ACID-BASE REGULATION DURING SPRINT EXERCISE IN HORSES FED LECITHIN

by

Lynn Elizabeth Taylor

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Approved:

D. S. Kronfeld, Chairperson

N. E. Jack

L. A. Lawrence

J. W. Rankin

D. Sklan

J. H. Williams

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Animal and Poultry Sciences

(Abstract)

The effects of exercise, training, and a supplemental lecithin/corn oil diet on acid-base homeostasis and blood gases in arterial and central venous blood were examined during repeated sprint exercise in horses. Differences between temperature measurement sites for the adjustment of pH and blood gases during exercise was also investigated.

The independent variables, strong ion difference (SID), total weak electrolytes ($[A_{tot}]$), and pCO_2, had different effects on the dependent variables, $[H^+]$ and $[HCO_3^-]$, and these influences changed depending on blood sampling site (arterial or central venous), and exercise intensity. Data supporting the involvement of the chloride shift during repeated sprint exercise in the horse was observed for the first time.

Training resulted in increased plasma $[Na^+]$, $[K^+]$, [SID], albumin, free fatty acids, and beta-hydroxybutyrate concentrations, and decreased blood lactate ($[Lac^-]$), plasma $[Cl^-]$, $[H^+]$, cholesterol, and heart rate during exercise.

Horses consuming the corn oil/lecithin supplemented diet had a higher $pvCO_2$, $[HCO_3^-]$, $[Cl^-]$, cholesterol, and glucose, and lower blood $[Lac^-]$, $[H^+]$, and triglycerides during exercise. The sprint training and corn oil/lecithin diet may act synergistically to enhance performnace in horses by maintaining a lower $[H^+]$ during high intensity exercise.

There were differences between skin, rectal, blood, and muscle temperatures during incremental exercise and recovery in horses. The pH and blood gases adjusted to rectal, blood, and muscle temperatures were also different during exercise and recovery. Muscle and blood temperature may be predicted from rectal or skin temperature during exercise, and from skin temperature during recovery.

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General Introduction

Exercise-induced fatigue, which involves central (brain) and peripheral factors is complex. It may result from the accumulation of metabolic products, such as hydrogen ions (H^+), ammonia (NH_4), carbon dioxide (CO_2), and inorganic phosphate (P_i) or from reduced availability of substrates such as fat, glycogen and glucose, and depletion of phosphocreatine (PCr), and ATP (Maclaren et al., 1989). This study will focus on H^+ accumulation, which has several detrimental effects, including the alteration of activities of key glycolytic enzymes, and a direct negative effect on skeletal muscle contraction (Mainwood and Renaud, 1985).

The changes in body temperature during exercise must also be considered in the evaluation of blood gases and acid-base balance (Jones et al., 1989). Partial pressures of blood gases (pO_2 and pCO_2) and dissociation constants of water, bicarbonate ion, and plasma proteins will change with varying $[H^+]$ and temperature (Fedde, 1991). Rectal temperature may not be indicative of temperature changes in the central blood and muscle, which are the primary sites of focus for interpretation of metabolism during exercise.

Whole body acid-base balance must be maintained during exercise, and perturbations in $[H^+]$ are countered by the interaction of arterial and venous plasma, erythrocytes, and

skeletal muscle. A physicochemical approach (Stewart, 1981; 1983) to the evaluation of acid-base balance during exercise may be more appropriate than previous methodology which has focused on the role of bicarbonate (HCO_3^-) ions. Contributions of the independent variables, pCO_2, strong ion difference ([SID]), and total weak electrolytes ($[A_{tot}]$) to the changes in $[H^+]$ and $[HCO_3^-]$, can then be quantified (Heigenhauser et al., 1990).

Enhanced fat oxidation during exercise may also affect acid-base balance, as fat produces less CO_2 per ATP generated compared to glucose (Ferrannini, 1988). Increased utilization of fat may be beneficial in sparing blood glucose (Hargreaves et al., 1991), and muscle and liver glycogen (Rennie et al., 1976). Combining a fat-supplemented diet with training has been termed fat adaptation (Kronfeld and Downey, 1981), as these factors may have a synergistic effect on exercise metabolism (Simi et al., 1991).

Dietary lecithins are comprised mainly of phospholipids, such as phosphatidylserine and phosphatidylcholine, and may enhance exercise endurance capacity by altering fat transport and metabolism, and directly influencing muscle contraction (Jones et al., 1992; Sandage et al., 1992).

The plan of this research was:

1) to simultaneously examine changes in arterial and central venous blood gases, strong ions, $[H^+]$, and $[HCO_3^-]$

during repeated sprinting exercise in horses;

2) to determine if there is any evidence supporting a role for the chloride shift during exercise in the horse;

3) to examine the effects of sprint training and a lecithin/corn oil supplemented diet on arterial and central venous acid-base balance during repeated sprints;

4) to examine the effects of sprint training and a lecithin/corn oil supplemented diet on central venous blood glucose, and plasma free fatty acids, cholesterol, triglycerides, and beta-hydroxybutyrate during repeated sprints;

5) to examine the differences between rectal, blood, muscle, and skin temperature during incremental exercise in the horse, and how these differences influence adjustment of blood gases, pH, and $[HCO_3^-]$.

Review of Literature

Power sources during exercise

The conversion of chemical energy, which is stored in the phosphate bond of adenosine triphosphate (ATP), to kinetic energy and heat, drives the contraction of skeletal muscle during exercise (Sahlin, 1986). The demand for energy changes rapidly, and the processes for producing the energy required for muscular contraction are classified by the amount and rate of production of ATP, which must be continually resynthesized. The replenishment of ATP can be achieved by the creatine kinase reaction, the adenylate kinase (myokinase) reaction, glycolysis with production of lactate, complete oxidation of carbohydrates, and oxidation of free fatty acids (Sahlin, 1986; Fitts, 1992).

