have examined the ergogenic potential of $NaHCO_3$ loading on exercise performance have produced variable results. Comparison of results from these studies is difficult due to differences in exercise parameters (e.g., intensity, duration, total work, and repeated versus single bouts), $NaHCO_3$ loading regimens (e.g., total dose, routes of administration, and time from dosing to exercise),

and subject characteristics. Subjects with a high pre-existing anaerobic capacity and/or muscle buffering capacity may not benefit from $NaHCO_3$ loading regimens, although several investigators have reported positive effects on performance in trained athletes (28,123).

Effects of $NaHCO_3$ ingestion on pre-exercise acid-base balance

Human studies in which $NaHCO_3$ solutions have been administered either orally or intravenously have documented pre-exercise increases in both resting blood ph (5,28,46,52,56,58,60,64,72,97,116,122,123) and HCO_3^- (5,11,45,46,52,58,60,64,72,97,123). Increased resting blood pH (30,31,67) and HCO_3^- concentration (30,31) have been observed following $NaHCO_3$ ingestion in equine.

The magnitude of the pre-exercise change in ph and/or HCO_3^- induced by $NaHCO_3$ ingestion, which may be critical in the effectiveness of alkalinizing agents, may be affected by the total dose, route of administration, and latent time from dosing to the exercise task. Most human investigations have utilized a total $NaHCO_3$ dose ranging from 100 to 400 mg·kg body weight^{-1}. In a meta-analysis (80) of human $NaHCO_3$ loading studies, the effects of $NaHCO_3$ ingestion on blood acid-base homeostasis were reported. A mean increase in blood pH of 0.07 ± 0.02 and HCO_3^- of 5.3 ± 1.4 mmol·l^{-1} was reported for investigations utilizing a 300 mg·kg body wt^{-1} dose (most

common total dose reported). Investigations using a dose of 200 mg·kg body wt^{-1} reported a mean increase of 0.06 ± 0.02 and 4.4 ± 1.1 mmol·l^{-1} for blood pH and HCO_3^-, respectively. There were significant, but "only moderate associations" reported between the increases in blood pH and HCO_3^- ($r=0.65$, $p<0.01$), increases in blood pH and the total dose administered ($r=0.42$, $p<0.05$), and increases in blood HCO_3^- and the total dose administered ($r=0.53$, $p<0.05$). The administration of the $NaHCO_3$ dose in the investigations reviewed, ranged from a single bolus to intermittent administration over periods up to three hours (80). In addition, the time between dosing to the exercise task ranged from minutes up to three hours (80). Even with the same dosing protocol (total dosage and timing of ingestion), there seems to be considerable variability in the reported changes in pre-exercise blood pH and HCO_3^-. This variability may be attributed to several factors including the amount of fluid ingested during the dosing regimen and the possible influence of ions in either the $NaHCO_3$ dose (Na^+) itself or the various placebo solutions (Na^+, Cl^-, Ca^{++}) used (80).

Investigations of equine athletes have reported using total doses which range from 300 to 600 mg·kg body $weight^{-1}$ and administered one to four hours before exercise. Greenhaff et al. (31) examined the effects of a 600 mg·kg^{-1} dose of $NaHCO_3$ administered via a nasogastric tube in thoroughbred

horses. Blood samples were obtained over a 24-hour period. Venous blood pH was increased at two hours post-dose, peaked at eight hours, and remained elevated through twelve hours post-dose. Blood HCO_3^- was elevated thirty minutes post-dose, peaked between three and five hours, and remained elevated through twelve hours. Base excess followed a similar pattern, peaking at three hours post-dose and remained elevated at twelve hours post-dose. The authors suggest that $NaHCO_3$ dosing in equine athletes may be more effective with larger total doses (600 $mg \cdot kg^{-1}$) administered at least three hours prior to competition.

The initial human investigations (17,19,55,78) which reported no effect of alkalinizing agents on performance utilized much lower (<100 $mg \cdot kg^{-1}$) $NaHCO_3$ doses. A study by Horswill et al. (45) was designed to determine the oral threshold dosage of $NaHCO_3$ to significantly alter blood HCO_3^- levels. Human subjects received either a NaCl placebo or a 0.10 g, 0.15 g, or 0.20 $g \cdot kg$ body $weight^{-1}$ oral dose of $NaHCO_3$. Plasma HCO_3^- was elevated one hour post-ingestion in the 0.15 and 0.20 $g \cdot kg^{-1}$ trials and remained unchanged in the 0.10 $g \cdot kg^{-1}$ suggesting that total doses below 0.15 $g \cdot kg^{-1}$ may not significantly enhance blood buffer capacity. The use of doses which exceed 300 $mg \cdot kg^{-1}$ are generally believed to result in acute gastrointestinal reactions, although at least one investigation utilized a 400 $mg \cdot kg^{-1}$ dose (28) with only minor

gastrointestinal distress reported after the exercise bout.

Post-exercise blood and muscle lactate accumulation

The most common hypothesis concerning the ergogenic potential of $NaHCO_3$ involves the enhanced efflux of lactate and/or H^+ ions from the cell. Lactate efflux is typically documented by comparing the blood lactate accumulation after a standard exercise task in a control trial and $NaHCO_3$ trial. In a meta-analysis review (80) of 35 human studies reporting values for post-exercise blood lactate accumulation, 27 reported higher post-exercise blood lactate accumulation following $NaHCO_3$ ingestion, although these values were obtained at a variety of post-exercise intervals. An increased blood lactate accumulation in these investigations is usually interpreted as an indicator of increased anaerobic metabolism and/or muscle lactate efflux (assuming that blood lactate clearance has not changed). The relationship between blood lactate accumulation and performance was also examined. Because of the variety of performance tasks used in the investigations included in the review, a performance effect size was calculated for each study. The performance effect size for each individual study was defined as the difference between the placebo and $NaHCO_3$ trial means divided by the pooled standard deviation of the two trials. Each study effect size was also weighted for sample size by multiplying

the study effect size by the sample size then dividing by the total number of individual studies. According to the authors, the weighted performance effect size generated by this procedure allows for comparisons of outcomes measured in different scales. The relationship ($r=0.13$, $p>.10$) between the magnitude of the increase in post-exercise blood lactate accumulation and the performance effect size was not significant.

Following $NaHCO_3$ ingestion, investigators have reported increases in exercise task performance despite no change in blood lactate accumulation (5,11) and conversely, no effect on performance despite increased blood lactate accumulation (60,116). Higher post-exercise blood lactate accumulation has also been reported in horses run to fatigue on a treadmill following $NaHCO_3$ ingestion (67). Conversely, horses running over a 1.6 km distance exhibited no differences in post-exercise blood lactate levels between a control and $NaHCO_3$ trial (66).

Very few investigations have reported post-exercise muscle lactate accumulation. Muscle lactate accumulation after a cycle ergometer ride to exhaustion (at 375 watts) was higher (32 vs 18 $mmol \cdot kg\ wet\ wt^{-1}$) in a $NaHCO_3$ trial (5). Following a cycling exercise task to exhaustion at 95% of VO_2max, muscle lactate was higher in an alkalosis trial ($17.10 \pm 2.50\ umol \cdot g^{-1}$) when compared to either a control trial

(14.67 ± 1.50 umol·g^{-1}) or an acidosis trial (12.21 ± 1.41 umol·g^{-1}) (116). If $NaHCO_3$ ingestion does accelerate lactate efflux, then muscle lactate accumulation should be reduced, not increased, assuming that lactate production and oxidation is unchanged. Measurements of muscle lactate accumulation, however, represent the net effect of lactate production, lactate efflux, and/or oxidation. In addition, increases in time to exhaustion were reported in both investigations (5,116) which may explain the increased muscle lactate accumulation. An increase in total work, and thus lactate produced via metabolism, may lead to greater muscle lactate accumulation, assuming that an increased lactate efflux could not completely compensate for the increased production. In both investigations, blood lactate accumulation was reported to be similar in the control and $NaHCO_3$ trials, suggesting that efflux had not been enhanced (assuming that blood compartment clearance mechanisms were not altered). In contrast, Costill et al. (11) reported no difference in either muscle or blood lactate accumulation between a control and $NaHCO_3$ trial after five, one-minute maximal cycling bouts with the last bout to exhaustion. Despite similar lactate accumulation, the $NaHCO_3$ trial time to exhaustion was significantly increased by 47 seconds (+42%) when compared to control.

Few studies have examined the effects of $NaHCO_3$ ingestion on the time course of blood or muscle lactate accumulation throughout recovery from exercise. If muscle lactate efflux is enhanced by $NaHCO_3$ ingestion, blood lactate accumulation throughout a standard recovery period may increase, although it is possible that blood lactate clearance mechanisms may mask or alter the blood lactate responses. Similar blood lactate concentrations ($NaHCO_3$ vs control trial) have been reported throughout a 30-minute recovery in thoroughbreds exercised at high speeds for two minutes on a treadmill (30), whereas a greater decline in blood lactate was reported for standardbreds recovering over 30 minutes from a 1.6 km run (66). Several investigations in humans have reported no differences in recovery blood lactate accumulation (11,45,60,81), although Katz et al. (58) reported higher post-exercise blood lactate accumulation from minutes 9 through 20 during a 30-minute recovery period following $NaHCO_3$ ingestion. In addition, plasma lactates have been reported to be elevated throughout a 30-minute recovery after cycling to exhaustion (56).

The post-exercise time course of muscle lactate accumulation has not been extensively examined. If muscle lactate efflux is enhanced following $NaHCO_3$ ingestion, then the decline in post-exercise muscle lactate may be accelerated. Muscle lactate measurements were reported to be

similar throughout five, one-minute cycling bouts (one minute rest intervals) and after the final bout in which subjects exercised to exhaustion (11).

Post-exercise blood and muscle acid-base balance

The post-exercise acid-base status of both blood and muscle have also been used as criteria for determining the effectiveness of $NaHCO_3$ loading regimens. The effect of $NaHCO_3$ ingestion on post-exercise blood pH has varied, with some investigators reporting higher post-exercise pH (11,28,45,58,60,64,88,123), whereas others have reported no effect on post-exercise blood pH (5,81). The higher post-exercise blood pH observed following $NaHCO_3$ ingestion seems to parallel the pre-exercise increase. A recent review (80) has illustrated that the difference ($NaHCO_3$ trial vs control) in post-exercise blood pH was similar to the pre-exercise difference following $NaHCO_3$ ingestion. Although the absolute decline in blood pH was similar, $NaHCO_3$ ingestion was associated with a higher blood lactate accumulation per unit change in blood pH, which, according to the authors, supports the hypothesis that lactate efflux has been enhanced (80). A larger performance effect size was also reported for those studies with higher post-ingestion blood pH, suggesting that the magnitude of the change in blood pH induced by the $NaHCO_3$ loading regimen is important. The association between changes

in pre-exercise blood pH and performance supports the contention that lactate and H^+ ion transport seem to be stimulated by a transmembrane proton gradient (95). A larger performance effect size was also associated with a lower post-exercise absolute blood pH. The contradictory performance effects reported for $NaHCO_3$ ingestion within the literature may be related to the magnitude of the acid-base disturbance generated by the specific exercise task used. Post-exercise blood pH also seems to be affected by $NaHCO_3$ ingestion, with a higher post-exercise blood pH reported during the exercise recovery period (11,45,58,60).

Changes in blood HCO_3^-, base excess, and/or base deficit are other commonly reported indicators of altered blood acid-base status. A reduction in blood HCO_3^-, indicating an increased buffering of H^+ ions, and a concomitant increased base deficit are associated with metabolic acidosis. Base excess is the base (if a negative base excess) or acid (if a positive base excess) required in milliequivalents per liter to titrate a blood sample to a pH of 7.40, at 37°C, and at a PCO_2 of 40 mm Hg (14). Base deficit is also used to express the condition in which base is required to titrate the blood sample to standard conditions.

Conflicting results concerning blood HCO_3^- and base deficit responses following $NaHCO_3$ ingestion have occurred as some investigators have compared absolute post-exercise values

for blood HCO_3^- and base deficit, whereas others have compared the magnitude of change in blood HCO_3^- and base deficit between pre-exercise and post-exercise samples.

Following $NaHCO_3$ ingestion, higher absolute post-exercise blood HCO_3^- concentrations have been reported in both humans (11,45,62,64,123) and equine (30), although in some investigations these differences only occurred later in recovery (45). Other investigations have found no differences in absolute post-exercise blood HCO_3^- concentrations (5,28,66,72,81). Two investigations (5,11) have reported greater pre-exercise to post-exercise reductions in blood HCO_3^- concentration following $NaHCO_3$ ingestion. Because the pre-exercise blood HCO_3^- concentration is expected to increase following $NaHCO_3$ ingestion, comparing the pre- to post-exercise change in HCO_3^- concentration may be more appropriate than comparing absolute concentrations. In a review of 29 studies, Matson and Tran (80) reported a greater exercise-induced decline in blood HCO_3^- following $NaHCO_3$ ingestion (-13.4 ± 3.1 mmol$\cdot l^{-1}$) than placebo (-11.3 ± 2.6 mmol$\cdot l^{-1}$). When the change in blood HCO_3^- and performance effect size were compared, an inverse relationship was reported for studies utilizing both time to exhaustion ($r=-0.62$, $p>0.1$) and "highly exhaustive events" ($r=-0.54$, $p>0.1$). Post-exercise base excess values have been reported to be higher (less negative) following $NaHCO_3$ ingestion

(28,30,58,60). These same studies have also reported higher pre-exercise base excess values following $NaHCO_3$ ingestion. As with blood HCO_3^- concentration, the interpretation of post-exercise base excess values requires the realization that absolute post-exercise changes may differ from the pre- to post-exercise change in base excess.

Changes in blood PCO_2 assist in the characterization of acid-base disturbances and the resulting respiratory compensation which occurs in response to metabolic alkalosis or acidosis. Acute metabolic alkalosis results in a relative depression of alveolar ventilation, resulting in CO_2 retention, a decline in arterial blood pH and an increased blood HCO_3^- concentration. A state of metabolic acidosis exhibits opposite effects, as alveolar ventilation is stimulated, resulting in an increase in CO_2 removal, increase in arterial blood pH, and lower HCO_3^- concentration (14). An increased pre-exercise PCO_2 has been reported following $NaHCO_3$ ingestion (30,60), although the majority of investigations (28,52,58,64) have not observed significant differences. In addition, most investigations (28,30,52,58,64) have not found significant differences in post-exercise PCO_2 between $NaHCO_3$ and control trials.

Very limited information exists concerning the effect of $NaHCO_3$ ingestion on post-exercise muscle pH. Rupp et al. (97) reported no trial difference in immediate post-exercise muscle

pH following a cycling task to exhaustion. In a series of five, one-minute cycling bouts, the muscle pH immediately prior to the fifth bout was reported to be higher in the $NaHCO_3$ trial (6.86 ± 0.06 vs 6.72 ± 0.04), although the pH after the fifth bout (which continued to exhaustion) was similar to control (11). Using ^{31}P-nuclear magnetic resonance spectroscopy, Hood et al. (46) reported no differences in post-exercise or recovery (seven minute period) intracellular muscle pH following two minutes of exhaustive forearm exercise.

Effects on exercise performance

The potential performance benefit of increasing the extracellular buffer reserve via $NaHCO_3$ ingestion may be dependent on the metabolic demands determined by the specific exercise task chosen. If the benefits of $NaHCO_3$ ingestion are related to an effect on acid-base homeostasis, it seems that exercise tasks which have the greatest potential to induce disturbances in acid-base homeostasis are the tasks most likely to exhibit a $NaHCO_3$ induced effect on exercise performance.

The majority of investigations examining $NaHCO_3$ ingestion and exercise performance are limited to studies of human subjects, with a few investigations reported for equine athletes. The exercise tasks used in these investigations

vary widely with respect to intensity, duration, and whether subjects are exposed to single or repetitive bouts. Significant positive effects on performance have been reported by a number of investigators using a time to exhaustion criteria for a single exercise bout. $NaHCO_3$ ingestion increased time to exhaustion for cycling at 80% (84), 95% (52,56,97,116), 100% (72), and 125% of VO_2 max (5).

Running tasks have also been used with reductions reported for both 400-meter (28) and 800-meter (123) sprint times. The duration of these tasks ranged from approximately 60 seconds (5,28) up to 30 minutes (84), with the majority of studies in the range of approximately two to five minutes (52,56,72,97,116,123). In addition, two investigations (59,66) have reported significant ($p<0.1$) reductions in sprint times in equine running over a 1.6 km distance following $NaHCO_3$ ingestion.

Other investigators have found no significant effect of $NaHCO_3$ ingestion on subsequent performance. No effect was found on the time to exhaustion in a progressive cycling task (64), a ride to exhaustion at either 100% (69) or 125% of VO_2 max (58), in measurements of power output in a 30-second maximal cycling test (81), for mean and peak power output during a 30-second maximal cycling test (51), sprint time for a 400-meter run (60), or total work performed in a two-minute maximal cycle test (45).

Investigations using tasks involving upper body work have reported no significant performance effects following $NaHCO_3$ ingestion (7,61). In addition, Lawrence et al. (67) reported no effect on treadmill time to fatigue in horses.

Several investigations have examined the effects of $NaHCO_3$ feedings on performance and acid-base balance during repeated bouts of exercise. The performance tasks in these studies were designed to provide a longer total duration at a higher intensity. If $NaHCO_3$ ingestion is associated with an accelerated lactate and H^+ ion efflux from muscle, more complete muscle acid-base recovery may occur in the rest intervals resulting in enhanced performance in the subsequent bouts. This type of performance test may have direct application for athletes who are training and/or competing under conditions which require multiple bouts of high intensity exercise over short time periods. In an investigation by Costill et al. (11), subjects performed five, one-minute cycling bouts at 125% of VO_2 max with a 1:1 work rest ratio. The fifth bout was continued until voluntary exhaustion. Cycling time during the final bout was increased by 42% in the $NaHCO_3$ trial. Lavender and Bird (68) reported that in eight of ten, ten-second maximal cycling sprints (50 seconds rest between bouts), the average power output was higher in the $NaHCO_3$ trial (exceptions were sprints one and four). Using a rank order correlation procedure, the power

output difference increased significantly ($r=0.821$, $p<0.01$) as the number of sprint repetitions increased. Peak power output, however, was significantly elevated only in sprints two and ten. A similar effect has been reported for an interval swimming task. Faster 100-meter swim times were reported for the fourth and fifth intervals in five, successive 100-meter swims (26). Other investigators utilizing interval exercise designs have reported no effects on performance in repeat one mile rowing tasks (93), in mean or peak power output in three, 30-second maximal cycling bouts (88), or time to exhaustion in the fifth bout of five, one-minute cycling bouts at 125% of VO_2 max (122).

Because of the variety of exercise tasks and $NaHCO_3$ loading regimens reported in the literature, comparisons of physiological responses and exercise performance between studies are tenuous. Matson and Tran (80) have provided some insight with their meta-analysis procedure of 35 human studies. Their analysis indicates that $NaHCO_3$ ingestion was associated with a mean exercise performance effect size that was 0.44 standard deviation units above that of the control trial. When investigations were subdivided into groups based on the performance task criteria used, the largest effect size (0.89) was found in those studies using a time to exhaustion protocol. A smaller effect size was reported for tasks measuring total work or power over specified time periods

(0.29), and performance times (0.32) for specified exercise tasks.

Summary - effects of $NaHCO_3$ on responses to exercise

In summary, $NaHCO_3$ ingestion is generally associated with significant increases in pre-exercise blood buffer reserve based on increases in blood pH (5,28,30,31,46,52,56,58,60, 64,67,72,97,116,122,123), base excess (28,30,58,60), and HCO_3^- concentration (5,11,30,31,45,46,52,58,60,64,72,97,123). The magnitude of the post-ingestion increase in blood pH and HCO_3^-, which may determine the effectiveness of the $NaHCO_3$ regimen, exhibits a significant positive relationship to the total dose administered (80).

Isolated and in situ tissues studies have demonstrated enhanced lactate efflux in muscle tissue stimulated in perfusates with high vs low HCO_3^- concentrations (42,75,76,108). Evidence for a H^+ ion linked lactate-monocarboxylate plasma membrane carrier exists, suggesting the co-transport of lactate and H^+ ions (18,62,77,79,90). Furthermore, this transport process appears to be stimulated by the proton gradient across the sarcolemmal membrane (95). The ergogenic potential of $NaHCO_3$ ingestion may be related to the observed post-ingestion increase in extracellular buffer capacity, which may result in the maintenance and/or development of a greater intracellular-extracellular proton

gradient, thus accelerating or maintaining H^+ ion and lactate efflux. The enhanced efflux of H^+ ions and lactate would result in maintenance of intracellular homeostasis and thus maintain and/or improve muscle function.

Higher post-exercise blood lactate accumulation following HCO_3 ingestion has also been reported (52,56,72,84,97,116, 123), whereas others have reported no treatment differences (5,11,45,60,69,81,88). The magnitude of the increase in blood lactate accumulation in the $NaHCO_3$ condition, however, was not significantly correlated with the performance effect size (80). If muscle lactate efflux is increased by $NaHCO_3$ ingestion, a decline in post-exercise muscle lactate accumulation may be expected, assuming that other factors which affect lactate accumulation such as production or oxidation have not been altered. The available data do not support this, however, as post-exercise muscle lactate has been reported to be elevated (5,116) or not affected by $NaHCO_3$ ingestion (11). Post-exercise blood acid-base status following $NaHCO_3$ is typically characterized by a higher absolute pH (11,28,45,58,60,64,88,123) which appears to exist throughout exercise recovery (11,45,58,60). Blood HCO_3^- concentration (11,30,45,60,64,123) and base excess values are also typically higher following $NaHCO_3$ exercise trials (28,30,58,60), although these differences appear to reflect the existing higher pre-exercise values. However, a greater

pre-exercise to post-exercise change in blood HCO_3^- concentration has been reported (5,11,80) suggesting that $NaHCO_3$ ingestion is associated with greater buffering of blood H^+ ions.

The general hypothesis concerning the ergogenic potential of $NaHCO_3$ ingestion is based on the concept that the metabolic and contraction functions of muscle are enhanced and/or sustained by the maintenance of a more favorable intracellular environment. This is believed to be related to the enhanced ability to maintain intracellular acid-base homeostasis. Few studies have attempted to document the effects on intracellular acid-base balance. The investigations which have measured muscle pH have reported no significant post-exercise differences in muscle pH (11,46,97), although two of these investigations reported significant increases in exercise time to exhaustion (11,97).

Conflicting results have also been reported for the effect of $NaHCO_3$ ingestion on performance measurements. Both a positive effect (5,28,52,56,59,66,72,84,97,116,123) and no effect (7,45,51,58,60,61,64,67,69,81) have been reported for a variety of exercise tasks. Exercise tasks which have a greater dependence on anaerobic metabolism and subsequently generate a more severe acid-base disturbance appear to be more likely to be positively affected by pre-exercise $NaHCO_3$ ingestion (80).

PAPER 1. ADAPTATIONS OF MUSCLE GLYCOLYSIS TO SPRINT TRAINING IN RACING GREYHOUNDS

Title: Adaptations of muscle glycolysis to sprint training in racing greyhounds.

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ABSTRACT

The purpose of this investigation was to examine the effects of two sprint training regimens on muscle glycolysis in racing greyhounds. Training consisted of either a low volume regimen (LV) of 12 400-meter sprints during a six-week period, or a high volume regimen (HV) totalling 30 sprints. Before and after training, 400-meter trial sprints were used to assess muscle glycogen utilization, blood and muscle lactate accumulation, and muscle buffer capacity.