Creatine Kinase Reaction. With the onset of high intensity or supramaximal exercise of short duration, phosphocreatine (PCr) combines with adenosine diphosphate (ADP) to produce creatine and ATP, which is controlled by creatine kinase (CK). The CK reaction is crucial in controlling the ATP/ADP ratio, and provides metabolic capacitance, allowing changes in the peak rates of ATP synthesis during high and low energy demand (Sweeney, 1994).

The PCr stores in muscle may decline by 10% during the first 30 sec of exercise (Spriet et al., 1987), with a larger decrease seen in fast twitch muscle (Ivy et al., 1987). This disproportionate decline in muscle fibers probably reflects the three to four-fold higher ATP utilization rate in fast twitch fibers (Spriet, 1990). The decrease in intramuscular ATP and PCr has been observed in horses immediately after high intensity exercise (Valberg, 1987)

Adenylate Kinase Reaction. The other reaction that is important during brief, high-intensity exercise is the formation of ATP and adenosine monophosphate (AMP) from ADP, which is catalyzed by adenylate kinase (AK). This reaction, and the CK reaction are driven by the increased levels of ADP in muscle during short-term, intense exercise (Fitts, 1992). During sub-maximal exercise, the CK reaction limits the available ADP, thus lowering the activity of the AK reaction.

Glycolysis to Lactate. The formation of lactate from glucose-6-phosphate via glycolysis can also provide ATP for muscular contraction. Glucose-6-phosphate originates from glucose in blood or from stored glycogen and is converted to pyruvate by the glycolytic pathway, generating 2 nicotinamide adenine dinucleotide (NADH), which are oxidized during lactate formation, and 2 ATP per glucose (Stryer, 1988). The maximal power that can be generated from glycolysis to lactate is less

than that produced via the MK and AK reactions (Sahlin, 1986). Oxygen-limiting metabolism can raise muscle lactate production, but is not the sole cause for increased lactate concentration (Stainsby and Brooks, 1990). Lactate formation is possible under aerobic conditions, including rest, and is an important mechanism for tissues to share a carbon source for oxidation (Brooks, 1986).

Carbohydrate oxidation. Carbohydrate (CHO) is stored in the body primarily in the form of muscle and liver glycogen, and is available to sustain ATP production at moderate intensity work for as long as 90 minutes (Sahlin, 1986). The power generated by this process is only 20 - 25% of that supplied by anaerobic processes. Glucose is completely oxidized via glycolysis to pyruvate in the cytosol, which moves through the tricarboxylic acid (TCA) cycle and oxidative phophorylation inside the mitichondria, yielding 36 - 38 ATP per glucose (Stryer, 1988). Decreases in muscle glycogen of 25 - 30% (Essen-Gustavsson et al., 1991; Snow and Harris, 1991), and decreases in liver glycogen of 22 - 59% (Pagan et al., 1987) have been observed during exercise in horses.

Fat oxidation. Triacylglycerols (triglycerides, TG) are the primary storage form of free fatty acids (FFA), and are found in adipose and muscle tissue. The amount of stored FFA available for oxidation is not usually a limiting factor in

performance, but power output from this energy generating source is low, primarily due to a slow rate of delivery into the mitochondria (Sahlin, 1986). There appears to be a direct relationship between the plasma [FFA] and the rate of FFA oxidation (Hagenfeldt, 1975). An increase in plasma [FFA] during 50 minutes of sub-maximal exercise has been observed in horses (Essen-Gustavsson et al., 1991).

Diets for the Performance Horse

The primary sources of dietary energy for horses are carbohydrates and fats, since protein utilization is not considered efficient (Miller and Lawrence, 1988). The ratio of nutrients in the athletic diet can be altered in several ways, but there are four basic variations: 1) an increased amount of a maintenance diet; 2) diets which are energy and nutrient dense (more energy/unit of weight); 3) diets enriched for work only; and 4) diets enriched for work and stress (Kronfeld and Ferrante, 1992). Energy demand is the nutritional factor most influenced by exercise. Increasing the amount of a basal ration while keeping the nutrient content proprtional will provide adequate energy as a suitable diet in horses, but this strategy has limitations. A certain amount of roughage is required to maintain normal digestive function (Meyer, 1987), and this requirement may limit the application of low fiber, nutrient dense diets, especially for

long distance events. Diets enriched for work may be high in either carbohydrates or fats, the primary fuel sources.

Carbohydrate loading. Carbohydrate loading is the combination of training and a special diet designed to initially deplete then supercompensate glycogen storage just prior to an athletic event (Bergstrom et al., 1967). Glycogen sparing and glucose repletion during athletic events in humans can be achieved by ingestion of carbohydrates during and after exercise (Costill, 1985), and glycogen repletion after moderate exercise in horses may be effective (Brewster-Barnes et al., 1994). The muscle glycogen content in horses at rest is higher than in humans (Harris et al., 1974; Essen-Gustavsson et al., 1989; Julen et al., 1994), but the effects of diet, training, and exercise have been inconsistent, and carbohydrate loading has not been demonstrated effective in increasing exercise performance of horses (Frape, 1994).

Fat adaptation. Feeding high fat diets to horses may offer some advantages over more conventional diets. Carbohydrate loading is in effect preferred in all athletic horses from the replacement of forages with grain concentrates. An increased risk of laminitis and digestive disturbances is associated with feeding large amounts of carbohydrates (Clarke et al., 1990; Sprouse et al., 1987). Although some studies have shown slight decreases in dry matter and protein digestibilty by

horses upon inclusion of 6 - 20 % fat in the diet, the benefits for maintenance, growth, and exercise have been well documented (Potter et al., 1992). Fat adaptation is the combination of training and simulataneous consumption of a fat supplemented diet (Kronfeld and Downey, 1981; Miller et al., 1984; Simi et al., 1991). The primary advantage to fat adaptation is an increased reliance on fat oxidation for energy during exercise (Holloszy et al., 1986), which can be demonstrated by training, high fat diets, or the combination of both. After 12 weeks of training, muscle glycogen utilization was 41% lower, while muscle triglyceride usage doubled in men exercising at 64% VO_2max (Hurley et al., 1986).

Elevation of plasma [FFA] in earlier studies resulted in decreased carbohydrate utilization and muscle glycogen sparing during exercise in rats (Rennie et al., 1976; Hickson et al., 1977) and humans (Costill et al., 1977). Although some studies have failed to show these effects (Auclair et al., 1988; Hargreaves et al., 1991), the discrepencies may be the result of differences in exercise intensity, and the methods used to raise the plasma [FFA] (Vukovitch et al., 1993).

Feeding a high fat meal prior to exercise at 65% of maximal oxygen consumption (VO_2max) lowered respiratory exchange ratio, and increased FFA uptake by the leg in human subjects (Jansson and Kaijser, 1982). Consumption of a high fat diet for either 1 or 5 weeks in rats decreased glycogen breakdown during exercise, and increased B-oxidation enzyme activities.

The high fat diet also significantly prolonged a run to exhaustion when compared to rats consuming a normal diet, despite lower muscle and liver glycogen concentrations in the rats consuming the high fat diet (Miller et al., 1984). Increased B-oxidative enzyme activity was observed in rats consuming a high fat diet (Nemeth et al., 1992), and this was coupled with a decrease in phosphofructokinase (PFK) activity, a key glycolytic enzyme. The infusion of a fat emulsion or a fat feeding to raise FFA levels spared muscle glycogen in men during cycling at 70% VO_2max (Vukovitch et al., 1993)

Enhanced exercise capacity was observed in rats due to the additive effects of a high fat diet combined with endurance training using a 2 (high fat or normal diet) by 2 (sedentary or trained) factorial experiment (Simi et al., 1991). After consumption of a high fat diet and training for 12 weeks, rats had a greater endurance capacity, increased VO_2max, and a higher oxidative capacity of red skeletal muscle. The individual effects of training and diet were synergistic in resulting in superior performance in these rats as compared to all other groups. Plasma [FFA] and VO_2max were higher in trained runners consuming a high fat diet as compared to runners consuming normal, or high protein diets (Muoio et al., 1994).

A recent investigation suggested a role for albumin in enhanced exercise performance because it transports fatty acids to the muscle (McClelland et al., 1994). In vivo

studies showed that, although plasma albumin concentrations were similar between trained goats (sedentary species) and trained dogs (aerobic species), the dogs always had higher plasma [FFA] at 40, 60, and 85% VO_2max. This was attributed to a 50% increased "loading" of FFA by canine albumin, which was confirmed in vitro.

Studies conducted on horses have revealed conflicting results. A glucose-sparing effect has been observed in horses during long term, aerobic exercise (Hintz et al., 1978; Hambleton et al., 1980), with no differences in performance. Investigators have reported increased (Hambleton et al., 1980; Scott et al., 1992), decreased (Pagan et al., 1987) or no difference (Hintz et al., 1978) in resting muscle glycogen. If increases in muscle glycogen are found with the consumption of a fat supplemented diet, these differences have been lost at the end of exercise, suggesting greater utilization of muscle glycogen during exercise (Oldham et al., 1990; Scott et al., 1992).

Lecithins

Origin. Lecithin is the trivial scientific name for the phospholipid phosphatidylcholine (PtdCh), but in the food industry, lecithin refers to a mixture of polar and neutral lipids, which usually contain other phosphatides in addition to PtdCh (Wurtman, 1979). There are many sources of lecithin

in the diet, but the primary sources are soybeans, eggs, liver, and peanuts, with commercial lecithins utilized for farm and lab animals originating primarily from soybeans, rapeseed, and corn. Lecithin from soybeans is preferred as a dietary source, due to the high level of polyunsaturated fatty acids, and PtdCh (Bonacker, 1988).

The soybeans are cleaned and flaked, and soaked in solvent before filtration and distillation, which results in an oil containing 2-3% crude lecithin. The lecithin is then precipitated and separated to yield an oil consisting of 60-70% phospholipid, of which 30% can be PtdCh, and 30% soybean oil. Common commercial grades of lecithins also contain considerable amounts of palmitic, stearic, oleic, linoleic, and linolenic acids.

Phospholipids are hydrolyzed in the intestinal lumen by phospholipases to yield FFA, diglycerides, phosphatidic acid, and choline, which can be utilized for lipid and carbohydrate synthesis, or be metabolized further (Roberts and Dennis, 1989).

Animal diets. Studies on the effects of addition of lecithin to animal diets have been mixed, but most show improvement in the digestibility of other fats upon the inclusion of lecithin, especially fats that are relatively poorly digested, such as long chain, highly saturated fats. The digestibility of tallow was improved with the addition of

lecithin to the diet in pigs (Jones et al., 1992), and chicks (Polin, 1980). Feeding lecithin to ram lambs increased the content of polyunsaturated fatty acids in the carcass, which may be more desirable to consumers (Lough et al., 1992). Milk fat percentage and milk fat yield were higher in cows when abomasally infused with soy lecithin compared to water (Grummer et al., 1987).

Lipid metabolism. Lecithins have been of interest in model studies for humans because of their ability to alter lipid metabolism. Rats fed a corn oil diet supplemented with soy lecithin had a significant lowering of plasma cholesterol when compared to phosphatidylinositol (PI) supplementation (Ishida et al., 1988). This effect was attributed to the phosphatidylethanolamine (PtdE), which reduces hepatic cholesterol secretion, and increases uptake of high density lipoproteins by the liver.