Training increased ($p<0.05$) the post-sprint blood lactate accumulation in both LV (15.0 ± 1.4 vs 17.6 ± 1.1 $mmol \cdot l^{-1}$) and HV (14.0 ± 1.4 vs 17.3 ± 1.4 $mmol \cdot l^{-1}$). Post-sprint muscle lactate accumulation was not affected by either training regimen. Post-sprint muscle pH was similar before and after training in LV (6.78 ± 0.03 and 6.80 ± 0.03) and HV (6.81 ± 0.02 and 6.83 ± 0.01). Glycogen utilization was increased ($p<0.05$) following training in HV (16.3 ± 2.8 vs 25.7 ± 3.0 mmoles glucose·kg wet wt^{-1}), and remained unchanged (17.6 ± 2.7 vs 16.1 ± 3.3 mmoles glucose·kg wet wt^{-1}) in LV. The increased glycogen utilization in HV was associated with a 14% increase ($p<0.05$) in maximal phosphofructokinase (PFK) activity (43.3 ± 1.3 to 47.4 ± 1.4 $mmol \cdot kg$ body $wt^{-1} \cdot min^{-1}$).

In vivo muscle buffer capacity was not significantly increased by training in LV (57.1 ± 4.4 vs 58.3 ± 3.8 Slykes), while a trend ($p=0.09$) towards an increased buffer capacity

occurred in HV (58.4 ± 4.6 vs 66.7 ± 5.8 Slykes). The training-induced increase in glycogen utilization and PFK activity in HV suggests an enhanced glycolytic capacity associated with the higher training volume.

Index terms: glycogen; metabolism; dog; buffer capacity

INTRODUCTION

Modern greyhound racing, now an international sport industry, consists of competitive track races which typically range in length from 300 to 800 meters, but may reach approximately 1000 meters in the greyhound equivalent of the "marathon". These running events may last from 15 to 60 s in duration and at occur at average running speeds in excess of 18 $m \cdot s^{-1}$ (~65 $km \cdot h^{-1}$) over the shorter race distances.

Short term, high intensity exercise tasks require rapid resynthesis of ATP via stored muscle phosphagens (ATP and phosphocreatine) and the glycolytic pathway, resulting in the accumulation of lactate and hydrogen (H^+) ions in blood and muscle (14,21,33). Greyhounds exhibit a high potential for phosphocreatine utilization, as evident by a higher total activity of creatine phosphokinase (CPK) in comparison to that of other species such as humans, horses, and other canine breeds (33). This high CPK activity probably contributes to the rapid acceleration exhibited by racing greyhounds at the onset of a race. Because the concentration of endogenous phosphagen substrates in skeletal muscle is similar and relatively limited in all three species (33), the metabolic burden of continued ATP resynthesis would likely be shifted to glycolysis shortly after the onset of high intensity exercise.

The contribution of glycogen-derived ATP resynthesis in short term, high intensity exercise has been investigated in

humans. It appears, based on determinations of muscle lactate accumulation, that rapid muscle glycolysis in humans occurs within seconds after the onset of high intensity exercise (2,17). Glycolytic potential in greyhounds appears to be considerably higher than that in their human counterparts, based on the two- to three-fold higher activity of glycogen phosphorylase and phosphofructokinase activities (33). Significant declines in muscle glycogen (7) and muscle pH (7) and a concomitant rise in muscle (7) and blood lactate accumulation (7,15,22,24,26) also suggest that glycogenolysis and subsequent anaerobic glycolysis appear to play a major metabolic role in the sprinting greyhound.

Human athletes who participate in athletic events requiring short term, supramaximal exercise commonly engage in training regimens of high intensity, repetitive bouts of exercise that mimic the competitive event itself. Physiological adaptations to sprint training in humans include increased contribution of anaerobic glycolysis to ATP resynthesis (21), activity of glycolytic regulatory enzymes (16,25,30), muscle (16,21) and blood lactate accumulation (13,16,18,21), and muscle buffer capacity (30). In contrast, efforts to enhance the performance of greyhound athletes have focused primarily on selective breeding practices, rather than the application of scientifically based training principles. Although some greyhound trainers employ formal regimens of

exercise, most greyhounds are not exposed to chronic, repetitive sprint training prior to their racing career.

Information supporting whether or not greyhound athletes exhibit biochemical adaptations to formal exercise regimens is unavailable. The purpose of this investigation was to examine the effects of two sprint training regimens on muscle glycolysis in the biceps femoris muscle of racing greyhounds.

MATERIALS AND METHODS

All protocols and procedures used in this investigation were reviewed and approved by the Iowa State University Committee on Animal Care.

Fifteen healthy adult greyhounds (ages 14 to 48 months, mean 29.3 ± 1.8 months), all with previous track experience, were randomized by gender and age into one of two training groups. Four weeks before the training study began, all dogs were placed on a standard dry food diet[1] and housed in similar outside runs to minimize any influence of previous diet, exercise patterns, or trainer handling.

Animals (n=8, 5 females, 3 males) in the low volume training group (LV) performed one 400-meter sprint, two days per week for six weeks. The LV training would simulate the volume of high intensity exercise to which most greyhounds are exposed to. Animals (n=7, 4 females, 3 males) in the high volume training group (HV) performed the following interval sprint training program: Week 1 - one 400-meter sprint, two days/week, weeks 2 and 3 - two 400-meter sprints, two days per week, weeks 4 and 5 - three 400-meter sprints, two days/week, and week 6 - four 400-meter sprints, two days/week. Repetitive sprints were separated by a two minute inactive rest period. All training occurred on Monday and

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[1] Science Diet Maintenance®, Hills Division of Colgate Palmolive Corp., Topeka, KA

Thursday mornings (8:00 to 11:00 a.m.) with animals fasted (feed removed at 6:00 p.m. of the previous night).

Blood and muscle samples were obtained before and after a 400-meter sprint trial prior to and after the six-week training period. All sprint trials were conducted during the morning with the animal off feed (food removed at 6:00 p.m. of previous night). Post-training sprint trials were conducted within three days of the last training session for each animal. Pre- and post-sprint venous blood samples were obtained by venipuncture of the left external jugular with the animal in right lateral recumbency. The pre-sprint blood sample was taken in the laboratory five minutes after the pre-sprint muscle biopsy was obtained. Post-sprint blood samples were obtained five minutes after the completion of the sprint trial with the animal resting in lateral recumbency. Following the removal of the post-sprint biopsy sample, the incision site was sutured[2] and a topical antibiotic[3] was applied to reduce the potential of post-biopsy infections

A one ml blood sample was deproteinized in two ml of cold 8% perchloric acid, centrifuged (4,000xg for 10 minutes) and the supernatant was stored at -5°C until determination of

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[2] 00 Ethibond polyester suture, Ethicon Inc., Somerville, N.J.

[3] Panalog®, Solvay Veterinary Inc., Princeton, N.J.

[4] Beckman DU-5 Spectrophotometer, Beckman Instruments Inc., Irvine, CA

lactic acid concentration by spectrophotometric[4] analysis at the conclusion of the study (20). Hemoglobin concentration (Hgb) was determined in triplicate by using a hemoglobin-cyanmethemoglobin method (10). Packed cell volume (PCV) was determined in triplicate by using standard microhematocrit techniques. Five to ten ml of whole blood was placed on ice, and serum was harvested for analysis of total protein (TP) and serum osmolality (Osm)[5].

After injection of a local anesthetic[6] under the skin, a skin incision was made over the midsection of the left biceps femoris muscle. A pre-sprint muscle biopsy was removed by using a six mm percutaneous biopsy needle[7] and immediately frozen (within five seconds of excision) and stored in liquid nitrogen until analysis.

The incision site was sutured and the animals were transported (~20 miles) to the track site. Each dog was run individually, and after being placed in a starting box, sprinted after a mechanical hare for a 400 meter distance. The dog was caught within four to five seconds of the conclusion of the trial sprint, and a post-sprint biopsy was immediately obtained through the pre-exercise incision site

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[5] Roche Biomedical Laboratories, Kansas City, MO

[6] 2% Lidocaine®, Elkins-Sinn Inc., Cherry Hill, NJ

[7] Bergstrom biopsy needle

but lateral to the pre-sprint sample and at the same approximate muscle depth.

The post-sprint biopsy samples were frozen (within five seconds of excision) and stored in liquid nitrogen until determinations of muscle pH, glycogen, lactate, and maximal phosphofructokinase (PFK) activity were made at the conclusion of the study. All visible blood and connective tissue was removed, and muscle samples were weighed on a microbalance[8] at -20°C prior to analysis.

Intracellular pH was estimated by using the homogenate technique described by Costill et al. (6). The pH of the homogenate was measured at 38° C with a Radiometer BMS 3MK2 Blood Micro System coupled to a PHM-73 pH/blood gas monitor[9] which was calibrated after every fifth sample by using a two-point (6.840 and 7.383) calibration procedure. Muscle lactate was determined by fluorometric[10] enzymatic analysis (20).

In vivo muscle buffer capacity was calculated from muscle pH and lactate determined before and immediately after the 400-meter trial sprints. The ratio of the change (pre-sprint to post-sprint) in muscle lactate to muscle pH, expressed in units of mmoles lactate$\cdot kg^{-1}\cdot pH^{-1}$ or "Slykes", was used to

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[8] Mettler AE 163 microbalance, Mettler Instrument Corp., Highstown, N.J.

[9] Radiometer, Copenhagen, Denmark

[10] Ratio 2 Fluorometer, Farrand Optical Co., Valhalla, N.Y.

estimate muscle buffer capacity (35).

Fluorometric[10] determinations of muscle glycogen were made according to the methods of Passoneau and Lauderdale (23) and are expressed as mmoles glucosyl units·kg wet wt^{-1}. Maximal phosphofructokinase activities were determined by fluorometric[10] analysis (20) and are expressed as mmoles of substrate degraded·kg wet wt^{-1}·$minute^{-1}$.

To determine the effect(s) of transporting the animals between the laboratory (site of pre-sprint sampling) and the track (site of the trial sprint and subsequent post-sprint sampling), muscle and blood samples were obtained (as previously described) from eight animals at the laboratory and after transport to the track. These samples were used to determine blood lactate, hemoglobin, packed cell volume, muscle lactate, and muscle pH.

The effects of the six-week training programs were analyzed by using an ANOVA procedure with two training levels (LV and HV) and two pre-sprint to post-sprint differences (before and after training). When a significant interaction F ratio was found, a Student-Newman-Keuls multiple range test procedure was used to identify significant mean differences. The parameters examined before and after transport were assessed for significant differences with a paired t-test procedure. All statistical analysis involving pH measurements were performed with H^+ ion concentrations and have been

converted back to pH units for purposes of presentation. The level of probability was set at $p<0.05$ to reject the null hypothesis. All results are expressed as mean ± standard error of mean.

RESULTS

Sprint performance time was not significantly different in LV before (26.44 ± 0.10 s) or after training (26.38 ± 0.14 s). The difference in performance time in HV before (26.57 ± 0.08 s) and after training (26.29 ± 0.12 s) approached statistical significance (p=0.07).

Transport to the track site had no significant effect on blood lactate, Hgb, PCV, muscle pH, or muscle lactate (Table 1). Whole blood Hgb, PCV, TP and Osm data are presented in Table 2.

Table 1 Select muscle and blood parameters before and after transport to the track site

	Before	After
Blood Lactate ($mmol \cdot l^{-1}$)	1.4 ± 0.2	1.9 ± 0.2
Muscle Lactate ($mmol \cdot kg\ wet\ wt^{-1}$)	4.6 ± 0.3	4.8 ± 0.4
Muscle pH	6.98 ± 0.01	6.97 ± 0.01
Hemoglobin ($g \cdot d^{-1}$)	18.4 ± 0.4	19.3 ± 0.6
Packed Cell Volume (%)	58.6 ± 0.9	60.4 ± 0.8

Table 2 Hemoglobin, packed cell volume, serum total protein, and serum osmolality before (pre) and after (post) a 400 meter sprint before and after training

	Low Volume Training (LV)					
	Pre-Training			Post-Training		
	Pre	Post	Δ	Pre	Post	Δ
Hemoglobin ($g \cdot dl^{-1}$)	19.7 ± 0.4	21.5 ± 0.6*	1.8 ± 0.5	20.1 ± 0.5	23.2 ± 0.5*	3.1 ± 0.3†
Packed Cell	57.4 ± 1.4	61.5 ± 1.5*	4.1 ± 1.1	59.4 ± 1.4	69.1 ± 1.0*	9.7 ± 3.2†
Total Protein ($g \cdot dl^{-1}$)	5.5 ± 0.1	6.2 ± 0.2*	0.7 ± 0.2	5.9 ± 0.1	6.8 ± 0.1*	0.9 ± 0.1
Osmolality ($mosmoles \cdot l^{-1}$)	313 ± 7	324 ± 6*	11 ± 5	327 ± 1	342 ± 3*	15 ± 3
	High Volume Training (HV)					
Hemoglobin ($g \cdot dl^{-1}$)	18.9 ± 0.5	20.6 ± 0.5*	1.7 ± 0.3	19.8 ± 0.5	23.1 ± 0.4*	3.3 ± 0.6†
Packed Cell Volume (%)	57.5 ± 2.5	62.8 ± 2.3*	5.3 ± 1.2	61.7 ± 2.0	70.1 ± 0.8*	8.4 ± 1.5†
Total Protein ($g \cdot dl^{-1}$)	5.7 ± 0.1	6.3 ± 0.2	0.6 ± 0.2	6.1 ± 0.1	6.9 ± 0.1*	0.8 ± 0.2
Osmolality ($mosmoles \cdot l^{-1}$)	315 ± 2	326 ± 7*	11 ± 6	330 ± 3	345 ± 4*	15 ± 4

* Significant ($p<0.05$) difference between pre-sprint and post-sprint means
† Significant ($p<0.05$) difference between Δ pre-training and post-training means

In both training groups, increases ($p<0.05$) in Hgb, PCV, TP, and Osm occurred during the sprint trials before and after training. There were no training group differences before or after training in the pre- or post-sprint Hgb, PCV, TP, or Osm. In both training groups, there was an increase ($p<0.05$) in the pre-sprint to post-sprint change in HgB and PCV at post-training when compared to the pre-training response (Table 2). There were no differences in the pre-sprint to post-sprint change in TP and Osm before versus after training.

Blood lactate responses to the 400-meter trial sprints are presented in Figure 1. Resting blood lactate concentrations were similar for both groups before and after training. Although there was no effect of training level on blood lactate accumulation, pooled data revealed a significant ($p<0.05$) increase in post-training blood lactate accumulation over pre-training values. Post-training blood lactate accumulation was increased ($p<0.05$) by 17% in LV (15.0 ± 1.4 vs 17.6 ± 1.1 $mmol \cdot l^{-1}$) and 24% in HV (14.0 ± 1.4 vs 17.3 ± 1.4 $mmol \cdot l^{-1}$).

There were no differences in resting muscle lactate concentrations between training groups before or after training. Muscle lactate accumulation (Figure 2) increased ($p<0.05$) during the 400-meter sprint trials, but was unaffected by training level (14.5 ± 1.7 vs 14.8 ± 1.6, 13.2 ± 1.3 vs 13.5 ± 1.2 $mmol \cdot kg$ wet wt^{-1} in LV and HV respectively).

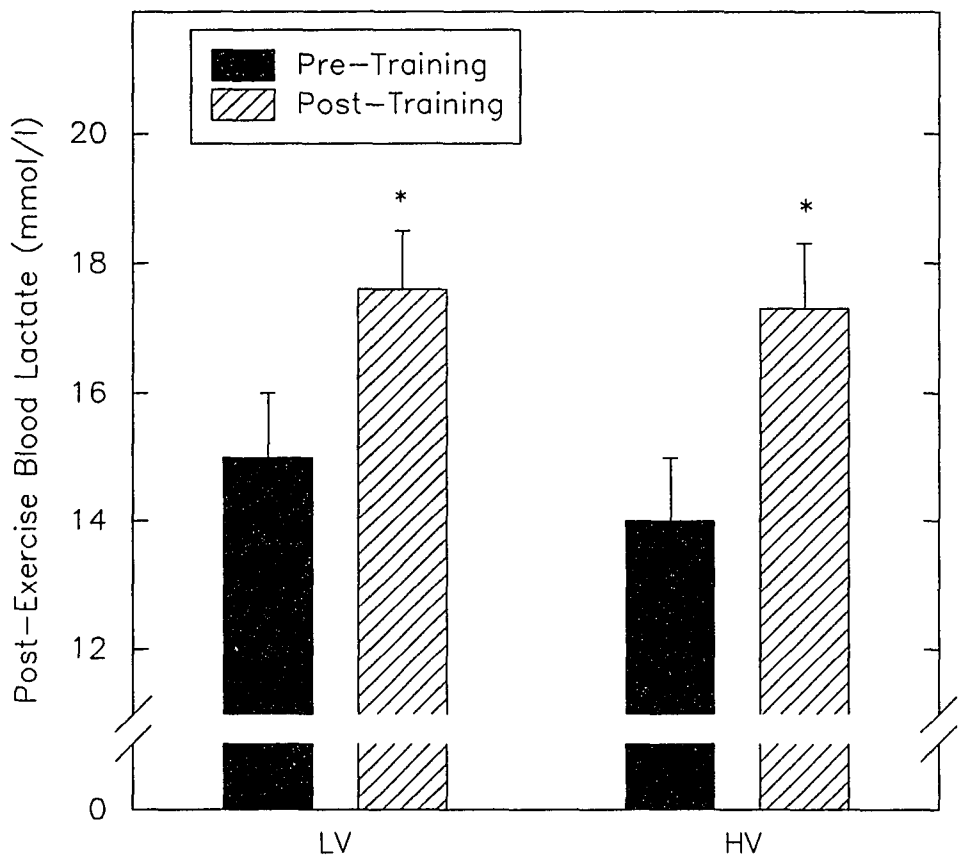

Figure 1 Post-exercise blood lactate accumulation before and after sprint training regimens in greyhounds

*** Significant ($p<0.05$) difference between post-training and pre-training means**

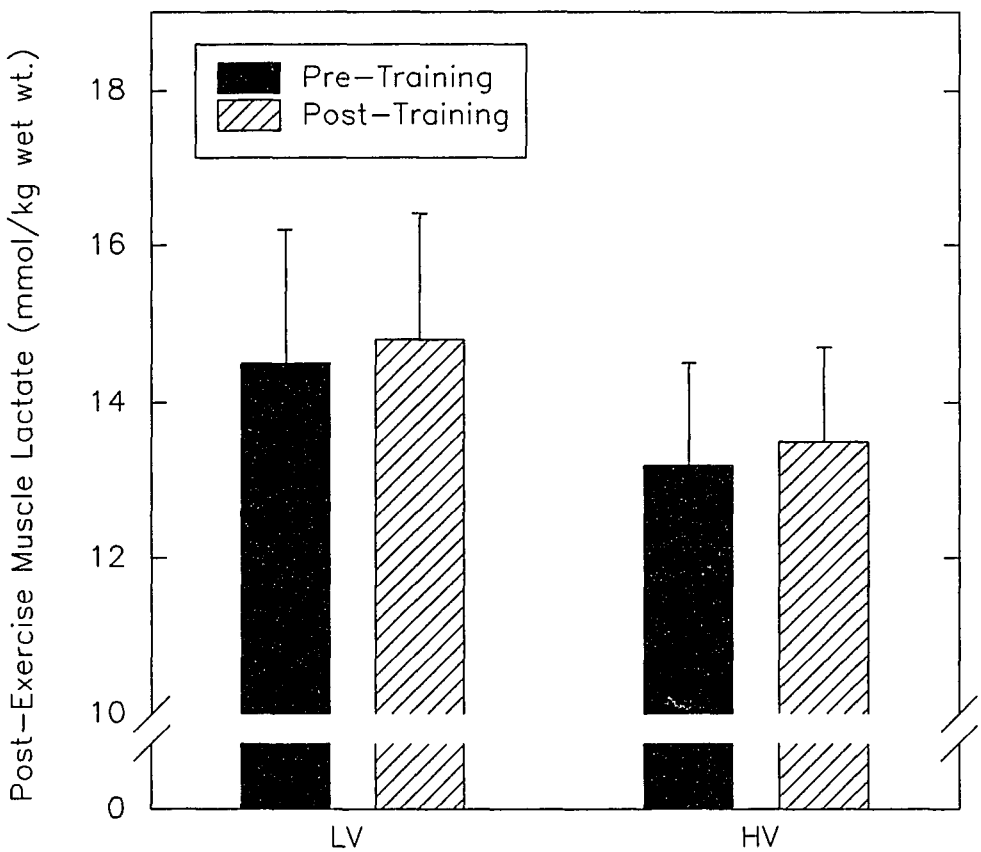

Figure 2 Post-exercise muscle lactate accumulation before and after sprint training regimens in greyhounds

Pre-exercise muscle pH values were similar for both groups before (6.95 ± 0.01 vs 6.96 ± 0.01) and after training (6.96 ± 0.02 vs 6.97 ± 0.01 for LV and HV, respectively). Muscle pH decreased significantly ($p<0.05$) during the 400-meter sprint trials in both training groups prior to and after training. There was no difference between the training groups (6.78 ± 0.03 vs 6.80 ± 0.03, and 6.81 ± 0.02 vs 6.83 ± 0.01, pre and post-training in LV and HV, respectively) in post-sprint muscle pH (Figure 3).

Pre-training muscle buffer capacity was similar in LV and HV (Table 3). Training did not result in a significant increase in muscle buffer capacity for LV or HV, although a trend ($p=0.09$) towards an increased muscle buffer capacity was noted in HV following training (Table 3).

Table 3 Muscle buffer capacity in Slykes before and after sprint training in greyhounds (mmol lactate·kg^{-1}·pH^{-1}).

	Low Volume Group (n=6)	High Volume Group (n=6)
Pre-training	57.1 ± 4.4	58.4 ± 4.6
Post-training	58.3 ± 3.8	66.7 ± 5.8

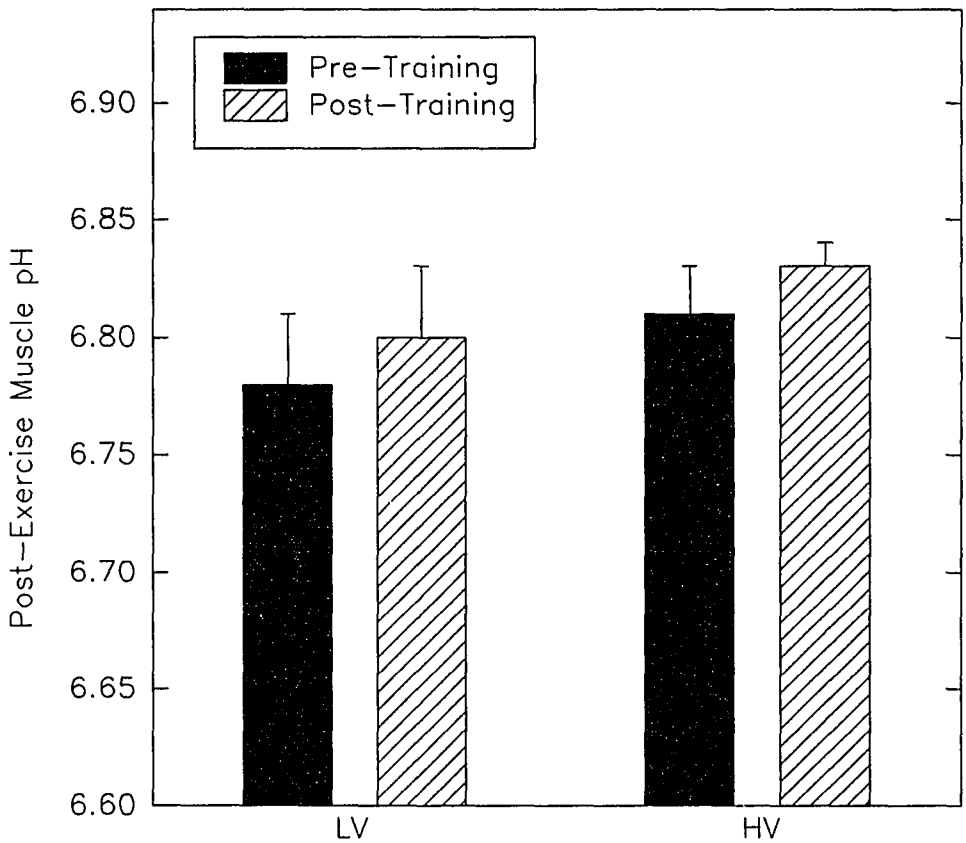

Figure 3 Post-exercise muscle pH before and after sprint training regimans in greyhounds

Phosphofructokinase activity was similar at pre-training in both groups. Comparison of PFK activity post-training revealed a significant ($p<0.05$) increase (14%) in HV, while LV remained unchanged (Table 4).