Membrane function. The PtdCh found in lecithins is important in human nutrition because choline is required in many animal and human diets (Zeisel and Canty, 1993). Choline phospholipids found in cell membranes play an imporatant role as second messengers in many cascade reactions, and may elicit protective effects against hepatic cancers (Newberne and Rogers, 1986). In cholinergic neurons in the brain, lecithin serves as a structural support and a reservoir for sythesis of

the neurotransmitter, acetylcholine (Ach), and this reservoir and synthesis can be enhanced by increased dietary choline (Wurtman et al., 1990).

Exercise performance. Early studies on choline and human athletic performance revealed that exercise caused a decrease in plasma choline, but this decrease was avoided by the consumption of either a choline-, or a lecithin-enriched meal (Hirsch et al., 1978). The decrease in plasma choline was also observed recently in marathon runners (Conlay et al., 1992), and could contribute to poor performance if Ach release is impaired. Triathletes who took a lecithin supplement prior to exercise had increased plasma choline during exercise, as compared to controls given a placebo (von Allworden et al., 1993). The administration of choline citrate to long distance runners significantly increased plasma choline, and decreased the mean run time during a race when compared to controls (Sandage et al., 1992).

Regulation of Metabolism

Carbohydrate oxidation. The inputs to glycolysis all converge at glucose-6-phosphate (G6P). Glycogen is split by phosphorylase to yield G6P. Glucose is phosphorylated to G6P by hexokinase, which is regulated by feedback inhibition. The next step is the conversion of G6P to fructose-6-phosphate

(F6P), and then F6P is converted to fructose 1,6-diphosphate, which is controlled by phosphofructokinase (PFK). This step is irreversible, and depends on substrate availabilty. The activity of PFK may have a critical role in coupling glycolysis to the rate of lactate accumulation, the cytosolic phosphorylation state (ATP/ADP ratio), and TcA cycle activation state via inhibition by citrate (Stanley and Connett, 1991).

Skeletal muscle contraction increases the rate of glucose transport into muscle fibers, and hepatic glucose production is controlled by the activities of glucagon and insulin, stimulation by epinephrine and norepinephrine, and an increase in the release and capacity to synthesize glucose by cortisol (Stanley and Connett, 1991). Glucose is transported into muscle primarily by the insulin-regulated GLUT-4 transporter (James et al., 1988), and treadmill exercise in rats increased the number of glucose transporters, and glucose uptake in hindlimb muscle (Douen et al., 1989).

Glycogen breakdown in muscle is controlled by the activity od glycogen phosphorylase, which has an active (a) form and an inactive (b) form. Activation of phosphorylase is controlled by phosphorylase kinase, which in turn is activated by Ca^{++}, high pH levels, and beta-adrenergic receptor stimulation (Brostrom et al., 1971). Glycogen is the main supplier of substrate during early and short term exercise, and can be maintained via epinephrine release (Drummond et al., 1969).

During reductions in muscle glycogen storage due to diet or prolonged exercise, blood glucose will contribute more to the substrate pool (Issekutz, 1984).

Fat oxidation. Free fatty acids (FFA) derived from triglyceride (TG) hydrolysis in adipose tissue are the major source of energy during exercise, and their release is stimulated by homone-sensitive lipase (HSL), and down-regulated by insulin. The activity of HSL is mediated through cylic AMP-dependent protein kinase. There is evidence suggesting a role for single signal control of HSL and lipoprotein lipase (LPL), which would function as a unit in muscle and the capillary beds to meet energy demands (Oscai et al., 1990). There are considerable reductions in intramuscular TG during prolonged exercise, and it is possible that these stores are replenished by plasma TG, under the control of LPL (Blanchette-Mackie et al., 1986).

Fatty acids are activated on the outer mitochondrial membrane in muscle by the formation of CoA esters by acyl CoA synthetase. Long chain activated fatty acids are carried into the mitchondria by carnitine, after formation of acyl carnitine esters by carnitine acetyltransferase I. The saturated acyl CoA is degraded by a repeated, four step procedure: 1) oxidation by flavin adenine dinucleotide (FAD); 2) hydration; 3) oxidation by NAD^+; and 4) thiolysis by CoA. The fatty acid chain is shortened by 2 carbon units at a time,

generating $FADH_2$, NADH, and acetyl CoA units, and this process is termed beta-oxidation (Foster, 1984). Acetylcarnitine can serve as a storage form of acetyl groups, and muscle acetylcarnitine is increased during brief, high intensity exercise in humans (Sahlin, 1990), and horses (Carlin et al., 1990).

Integration. The balance between carbohydrate and lipid utilization during exercise in healthy, fed individuals is under various regulatory influences, and changes to meet increasing, or decreasing energy needs. This balance can be altered by substrate input, enzymatic feedback and regulation, diet, and training status. Training promotes lipid oxidation during moderate and mild intensity exercise, but increasing exercise intensity demands higher rates of glycogenolysis. Substrate utilization at any point depends on the interaction between exercise intensity-induced reponses, and exercise training-induced reponses, and a "crossover point" will be encountered (Brooks and Mercier, 1994). This point is the power output at which energy from CHO-derived fuels predominates over energy derived from lipid sources.

The TCA cycle is the entry point for acetyl units, whether derived from carbohydrate or fat sources. Pyruvate dehydrogenase (PDH) is a multiple enzyme and important regulator of the balance of preferential fuel utilization (Randle, 1986; Jones and Heigenhauser, 1992). Increased

ratios of acetyl CoA/CoA and $NADH/NAD^+$ will inactivate the activity of the PDH complex by activating PDH kinase (Wieland, 1983). Increased pyruvate and ADP inhibit PDH kinase, and increased intracellular Ca^{2+} and Mg^{2+} activate PDH phosphatase, which increases the complex activity.