Table 4 Maximal phosphofructokinase activity before and after sprint training in greyhounds ($mmol \cdot kg^{-1} \cdot min.^{-1}$)

	Low Volume Group (n=6)	High Volume Group (n=6)
Pre-training	45.4 ± 2.2	41.4 ± 1.4
Post-training	43.3 ± 1.3	47.4 ± 1.4*†

* Significant ($p<0.05$) difference between pre-training and post-training means.
† Significant ($p<0.05$) difference between training groups

Pre-exercise muscle glycogen content was similar before and after training in both LV (57.5 ± 2.5 vs 60.8 ± 2.9 mmoles·kg wet wt^{-1}) and HV (62.1 ± 2.9 vs 67.9 ± 3.9 mmoles·kg wet wt^{-1}).

Glycogen utilization, defined as the post-sprint minus pre-sprint glycogen difference, is presented in Figure 4. Muscle glycogen utilization in LV was similar before (17.6 ± 2.7 mmoles·kg wet wt^{-1}) and after training (16.1 ± 3.3 mmoles·kg wet wt^{-1}). In contrast, glycogen utilization was significantly ($p<0.05$) increased after training in HV (25.7 ±

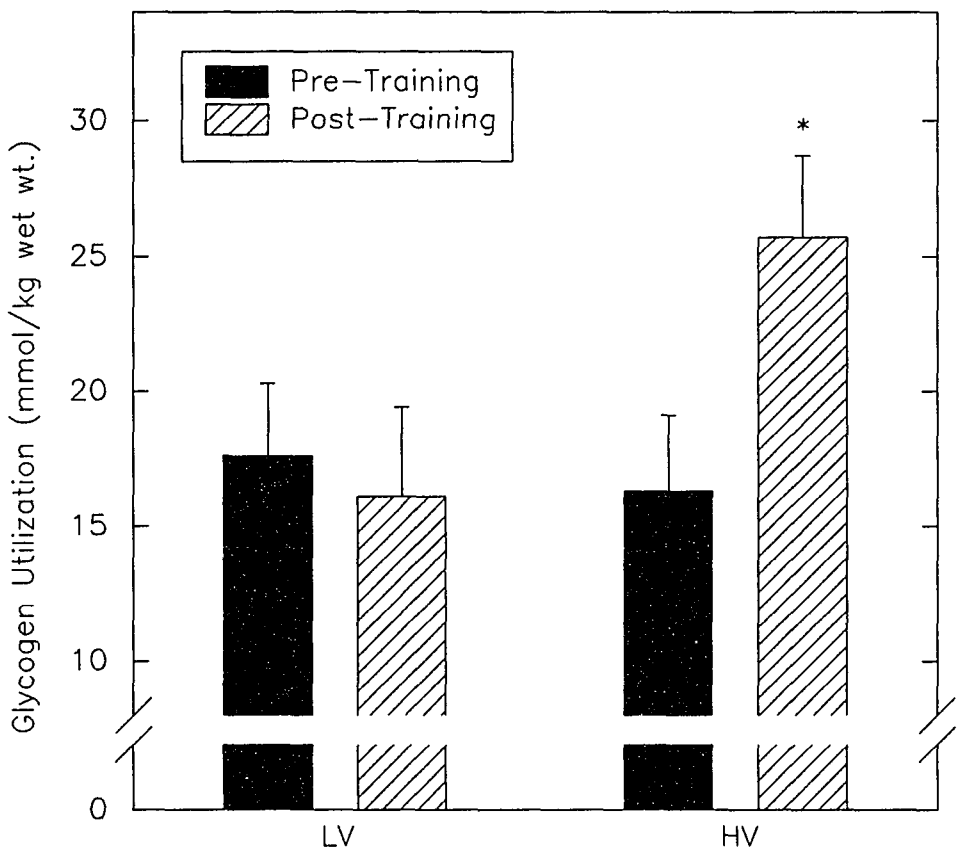

Figure 4 Muscle glycogen utilization before and after sprint training regimens in greyhounds

*** Significant ($p<0.05$) difference between post-training and pre-training means**

3.0 mmoles·kg wet wt^{-1}) when compared to before training (16.3 ± 2.8 mmoles·kg wet wt^{-1}).

DISCUSSION

The major finding of this investigation was a training-induced increase in maximal PFK activity and glycogen utilization in the higher volume (HV) training group. These adaptations suggest an enhanced glycolytic capacity in the greyhounds trained at the higher sprint volume.

Measurement of muscle PFK activity has been used as a general index of glycolytic potential and to document a training effect on anaerobic glycolysis. Significant increases in PFK activity following high intensity training regimens have been reported in human subjects (8,16,25,30) and rats (9). The PFK activities observed in this study are higher than those reported for sprint-trained humans (16,25,30), although considerably lower than the activity of 76 $mmol \cdot min^{-1} \cdot kg$ wet wt^{-1} reported for greyhound muscle by Snow and Harris (33). The lower PFK activities obtained in this investigation may reflect a difference in the training status of the animals sampled or variations in methodology.

A significant exercise-induced reduction in muscle glycogen content supports the reliance of the sprinting greyhound on glycolysis. A 59% reduction in muscle glycogen content has been reported in greyhounds sprinting over an 800 meter distance (7). In contrast, Rose and Bloomberg (26) reported no significant change in muscle glycogen content in greyhounds sprinting over 400 meters, despite significant

increases in muscle and blood lactate accumulation. Sprint training in human subjects has been reported to significantly increase the contribution of anaerobic glycolysis to total ATP resynthesis during high intensity exercise tasks (21). In the current investigation, the increased rate of glycogen utilization following training in the HV group supports an increase in glycolytic rate.

The marked decline in muscle glycogen was accompanied by a significant rise in blood and muscle lactate accumulation and a decline in muscle pH during all the sprint trials. An increase in blood and muscle lactate accumulation is typically associated with an increased glycolytic rate. The post-exercise blood and muscle lactate accumulation in this investigation was less than that reported for greyhounds sprinting over a similar distance (26), although these variations could reflect differences in training status of the animals and sampling protocols.

Because blood lactate accumulation is expressed relative to vascular volume, a training-induced increase in blood lactate accumulation will be influenced by training-induced changes in vascular volume. In greyhounds, sprint exercise is associated with transient increases in Hgb and PCV (7,15,26) which may be the result of a sympathetic-induced splenic contracture (7) and/or plasma volume shifts resulting in hemoconcentration. In both training groups, significant

increases in Hgb, PCV, TP, and Osm were observed during all the sprint trials, suggesting that an acute, exercise-induced hemoconcentration was present. Following training, both groups exhibited a significant and similar increase in the pre- to post-exercise change in Hgb and PCV, supporting the possibility that a greater hemoconcentration occurred following training, thus contributing to the observed training-induced increase in blood lactate accumulation. Training did not, however, affect the pre- to post-sprint change in either TP or Osm, suggesting that exercise-induced changes in vascular volume were similar before and after training.

An increase in post-exercise blood (13,16,18,29,30) and muscle (21,30) lactate accumulation has been documented in humans after high intensity training. In the current investigation, post-exercise muscle lactate accumulation was not affected by training at either level, however, post-exercise blood lactate accumulation was increased following both training regimens. The increased blood lactate accumulation following training, coupled with no further increase in muscle lactate accumulation may indicate an increase in the transmembrane efflux of lactate (27). A training-induced increase in metabolite efflux from the muscle could explain the lack of an increase in muscle lactate accumulation despite the increased muscle glycogen

utilization. Recent work (28) with preparations of rat sarcolemmal vesicles, however, have not demonstrated a training effect on lactate transport rate or capacity. In addition, muscle and blood lactate accumulation represent the net effect of production, clearance, and oxidation by the muscle itself (14). Therefore any conclusions concerning specific adaptations based on lactate accumulation alone, are tenuous. The LV training group did not exhibit any change in glycogen utilization, muscle lactate accumulation, or PFK activity, making it difficult to explain the increase in blood lactate accumulation in this group.

An increased glycogen utilization without an increase in muscle lactate accumulation following training may suggest some alternative fate for the additional pyruvate formed. A high alanine aminotransferase activity (two to three times the activity in human muscle) suggests that greyhound muscle possesses a high capacity to transaminate pyruvate to form alanine, thus providing an alternative end product for glycolysis (33). An increased rate of alanine formation and a decrease in lactate and H^+ ion accumulation, would be advantageous to the sprinting animal as intracellular acidosis could be reduced, possibly delaying the onset or rate of fatigue. Blood and/or muscle alanine concentrations were not determined in this investigation.

Canine skeletal muscle, including greyhounds, appears to

consist primarily of type IIA, fast twitch fibers with high oxidative and glycolytic capacity (1) and no classical type IIB fibers (4,31). Increased disposal of pyruvate via conversion to acetyl CoA and subsequent oxidation may have occurred following training; however, this type of metabolic adaptation is normally associated with endurance rather than sprint training. Significant increases in oxidative enzyme activities have been documented following high intensity training regimens (12,16,25). It is also possible that muscle lactate disposal and accumulation were influenced by training induced changes in the rate of muscle lactate oxidation.

The decline in muscle pH associated with the 400-meter sprint task was not affected by either training regimen. High intensity training in humans has also been reported to have no effect on post-exercise muscle pH (21,30). Muscle buffer capacity was assessed by determining the ratio of the change in muscle lactate to change in muscle pH. The range of pre-training and post-training muscle buffer capacity values (57 to 67 Slykes) in the current study were slightly higher than values of 45 to 55 Slykes reported for greyhounds by other investigators (7,11). These differences may be related to methodology, muscle groups studied, or timing of post-sprint biopsy sample collections. Training did not significantly alter buffer capacity, although as mentioned previously, a trend towards significance ($p=0.09$) was noted in HV. Human

muscle buffer capacity has been reported to both increase (30) and remain unchanged (21) following high intensity training. A failure to induce an adaptation in muscle buffering capacity despite significant increases in glycolytic rate may lend support to an increased rate of transamination of pyruvate to alanine. It is also possible that the exercise stimulus was insufficient to induce adaptations in muscle buffer capacity.

The current investigation provides evidence that a formal sprint training regimen induces skeletal muscle adaptations which may benefit the sprinting greyhound. These metabolic adaptations occurred, however, without a concomitant measureable change in performance (i.e., a significant reduction in 400-meter sprint time). Other investigators (13,16) have reported similar discrepancies between physiological adaptations and performance markers and suggested that the time course of sprint-induced cellular adaptations may precede measurable changes in performance. It is also possible that the training volume used in this investigation was not sufficient to induce measureable changes in performance. The training volume required to induce the metabolic adaptations observed in this study, however, was less than that typically used in investigations examining sprint-induced adaptations in humans (16,25,30).

In summary, greyhounds exposed to a six-week sprint training regimen (HV) exhibited significant increases in

muscle glycogen utilization, maximal PFK activity, and post-exercise blood lactate accumulation. These results support a training-induced increase in anaerobic capacity. Post-training 400-meter sprint time and an increase in calculated muscle buffer capacity approached statistical significance in the HV training group. With the exception of an increased post-exercise blood lactate accumulation, the LV training regimen had no effect on the muscle lactate accumulation, change in muscle pH, or muscle glycogen utilization associated with a 400-meter sprint task. In addition, the LV training regimen had no effect on maximal PFK activity or calculated muscle buffer capacity. It appears that greyhounds exhibit similar metabolic adaptations associated with sprint training in other species, and, although selectively bred for sprint racing performance, these athletes may benefit from the incorporation of formal sprint training regimens into the overall training strategy.

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PAPER 2. EFFECTS OF SODIUM BICARBONATE LOADING ON ACID-BASE BALANCE, LACTATE ACCUMULATION, AND PERFORMANCE IN RACING GREYHOUNDS

Effects of $NaHCO_3$ loading on acid-base balance, lactate accumulation, and performance in racing greyhounds.

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ABSTRACT

The purpose of this investigation was to examine the effects of $NaHCO_3$ administration (300 $mg \cdot kg^{-1}$, gastric bolus) on muscle and blood lactate accumulation, acid-base balance, and performance in greyhounds sprinting over a 603.5 m distance. Ten greyhounds performed a $NaHCO_3$ and control trial in a crossover design. Arterial blood samples, obtained at pre-dose, pre-sprint, and post-sprint intervals, were analyzed for lactate, pH, blood gases, HCO_3^- (bicarbonate), and base excess (BE). Muscle biopsies, obtained at pre-dose, pre-sprint, and post-sprint were analyzed for lactate and pH.

Pre-sprint differences ($p<0.05$) were found between control and $NaHCO_3$ for blood pH (7.42 ± 0.01 vs 7.47 ± 0.01), HCO_3^- (23.5 ± 0.3 vs 28.4 ± 0.4 $meq \cdot l^{-1}$), BE (0.2 ± 0.3 vs 5.0 ± 0.3 $meq \cdot l^{-1}$) and $PaCO_2$ values (37.5 ± 0.6 vs 40.4 ± 0.8 mm Hg).

Peak post-exercise (2 min) blood lactate accumulation was increased ($p<0.05$) following $NaHCO_3$ ingestion (20.4 ± 1.6 $mmol \cdot l^{-1}$) when compared to control (16.9 ± 1.3 $mmol \cdot l^{-1}$), whereas peak post-exercise (30 s) muscle lactate accumulation was similar in both trials (17.7 ± 1.8 vs 16.2 ± 1.6 $mmol \cdot kg^{-1}$ wet wt. for $NaHCO_3$ and control trials, respectively). Peak post-exercise (2 min) blood hydrogen ion (H^+) accumulation was similar in both trials (53.6 ± 2.4 vs 56.4 ± 2.0 $nmol \cdot l^{-1}$ for $NaHCO_3$ and control trials, respectively). The $NaHCO_3$ trial

produced a greater ($p<0.05$) peak reduction in arterial BE (-18.5 ± 1.4 vs -14.1 ± 0.8 meq·l^{-1}) and HCO_3^- (-17.4 ± 1.2 vs -12.8 ± 0.7 meq·l^{-1}) from pre-sprint to post-exercise (2 min sample). Peak post-exercise (30 s) muscle H^+ accumulation was higher ($p<0.05$) in the $NaHCO_3$ trial (158.8 ± 8.8 nmol·l^{-1}) compared to that of control (137.0 ± 5.3 nmol·l^{-1}). Muscle buffer capacity was not different between trials as calculated by the Δmuscle lactate/Δmuscle pH. There was a reduced ($p<0.05$) muscle H^+ recovery half time (7.2 ± 1.6 vs 11.3 ± 1.6 min) and time to reach pre-dose values (22.2 ± 2.4 vs 32.9 ± 4.0 min) in the $NaHCO_3$ trial compared to control trial. No trial differences were found for blood H^+ recovery or lactate (muscle and blood) recovery curves. Performance times were not different between trials, although there was a trend ($r=.55$, $p=0.09$) for animals with lower control trial muscle buffer capacity to exhibit greater improvements in sprint performance after $NaHCO_3$ ingestion.

In this investigation, a 300 mg·kg^{-1} dose of $NaHCO_3$ increased post-exercise blood lactate accumulation, but did not reduce the magnitude of the muscle or blood acid-base disturbance associated with a 603.5 m sprint task or significantly impact on performance times.

Index terms: sodium bicarbonate; acid-base balance; dog

INTRODUCTION

The ability to generate and sustain high locomotory speeds requires a metabolic strategy which will support a high muscle power output. The inability to generate or maintain a specified or desired power output is commonly used as a definition of fatigue.

High intensity, short duration exercise bouts are associated with high glycolytic rates and rapid accumulation of muscle and blood lactate. A concomitant rise in intracellular H^+ ion concentration may contribute to muscular fatigue via several proposed mechanisms including inhibition of rate limiting metabolic enzymes such as phophofructokinase (4,41) and phosphorylase (4,36), inhibition of calcium ion release from sarcoplasmic reticulum (7,8), reduced binding of calcium to troponin (7,8), and alterations in neural impulse propagation (5,38).

The potential role of pH disturbance in muscle fatigue has led to investigations in which attempts have been made to acutely alter buffering capacity before an exercise bout, primarily by ingestion of solutions containing sodium bicarbonate ($NaHCO_3$). Although the mechanism(s) by which $NaHCO_3$ may alter acid-base status is debated, it is generally accepted that minimizing the acid-base disturbance associated with exercise metabolism is beneficial. Administration of $NaHCO_3$ results in an acute elevation of blood bicarbonate

concentration (1,2,14,17,19,20,24,35, 43) and pH (1,11,16,17,19,20,24,35,40,43), thus augmenting the extracellular (blood) buffer reserve. Several investigations lend support to the hypothesis that increasing extracellular buffer reserve may alter intracellular events by affecting lactate or H^+ ion efflux rates (13,25,26).

Transmembrane co-transport of lactate and H^+ ions is facilitated by the presence of a family of H^+-linked lactate-monocarboxylate carriers which exist in the plasma membranes of different cell types (32). The transport process appears to be stimulated by a proton gradient across the sarcolemmal membrane, resulting in the acceleration of lactate and H^+ ion efflux from muscle as intracellular pH decreases during high intensity exercise (34). If the increase in extracellular or intracellular H^+ ion concentration plays an important role in the extent or rate of muscular fatigue, altering extracellular buffer reserve may influence the intracellular events contributing to fatigue.

The effect of $NaHCO_3$ ingestion on exercise performance has been equivocal. Protocols have varied in terms of total dosage, route of administration, latent time from dosing to exercise, animal species, performance criteria, and exercise parameters (intensity, duration, total work, single versus repeated bouts), making direct comparison of results difficult. Humans performance in a variety of exercise tasks

has been reported to be enhanced (1,2,10,11,16,21,24,40,43) or unchanged (14,17,19,20,28,30) by $NaHCO_3$ loading regimens. Exercise performance has been reported to improve in race horses following $NaHCO_3$ loading (18,22).

Greyhounds participate in a variety of competitive events which range in duration from 15 to 60 sec and attain running speeds in excess of 18 $m \cdot sec^{-1}$ (~65 $km \cdot h^{-1}$). Significant increases in muscle (6,33) and blood lactate accumulation (6,15,29,31,33), and marked acid-base disturbances (6,15) have been reported in greyhounds running race distances of 400 to 800 meters. The magnitude of the lactate accumulation and acid-base disturbance in greyhounds is much greater than in their human or equine counterparts exercising over similar distances or duration (37). In addition, some greyhounds exhibit exertional rhabdomyolysis, a syndrome associated with exercise-induced muscle trauma. It has been suggested that acidosis may contribute to this syndrome and that a reduction in the acid-base disturbance associated with exercise may prove beneficial (9).

The responses to sprint exercise in the greyhound may potentially be influenced by acute alterations in blood buffer reserve. Therefore, the purpose of this investigation was to examine the effects of $NaHCO_3$ loading on muscle and blood lactate accumulation, acid-base balance, and exercise performance in racing greyhounds.

MATERIALS AND METHODS

All protocols and procedures used in this investigation were reviewed and approved by the Iowa State University Committee on Animal Care. Four weeks before the study began, all animals were placed on an standard commercial dry food diet[1] and housed in similar inside runs to minimize the influence of previous diet, exercise patterns, or trainer handling.

Ten (four male, six female) adult greyhounds (mean age 31 ± 2 months), all with previous track experience, completed a 603.5 meter sprint after both a control and $NaHCO_3$ solution. Animals were randomly assigned in a crossover design with seven to ten days between the two trials. Trials were conducted in the morning (8:00 a.m. - 12:00 p.m.) with animals fasted overnight (feed removed by 6:00 p.m. the previous night). During the 4 weeks prior to the experimental trials, animals performed five practice runs to familiarize them with the track and lure device.

In a preliminary pilot study of five animals receiving a 300 $mg \cdot kg^{-1}$ dose of $NaHCO_3$ in a single gastric bolus, resting arterial blood bicarbonate concentrations, pH, and base excess were significantly ($p<0.05$) elevated above pre-dose levels beginning at 30 minutes post-dose and persisting throughout a

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[1] Science Diet Maintenance®, Hills Division of Colgate Palmolive Corp., Topeka, KA

four-hour sampling period. In addition, no overt gastrointestinal or muscular disturbances were noted during these preliminary dosing trials.

In the present investigation, the $NaHCO_3$ solution consisted of a 300 mg·kg body weight^{-1} dose of $NaHCO_3$ dissolved in 200 ml of warm water and administered via a gastric tube in a single bolus. The control solution consisted of 200 ml of warm tap water. Administration of solutions occurred 70 minutes prior to the sprint trials. Animals sprinted over a 603.5 meter distance after a mechanical lure on a 300 meter mile oval track.

Blood and muscle sampling intervals are illustrated in Figure 1. With the animal in left lateral recumbency, a needle drawn pre-dose (PD) arterial blood sample was obtained from the left femoral artery. After injection of a local anesthetic[2] under the skin, a skin incision was made over the midsection of the right biceps femoris muscle. A PD sample of muscle tissue (75 to 100 mg) was surgically removed and immediately frozen (within two to three seconds of excision) and stored in liquid nitrogen until analysis. The incision site was sutured and the appropriate trial solution was administered. A second incision site was made over the midsection of the left biceps femoris for post-exercise

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[2] 2% Lidocaine®, Elkins-Sinn Inc., Cherry Hill, NJ

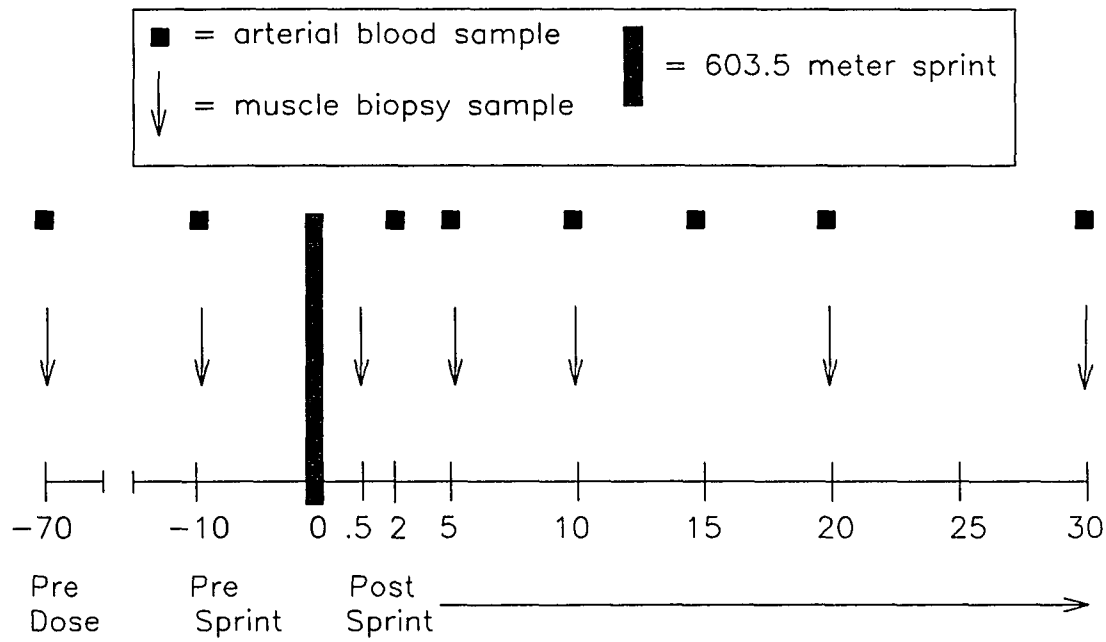

Figure 1 Sampling intervals for muscle biopsy and arterial blood samples

muscle biopsies and loosely sutured to facilitate rapid post-exercise sampling. The animal was transported to the track site (~1 km) and remained in a pen until ten minutes before (60 minutes post-dose) the scheduled sprint time.

At ten minutes prior to the scheduled sprint time, a pre-sprint (PS) arterial blood sample and muscle biopsy were obtained from the left femoral artery and right biceps femoris muscle, respectively. The animal was exercised at 70 minutes post-dose. This ten-minute differential between pre-sprint sampling and the sprint trial was necessary to facilitate collection of pre-sprint samples and minimize the hematoma at the arterial puncture site.

Animals sprinted over a 603.5 meter distance and were caught within 10 to 15 seconds for post-exercise sampling. With the animal in right lateral recumbency, post-exercise muscle biopsies were obtained at 30 seconds, 5, 10, 20, and 30 minutes post-sprint. Arterial blood samples were obtained at 2, 5, 10, 15, 20, and 30 minutes post-sprint via a 20 gauge, two inch teflon catheter[3] which was placed in the right femoral artery. The animal remained in left lateral recumbency throughout the recovery sampling period after which the catheter was removed and the incision sites sutured.

A three-ml blood sample was anaerobically collected in

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[3] Angiocath, radiopaque teflon catheter, Deseret Medical Inc., Benton Dickinson and Company, Sandy, UT

heparinized glass syringes, and the tube was capped, and placed on ice until analysis of arterial PO_2, PCO_2, and pH. All blood gas measurements were determined within 60 minutes of collection on a Instrumentation Laboratories Model 813 pH/blood gas analyzer[4]. Blood gas analysis was determined in an autocalibration mode in which the original calibration settings are automatically reset between each successive experimental sample. Blood bicarbonate (HCO_3^-) and base excess (BE) were calculated from the PCO_2 and pH measurements.

One ml of whole blood was deproteinized in two ml of cold 8% perchloric acid, centrifuged (4000xg for 10 minutes) and the supernatant stored at -5°C until determination of lactic acid concentration by spectrophotometric[5] analysis (23) at the conclusion of the study. Hemoglobin (Hgb) was determined by the cyanmethemoglobin method (12), and packed cell volume (PCV) was determined by the microhematocrit method. Approximately 5-10 ml of whole blood was placed on ice and serum was harvested for analysis of standard blood chemistries[6] at the PD, PS, 2, and 30 minute sampling intervals.

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[4] Model 813 pH/blood gas analyzer, Instrumentation Laboratories Inc., Lexington, MA

[5] Spectronic® 70, Bausch and Lomb, Analytical Systems Division

[6] Roche Biomedical Laboratories, Kansas City, MO

The serum strong ion difference (SID) was calculated from the following equation: ([sodium] + [potassium] + [diffusible calcium]) - ([chloride] + [lactate]) (31). The diffusible calcium concentration was calculated from total serum calcium and protein using the relationship as described by Zeisler (44).

All visible blood and connective tissue were removed, and muscle samples were weighed on a microbalance[7] prior to analysis. Intracellular muscle pH was estimated by using the homogenate technique as described by Costill, et. al. (3). The pH of the homogenate was measured at 38°C with a Radiometer BMS 3MK2 Blood Micro System coupled to a PHM-73 pH/blood gas monitor[8] which was calibrated every fifth sample using a two-point (6.840 and 7.383) calibration procedure. Muscle lactate concentration was determined by fluorometric[9] enzymatic analysis (23). All assay samples and standards were analyzed in duplicate. All samples (both trials) from a specific subject were analyzed within the same assay batch at the conclusion of the study to decrease interassay variance.

The effects of the treatments were tested by using a two-factor ANOVA (treatment X sample interval) with repeated

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[7] Mettler AE 163 microbalance, Mettler Instrument Corp., Highstown, N.J.

[8] Radiometer, Copenhagen, Denmark

[9] Ratio 2 Fluorometer, Farrand Optical Co., Valhalla, N.Y.

measures on sampling intervals. The effects of the $NaHCO_3$ ingestion were assessed by analyzing the PD and PS samples. The effects of the exercise task were assessed by analysis of the PS and the immediate post-exercise sampling interval. Recovery was assessed by analysis of the recovery time intervals. A Student-Newman-Keuls multiple range test procedure was used to identify significant mean differences.

Post-exercise H^+ ion and lactate accumulation were characterized in both muscle and blood by non-linear, least squares regression analysis to the function [H^+ or lactate] = [pre-dose] + ([maximal Δ in H^+ or lactate] $exp^{(-bt)}$), where b = rate constant and t = time. Correlation coefficients were used to describe the relationship between actual data and the monoexpotential curve fit. The restoration of lactate and H^+ ion concentrations to pre-dose levels were characterized by calculation of a $t^{1/2}$ time and a t^{PD} time. The $t^{1/2}$ time was defined as the time required to return the concentration to one half of the difference between the peak post-exercise and pre-dose concentrations. The t^{PD} time was defined as the time required to return the concentration to pre-dose levels. *In vivo* muscle buffer capacity was calculated from the pre-sprint and 30 second post-sprint muscle pH and lactate values. The ratio of the change (pre to post-sprint) in muscle lactate to muscle pH, expressed in units of mmoles lactate$\cdot kg^{-1}\cdot pH^{-1}$ or "Slykes" was used to estimate muscle buffer capacity (39). A

paired t-test procedure was used to compare $t^{1/2}$, t^{PD}, muscle buffer capacity, and performance sprint times between trials.

All statistical analysis involving pH measurements were performed with H^+ ion concentrations. The level of probability was set at $p<0.05$ to reject the null hypothesis. All results are expressed as mean ± standard error of mean.

RESULTS

Administration of the $NaHCO_3$ solution resulted in significant ($p<0.05$) increases in arterial blood pH, BE, and HCO_3^- (Table 1). Pre-sprint $PaCO_2$ exhibited a tendency ($p=0.09$) to be elevated in the $NaHCO_3$ trial when compared to control. There were small but significant ($p<0.05$) increases in muscle pH, Hgb, and PCV from PD to PS in both trials. Resting muscle lactate, blood lactate accumulation, and PaO_2 were not affected by the $NaHCO_3$ loading regimen.

Table 2 illustrates the changes in arterial serum ions and SID. A significant ($p<0.05$) increase in PD serum sodium (Na^+) was observed in the $NaHCO_3$ trial with respect to the pre-sprint value for the control trial. In both trials, serum Na^+ was significantly ($p<0.05$) elevated at the two-minute post-exercise sample and had decreased by 30 minutes post-exercise, although the treatment difference remained. Serum potassium (K^+) significantly ($p<0.05$) decreased from PD to PS, tended to be lower ($p=0.08$) than control from PS to two minutes post-exercise, with no difference over the two to 30 minutes recovery interval. No treatment differences in serum chloride (Cl^-) or calculated diffusible calcium (Ca^{++}) occurred following the administration of solutions, although Cl^- significantly decreased from PD to PS and post-exercise Ca^{++} levels were significantly elevated with respect to the PS level. The SID was significantly ($p<0.05$) elevated at the PS

Table 1 Select arterial blood and muscle parameters before (pre-dose) and 60 minutes after (pre-sprint) a 300 $mg \cdot kg^{-1}$ gastric bolus of $NaHCO_3$ and a control solution.

	Control		$NaHCO_3$	
	Pre-Dose	Pre-Sprint	Pre-Dose	Pre-Sprint
Blood pH	7.42 ± 0.01	7.42 ± 0.01	7.41 ± 0.01	7.47 ± 0.01*†
Base Excess ($meq \cdot l^{-1}$)	0.8 ± 0.3	0.2 ± 0.3	0.6 ± 0.3	5.00 ± 0.3*†
HCO_3^- ($meq \cdot l^{-1}$)	24.5 ± 0.3	23.5 ± 0.3	24.2 ± 0.3	28.4 ± 0.4*†
PO_2 (mmHg)	94.4 ± 1.9	92.9 ± 2.0	97.7 ± 2.2	92.7 ± 2.0
PCO_2 (mmHg)	39.4 ± 0.6	37.5 ± 0.6	39.3 ± 0.5	40.4 ± 0.8
Blood Lactate ($mmol \cdot l^{-1}$)	1.9 ± 0.0	2.1 ± 0.2	1.8 ± 0.2	1.9 ± 0.2
Muscle pH	7.08 ± 0.02	7.11 ± 0.01*	7.06 ± 0.01	7.13 ± 0.02*
Muscle Lactate ($mmol \cdot l^{-1}$)	3.0 ± 0.3	3.2 ± 0.3	2.9 ± 0.3	3.2 ± 0.4
Hemoglobin ($g \cdot dl^{-1}$)	18.9 ± 0.7	20.6 ± 0.6*	18.8 ± 0.4	20.5 ± 0.8*
Packed Cell Volume (%)	53.6 ± 1.6	56.6 ± 1.5*	52.6 ± 1.2	54.9 ± 1.8*

* = $p<0.05$ from pre-dose to pre-sprint
† = $p<0.05$ from $NaHCO_3$ to control trial

Table 2 Select arterial serum ions and strong ion difference (SID) at pre-dose (PD), pre-sprint (PS), two minutes post-exercise, and 30 minutes post-exercise following a 300 $mg \cdot kg^{-1}$ gastric bolus of $NaHCO_3$ (Alk) and control solution.

		PD	PS	2	30
Na^+ ($meq \cdot l^{-1}$)	Con	153.1 ± 0.6	153.7 ± 0.5	160.2 ± 1.1*	153.6 ± 2.2*
	Alk	153.7 ± 1.2	157.6 ± 0.8†*	164.7 ± 3.5*†	156.8 ± 1.0†*
K^+ ($meq \cdot l^{-1}$)	Con	4.8 ± 0.1	4.7 ± 0.1	4.5 ± 0.1*	4.5 ± 0.1
	Alk	4.8 ± 0.1	4.5 ± 0.1*	4.4 ± 0.1	4.3 ± 0.1
Ca^{++} ($meq \cdot l^{-1}$)[a]	Con	2.2 ± 0.1	2.2 ± 0.1	2.5 ± 0.0*	2.5 ± 0.0
	Alk	2.3 ± 0.1	2.3 ± 0.0	2.4 ± 0.0*	2.5 ± 0.1
Cl^- ($meq \cdot l^{-1}$)	Con	118.0 ± 0.9	115.9 ± 0.7*	118.2 ± 1.4	116.9 ± 0.9
	Alk	118.9 ± 0.7	116.1 ± 0.8*	117.2 ± 0.6	116.2 ± 1.0
SID ($meq \cdot l^{-1}$)	Con	40.1 ± 1.0	42.7 ± 1.2	33.4 ± 1.9*	40.9 ± 0.6*
	Alk	40.1 ± 1.1	45.5 ± 1.0†*	33.9 ± 1.1*	43.5 ± 0.9*

a = Ca^{++} (diffusible calcium)
* = $p<0.05$ from when compared to previous within treatment sampling interval
† = $p<0.05$ from $NaHCO_3$ to control trial

sample with respect to PD, decreased during exercise (PS to 2 minute), and increased during the recovery interval (two to 30 minutes) with the only treatment difference occurring from the PS to two-minute post-exercise interval.

Significant ($p<0.05$) increases in both muscle and blood lactate accumulation occurred in both trials over the sprint task. Post-exercise blood and muscle lactate accumulation are presented in Figure 2. The highest measured blood lactate accumulation, which occurred two minutes after exercise in both trials, was significantly ($p<0.05$) higher in the $NaHCO_3$ trial (20.4 ± 1.6 mmol$\cdot l^{-1}$) vs control (16.9 ± 1.3 mmol$\cdot l^{-1}$). The highest muscle lactate accumulation, which occurred 30 seconds after exercise, was similar in both trials (17.7 ± 1.8 vs 16.2 ± 1.6 mmol$\cdot l^{-1}$ for $NaHCO_3$ and control, respectively).

Post-exercise blood and muscle lactate accumulation was used to characterize the time course of recovery to pre-exercise conditions (Figures 3 and 4, respectively). The $t^{1/2}$ times for blood lactate were similar for control (14.5 ± 0.9 min) when compared to the $NaHCO_3$ trial (13.9 ± 1.8 min). There were no differences in blood lactate t^{PD} times for control (31.7 ± 2.5 min) when compared to the $NaHCO_3$ trial (35.4 ± 4.8 min). Post-exercise muscle lactate accumulation $t^{1/2}$ times (10.8 ± 1.6 vs 14.5 ± 1.7 min for control vs $NaHCO_3$, respectively) and t^{PD} times (38.4 ± 5.3 vs 40.3 ± 4.4 min for control vs $NaHCO_3$, respectively) were similar.

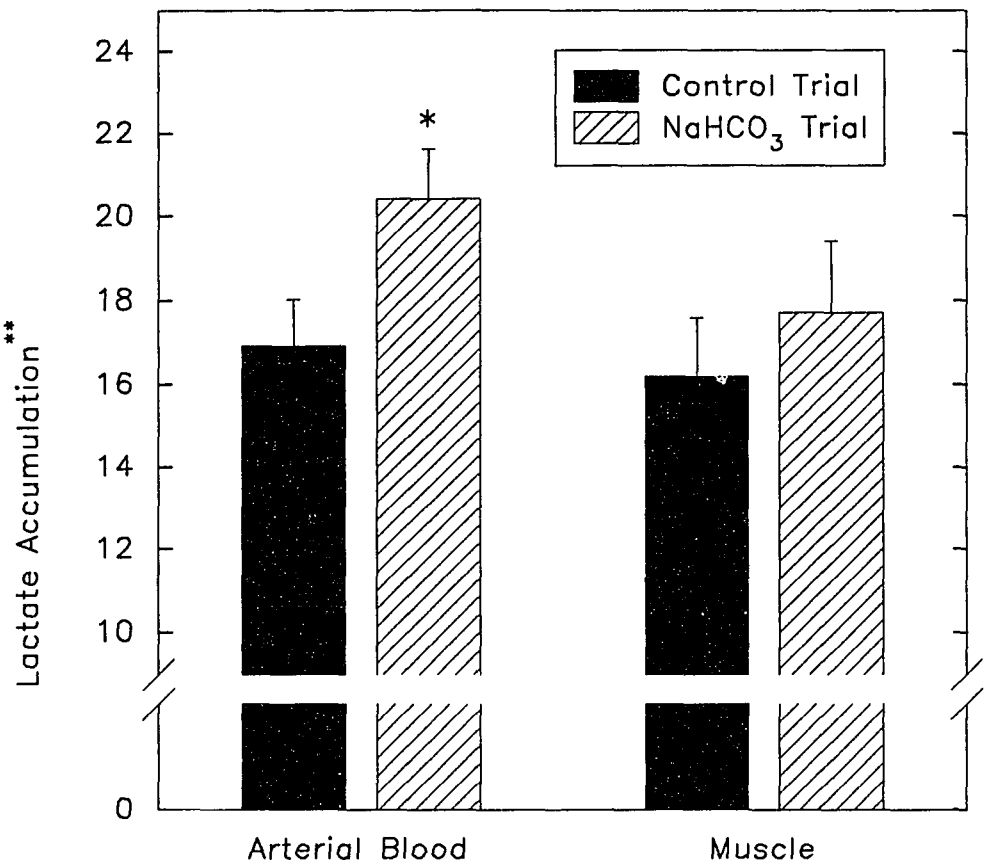

Figure 2 Two-minute post-exercise arterial blood and 30-second post-exercise muscle lactate accumulation

** Lactate concentration expressed as mmol·l^{-1} in blood and mmol·kg wet wt^{-1} for muscle

* Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial

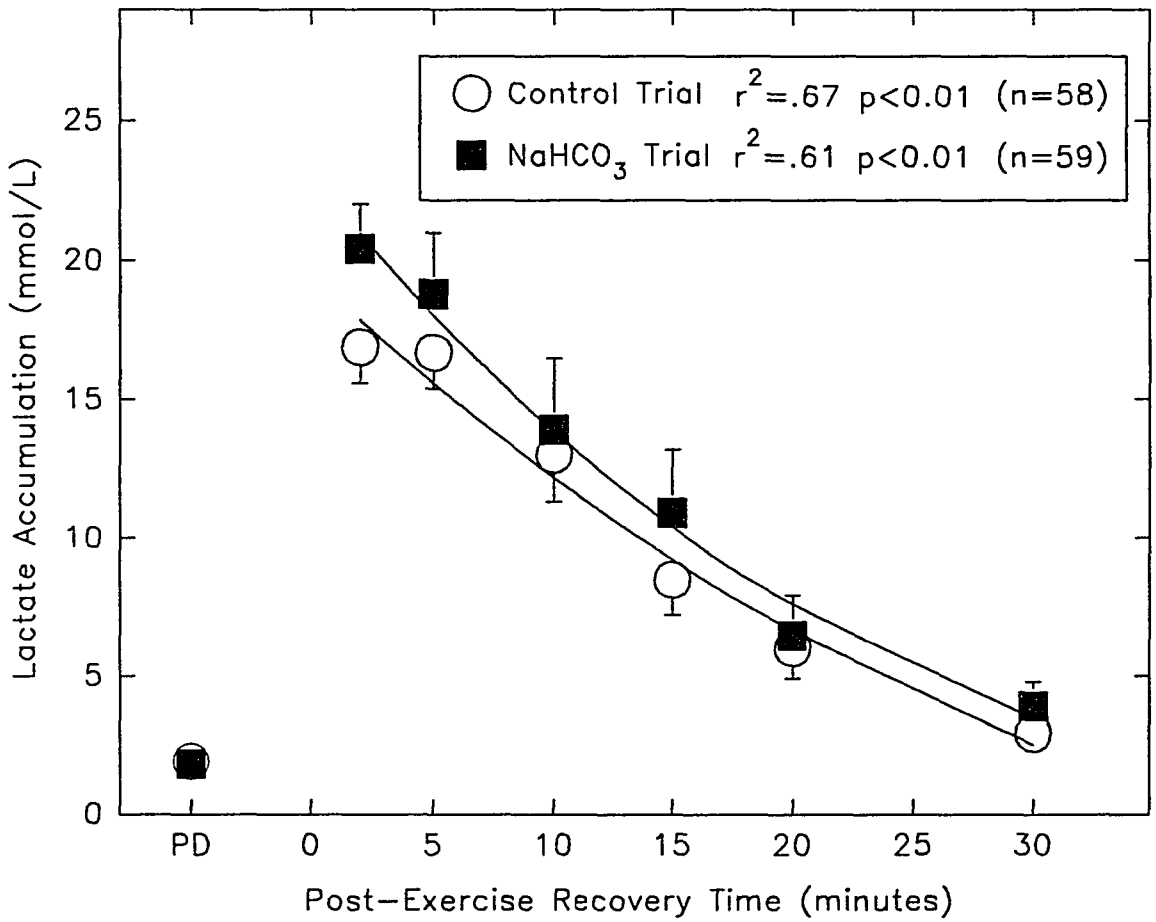

Figure 3 Post-exercise arterial blood lactate concentration. Data points represent means of 10 animals. Correlation coefficients shown for monoexpotential curve fit.

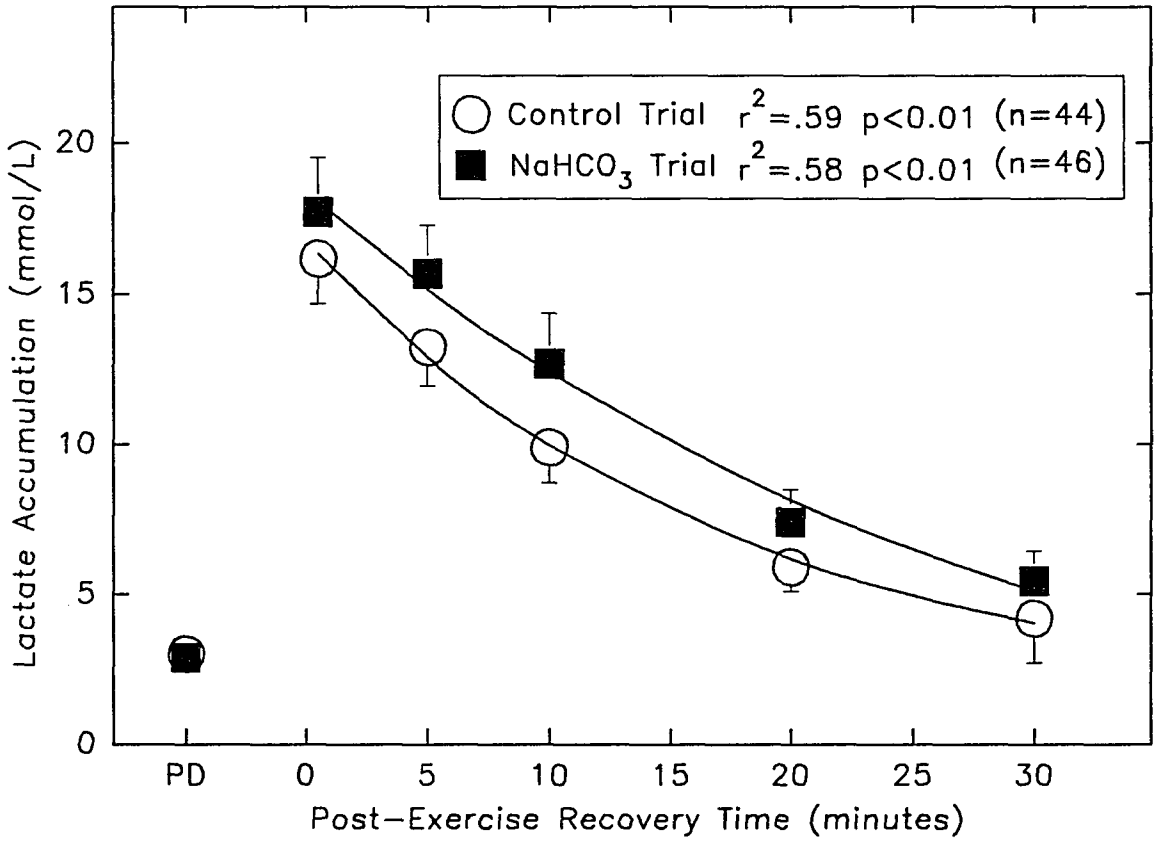

Figure 4 Post-exercise muscle lactate concentration. Data points represent means of 10 animals. Correlation coefficients shown for monoexpotential curve fit.