Evaluation of Acid-base Balance

Conventional approach. Selective membrane electrodes for measuring pO_2 and pCO_2 as well as pH became available commercially in the early 1960's. Since that time, the most common approach to acid-base evaluation in physiology and medecine has emphasized the bicarbonate buffer system, as described by the Henderson-Hasselbach equation:

$$pH = pK'a + \log ([HCO_3^-]/[\alpha pCO_2])$$

where pK'a is the apparent dissociation constant of the carbonic acid-bicarbonate system, and α = the solubiblity coefficient for CO_2 in plasma. The pK'a for normal plasma is 6.1, and α = 0.0301 mmol/L/mm Hg in plasma at normal body temperature (Severinghaus, 1971). This equation can be used to calculate $[HCO_3^-]$ after direct measurement of pCO_2 and pH ($[H^+]$), but it does not describe how changes in other variables, such as water shifts, strong ions (sodium, potassium, magnesium, calcium, chloride, lactate) and plasma

proteins, affect acid-base balance. This is especially important in the horse because of the significant water, protein, and ion losses in sweat during certain types of exercise (Kerr and Snow, 1983; Andrews et al., 1994; Ecker and Lindinger, 1994). The Henderson-Hasselbach equation is misleading because it fails to identify independent and dependent variables, and incorrectly implies that $[H^+]$ is partly determined by $[HCO_3^-]$ (Reeves, 1983).

Physicochemical approach. Another more comprehensive approach to acid-base evaluation was reintroduced by Stewart (1981, 1983), which involved the six 'bicarbonate equations' and rules originally taught at Tufts University in Boston (Schwartz and Relman, 1963). The $[H^+]$ and $[HCO_3^-]$ are dependent variables, and are determined by the independent variables, pCO_2, strong ion difference ([SID]), and total weak electrolyte concentration ($[A_{tot}]$). Strong ions are fully dissociated in aqueous solutions, and the [SID] is the sum of the strong cations minus the sum of the strong anions. The six equations which summarize this approach must account for the maintenance of dissociation equilibria, the maintenance of electrical neutrality, and the conservation of mass.

1) The dissociation of water into its component ions, can be described by the law of mass action at equilibrium where K′ w is the ion product of water:

$$[H^+] \times [OH^-] = K'_w$$

2) Solutions containing strong (fully dissociated) ions must maintain electrical neutrality, and can be described by the [SID], which is an independent variable:

$$[SID] + [H^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] = 0$$

3) Weak electrolytes are only partly dissociated in solution, and the equilibrium of the dissociated ($[H^+]$, $[A^-]$) and undissociated ([HA]) species must be maintained:

$$[H^+] \times [A^-] = K_A \times [HA]$$

4) The changes in the total concentration of weak electrolytes ($[A_{tot}]$) occurs slowly, but must also be considered. The $[A_{tot}]$ is an independent variable, because it only changes by mass transfer of dissociated or undissociated species into, or out of solution, and the conservation of mass must be maintained:

$$[HA] + [A^-] = [A_{tot}]$$

5) Plasma pCO_2 is maintained primarily by the lungs, making it an open system, and an independent variable. The

CO_2 in body fluids is present as dissolved CO_2 (dCO_2), carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbonate ion (CO_3^{2-}). The first equation describing this system is the formation of bicarbonate ion, where K_c is the dissociation constant:

$$[H^+] \times [HCO_3^-] = K_c \times pCO_2$$

6) The $[HCO_3^-]$ further dissociates, where K_3 is the dissociation constant:

$$[H^+] \times [CO_3^{2-}] = K_3 \times [HCO_3^-]$$

These six equations summarize the physicochemical principles of water, solutions of strong ions, weak acids, and CO_2, and the laws of electrical neutrality, conservation of mass, and maintenance of equilibria. Solving these equations simultaneously, one equation can be obtained with three independent variables ([SID], $[A_{tot}]$, pCO_2) that can be measured directly, four dissociation constants, and one unknown, dependent variable, $[H^+]$:

$$[H^+]^4 + ([SID] + K_a) \times [H^+]^3 + (K_a \times ([SID] - [A_{tot}]) - ((K_c \times pCO_2) + K' w)) \times [H^+]^2 - (K_a ((K_c \times pCO_2) + K' w) - (K_3 \times K_c \times pCO_2)) \times [H^+] - K_a \times K_3 \times K_c \times pCO_2 = 0$$

Comparison. The Henderson-Hasselbach equation is physicochemically correct, but any physiological interpretation that implies that [HCO_3^-] partly determines [H^+] is incorrect. Also, the Henderson-Hasselbach equation neglects the effects of [SID] and [A_{tot}] on [H^+] (Ferrante and Kronfeld, 1994; Kronfeld et al., 1995). It may also overlook important changes in [SID] that are due to dilutional and concentration effects of water shifts (Leith, 1990; Fencl and Rossing, 1989).

The relevant strong ions in the body may be Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, Lactate (Lac^-), and phosphate, depending on the site of evaluation (plasma, erythrocyte, muscle). An initial increase in [SID] means that the [cations] exceeds the [anions]. The body will respond by decreasing the positive ions (H^+), and increasing the negative ions (HCO_3^-) to maintain electrical neutrality.

The important components of [A_{tot}] in the body are the plasma proteins, albumin and globulin, and phosphate. Phosphate is more important in clinical cases such as renal failure, but comprises only 5% of normal plasma [A_{tot}] (de Morais, 1992). An increase in [A_{tot}] results in an increase in the dissociated anion, A^-, to conserve mass. To maintain electrical neutrality, the body responds by increasing [H^+], and decreasing [HCO_3^-].

The physicochemical approach has limitations, the primary one being the problems in directly quantifying [A_{tot}] and the

equilibrium constants (Cameron, 1989). Total weak acid is difficult to measure, so plasma albumin or total protein are used to estimate [A_{tot}], because protein is the primary weak acid in blood plasma (Figge et al., 1991). The numerous equilibrium 'constants' of weak acids will change, because they are dependent on osmolarity, temperature, and the concentration of other ions (Edsall and Wyman, 1958).