In both trials, blood and muscle H^+ ion accumulation increased significantly ($p<0.05$) during the exercise task. The two minute post-exercise blood H^+ ion accumulation (Figure 5), was similar (53.6 ± 2.4 vs 56.4 ± 2.0 $nmol \cdot l^{-1}$) for $NaHCO_3$ and control, respectively. In addition, there was a similar change in blood H^+ ion concentration from PS to two min post-exercise in both trials (20.0 ± 1.4 vs 18.7 ± 1.5 $nmol \cdot l^{-1}$ for $NaHCO_3$ and control, respectively). The 30 sec post-exercise muscle H^+ ion concentration (Figure 5) was significantly ($p<0.05$) higher in the $NaHCO_3$ trial (158.8 ± 8.8 $nmol \cdot l^{-1}$) when compared to control (137.0 ± 5.3 $nmol \cdot l^{-1}$). Post-exercise blood and muscle H^+ ion accumulation was used to characterize the time course of recovery to pre-exercise conditions (Figures 6 and 7, respectively). The $NaHCO_3$ trial post-exercise muscle H^+ ion $t^{1/2}$ (7.2 ± 1.2 min) and t^{PD} times (22.2 ± 2.4 min) were significantly ($p<0.05$) reduced when compared to the control trial (11.3 ± 1.6 and 32.9 ± 4.0 minutes for $t^{1/2}$ and t^{PD} times, respectively). There were no differences in post-exercise blood H^+ ion $t^{1/2}$ times (9.7 ± 1.4 vs 9.5 ± 1.4 min for $NaHCO_3$ and control, respectively) or t^{PD} times (20.4 ± 2.5 vs 20.8 ± 2.7 minutes for $NaHCO_3$ and control, respectively).

In addition, the calculated *in vivo* muscle buffer capacity was similar in both trials (45.1 ± 5.5 vs 52.4 ± 6.3 $mmol\ lactate \cdot kg^{-1} \cdot pH^{-1}$ for $NaHCO_3$ vs control, respectively.

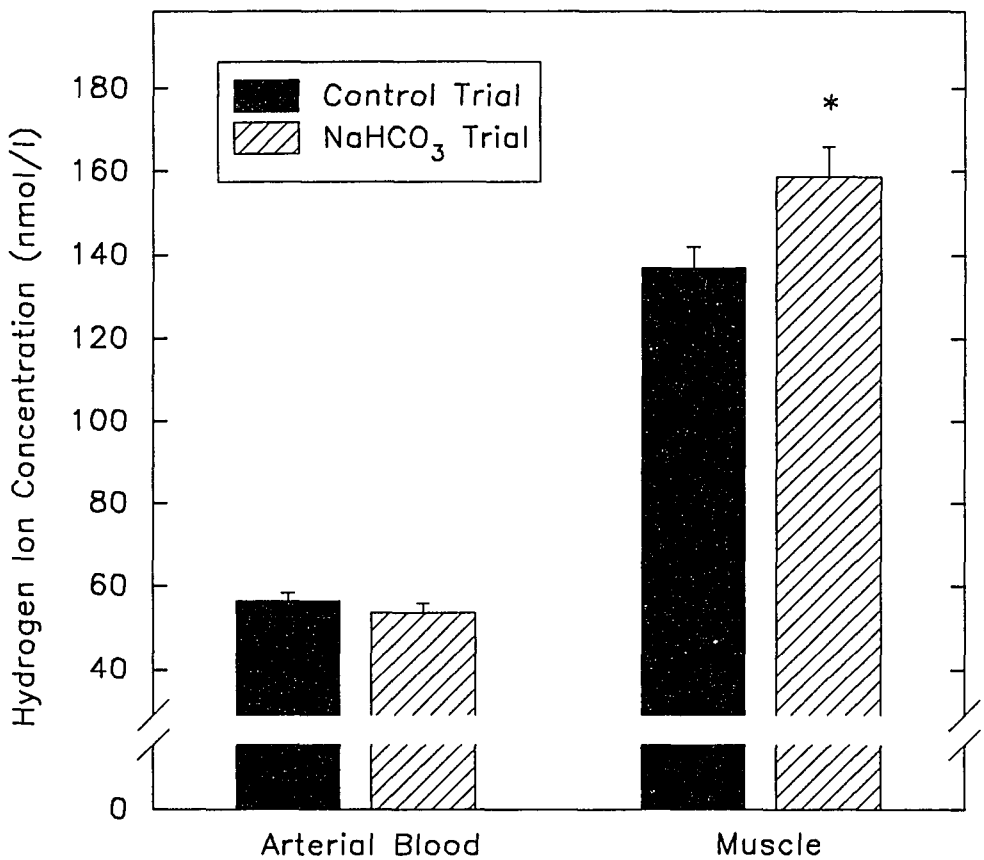

Figure 5 Two minute post-exercise arterial blood and 30-second post-exercise muscle hydrogen ion accumulation

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

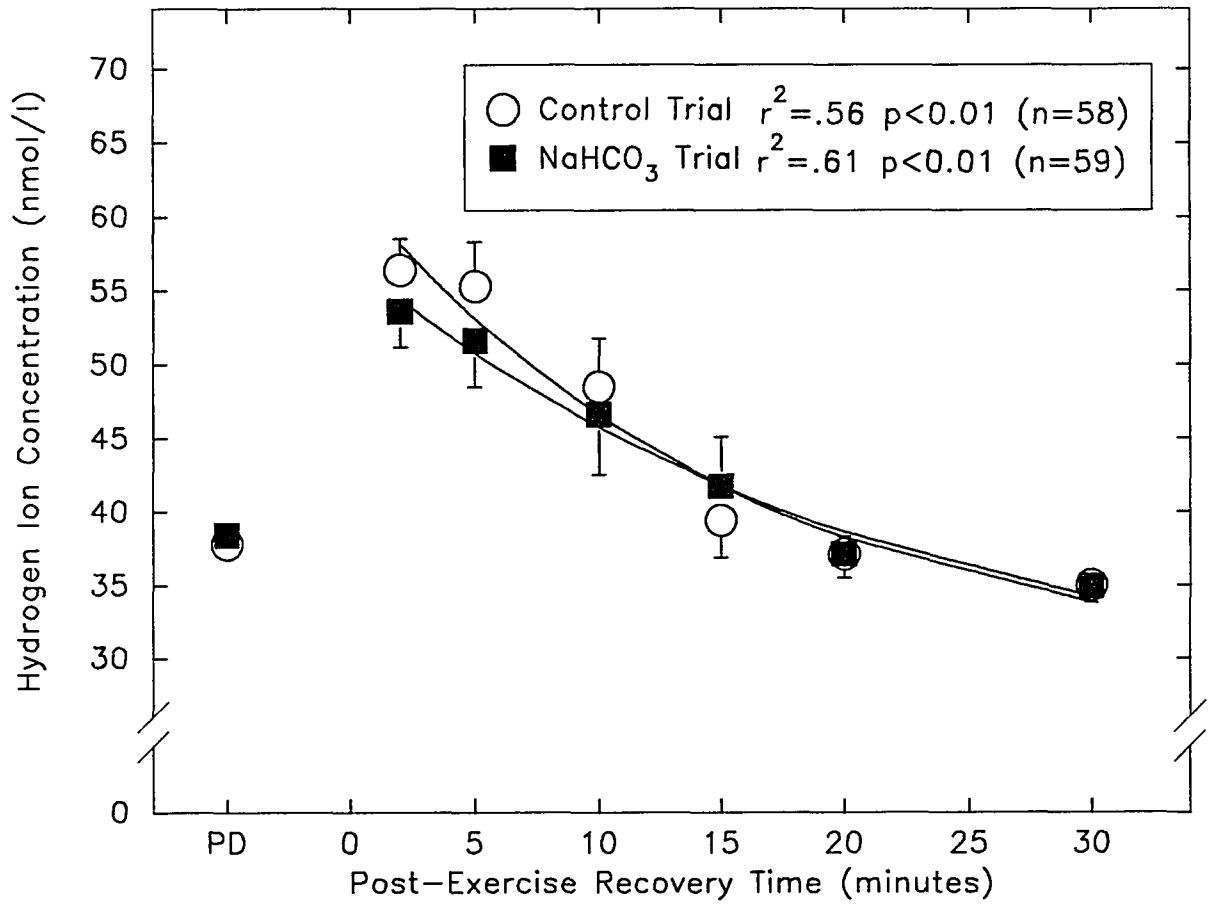

Figure 6 Post-exercise arterial blood hydrogen ion concentration. Data points represent means of 10 animals. Correlation coefficients shown for monoexpotential curve fit.

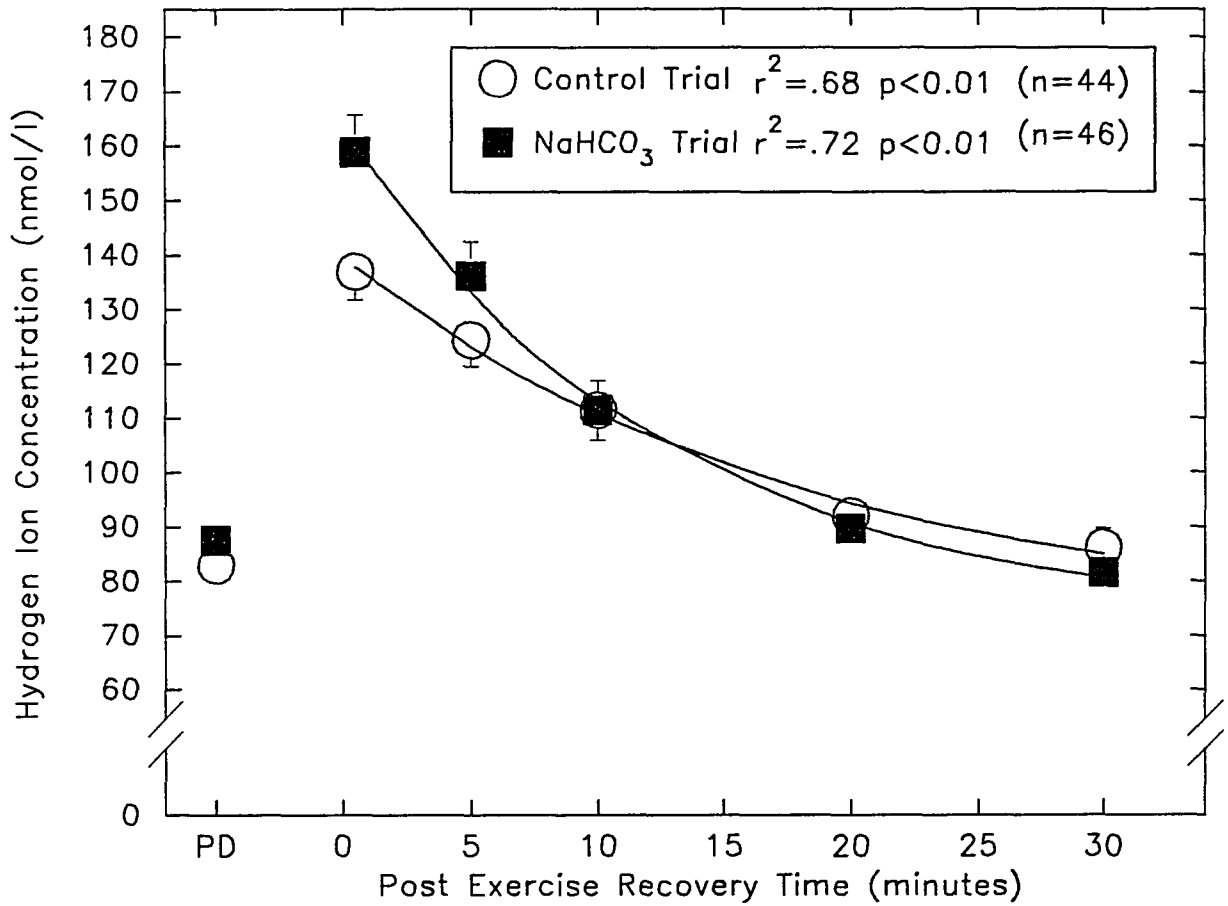

Figure 7 Post-exercise muscle hydrogen ion concentration. Data points represent means of 10 animals. Correlation coefficients shown for monoexpotential curve fit.

Post-exercise arterial blood gas, Hgb, and PCV data are presented in Table 3. In both trials, significant ($p<0.05$) declines in $PaCO_2$, BE, and HCO_3^- and increases in PaO_2, Hgb, and PCV occurred from PS to two minutes post-exercise with values returning towards pre-exercise levels throughout the remainder of the recovery period. The $NaHCO_3$ trial, however, produced a significantly ($p<0.05$) greater peak reduction in arterial BE (-18.5 ± 1.4 vs -14.1 ± 0.8 meq$\cdot l^{-1}$) and HCO_3^- (-17.4 ± 1.2 vs -12.8 ± 0.7 meq$\cdot l^{-1}$) from pre-sprint to the initial post-exercise sampling point (Figure 8). This difference was only observed at the initial sampling interval with no significant differences throughout the remainder of the recovery period.

Mean performance sprint times for the 3/8 mile sprint task were not significantly different between the $NaHCO_3$ (46.29 ± 1.1 s) and control (47.76 ± 0.9 s) trials. In six of the ten animals, there were reductions in individual sprint times (Figure 9). The magnitude of change in blood HCO_3^- and pH at the PS sample in the $NaHCO_3$ trial and control trial was not related to the change in individual performance times ($r=.54$, $p>.10$ and $r=.25$, $p>.10$ for HCO_3^- and pH, respectively). There was a trend for animals with lower control trial muscle buffer capacity to exhibit greater reductions in sprint times during the $NaHCO_3$ trial ($r=.55$, $p=0.09$).

Table 3 Post-exercise arterial blood gas parameters, hemoglobin, and packed cell volume following the $NaHCO_3$ (Alk) and control (Con) trial.

		2	5	10	15	20	30
$PaCO_2$ (mm Hg)	Con	25.1 ± 1.4	23.1 ± 1.9	25.6 ± 1.9	28.0 ± 1.8	31.2 ± 1.9	34.3 ± 1.5
	Alk	24.6 ± 1.9	25.4 ± 1.8	29.9 ± 1.9	32.1 ± 1.4	35.3 ± 1.2	38.1 ± 0.7
PaO_2 (mm Hg)	Con	106.8 ± 2.5	108.4 ± 1.6	100.0 ± 2.8	93.8 ± 2.8	86.9 ± 3.6	86.5 ± 3.3
	Alk	109.1 ± 1.8	105.1 ± 3.3	91.4 ± 1.9	86.1 ± 4.1	81.5 ± 3.7	80.7 ± 2.3
BE ($meq \cdot l^{-1}$)	Con	-14.0 ± 0.8	-13.7 ± 1.5	-9.6 ± 2.0	-4.2 ± 1.7	-1.6 ± 1.4	1.2 ± 0.4
	Alk	-13.0 ± 1.2	-11.4 ± 1.8	-6.6 ± 2.5	-3.4 ± 2.3	0.3 ± 1.6	3.4 ± 0.9
HCO_3^- ($meq \cdot l^{-1}$)	Con	10.7 ± 0.6	10.5 ± 1.6	13.6 ± 1.6	17.6 ± 1.4	20.3 ± 1.4	23.3 ± 0.5
	Alk	11.1 ± 1.1	12.5 ± 1.5	16.9 ± 2.0	19.6 ± 1.8	23.3 ± 1.4	26.3 ± 0.8
Hgb ($g \cdot dl^{-1}$)	Con	21.6 ± 0.4	21.2 ± 0.4	20.3 ± 0.4	19.5 ± 0.4	18.4 ± 0.5	17.8 ± 0.5
	Alk	21.8 ± 0.6	21.5 ± 0.5	20.4 ± 0.7	20.1 ± 0.8	19.1 ± 0.5	18.4 ± 0.6
PCV (%)	Con	59.3 ± 1.4	60.0 ± 1.7	58.0 ± 1.7	54.5 ± 1.4	52.5 ± 1.5	50.4 ± 1.7
	Alk	59.1 ± 1.7	58.9 ± 1.5	57.3 ± 1.9	55.3 ± 1.8	52.6 ± 1.7	50.3 ± 1.7

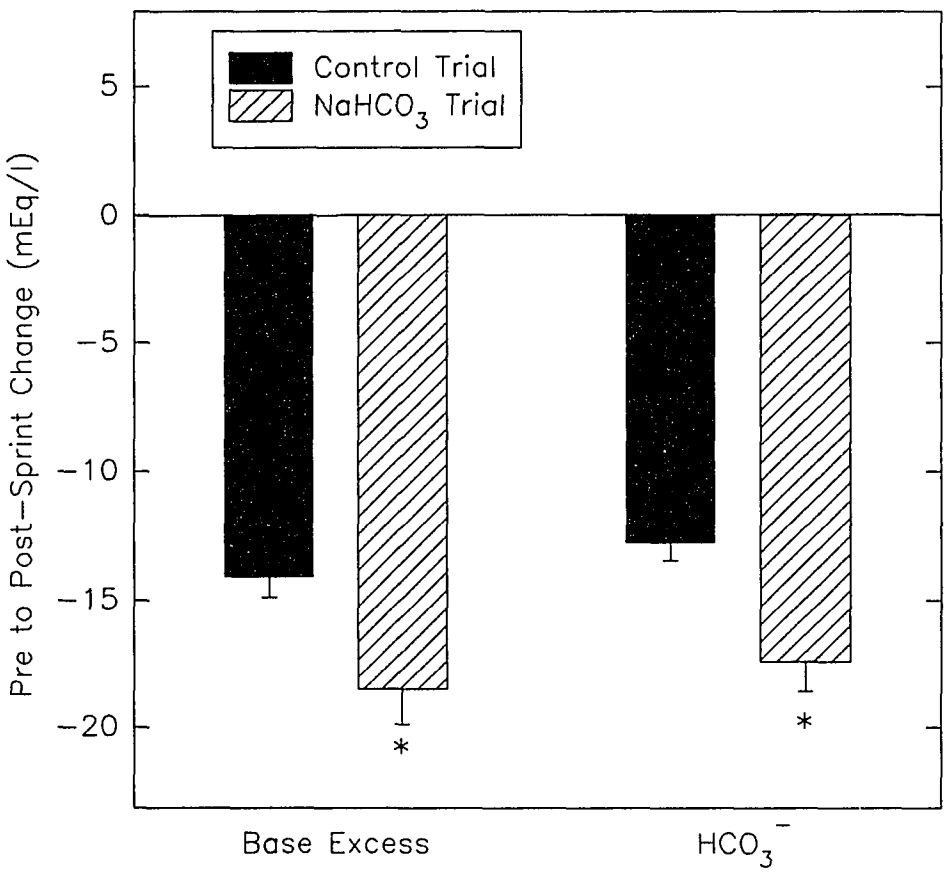

Figure 8 Pre-sprint to post-sprint change in arterial blood base excess and HCO_3^-

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

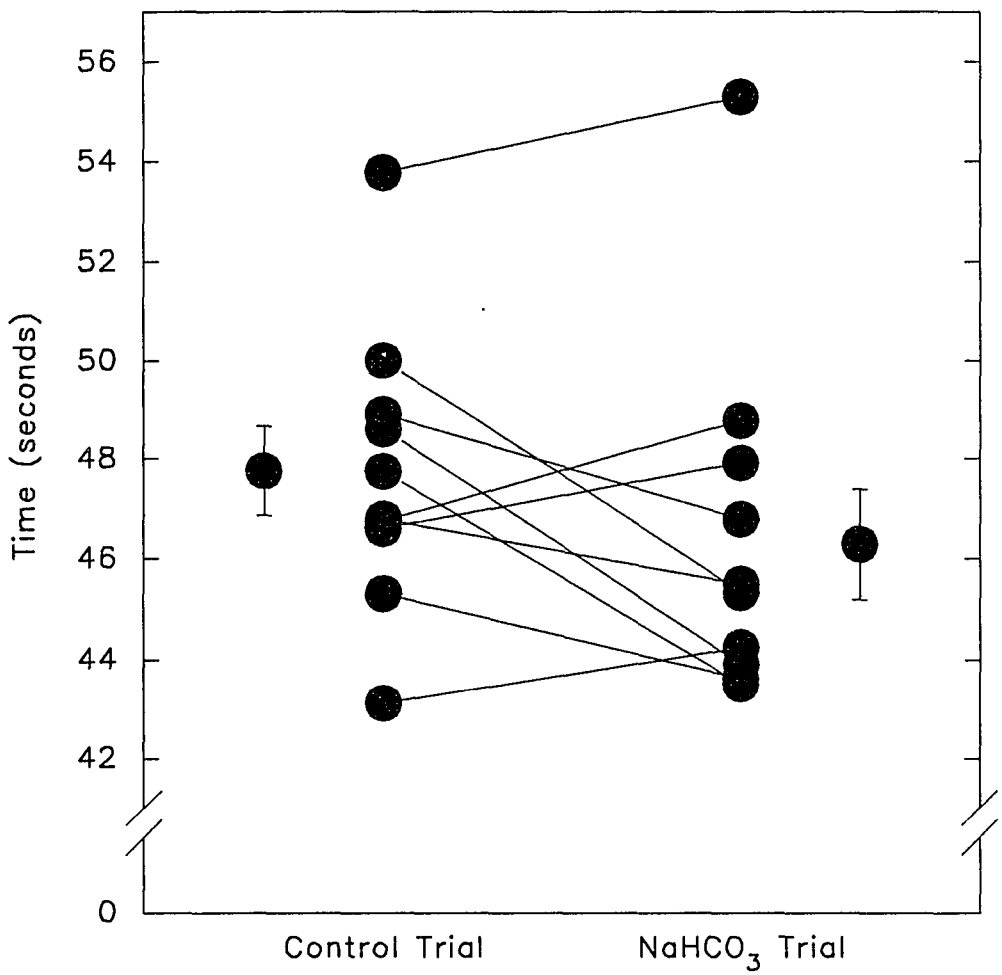

Figure 9 Control trial and $NaHCO_3$ trial individual performance times for a 603.5 meter sprint

DISCUSSION

In this investigation, $NaHCO_3$ ingestion did not significantly impact on the performance time in greyhounds sprinting over a 603.5 meter distance. The $NaHCO_3$ trial was associated with a significant increase in the two min post-exercise blood lactate accumulation and 30 sec muscle H^+ ion accumulation. The $NaHCO_3$ trial did not reduce the intracellular acid-base disturbance associated with the exercise task based on muscle buffer capacity determinations, although the $NaHCO_3$ trial was associated with an accelerated rate of muscle acid-base restoration based on a significant reduction in the muscle H^+ ion $t^{1/2}$ and t^{PD} times.

The potential ergogenic effect of $NaHCO_3$ loading regimens has been attributed to an enhancement of extracellular buffer reserve. This increased extracellular buffer reserve may subsequently affect rates of lactate and/or hydrogen ion efflux (13,25,26), thus minimizing the intracellular pH disturbance and thus reduce pH-related impairment of muscle function (4,5,7,8,36,38,41). The effectiveness of $NaHCO_3$ regimens may be related to the degree of alkalosis induced prior to exercise. In a recent meta-analysis (27) of human $NaHCO_3$ loading studies, a mean increase in blood pH of 0.07 ± 0.02 and HCO_3^- of 5.3 ± 1.4 $mmol \cdot l^{-1}$ was reported for investigations using a 300 $mg \cdot kg^{-1}$ dose. These responses were slightly higher than the 0.06 ± 0.005 unit increase in pH and

4.4 ± 0.4 meq·l^{-1} increase in HCO_3^- observed in the present study, although these are interspecies comparisons.