The primary advantage of the physicochemical approach is the ability to identify independent and dependent variables, thereby enhancing the understanding of interactions between plasma, red cells, and muscle cells. The movement of H^+ and HCO_3^- between compartments does not directly affect the [H^+] and [HCO_3^-]: these change according to changes in the independent variables (Weinstein et al., 1991). The differentiation between the two types of variables also means that the contributions of each independent variable to the changes in [H^+] and [HCO_3^-] can be quantified (Heigenhauser et al., 1990; Lindinger et al., 1992). Studies have confirmed the practical use of the physicochemical method by comparing measured vs. calculated values of [H^+], and by partitioning the contributions of each independent variable to the changes in [H^+] and [HCO_3^-] in dogs (Stainsby and Eitzman, 1988; Pieschl et al., 1992), rats (Lindinger and Heigenhauser, 1988; Lindinger and Heigenhauser, 1990), humans (Weinstein et al., 1991), and horses (Ferrante and Kronfeld, 1994). This approach has also been advocated for acid-base evaluation in

animals in the veterinary clinical setting (Eicker, 1990; Kohn, 1990; Leith, 1990; Geiser et al., 1994; Whitehair et al., 1995).

Another advantage to the physicochemical approach is the use of proper terminology (Reeves, 1983). The classical approach describes four basic types of disturbances: metabolic acidosis and alkalosis, which refer to a primary decrease or increase, respectively, in $[HCO_3^-]$; and respiratory acidosis and alkalosis, which refer to a primary increase or decrease, respectively, in $paCO_2$ (Harrington et al., 1982). This terminology incorrectly implies that the changes in $[HCO_3^-]$ are strictly 'metabolic', and are not affected by changes in pCO_2]. The independent variable, [SID], is a measure of metabolic status because it is not affected by changes in pCO_2. The pCO_2 is not just a respiratory measurement. While arterial pCO_2 reflects the adequacy of alveolar ventilation, CO_2 production by working muscle is more clearly manifested in venous pCO_2.

Changes in Blood Gases During Exercise in the Horse

$\underline{pCO_2}$. Many of the changes in blood gases observed during exercise in the horse are unique to the species, such as larger initial decreases in $paCO_2$ during mild and moderate exercise in ponies compared to humans (Forster and Pan, 1994). This may be due to a decreased response of chemoreceptors to

match alveolar ventilation to CO_2 production in the equine, or ventilatory drive and metabolic rate may be more closely matched in humans (Hodgson et al., 1990). Other studies have observed a marked hypoventilation and hypercapnia (increased $paCO_2$) at maximal exercise in horses, which does not occur in humans (Landgren et al., 1991), and may be due to ventilation-perfusion inequalities or limitations (Bayly et al., 1983). There is evidence that the breathing and stride frequency cycles are closely related in the exercising horse, but certain types of exercise will cause decoupling (Landgren and Gillespie, 1989; Evans et al., 1994).

Increases in venous plasma CO_2 result from diffusion from working muscle. Due to the lack of carbonic anhydrase in plasma, the CO_2 moves quickly into the erythrocyte, where it is hydrated to HCO_3^- and H^+. The H^+ combines with Hb, and the HCO_3^- moves out of the red cell in exchange for plasma Cl^-; this 'chloride shift' is reversed in arterial blood at the lung (Molony and Jacobson, 1986). This mechanism facilitates the transport of CO_2, and has been implicated in the exercising horse, but there are few studies which have simultaneously evaluated arterial and central venous acid-base balance during exercise (Carlson et al., 1992).

$\underline{pO_2}$. Increased oxygen demands are met in the horse through several mechanisms. Cardiac output can increase dramatically in the horse, due primarily to a seven fold increase in heart

rate at maximal exercise (Taylor et al., 1987). Pulmonary diffusing capacity and alveolar-capillary pO_2 difference were larger in racehorses compared to steers, resulting in a greater oxygen uptake, and adaptations in muscle capillary and mitochondrial density could contribute to high levels of muscle oxygen consumption (Constantinopol et al., 1989).

Changes in maximal oxygen uptake (VO_2max) in ponies resulted from an increased hemoglobin concentration ([Hb]) due to splenic contraction, and differences in oxygen saturation as compared to other species (Wagner, 1994). The increased [Hb] can augment VO_2max by 30% in the horse, but the large increases in temperature and $[H^+]$ cause oxygen desaturation. The diverging ventilatory response (increased $paCO_2$ and decreased paO_2) in ponies resulted in peak oxygen transport only at submaximal exercise, as compared to maximal exercise in humans (Wagner, 1995).

Muscle Fatigue and $[H^+]$

Fatigue has been defined as a failure to maintain the expected or required power output, and requires the maintenance of both force and velocity (Edwards, 1983). The accumulation of $[H^+]$ in muscle has often been implicated as the primary causative factor of fatigue, but this may not be a direct effect, and fatigue may have more than one component (Mainwood and Renaud, 1984). A combination of variables could

be responsible for muscle fatigue, as shown by studies reporting no correlation between $[H^+]$ and changes in force production (Vollestad and Sejersted, 1988; Wilson et al., 1988). Although the second phase of force recovery has been significantly correlated with $[H^+]$, the initial phase of recovery is independent of $[H^+]$ (Metzger and Fitts, 1987).

Muscle fatigue probably results from both central and peripheral events, such as changes in neural conduction, or alteration of other metabolites, such as phosphate and calcium, which may be mediated through $[H^+]$ (Luckin et al., 1991). Changes in $[H^+]$ itself are directly related to the hydrophilic amino acid, histidine, which contains an imidazole group that can reversibly bind protons over the physiological pH range (Jennings, 1993). Supplying protons to enzymes that contain histidine imidazole groups will promote binding or buffering, change the net charge, affect conformation, and alter the function of enzymes (Reeves, 1985).