Based on Stewart's (39) quantitative approach to determination of acid-base status of biological fluids, the concentration and translocation of strong ions directly influence the distribution of H^+ ion. In this investigation, the administration of $NaHCO_3$ was associated with a small, but significant increase in serum Na^+ and a trend towards a lower serum K^+. There was an increase in serum SID following the administration of $NaHCO_3$; however, this increase was not significantly different from the PS SID in the control trial and post-exercise SID values were similar between trials.

In a meta-analytical review (27) of 35 human studies reporting values for post-exercise blood lactate accumulation, 27 reported higher post-exercise blood lactate accumulation following $NaHCO_3$ loading regimens. An increased blood lactate accumulation in these investigations is usually interpreted as an indicator of increased anaerobic metabolism and/or muscle lactate efflux. In the current investigation, the two minute post-exercise blood lactate accumulation was significantly increased following $NaHCO_3$ loading. This difference, however, was only transient as blood lactate accumulation from 5 minutes to 30 minutes post-exercise were similar based on similar $t^{1/2}$ and t^{PD} times.

Post-exercise muscle lactate accumulation was similar in

both trials. In other investigations involving human subjects, metabolic alkalosis has been associated with an increase in peak post-exercise muscle lactate (1,40) or no difference (2), although these investigations employed a variety of exercise tasks. In the current study, the increase in peak blood lactate accumulation coupled with a similar muscle lactate accumulation may suggest an increased production of lactate in the $NaHCO_3$ trial; however, conclusions based on lactate accumulation are tenuous as muscle accumulation is the net consequence of production, clearance, and oxidation.

The effect of $NaHCO_3$ ingestion on post-exercise blood pH has also varied, with some investigators reporting higher post-exercise blood pH (2,11,14,17,19,20,30,43) or no treatment differences (1,28). The post-exercise blood pH difference between $NaHCO_3$ and control trials appears to parallel the pre-exercise difference produced by the $NaHCO_3$ dosing protocol (27). In this investigation, based on similar peak blood H^+ ion accumulation, pre-sprint to post-sprint change in H^+ ion accumulation, $t^{1/2}$, and t^{PD} times, there was no difference in the magnitude of disturbance or rate of recovery of blood acid-base balance. A greater peak reduction in arterial blood HCO_3^- and BE in the $NaHCO_3$ trial, however, suggests that greater blood H^+ ion buffering occurred during exercise and in the initial recovery period. Similar results

have been reported in both individual investigations (1,14) and a recent review (27). A similar post-exercise blood pH, coupled with the greater reduction in blood HCO_3^- and BE and increased blood lactate accumulation, suggests that the buffer capacity of the arterial blood was enhanced in the $NaHCO_3$ trial.

Little information has been reported concerning effects of $NaHCO_3$ loading regimens on muscle pH. Rupp et al. (35) reported no treatment difference in pre- or post-exercise muscle pH following a cycling task to exhaustion. In a series of five, one minute cycling bouts, muscle pH immediately prior to the fifth bout was significantly higher in the $NaHCO_3$ trial compared to that of control, although the pH after the fifth bout (which continued to exhaustion) was similar in both trials (2). In the present investigation, the muscle H^+ ion accumulation at the 30 sec post-exercise interval was significantly higher in the $NaHCO_3$ trial compared to that of control. An increased accumulation of muscle H^+ ions during an exercise task over a fixed running distance could occur if the production of H^+ ions increased and/or there was a decrease in muscle buffering mechanisms. The increase in blood lactate accumulation coupled with no significant change in muscle lactate accumulation during the $NaHCO_3$ trial does suggest that lactate, and thus H^+ ion production may have been higher in the $NaHCO_3$ trial. The greater reduction in blood BE

and HCO_3^- from the pre-sprint to the two minute post-exercise sample also suggests that more H^+ ions were generated during the $NaHCO_3$ trial. If muscle buffering mechanisms, including the rate of H^+ efflux, did not increase proportionally, then muscle H^+ ion accumulation could conceivably be higher.

The increased muscle H^+ ion accumulation coupled with no difference in muscle lactate accumulation suggests that $NaHCO_3$ ingestion did not effectively enhance intracellular muscle buffer capacity during exercise and the initial recovery period. The reduction in post-exercise muscle hydrogen ion accumulation $t^{1/2}$ and t^{PD} times in the $NaHCO_3$ trial suggests that $NaHCO_3$ ingestion may reduce the time required to reach the pre-exercise acid-base status. This acceleration in muscle acid-base restoration, however, may be a consequence of the initially higher H^+ ion accumulation in the $NaHCO_3$ trial, although comparisons of muscle pH recovery following various levels of exercise induced pH disturbance have not been reported. The acceleration of muscle acid-base restoration may be beneficial when exposing greyhounds to repeated bouts of exercise as shown by positive effects on performance reported in human investigations using repeated bout protocols (2,10,21). Despite the accelerated time course of muscle acid-base recovery, if $NaHCO_3$ administration increases the initial post-exercise accumulation of muscle H^+ ions, its use in reducing the occurrence and/or severity of post-exercise

muscle trauma is questionable and requires further investigation.

The lack of a significant effect on performance may be related to the metabolic demands of the exercise task chosen, the total dosage and time course of the $NaHCO_3$ loading regimen, and the potential variability in individual subject responses to ingestion of $NaHCO_3$. The treatment effect appears to be greater in those investigations utilizing a test criteria involving exercise time to exhaustion as opposed to a performance time for a specified exercise task (27). In this investigation, a greater muscle acid-base disturbance in the $NaHCO_3$ trial without a concomitant decrement in performance suggests that the acid-base disturbances which occur in this type of exercise task may not be limiting to muscle function.

In addition, a pre-existing high muscle buffer capacity and/or anaerobic capacity may minimize the ergogenic potential of $NaHCO_3$ ingestion, although improved performance in trained athletes has also been reported (11,43). In the present study, an inverse relationship ($P=0.09$) was observed between control skeletal muscle buffer capacity and the individual changes in sprint times, supporting the concept that $NaHCO_3$ may be more beneficial in those athletes exhibiting limitations in pre-existing muscle buffer capacity.

In summary, a 300 $mg \cdot kg^{-1}$ dose of $NaHCO_3$ given 70 minutes prior to exercise, increased post-exercise blood lactate

accumulation, but did not reduce the magnitude of the muscle or blood acid-base disturbance associated with a 603.5 meter sprint task or significantly impact on performance times.

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GENERAL CONCLUSIONS

The first investigation presented in this dissertation examined the effects of a six-week sprint training regimen on the metabolic responses to a standard exercise task (400-meter sprint). Two levels of training were designated. The low volume (LV) training group completed two 400-meter sprints per week over a six-week period. It was believed that the LV regimen would simulate the volume of high intensity exercise to which most greyhounds are exposed. The high volume (HV) training group progressed from two to eight 400-meter sprints per week over the six-week training period. Although these exercise regimens represent a minimal training volume, it is generally believed that greyhounds do not require or tolerate repetitive bouts of high intensity exercise (3,25,111). Several physiological disorders, believed to be associated with "excessive" exercise training stress, appear to support this belief (25,111).

Although the mechanisms underlying muscle fatigue are debated, attaining high locomotory speed requires a muscle metabolic strategy which allows for rapid ATP resynthesis. Based on comparisons to human and equine athletes, several characteristics of greyhound muscle suggest a high anaerobic glycolytic potential. Greyhound muscle exhibits high glycogenolytic and glycolytic enzyme activities (106) coupled with a relatively homogenous high myosin ATPase muscle fiber

type (34). Significant exercise induced muscle (20,94) and blood lactate accumulation (20,50,86,89,94,107), glycogen utilization (20), and acid-base disturbance (20,50) supports the importance of anaerobic glycolysis in the sprinting greyhound. These muscle characteristics and metabolic responses to exercise may represent adaptations associated with generations of selective breeding and/or may be training induced. In addition, glycolytic potential in greyhounds appears to be higher than expected based on the positive relationship between body mass and anaerobic enzyme activity reported across a number of mammalian species (44). This raises the question of whether greyhounds represent a canine breed which have approached a physiological limit with respect to muscle metabolic function. Currently, limited information exists concerning whether greyhounds will adapt to chronic repetitive sprint training regimens and if training will subsequently affect sprint performance.

In this investigation, the HV training regimen increased muscle glycogen utilization and maximal phosphofructokinase (PFK) activity. The increased PFK activity suggests a enhanced muscle glycolytic potential, while the increased glycogen utilization supports the contention that this potential is being utilized. This assumes that an increased glycogen utilization represents an increase in substrate flux through glycolysis resulting in ATP resynthesis, not an

increased accumulation of glycolytic intermediates. Increases in PFK activity following high intensity training have been reported in humans (8,16,25,30) and rats (9).

Both training regimens were associated with significantly higher post-exercise blood lactate accumulation in the post-training 400-meter sprint trial. Following high intensity training in humans, post-exercise blood lactate accumulation has been reported to increase (47,53,63,101,103,104), although others have found no change (83,85,91). An increased blood lactate accumulation in these investigations is usually interpreted as an indicator of increased anaerobic metabolism and/or muscle lactate efflux. Although a decline in blood lactate clearance could explain the increased blood lactate accumulation, there is no evidence suggesting that sprint training decreases blood lactate clearance rate.

Post-exercise muscle lactate accumulation is higher following high intensity training in humans (85,103). In the current investigation, the increased blood lactate accumulation was not associated with a significant increase in muscle lactate accumulation in either training group. The increased glycogen utilization in the HV group should, in theory, lead to an increase in post-exercise muscle lactate accumulation. Possible explanations include an increased clearance rate of muscle lactate via increased oxidation and/or efflux to the blood and an increase in the formation of

muscle alanine. The HV group did exhibit an increase in post-exercise blood lactate accumulation, suggesting that muscle lactate efflux may have been enhanced. However, post-exercise blood lactate accumulation was also increased in the LV group without a concomitant increase in PFK activity or glycogen utilization. Whether training affects lactate transport across the sarcolemmal membrane has only recently been addressed. Treadmill training in rats did not alter lactate transport rate or capacity in sarcolemmal vesicle preparations (96), although these results represent muscle tissue with a relatively heterogeneous fiber type.

Additional investigations, in which other potential fates of glycogen derived glucosyl units are characterized, such as formation of alanine, formation of glycolytic intermediates, and oxidation of lactate, may help to clarify the discrepancies observed in the present investigation. Muscle and blood lactate accumulation represent the net effect of production, clearance, and oxidation (14); therefore any conclusions concerning specific adaptations based on lactate accumulation alone are tenuous.

Metabolic processes and subsequent physiologic function may be impaired by the inability to maintain cellular homeostasis, especially acid-base homeostasis, thus limiting performance in short term, high intensity exercise tasks. A rise in intracellular H^+ ion concentration may contribute to

muscular fatigue via several mechanisms, including inhibition of rate limiting enzymes such as phosphofructokinase (13,120) and phosphorylase (13,98), inhibition of calcium ion release from the sarcoplasmic reticulum (21,23), reduced binding of calcium to troponin (21,23), and alterations in neural impulse propagation (15,113).

The ability to resist changes in pH despite the increase in H^+ ion production may be a significant factor limiting performance in high intensity exercise tasks. An increased glycolytic rate will result in an increased rate of intracellular H^+ ion accumulation (assuming that pyruvic acid is reduced to lactic acid with subsequent dissociation of lactic acid). Following high intensity training regimens in humans, significant increases in muscle lactate accumulation have been reported with no further change in post-exercise muscle pH (85,103). The ratio of the pre-exercise to post-exercise change in muscle lactate concentration to the change in muscle pH has been used as an index of *in vivo* muscle buffer capacity. An increase in muscle lactate accumulation without a proportional change in pH suggests that the ability to buffer H^+ ions has been increased. Higher muscle buffer capacities have been reported in anaerobically trained human athletes (82,99), and high intensity training has been shown to significantly increase muscle buffer capacity (103).

In the present study, the decline in muscle pH

associated with the 400-meter sprint task was not affected by either sprint training regimen. Training did not significantly alter the calculated muscle buffer capacity (Δmuscle lactate/Δmuscle pH), although a trend ($p=0.09$) towards an increased muscle buffer capacity was found in the HV group. Based on species comparisons of the greyhound muscle buffer capacities obtained in this investigation and those reported by others (20,36), superior muscle buffering capacity does not seem to explain the superior sprinting ability of the greyhound. Because of the relatively high anaerobic potential and a relatively moderate muscle buffer capacity, training induced increases in muscle buffer capacity may represent a potential strategy for enhancing performance in the greyhound athlete.

Despite conflicting results concerning the physiological adaptations associated with high intensity training programs, most human investigations have reported enhanced exercise performance (12,47,83,91,103,104,119).
In the current study, 400-meter sprint times were not significantly reduced in either training group, although the post-training sprint time approached statistical significance ($p=0.07$) in the HV training group. Other investigators have reported similar discrepancies between physiological adaptations and performance markers in humans (47,53), suggesting that the time course of sprint-induced cellular

adaptations may precede measurable changes in performance. In addition, it is also possible that the training volume used in this investigation was not sufficient to induce measurable changes in performance.

In summary, greyhounds exposed to a six-week sprint training regimen (HV) exhibited significant increases in muscle glycogen utilization, maximal PFK activity, and post-exercise blood lactate accumulation. These changes support a training induced increase in muscle glycolytic capacity. No changes in post-exercise muscle lactate accumulation or muscle pH were observed in the HV group. Post-training 400-meter sprint time and an increase in calculated muscle buffer capacity approached statistical significance in the HV training group. With the exception of an increased post-exercise blood lactate accumulation, the LV training regimen had no effect on the observed parameters, suggesting that the lower volume of training did not significantly affect glycolytic capacity. Greyhounds, although selectively bred for racing performance, exhibit similar metabolic adaptations associated with sprint training in other species and may benefit from the incorporation of formal sprint training regimens into the overall training strategy. Additional research may help to define the training variables (frequency, duration, intensity) which may prove to enhance performance without adding undesirable stress to the animal. The use of

formal training programs may also help to optimize the existing genetic potential of each animal, thereby reducing the need for mass breeding practices.

Because of the potential role of exercise induced acid-base disturbances in muscle fatigue and the relatively moderate muscle buffer capacity exhibited by greyhounds, the second investigation presented in this dissertation was undertaken. This study was designed to examine the effects of acute $NaHCO_3$ ingestion on lactate accumulation, acid-base balance, and performance in greyhounds sprinting over a 603.5 meter distance.

Isolated and in situ tissues studies have demonstrated enhanced lactate efflux in muscle tissue stimulated in perfusates with high vs low HCO_3^- concentrations (42,75,76,108). Evidence for a H^+ ion linked lactate-monocarboxylate plasma membrane carrier exists, suggesting the co-transport of lactate and H^+ ions across the sarcolemmal membrane. Furthermore, this transport process appears to be stimulated by the proton gradient across the sarcolemmal membrane (95). The ergogenic potential of $NaHCO_3$ ingestion may be related to the observed post-ingestion increase in extracellular buffer capacity, which may result in the maintenance and/or development of a greater intracellular-extracellular proton gradient, thus accelerating or maintaining H^+ ion and lactate efflux. The enhanced efflux of

H^+ ions and lactate would result in maintenance of intracellular homeostasis and thus maintain and/or improve muscle function.

In addition, some greyhound athletes exhibit exertional rhabdomyolysis, a syndrome associated with exercise induced muscle trauma. It has been suggested that acidosis contributes to this condition and that a reduction in the acid-base disturbance associated with exercise may prove beneficial (25).

Sodium bicarbonate ingestion in both humans and equine has been reported to result in significant increases in pre-exercise blood buffer reserve based on increases in blood pH (5,28,30,31,46,52,56,58,60,64,67,72,97,116,122,123), base excess (28,30,58,60), and HCO_3^- concentration (5,11,30,31,45,46,52,58,60,64,72,97,123). The effectiveness of $NaHCO_3$ regimens may be related to the degree of alkalosis induced prior to exercise. In a recent meta-analysis (80) of human $NaHCO_3$ loading studies, a mean increase in blood pH of 0.07 ± 0.02 and HCO_3^- of 5.3 ± 1.4 $mmol \cdot l^{-1}$ was reported for investigations using a 300 $mg \cdot kg^{-1}$ dose. These responses were slightly higher than the 0.06 ± 0.005 unit increase in pH and 4.4 ± 0.4 $meq \cdot l^{-1}$ increase in HCO_3^- observed in the present study, although these are interspecies comparisons.

Based on Stewart's (114) quantitative approach to determination of acid-base status of biological fluids, the

concentration and translocation of strong ions directly influence the distribution of H^+ ions. It is possible that any effects associated with the administration of $NaHCO_3$ are related to changes in serum ions such as Na^+ or K^+. In this investigation, the administration of $NaHCO_3$ was associated with a small, but significant increase in serum Na^+ and a trend ($p=0.08$) towards a lower serum K^+. There was an increase (pre-dose to pre-sprint) in the serum strong ion difference (SID) following the administration of $NaHCO_3$ however, this increase was not significantly different from the pre-sprint SID in the control trial and post-exercise SID values were similar between trials. Although the changes in these ion concentrations may be influencing the observed changes in acid-base status, the changes in SID were similar in both trials, suggesting that the differences between trials were related to other factors.

Higher post-exercise blood lactate accumulation following $NaHCO_3$ ingestion has been reported (52,56,72,84,97,116,123) in humans. This increase is usually interpreted as an indicator of increased anaerobic metabolism and/or muscle lactate efflux. In the current investigation, peak post-exercise blood lactate accumulation was significantly increased in the $NaHCO_3$ trial. This difference, however, was only transient as blood lactate accumulation throughout the remainder of the recovery period was similar based on similar $t^{1/2}$ and t^{PD} times.

Peak post-exercise muscle lactate accumulation and the decline in muscle lactate over the 30 minute recovery period was not affected by $NaHCO_3$ ingestion. If muscle lactate efflux is increased by $NaHCO_3$ ingestion, a decline in post-exercise muscle lactate accumulation may be expected. The available data do not support this, however, as post-exercise muscle lactate has been reported to be elevated (5,116) or not affected by $NaHCO_3$ ingestion (11). It is possible that $NaHCO_3$ ingestion may result in an increased muscle lactate production, which is associated with an increase efflux and thus an increased blood lactate accumulation. Muscle lactate accumulation would be dependent on the balance between the increased production and the rate at which lactate clearance occurs, leading to either an increase (production > clearance) or no change (production = clearance) in muscle lactate accumulation. It is interesting to note that in the two human studies (5,116) that have reported an increased post-exercise muscle lactate accumulation following $NaHCO_3$ ingestion, blood lactate accumulation was not affected, suggesting no change in muscle lactate efflux (assuming no change in blood lactate clearance).

The post-exercise acid-base status of muscle and blood has been used as a criteria for determining the effectiveness of $NaHCO_3$ loading regimens. Most investigators have reported higher post-exercise blood pH

(11,28,45,58,60,64,88,123) following $NaHCO_3$ ingestion, which appears to parallel the pre-exercise difference (80). In the current investigation, based on similar two minute post-exercise blood H^+ ion accumulation, pre-sprint to post-sprint change in blood H^+ ion accumulation, $t^{1/2}$, and t^{PD} times, there was no difference in the magnitude of disturbance or rate of recovery of blood acid-base balance. A greater peak reduction in arterial blood HCO_3^- and BE in the $NaHCO_3$ trial, however, suggests that greater blood H^+ ion buffering occurred during exercise and in the initial recovery period. Similar results for the pre to post-exercise change in HCO_3^- and BE have been reported by others (5,45,80).

Post-exercise muscle pH has been reported to be similar ($NaHCO_3$ vs control) following a single exhaustive bout of cycling exercise (97), or after a series of five, one minute cycling bouts (fifth bout to exhaustion) (11). In the current study, the muscle H^+ ion accumulation at 30 sec post-exercise was significantly higher in the $NaHCO_3$ trial. This increase in muscle H^+ ion accumulation, coupled with the minimal change in muscle lactate accumulation, suggests that $NaHCO_3$ ingestion did not effectively enhance intracellular muscle buffer capacity during exercise or in the initial recovery period. The increase in blood lactate accumulation coupled with no significant change in muscle lactate accumulation during the $NaHCO_3$ trial does suggest that lactate, and thus H^+ ion

production may have been higher in the $NaHCO_3$ trial. If muscle buffering mechanisms, including the rate of H^+ ion efflux from muscle, did not increase proportionally, then muscle H^+ ion accumulation could conceivably increase. The reduction in post-exercise muscle H^+ ion accumulation $t^{1/2}$ and t^{PD} times in the $NaHCO_3$ trial suggests that $NaHCO_3$ ingestion may reduce the time required to reach pre-exercise muscle acid-base status. This acceleration in muscle acid-base restoration may be a consequence of the initially higher H^+ ion accumulation in the $NaHCO_3$ trial, although comparisons of muscle pH recovery following various levels of exercise induced pH disturbance with and without prior $NaHCO_3$ ingestion have not been reported. The acceleration of muscle acid-base restoration may be beneficial when exposing greyhounds to repeated bouts of exercise as shown by positive effects on performance reported in human investigations using repeated bout protocols (11,26,68). Despite the accelerated time course of muscle acid-base recovery, the increased post-exercise accumulation of muscle H^+ ions appears to contraindicate the use of $NaHCO_3$ to reduce the occurrence or severity of post-exercise muscle trauma.

The lack of a significant effect on performance may be related to the metabolic demands of the exercise task chosen, the total dosage and time course of the $NaHCO_3$ loading regimen, and the potential variability in individual subject

responses to ingestion of $NaHCO_3$. The magnitude of the change in blood HCO_3^- ($r=.54$, $p>.10$) and pH ($r=.25$, $p>.10$) at the pre-sprint sample was not related to the change in individual performance times. Treatment effect appears to be greater in those investigations utilizing a test criteria involving exercise time to exhaustion as opposed to a performance time for a specified exercise task (80). In this investigation, a greater muscle acid-base disturbance in the $NaHCO_3$ trial without a concomitant decrement in performance suggests that the acid-base disturbances which occur in this type of exercise task may not be limiting to muscle function.

A high pre-existing muscle buffer capacity and/or anaerobic capacity may minimize the ergogenic potential of $NaHCO_3$ ingestion, although improved performance in trained human athletes has been reported (28,123). In the present study, an inverse relationship ($r=.55$, $p=0.09$) was observed between control skeletal muscle buffer capacity and the reduction in sprint times, supporting the concept that $NaHCO_3$ ingestion may be more beneficial in those athletes exhibiting lower pre-existing muscle buffer capacity.

In summary, a 300 mg·kg body weight^{-1} dose of $NaHCO_3$ did not reduce the magnitude of the muscle or blood acid-base disturbance associated with a 603.5 meter sprint task or significantly impact on performance times. Greater blood lactate accumulation and pre- to post-exercise change in blood

HCO_3^- and BE suggest that lactate and H^+ ion efflux are higher following $NaHCO_3$ ingestion. In addition, the muscle H^+ ion accumulation at 30 seconds post-exercise was significantly higher in the $NaHCO_3$ trial. Although the recovery time course of muscle homogenate H^+ ion concentration was accelerated following $NaHCO_3$ administration, the higher post-exercise muscle H^+ ion concentrations attained would appear to minimize any potential post-exercise benefits of $NaHCO_3$ ingestion.