Increased $[H^+]$ decreases the developed force in cardiac muscle by interfering with the delivery of calcium ions (Ca^{++}) to the myofilaments, and/or the response of the myofilaments to the Ca^{++}, but the rate and magnitude of the responses depend on the origin of the acidosis (Orchard and Kentish, 1990). The skeletal muscle sarcoplasmic reticulum (SR) function can be altered in vitro at high $[H^+]$, resulting in reduced rates of calcium uptake and release (Nakamura and Schwartz, 1972). The rate and peak capacity of equine skeletal muscle SR to

take up Ca^{++} was depressed after maximal exercise to fatigue (Wilson et al., 1988; Byrd et al., 1989). The depressed uptake of Ca^{++} was correlated to a decrease in the activity of Ca^{++}-Mg^{++} stimulated ATPase activity, which may be related to both the muscle $[H^+]$ accumulation, and the large increase in muscle temperature.

Accumulation of $[H^+]$ in skeletal muscle during exercise will differ depending on the rate of hydrolysis of PCr, which utilizes protons (Mainwood et al., 1987), and the buffering capacity of the muscle. Horses may be able to tolerate the rise in $[H^+]$ associated with high intensity exercise better than other species by exploiting these variables. Muscle PCr was significantly reduced, and creatine was increased in Thoroughbred horses during short-term, maximal and supramaximal exercise (Rose et al., 1988). These responses were also observed in Thoroughbreds during the first of four, 600 meter maximal exercise bouts (Hodgson et al., 1987). Equine muscle tissue had the highest content of histidine dipeptides when compared to dogs and humans, which resulted in the greatest muscle buffering capacity (Harris et al., 1990).

Temperature Regulation During Exercise

Control of body temperature. Body temperature is a measure of heat content of the body, and homeothermic animals,

including mammals, maintain a body temperature that is primarily independent of the environmental temperature (Hardy, 1961). However, mechanisms regulating body temperature monitor the surroundings, and adjust the heat exchange to protect the narrow range within which body temperature is maintained (Stitt, 1993). The increase in body temperature that occurs with exercise is caused by the increase in internal heat production from the contracting muscle mass.

The exact mechanism responsible for the interaction between the heat produced, and the control of body temperature during exercise is not clear, but there are several theories. Input to the central nervous system (CNS) from cutaneous and muscle receptors, and CNS tissue ion alterations may play a major role (Myers, 1969). Changes in skin temperature in ponies were significantly correlated with breathing frequency, suggesting a role for cutaneous thermoreceptors in the horse (Kaminski et al., 1985). Depending upon environmental conditions, contributions of evaporative, conductive, convective, and radiative cooling will vary. Evaporation is the most efficient means of heat loss during exercise, and may be the only way to dissipate heat in hot environments (Astrand and Rodahl, 1970). The amount of heat produced by the exercising horse varies with intensity and duration, but 2,000 to 4,000 kcal of generated heat has been observed in Thoroughbred horses (Hodgson et al., 1993), and up to 20% of this remained as stored heat 30 minutes after exercise.

Thermoregulation was impaired in Thoroughbred horses primarily due to decreased transfer of heat from the core to the periphery (Naylor et al., 1993).

Temperature changes will vary with site of measurement, but there is debate concerning which site is most appropriate. Brain, or hypothalamic temperature is extremely sensitive to local temperature changes, but is a highly invasive measurement, and its regulation may be divergent from the control of body temperature (Baker and Hayward, 1967). Some researchers use tympanic temperature, but this may be affected by blood temperature returning from the surface of the head and face (Jessen and Kuhnen, 1992). Rectal or esophageal temperatures are used in human studies, with a measurement of blood leaving the heart (aortic temperature) considered more appropriate, but rarely justifiable (Neilsen and Neilsen, 1962).

Sites of measurement during exercise in the horse have included the rectum, superficial thoracic vein, carotid artery, pulmonary artery, and middle gluteal muscle (Pan et al., 1986; Bayly et al., 1989; Jones et al., 1989; Hodgson et al., 1993). Trained horses show reduced increases in pulmonary arterial temperature during exercise (Sexton et al., 1985), and in resting middle gluteal muscle temperature (Wilson et al., 1995). This may be due to improved heat loss through respiratory evaporation, and increased shunting of blood to the periphery.

Fluid Homeostasis. Water comprises a significant portion of the body, and the depletion of body water will adversely affect exercise performance. Water can be redistributed within the fluid-containing spaces, and this reservoir will minimize the effects of a water deficit (Sawka and Pandolf, 1990). Horses adapted to desert environments withstood 72 hours of dehydration (12% reduction in body mass) with no ill effects, primarily by limiting fluid loss from plasma at the expense of interstitial fluid volume, and intracellular or transcellular fluid (Sneddon, 1991). Another study revealed that the protection of plasma volume during dehydration and rehydration was aided by the maintenance of intravascular protein levels (Sneddon et al., 1992). Dehydration in horses increased plasma vasopressin and osmolality, and was accompanied by slight decreases in plasma aldosterone, while rehydration significantly increased aldosterone (Sneddon et al., 1993). The increased aldosterone upon rehydration was associated with an isotonic absorption of fluid across the gut epithelium.

Sweating Responses. In humans, sweating rate can change with alterations in ambient temperature, dew point temperature, radient heat load, air velocity, clothing, and activity level (Shapiro et al., 1982). An average sweating rate during military activity in the desert was 4.1 L every 24

hours, but can be as high as 3.7 L per hour, as was reported during an Olympic Marathon (Armstrong et al., 1986). Eccrine sweat glands are invovled in thermoregulation in humans, and are stimulated transiently by prostaglandins, but primarily by acetylcholine in the presence of Ca^{++} (Sato and Sato, 1981).

Exercise-induced sweating in the horse involves circulating epinephrine and sympathetic nervous activity, and equine sweat glands are follicle-associated, thus resembling apocrine glands in other species (Montgomery et al., 1982). Sweating rates in horses may approach 10 to 12 L per hour during prolonged exercise in hot environments (Carlson and Ocen, 1979). Perturbations in fluid and electrolyte balance can contribute to heat stroke, exhausted horse syndrome, and rhabdomyolysis (Carlson, 1985). Horses trained and raced in tropical climates may devleop partial, or complete anhidrosis, which is a decrease in sweat production, probably caused by prolonged overstimulation of the sweat glands (Jenkinson et al., 1982).