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APPENDIX A. PILOT STUDY - EFFECTS Of A 300 MG·KG BODY WEIGHT^{-1} DOSE OF SODIUM BICARBONATE ON GREYHOUND ARTERIAL BLOOD GASES, ELECTROLYTES, AND ACID-BASE BALANCE DURING A FOUR HOUR RESTING PERIOD

ABSTRACT

The purpose of this preliminary investigation was to examine the effects of a 300 mg·kg body weight^{-1} dose of $NaHCO_3$ on greyhound arterial blood gases, electrolytes, and acid-base balance. Five greyhounds were studied in a crossover design with a $NaHCO_3$ trial (single, gastric bolus dissolved in 200 ml of water) and a control trial (gastric bolus of 200 ml of water).

Femoral arterial blood samples were obtained prior to the dosing protocol (PD) and post-dose at ten minute intervals over a four hour period for analysis of blood gases (PCO_2 and PO_2), pH, bicarbonate (HCO_3^-), and base excess (BE). Packed cell volume (PCV) was determined at PD and at one hour intervals (60, 120, 180, and 240 minutes). Serum was harvested for the analysis of serum electrolytes (Na^+, K^+, Cl^-, Ca^{++}) and total protein concentration at PD, and at 60 and 240 minutes post-dose. Whole blood lactate was determined at PD, and at 60 and 240 minutes post-dose. The strong ion difference was calculated for the PD, 60 minute, and 240 minute time intervals.

Pre-dose variables were identical between trials for pH (7.41 ± 0.00 vs 7.42 ± 0.01, $NaHCO_3$ vs control, respectively), HCO_3^- (23.1 ± 0.3 vs 23.8 ± 0.5 meq·l^{-1}, $NaHCO_3$ vs control, respectively), and BE (-0.02 ± 0.2 vs 0.4 ± 0.5 meq·l^{-1}, $NaHCO_3$ vs control, respectively). By 30 minutes post-$NaHCO_3$ dose,

blood pH, HCO_3^-, and BE values were significantly elevated ($p<0.05$) in comparison to the control trial, and these differences persisted throughout the four-hour sampling period. From 30 to 240 minutes post-dose, the mean difference between the control and the $NaHCO_3$ trial for pH was 0.0439 units, 4.8 $meq \cdot l^{-1}$ for HCO_3^-, and 4.5 $meq \cdot l^{-1}$ for BE. The $NaHCO_3$ trial was associated with a small, but significant ($p<0.05$) elevation in blood PCO_2 and lower PO_2 compared to the control trial. No trial effects were found for serum electrolytes or blood lactate concentrations. There was a trend ($p=0.07$) towards a higher calculated SID in the $NaHCO_3$ trial. Both trials were associated with significant declines in serum total protein and PCV over the four hour period.

In summary, it appears that a 300 $mg \cdot kg$ body $weight^{-1}$ gastric bolus of $NaHCO_3$ is effective in elevating blood buffer reserve from 30 to 240 minutes post-dose. The changes in serum electrolytes (Na^+, Cl^-, K^+, and Ca^{++}) appear to be minimal, although serum K^+ levels did decrease over the sampling intervals in both trials and there was a tendency for an elevated SID in the $NaHCO_3$ trial. Without direct measurement of blood or plasma volume it is difficult to determine whether the changes in serum K^+, total protein, and PCV reflect actual decreases of these constituents in a constant volume compartment or simply reflect altered blood compartment volumes.

INTRODUCTION

One of the factors which may be limiting performance in high intensity exercise is the capacity to maintain muscle function as metabolites such as lactate and H^+ ions accumulate in muscle and blood. The ergogenic potential of sodium bicarbonate ($NaHCO_3$) loading regimens is thought to be related, in part, to their effects on extracellular (blood) buffer capacity and subsequent effect on transmembrane flux of these metabolites. Increases in blood pH, HCO_3^-, and BE would indicate an enhanced blood buffer reserve. Administration of $NaHCO_3$ in humans results in an acute elevation of blood HCO_3^- and pH (10), thus supporting an augmented extracellular buffer reserve.

The alterations in blood buffer reserve may be affected by the total dose administered, method of administration, and latent time from dosing to exercise. Most human investigations have utilized a total $NaHCO_3$ dose in the range of 200 to 400 mg·kg body weight^{-1}. The use of doses which exceed 300 mg·kg body weight^{-1} are generally believed to result in acute gastrointestinal disturbances and doses less than 300 mg·kg body weight^{-1} produce conflicting effects on performance, especially when single bout exercise protocols are utilized (10). Greenhaff, et. al., 1990 (3) concluded that a $NaHCO_3$ dose of 600 mg·kg body weight^{-1} may be more effective in equine athletes. The optimal latent period from

dosing to the exercise task appears to be approximately 90 minutes in humans (8), although a minimum latent period of three hours has been suggested for equine (3).

The mechanism(s) by which $NaHCO_3$ ingestion may alter acid-base balance is debated. According to Stewart (15), the concentration and translocation of strong ions (Na^+, K^+, Cl^-, Ca^{++}, and La^-), the partial pressure of CO_2, and the concentration of weak acids (typically estimated from either total protein or albumin and the total inorganic phosphorus concentration) determine the distribution of H^+ and HCO_3^- ions.

No information to date has been reported in the literature concerning the effectiveness or tolerance of greyhounds to $NaHCO_3$ loading regimens. The pre-race protocol required by the greyhound industry presents a potential limitation regarding the use of $NaHCO_3$. Animals are placed in a pre-race quarantine during which no intervention is allowed. Depending on the race schedule, an animal may be in quarantine from approximately 60 to 180 minutes prior to racing. Under these current restrictions, $NaHCO_3$ administration would have to produce an effect which lasts from 60 to 180 minutes.

This pilot study was conducted to determine the effectiveness of a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ on resting arterial blood gases, serum electrolytes, and acid-base parameters over a 240 minute time period.

MATERIALS AND METHODS

Five greyhounds were studied in a crossover design with five to seven days between the $NaHCO_3$ trial and control (Con) trial. All animals were fasted for 12 hours and inactive for a 48 hour period prior to dosing. The $NaHCO_3$ solution consisted of a 300 $mg \cdot kg^{-1}$ dose dissolved in 200 ml of warm tap water and delivered via a gastric tube in a single, gastric bolus. The control solution consisted of a 200 ml gastric bolus of warm tap water.

A three ml femoral artery blood sample was obtained prior to dose (PD) and post-dose at ten minute intervals over a four hour period. The pre-dose sample was needle drawn and the post-dose samples were obtained via a 20 gauge, two inch teflon catheter[1] which had been placed in the femoral artery. During the sampling period the animals remained in lateral recumbency without restraint or sedation.

Samples for blood gas analysis were collected anaerobically in heparinized glass syringes and immediately analyzed for PCO_2, PO_2, pH using an Instrumentation Laboratories Model 813 ph/blood gas analyzer[2]. The PCO_2 and PO_2 electrodes were calibrated with two commercial gas

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[1] Angiocath, Desert Medical Inc., Benton Dickinson and Company, Sandy, UT

[2] Model IL-813 H/blood gas analyzer, Instrumentation Laboratories, Lexington, MA.

mixtures, and the pH electrode was calibrated using a two point calibration procedure with commercial buffers (pH of 7.384 and 6.840). Once calibrated, the blood gas analyzer was placed in an autocalibration mode in which the original calibration settings are automatically reset between each successive experimental sample. Base excess, HCO_3^-, and total CO_2 were calculated (in board analyzer computer).

Packed cell volumes (PCV) were determined at the PD, 60, 120, 180, and 240 minutes sample intervals. Microhematocrit centrifugation was used to determine PCV on triplicate samples which were averaged. Blood serum was harvested (PD, 60, 240 minutes) for analysis of standard serum chemistries[3]. The serum strong ion difference (SID) was calculated from the following equation: ([sodium] + [potassium] + [diffusible calcium]) - ([chloride] + [lactate]) (13). The diffusible calcium concentration was calculated from total serum calcium using the relationship as described by Zeisler (17). One ml of whole blood was deproteinized in two ml of cold 8% perchloric acid and stored at 4°C until analysis of lactic acid concentration spectrophotometric[4] analysis (9).

The effects of treatments were tested using a two factor ANOVA procedure (treatment X sample interval) with repeated

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[3] Roche Biomedical Laboratories, Kansas City, MO.

[4] Spectronic® 70, Bausch and Lomb, Analytical Systems Division

measures on sampling intervals. A Student-Newman-Kuels multiple range test procedure was used to identify significant mean differences. The level of probability was set at $p<0.05$ to reject the null hypothesis. All results are expressed as means ± standard error of mean.

RESULTS

Analysis of variance revealed a significant ($p<0.05$) treatment effect and treatment by time interaction for blood HCO_3^- (Figure 1), pH (Figure 2) and BE (Figure 3). Pre-dose variables were identical between trials for pH (7.41 ± 0.00 vs 7.42 ± 0.01, $NaHCO_3$ vs control, respectively), HCO_3^- (23.1 ± 0.3 vs 23.8 ± 0.5 $meq \cdot l^{-1}$, $NaHCO_3$ vs control, respectively), and BE (-0.02 ± 0.2 vs 0.4 ± 0.5 $meq \cdot l^{-1}$, $NaHCO_3$ vs control, respectively). By 30 minutes post $NaHCO_3$ dose, blood pH, HCO_3^-, and BE values were significantly elevated ($p<0.05$) in comparison to the control trial, and these differences persisted throughout the four hour sampling period. Over the time period from 30 to 240 minutes post dose, the mean difference between the control and the $NaHCO_3$ trial for pH was 0.0439 units, 4.8 $meq \cdot l^{-1}$ for HCO_3^-, and 4.5 $meq \cdot l^{-1}$ for BE.

Arterial blood gases during the trials are shown in Figure 4. Analysis of blood PCO_2 data revealed a small, but significant ($p<0.05$) elevation in blood PCO_2 in the $NaHCO_3$ trial. A treatment effect ($p<0.05$) was also noted for blood PO_2, with the $NaHCO_3$ trial exhibiting lower PO_2 than in the control trial.

Serum electrolytes were examined at PD, 60 minutes, and 240 minutes post-dose. No treatment effect was observed for serum sodium (Na^+), chloride (Cl^-), or calculated diffusible

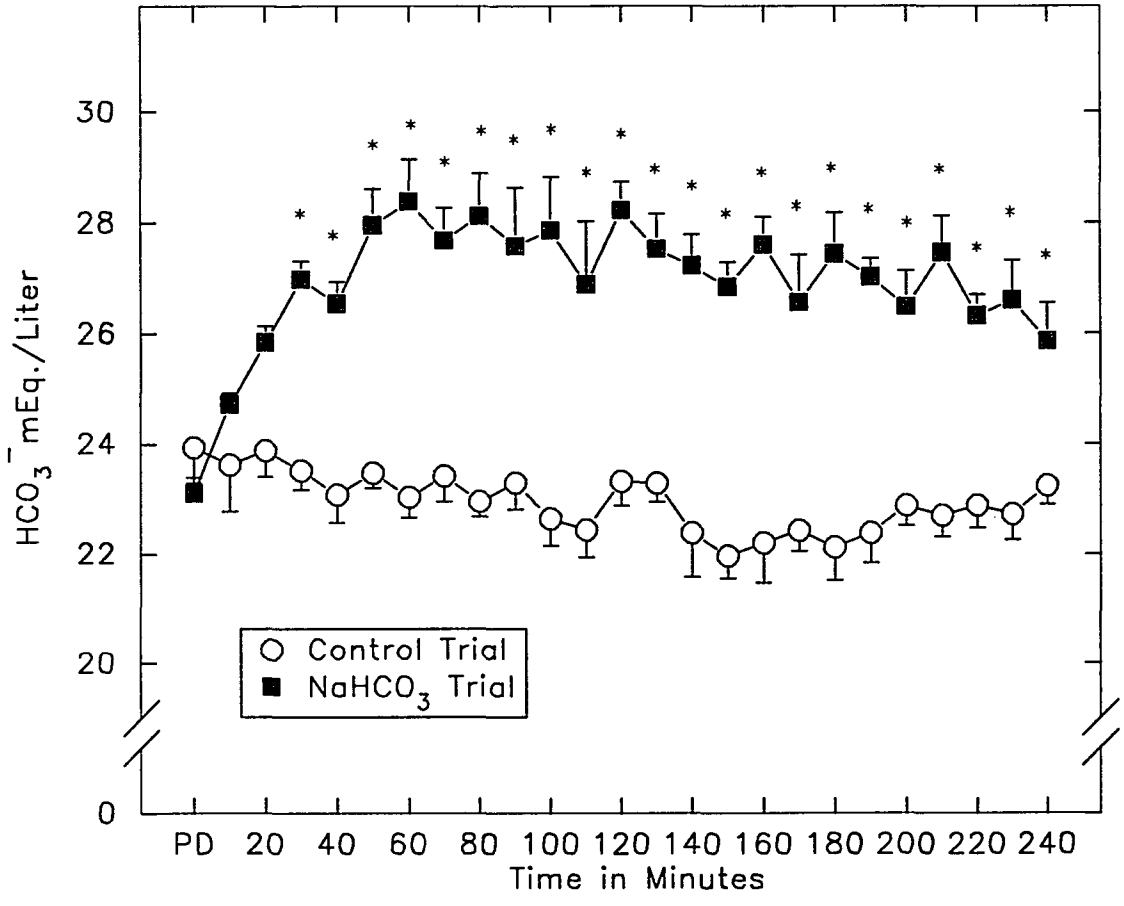

Figure 1 Arterial blood bicarbonate concentration following a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ vs control over a 4 hour period

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

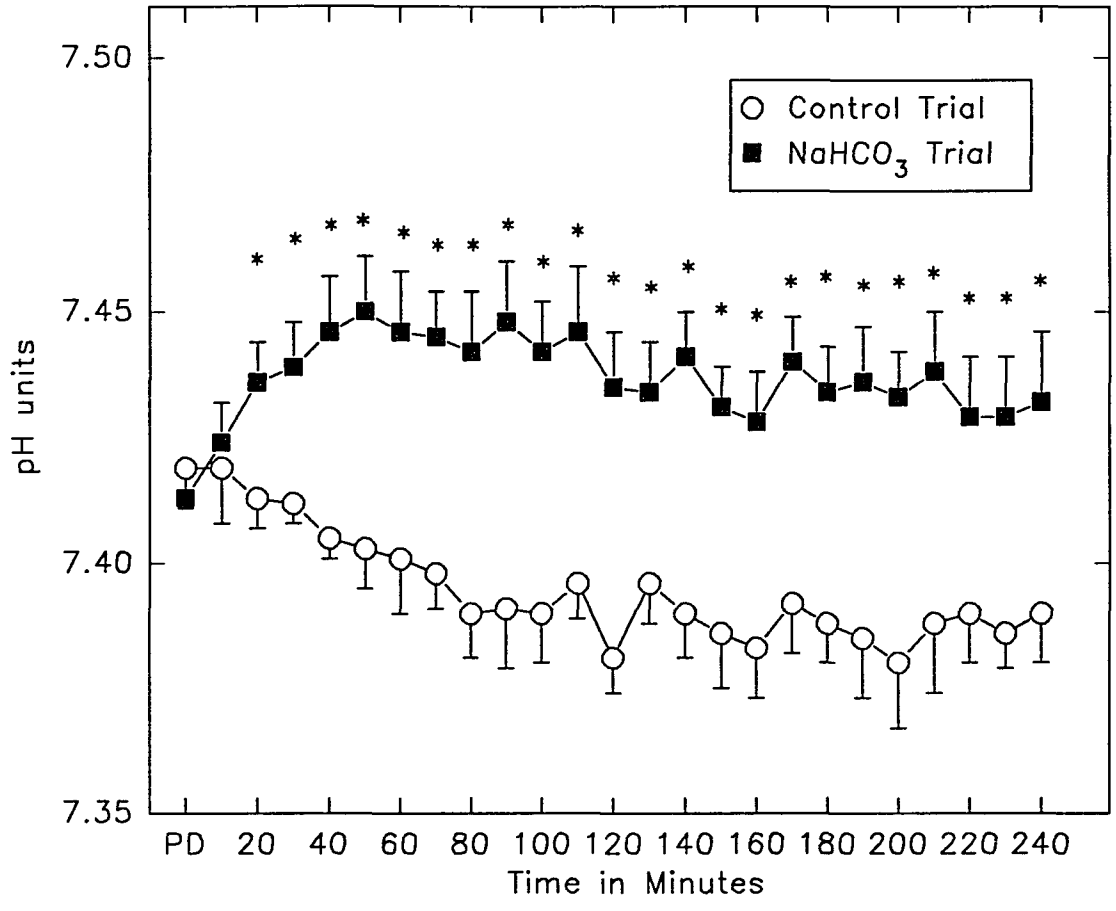

Figure 2 Arterial blood pH following a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ vs control over a 4 hour period

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

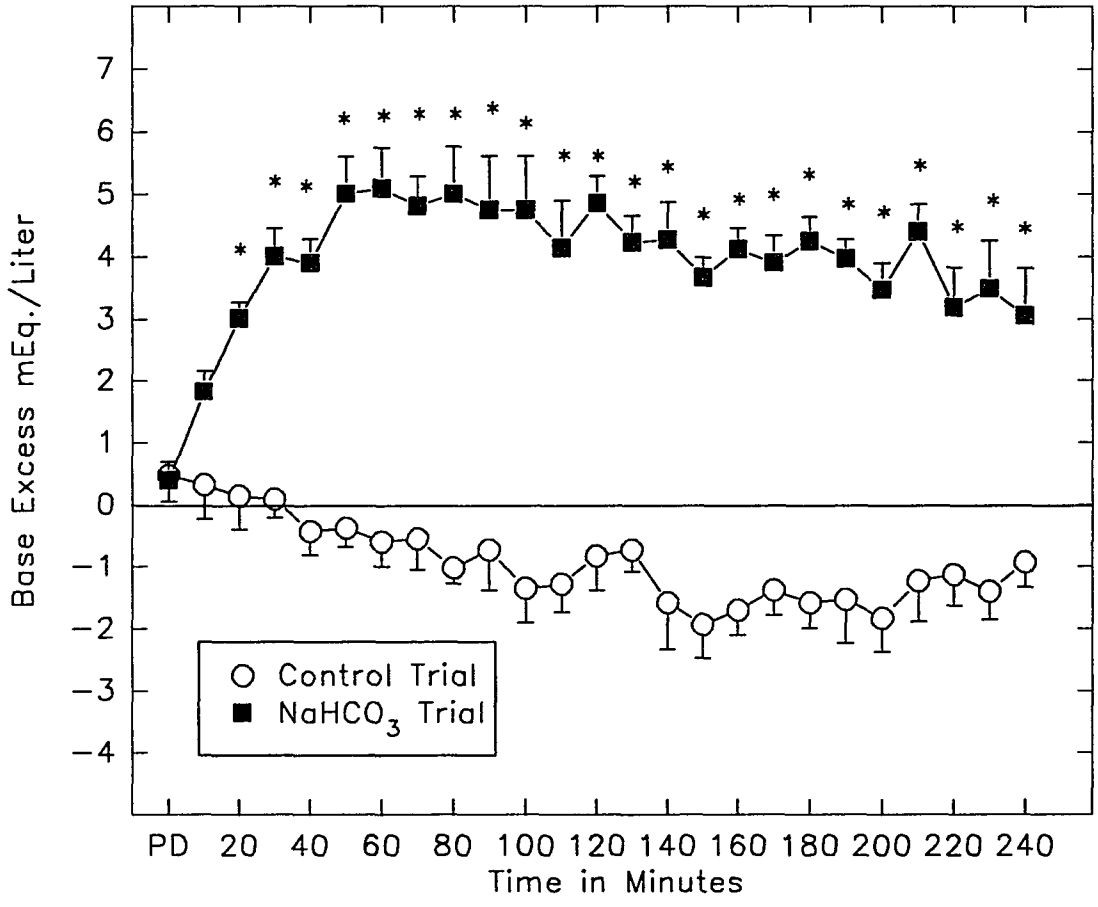

Figure 3 Arterial blood base excess values following a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ vs control over a 4 hour period

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

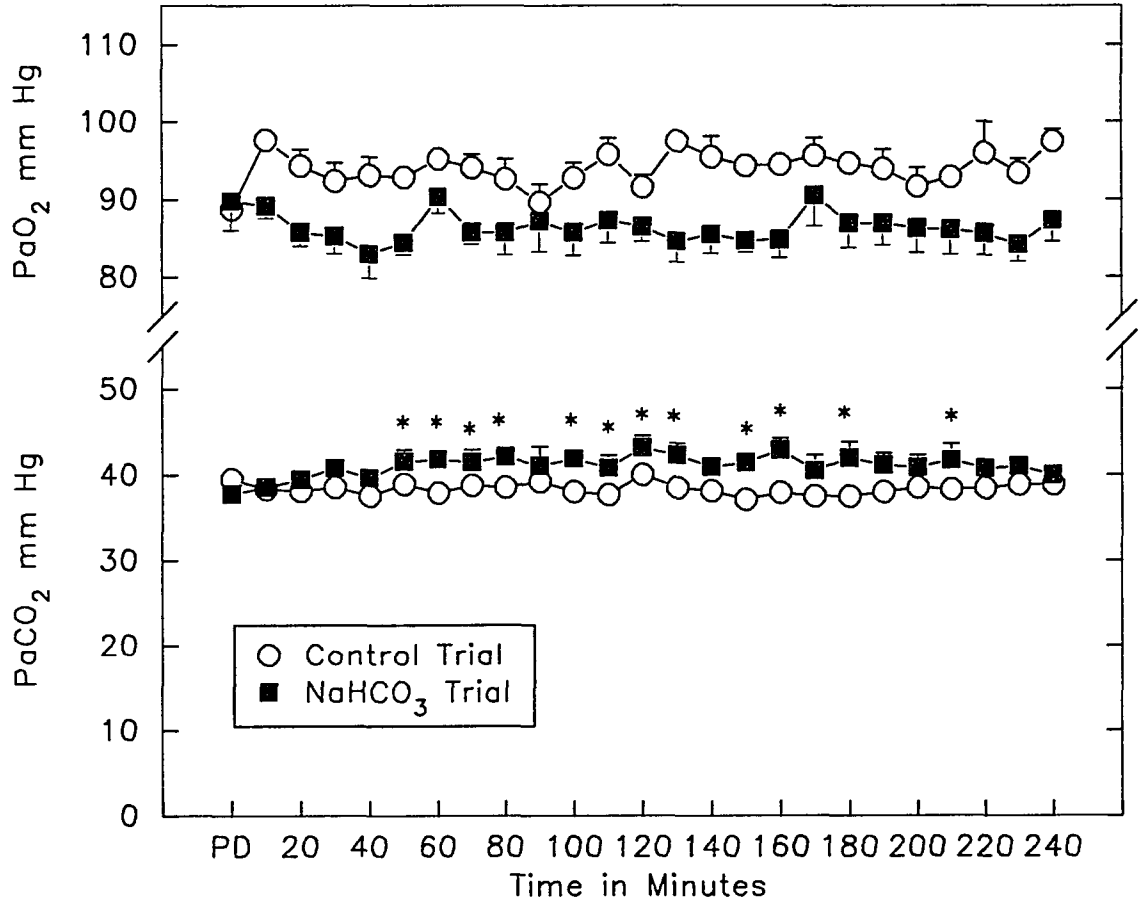

Figure 4 Arterial blood gases following a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ vs control over a 4 hour period

*** Significant ($p<0.05$) difference for control vs $NaHCO_3$ trial**

calcium (Ca^{++}). No treatment effects were found for serum potassium (K^{+}), however a significant ($p<0.05$) time effect was observed as levels decreased from the PD to the 240 minute post-dose sample. There was a trend ($p=0.07$) towards a higher calculated SID in the $NaHCO_3$ trial. Blood lactate concentrations were similar between trials (Table 1).

Total protein (TP) was significantly ($p<0.05$) higher throughout the control trial, and both treatments exhibited significant declines in total protein concentrations over the sampling intervals (Table 1). Packed cell volume was significantly higher ($p<0.05$) throughout the $NaHCO_3$ trial, and both treatments exhibited significant declines in PCV over the sampling intervals (Table 2). In addition, no overt gastrointestinal disturbances (diarrhea or vomiting) were noted during or within one hour after the dosing trials.

Table 1 Serum electrolytes, SID, total protein (TP), before and after a 300 $mg \cdot kg^{-1}$ gastric bolus of $NaHCO_3$ or control solution

	Control			$NaHCO_3$		
	PD	60	240	PD	60	240
Na^+ ($mEq \cdot L^{-1}$)	150.6 ± 1.1	148.6 ± 0.7	150.6 ± 0.4	150.4 ± 1.7	153.6 ± 1.2	152.4 ± 1.2
K^+ ($mEq \cdot L^{-1}$)	4.7 ± 0.02	4.5 ± 0.04*	4.3 ± 0.07*	4.7 ± 0.06	4.5 ± 0.07*	4.3 ± 0.18*
Cl^- ($mEq \cdot L^{-1}$)	116.6 ± 0.8	117.0 ± 0.8	117.8 ± 0.4	115.4 ± 0.8	117.2 ± 1.1	117.0 ± 1.2
Ca^{++} ($mEq \cdot L^{-1}$)[a]	2.3 ± 0.04	2.3 ± 0.03	2.5 ± 0.04	2.1 ± 0.14	2.3 ± 0.07	2.3 ± 0.17
Lactate ($mmol \cdot l^{-1}$)	1.8 ± 0.0	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.9 ± 0.2	1.8 ± 0.1
SID ($mEq \cdot L^{-1}$)	39.1 ± 0.5	36.7 ± 0.7	37.8 ± 0.6	39.8 ± 1.2	41.3 ± 1.5	40.1 ± 0.7
TP ($g \cdot dl^{-1}$)	6.1 ± 0.1	5.4 ± 0.1*	5.5 ± 0.1*	5.7 ± 0.1	5.2 ± 0.1*	5.1 ± 0.3*

* = $p<0.05$ from pre-dose
a = diffusible calcium

Table 2 Arterial packed cell volumes before and after a 300 $mg \cdot kg^{-1}$ gastric bolus of $NaHCO_3$ or control solution

Time	Pre-Dose	60	120	180	240
Control	55.8 ± 1.8	44.8 ± 1.5*	45.3 ± 1.7*	45.6 ± 2.3*	44.4 ± 1.6*
$NaHCO_3$	61.1 ± 1.2	52.8 ± 2.1*	51.2 ± 2.3*	49.4 ± 2.2*	48.3 ± 2.0*

* = $p<0.05$ from pre-dose

DISCUSSION

A 300 mg·kg body weight^{-1} dose of $NaHCO_3$ resulted in significant increases in blood buffer reserve by 30 minutes post-dose as indicated by elevated blood pH, HCO_3^-, and BE. The increase in HCO_3^- and BE in the $NaHCO_3$ trial indicates that an $NaHCO_3$ induced metabolic alkalosis was established. An alkalosis of metabolic origin has been reported in a number of studies in both human (10) and equine athletes (2,3) following $NaHCO_3$ ingestion. These changes in blood buffer reserve persisted throughout the four hour post-dose sampling period. The magnitude of the increase in both blood pH and HCO_3^- in this investigation was less than the mean increase reported for blood pH (0.07 ± 0.02) and HCO_3^- (5.3 ± 1.4 mmol·l^{-1}) in an analysis of human studies utilizing a 300 mg·kg^{-1} dose of $NaHCO_3$ (10). Direct comparison of these responses is tenuous, as it requires interspecies comparisons and the comparison of responses over different time intervals. The significant and persistent elevation in blood buffer reserve following the 300 mg·kg body weight^{-1} $NaHCO_3$ dose establishes the potential use of $NaHCO_3$ loading within the existing pre-race protocols used at many greyhound racing facilities.

The underlying mechanisms producing a $NaHCO_3$ induced alkalosis are debated. Based on Stewart's (15) quantitative approach to determination of acid-base status of biological fluids, several independent variables determine the

distribution and concentration of H^+ and HCO_3^- ions. These variables include the concentration and translocation of strong ions, the partial pressure of CO_2, and the concentration of weak acids (typically estimated from either total protein or albumin and total inorganic phosphate concentration). In addition to the direct role that blood ion balance may have on influencing the distribution of H^+ ions, alterations in electrolyte balance have been viewed as a primary factor affecting the performance potential of racing greyhounds (5,16) and in the underlying pathophysiology of some forms of exercise related muscle trauma (1). Following the dosing protocol used in this investigation, no significant treatment induced alterations were observed in serum Na^+, Cl^-, Ca^{++}, or K^+ ion concentrations. It has been suggested that a vascular metabolic alkalosis induces the efflux of H^+ ions from intracellular buffers to minimize the change in blood pH. This efflux of H^+ ions may then be coupled with movement of K^+ ions to intracellular compartments to maintain electroneutrality and thus decrease vascular K^+ ion concentrations (12). Serum K^+ concentrations did progressively decrease over the three sampling intervals, however, this decrease occurred in both the $NaHCO_3$ and control trials. Collectively, the serum ions (including the lactate concentration) can be used to determine the SID. Despite the similar serum ion concentrations, the treatment difference in

SID approached statistical significance suggesting that the net ion effect of $NaHCO_3$ ingestion is an elevated SID, which is associated with a reduction in H^+ ion concentration. However, the calculation of the SID in blood should include all strong ions present such as magnesium and sulfate, both which were not included in this calculation (11).

Respiratory compensation for metabolic alkalosis involves a reduction in alveolar ventilation to retain CO_2 thus effectively raising the H^+ ion concentration and minimizing the alkalosis. Ventilatory volumes and respiratory patterns were not determined during the dosing trials, however, the elevated $PaCO_2$ in the $NaHCO_3$ trial suggests that respiratory compensation was occurring.

Total protein concentrations decreased in both trials over the four hour period. The observed total protein concentration will be a net result of protein entry and clearance from the vascular space as well as the fluid volume in which the protein is distributed. If total protein turnover (entry vs clearance) is constant, then these changes in total protein concentration may reflect a "shift" of fluid from the extravascular space to the vascular compartment, thus "diluting" vascular constituents such as K^+ ions. Under physiological conditions of exercise induced or thermal stress, protein entry and loss from the vascular space are unbalanced (4) making changes in total protein a tenuous

marker of vascular fluid shifts. However, under the relatively short term, non-stressed conditions the animals were placed in during the dosing trials, the changes in total protein may reflect actual changes in vascular volume. The movement of fluids from the vascular space would be influenced by capillary hydrostatic pressures, which in turn are influenced by the magnitude of adrenergic stimulation. It is conceivable that as the animals progressed through the four hour trial period their level of excitement, and thus the "baseline" level of adrenergic stimulation was reduced decreasing transcapillary hydrostatic pressures. The decline in total protein concentration may also have acid-base implications. Hypoproteinemia, independent of other acid-base parameters, has been reported to result in alkalosis (14).

The pre-dose PCV for both $NaHCO_3$ (61.1 ± 1.2 %) and control (55.8 ± 1.8 %) are similar to pre-exercise or "rested" PCV reported by several investigators (6,7,13). The PCV values observed throughout the last three hours of both trials represent values which are lower than those normally reported for greyhounds. The gradual decline in PCV over the sampling intervals in both trials may also support a hemodilution effect due to a gain in vascular fluid volume. In addition, during exercise in canines, it is generally assumed that hemoconcentration occurs at least in part from adrenergic nervous system stimulation resulting in the release of the red

blood cell rich splenic contents. Conversely, it is also possible that the decline in PCV observed over these dosing trials resulted from reduced sympathetic stimulation and/or sequestering of red blood cells by the spleen.

In summary, based on elevated blood pH, HCO_3^-, and BE, a 300 mg·kg body weight^{-1} gastric bolus of $NaHCO_3$ significantly elevates blood buffer reserve from 30 to 240 minutes post-dose. This persistent elevation in blood buffer reserve makes the use of $NaHCO_3$ feasible within the current pre-race protocols. The changes in serum electrolytes (Na^+, Cl^-, K^+, and Ca^{++}) appear to be minimal, although serum K^+ levels did decrease over the sampling intervals in both trials and there was a tendency for an elevated SID in the $NaHCO_3$ trial. Without direct measurement of blood or plasma volume it is difficult to determine whether the changes in serum K^+, total protein, and PCV reflect actual decreases of these constituents in a constant volume compartment or simply reflect an increased plasma volume.

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APPENDIX B. ANALYTICAL METHODS FOR DETERMINATION OF MUSCLE AND BLOOD METABOLITES AND ACID-BASE PARAMETERS

Muscle Sample Collection Procedures

Sample Collection Procedures

All muscle samples were collected from the biceps femoris muscle, a hindlimb limb extensor located on the lateral aspect of the ("thigh") pelvic limb. The hair over the biopsy site was clipped and the skin cleaned using a Betadine®[1] solution followed by a 70% isopropyl alcohol rinse. After three repetitions of cleaning, 0.5 to 0.75 cc of a local anesthetic[2] was injected subcutaneously at the intended incision site. A one-half inch skin incision was made along the longitudinal axis of the muscle at the midpoint of the dorsal-ventral axis. Samples were obtained by two different methods as follows.

Investigation I With the sample notch closed, a six mm bore Bergstrom biopsy needle[3] was placed into the biceps femoris at a depth to approximate the middle of the muscle thickness. The needle was oriented so the sampling notch was perpendicular to the longitudinal axis of the fibers. Muscle tissue was aspirated into the sampling notch by suction from a large syringe and the cutting blade was closed. The sample

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[1] Purdue Frederick Corp., Norwalk, CT

[2] 2% Lidocaine®, Elkins-Sinn Inc., Cherry Hill, N.J.

[3] Bergstrom biopsy needle

was immediately placed and stored in liquid nitrogen until analysis. Direct pressure was applied for approximately five minutes to control any bleeding. The pre- sprint incision site was sutured[4] with a single stitch to facilitate rapid collection of the post-sprint biopsy sample. Post-sprint biopsy samples were obtained from the same incision site after removing the suture. The second sample was collected from a site which was approximately one to two inches lateral and cranial to the pre-sprint biopsy site. All samples were collected from the left biceps femoris muscle.

Investigation II Muscle biopsy samples were obtained by surgical removal of tissue across the surface of the muscle. A slightly larger incision was made (one inch) to facilitate the removal of multiple biopsies from the same incision site. The tissue was lightly grasped by forceps and removed by scalpel cuts. All samples were immediately placed and stored in liquid nitrogen until analysis. Direct pressure was applied for approximately five minutes to control any bleeding. The pre-dose incision site was sutured[4] with a single suture to facilitate collection of the pre-sprint biopsy sample from the same site. The pre-dose and pre-sprint samples were obtained from the right biceps femoris.

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[4] 00 Ethibond polyester suture, Ethicon Inc., Somerville, N.J.

A second incision site was also prepared on the left biceps femoris for the collection of post-exercise samples (30 sec., 5, 10, 20, and 30 minutes). Following the removal of all muscle samples, the incision sites were sutured and a topical antibiotic[5] was applied to reduce the potential of post biopsy infections.

Muscle pH Determination

General Principle of Assay

Intracellular muscle pH was estimated using a homogenate technique as described by Costill, et. al. (7). This procedure involves the homogenation of muscle tissue in an iodoacetate solution to prevent further glycolytic activity. The solution has no buffer capacity at physiologic pH, thus the H^+ ion content of the muscle will be reflected by the pH of the homogenate.

Procedure

All visible blood and connective tissue was removed and samples were weighed on a micro balance[6] at -20°C prior to analysis. A muscle sample (15-20 mg) was prepared by homogenating the tissue in 200 ul of a KCl (145 mM),

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[5] Panalog®, Solvay Veterinary Inc., Princeton, N.J

[6] Mettler AE 163 microbalance, Mettler Instrument Corp., Highstown, N.J.

NaCl (10 mM), and sodium iodoacetate (5 mM) solution. The homogenate was drawn into a 200 ul capillary pipette and immediately aspirated into a Radiometer BMS 3MK2 Blood Micro System coupled to a PHM-73 ph/blood gas monitor[7]. The gas analyzer was calibrated using a two point (6.840 and 7.383) calibration procedure. Samples were analyzed in duplicate and averaged. All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Blood Lactic Acid Determination

General Principle of Assay

Lactic acid in the blood sample is measured via the production of NADH in a reaction series (Lowry and Passonneau, 1974). The reaction involves the production of pyruvate via lactic acid dehydrogenase (LDH) and the stoichiometric production of NADH. NADH concentration can be determined by spectrophotometric[8] absorbance at 340 nm.

Procedure

One ml aliquots of whole blood for lactic acid determination were placed in two ml of cold 8% perchloric acid

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[7] Radiometer, Copenhagen, Denmark

[8] Beckman DU-5 Spectrophotometer, Beckman Instruments Inc. Irvine, CA

(PCA) and mixed to ensure deproteinization of the sample and prevent any further glycolysis in the red cell mass. The samples were centrifuged at 4,000xg for 10 minutes and the supernatant removed for analysis. The reagent cocktail includes glycine (0.32 M), hydrazine hydrate (0.32 M), NAD (2.4mM), and bovine heart LDH (15 U/ml) titrated to a pH of 9.9. All reaction tubes contained one ml of reagent with the addition of 20 ul of sample for the sample tubes, 20 ul of PCA for reagent blanks, and 20 ul of .5 mM lactate for standard tubes. The tubes were vortexed and allowed to incubate for 30 minutes in a water bath at 37°C. The spectrophotometer was zeroed using the blanks at 340 nm. Sample and standards were assayed in duplicate and averaged. All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Muscle Glycogen Determination

General Principle of Assay

Intramuscular glycogen is determined following the production of glucosyl units by acid homogenation, application of heat, and enzymatic hydrolysis of the muscle tissue sample. The glucose residues are the initial substrate for a reaction series in which the stoichiometric production of NADPH is measured using fluorometry.

Procedure

All visible blood and connective tissue was removed and samples were weighed on a micro balance[9] at -20°C prior to analysis. A 10-15 mg sample was homogenized in 1 ml of .03 N HCl at 0°C. The homogenate was transferred to a microcentrifuge tube and placed in 100°C water for 5 minutes. A 20ul sample of the homogenate was placed in a assay tube with 100 ul of 0.1 M acetate buffer. Blank tubes with 20 ul of distilled H_2O and standard tubes with 20 ul of a 0.55 mM glucose standard[10] were also prepared. Next 5 ul of an amyloglucosidase solution (10 ug/ml in 20 mM Tris buffer, pH 7.5, with 2% bovine serum albumin) was added to each assay tube. After 30 minutes at room temperature, 1 ml of diluted (1:1 in 50 mM Tris buffer) glucose reagent[10] was added to each assay tube and allowed to incubate in the dark for 15 minutes at room temperature. The fluorescence (F) of the assay tubes was determined on a fluorometer[11] and glycogen content, expressed as glucosyl units·$gwwt^{-1}$, was calculated as follows.

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[9] Mettler AE 163 microbalance, Mettler Instrument Corp., Highstown, N.J.

[10] Diagnostic reagent kit (glucose HK), Sigma Chemical Co., St. Louis, MO

[11] Ratio 2 Fluorometer, Farrand Optical Co., Inc., Valhalla, N.Y.

$$\frac{(F\ sample - F\ blank)}{(F\ standard - F\ blank)} \times 0.55\ mM\ /\ gram\ wet\ weight\ of\ sample$$

Samples were assayed in duplicate, blanks and standards were assayed in triplicate and averaged. All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Muscle Lactate Determination

General Principle of Assay

Lactic acid in the muscle sample is measured via the production of NADH in a reaction series (Lowry and Passonneau, 1974). The reaction involves the production of pyruvate via lactic acid dehydrogenase (LDH) and the stoichiometric production of NADH. The NADH concentration can be determined by fluorometric analysis.

Muscle Lactate Extraction

Muscle lactate must first be extracted from the muscle sample and isolated in a supernatant fluid. All visible blood and connective tissue was removed and samples were weighed ona micro balance[12] at -20°C prior to analysis. The frozen

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[12] Mettler AE 163 microbalance, Mettler Instrument Corp., Highstown, N.J.

muscle sample (10-15 mg) was placed on 200 ul of frozen 3N PCA. The samples were left at -5°C for 20 minutes, then moved to 5°C for 30 minutes. The section of muscle was removed and the sample was centrifuged at 4000xg for 10 minutes (4°C). Next, 100 ul of sample supernatant was frozen and 175 ul of 2N $KHCO_3$ was added. This was centrifuged at 4000xg for 60 minutes (4°C) to produce the muscle extract supernatant.

Procedure

The initial fluorescence (F_i) of all assay tubes was determined on a fluorometer[13] at 460 nm. A 50 ul aliquot of sample extract was added to 1 ml of a reagent cocktail (0.1 M amino methyl propanol, 0.5 mM NAD, 0.1 M hydrazine, and 10 U/ml bovine heart LDH, adjusted to pH 9.9). Reagent blanks (50 ul distilled H_2O) and standards (0.5 mM lactate) were also prepared. The tubes were vortexed and allowed to incubate in the dark at room temperature for 30 minutes. Following incubation, a final fluorescence (F_f) was determined for all assay tubes. Muscle lactate content, expressed as umoles·gwwt^{-1}, was calculated as follows.

$$\frac{\Delta F \text{ sample}}{\Delta F \text{ standard}} \times \text{std (mM)} \times \text{vol std (ml)} \times \text{DF} \times 1000/\text{sample wt mg}$$

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[13] Ratio 2 Fluorometer, Farrand Optical Co., Inc., Valhalla, N.Y.

ΔF sample = [F_f sample - F_i sample - (F_f blank - F_i blank)]

ΔF standard = [F_f standard - F_i standard - (F_f blank - F_i blank)]

DF = total dilution factor

The sample and standards were assayed in duplicate and averaged. All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Phosphofructokinase Determination

General Principle of Assay

Phosphofructokinase (PFK) activity was determined using a direct, kinetic method (Baldwin, et.al., 1973). This method involves a reaction series in which the initial step involves the phosphorylation of fructose-6-phosphate (F6P) by PFK.

The subsequent reactions require the conversion of NADH to NAD which can be detected as a decline in fluorescence[14] over time. The maximal rate of decline in NADH fluorescence represents the maximal rate at which the PFK in the sample can initiate the reaction series.

Procedure

First, a change in fluorescence was calibrated to a known

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[14] Ratio 2 Fluorometer, Farrand Optical Co., Inc., Valhalla, N.Y.

concentration of NADH. A small amount (~30-40 mg) of NADH is added to one ml of a 0.1 M potassium phosphate buffer. The NADH solution is subsequently diluted by placing 20 ul in one ml of 0.1 M potassium phosphate buffer. The absorbance[15] was determined and the concentration of NADH was calculated as follows.

$$\text{std NADH concentration (mM)} = \text{absorbance}/6.27 \times \frac{\text{total volume}}{\text{sample volume}}$$

The NADH standard was again diluted (1:100) in 0.1 M potassium phosphate buffer. The fluorescence of a one ml aliquot of potassium phosphate buffer was determined, 20 ul of diluted NADH standard was added, and the fluorescence was again determined. A change in fluorescence could then determined for a given concentration of NADH by the following calculation.

diluted standard (mM) x .02 ml/Δ fluorescence = um NADH/fluorescence unit

All visible blood and connective tissue was removed and muscle samples were weighed on a micro balance[16] at -20°C prior

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[15] Beckman DU-5 Spectrophotometer, Beckman Instruments Inc. Irvine, CA

[16] Mettler AE163 microbalance, Mettler Instrument Corp., Highstown, N.J.

to analysis. The sample (5-10 mg) was homogenized on ice with a 1:100 buffer solution (0.1 M potassium phosphate buffer pH 8.2, glutathione 10 mM, ATP .5 mM, $MgSO_4$ 5 mM, NaF 30 mM). The resulting homogenate was centrifuged at 4000xg for 10 minutes (4°C). Five ul of the supernatant was added to one ml of assay medium consisting of 50 mM (pH 8.2) glycylglycine buffer, 0.1 mM ATP, 1 mM F6P, 0.1 mM $MgCl_2$, 0.01% bovine serum albumin, 20 mM NADH, 14 mM cysteine, 0.08 U/ml aldolase, 0.08 U/ml triosphosphate isomerase, and 0.08 U/ml glycerophosphate dehydrogenase. The sample fluorescence was determined at 30 second intervals over a continuous five minute period and the greatest change in fluorescence over a one minute period was used to calculate PFK activity. The activity of PFK, expressed as $umoles \cdot gwwt^{-1} \cdot minute^{-1}$, was calculated as follows.

(Δfluorescence units/minute x umoles/fluorescence unit x DF)/2
DF = total dilution factor

The samples were assayed in duplicate and averaged. All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Hemoglobin Determination

General Principle of Assay

Hemoglobin concentration was determined using the cyanmethemoglobin procedure. This procedure involves the oxidation of hemoglobin to methemoglobin in the presence of potassium ferricyanide and subsequent formation of cyanmethemoglobin which can be determined by spectrophotometric[17] analysis at 540 nm.

Procedure

Blood samples which had been collected in heparinized syringes were used. A 20 ul sample of well mixed whole blood was added to five ml of Drabkin reagent[18] and vortexed. Reagent blanks (containing only Drabkins) and standard tubes (20 ul of a 20 $mg \cdot dl^{-1}$ standard) were also prepared. The tubes stood at room temperature for 30 minutes, were vortexed, then absorbance readings were determined. The alteration in sample absorbance is compared to the standard absorbance (and hemoglobin concentration) and a sample hemoglobin concentration was determined

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[17] Beckman DU-5 Spectrophotometer, Beckman Instruments Inc. Irvine, CA

[18] Diagnostic reagent kit (Drabkin), Sigma Chemical Co., St. Louis, MO

The samples were assayed in triplicate and averaged.

All samples from a specific subject were analyzed within the same sample batch to reduce interassay variance.

Packed Cell Volume Determination

Procedure

Heparinized blood was thoroughly mixed and samples were drawn by capillary action into microhematocrit tubes. The microhematocrit tubes were centrifuged[19] for 5 minutes at 11,000 rpm. The percentage of total fluid column length occupied by the red cell mass was determined (measured in millimeters) and used to estimate the percentage of the blood volume represented by red cell mass. The samples were assayed in triplicate and averaged.

Coefficients of Variation for Assays

Both intrassay (within assay) and interassay (between assays) coefficients of variation (CV) were calculated by the following equation;

CV = standard deviation/mean X 100.

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[19] Model MB, Micro-capillary centrifuge, International Equipment Company, Needham Hts. Mass.

In each assay batch, a standard was assayed in minimally in triplicate and an intrassay CV was determined for that batch using the repeated measures for the standard. A mean intrassay CV was generated by averaging the individual batch intrassay coefficients. An interassay CV was determined by using the individual assay standard means. In the PFK assay and packed cell volume determinations, experimental samples were replicated to produce a "standard". The CV for the assays used in this dissertation are shown in Table 1.