Control of sweat secretion and regulation of sweat content in the horse is not fully understood, but the electrolyte compostion is probably modified by secretory or resorptive processess within the sweat duct (Kerr and Snow, 1983). In contrast to humans, equine sweat is hypertonic to plasma for sodium, potassium, chloride, and protein. However, diet, sweat collection site, and means of induction of sweating will all contribute to the variation in sweat composition (Snow et

al., 1982; Kerr and Snow, 1983).

Temperature correction factors. Exercise-induced increases in body temperature alone may cause significant changes in blood gases and acid-base status. When blood temperature is increased, $[H^+]$, pCO_2, and O_2 release to tissues is increased, but O_2 affinity is decreased (Adams anf Hahn, 1982). The changes in $[H^+]$ are related to the hydrophilic amino acid, histidine, which contains an imidazole group that can reversibly bind protons over the physiological pH range (Jennings, 1993). Supplying protons to enzymes that contain histidine imidazole groups will promote binding or buffering, change the net charge, affect conformation, and alter the function of the enzyme (Reeves, 1985). This supply is regulated through ventilatory control of pCO_2, which will alter the $[H^+]$ in relation to the dissociation constants of protein so that during changes in body temperature their integrity, and hence, function, is protected - the 'alphastat hypothesis' of acid-base regulation (Reeves, 1972).

Hyperthermia in most mammals will increase oxygen release from blood, but it reduces blood oxygenation. Emptying of the splenic reserve in the horse increases the oxygen-carrying capacity, and equine hemoglobin shows a higher oxygen affinity than humans (Clerbaux et al., 1986). A lower P_{50} was observed in Thoroughbred horses when compared to humans and the Hanovarian breed of horse, indicating that the Thoroughbred

hemoglobin became 50% saturated at a lower pO_2 (Smale et al., 1994). Therefore, some horses could be incorrectly interpreted as being hypoxaemic when the actual percent of oxygen saturation is adequate.

Increases in $[H^+]$ alone will lower the oxygen saturation of blood - the Bohr effect. The acid Bohr effect is the uptake of protons on oxygenation below pH 6.0, and the alkaline Bohr effect is the physiologically important release of protons above pH 6.0 on oxygenation (Kilmartin, 1971). These pH and temperature-dependent changes in acid-base status require that blood gases and $[H^+]$ be corrected for the increases in temperature that are associated with exercise. Central blood, or muscle temperatures are the preferred sites for correction purposes in horses (Jones et al., 1989), but rectal temperature may be sufficient during short-term, low intensity exercise (Pan et al., 1986).

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Journal Article 1. Arterial and central venous acid-base status and strong ion difference during repeated sprints.

Abstract

Seven horses performed 6 1-min sprints separated by 4-min intervals at a walk, followed by a 30-min recovery period. Blood samples were taken at rest, after a sub-maximal warm up, during the last 15 sec of sprints 5 and 6, and at 5 and 30 min of recovery from the carotid artery (A) and right heart (central venous, V) to evaluate changes in blood gases and strong ions. At both sites, plasma $[H^+]$, pCO_2, pO_2, albumin ([Alb]), and strong ion concentrations ($[Na^+]$, $[K^+]$, $[Cl^-]$) were measured, and hematocrit (Hct), and blood lactate ($[Lac^-]$), and hemoglobin concentrations ([Hb]) were measured. Strong ion difference ([SID]) and bicarbonate concentration ($[HCO_3^-]$) were calculated, and [Alb] was used as an estimate of total weak acid $[A_{tot}]$. Between sites (A vs V) there were differences in $[H^+]$, pCO_2, $[HCO_3^-]$, $[Cl^-]$, [SID], $[Na^+]$, $[K^+]$, and $[Lac^-]$. During exercise, $[Cl^-]$ increased at A and decreased at V, while $[H^+]$, pCO_2, and $[HCO_3^-]$ decreased at A and increased at V. With exercise, $[Na^+]$, $[K^+]$, and $[Lac^-]$ increased at both sites, with $[Na^+]_v$ and $[K^+]_v$, and $[Lac^-]_a$ increasing to a greater extent.

Plasma $[SID]_a$ decreased due to a greater increase in $[Lac^-]_a$ compared to other strong ions, and $[SID]_v$ increased due to increased $[Na^+]_v$ and $[K^+]_v$, and decreased $[Cl^-]_v$. Plasma [Alb], and blood [Hb] and Hct increased with exercise, with no site differences. Results show that SID, $[A_{tot}]$, and pCO_2 have different effects on plasma $[H^+]$ and $[HCO_3^-]$ at sites A and V, and that the chloride shift is evident in the exercising horse.

horses; blood gases; strong ions; chloride shift;

Introduction

Exercise is associated with perturbations in acid-base balance that contribute to fatigue (Mainwood and Renaud, 1984). Conventional assessment of acid-base status during maximal exercise in the horse has included emphasized the bicarbonate buffer system, and has yielded inconsistent results (Harris and Snow, 1988; Cohen et al., 1993; Tate et al., 1993) and little information available on repeated, periodic maximal exercise (Geiser et al., 1992). Arterial blood samples must be obtained for interpretation of cardiopulmonary function, while a mixed central venous sample reflects muscle metabolism during exercise (Carlson, 1987).

A comprehensive physicochemical evaluation of acid-base status during exercise should include determination of the measurement of [SID], and [A_{tot}], in addition to blood gases, [H^+], and [HCO_3^-] (Stewart 1981, 1983). Strong ions are completely dissociated in aqueous solutions, and the [SID] is the difference between the sum of all the strong cations and anions. The independent variables, pCO_2, [SID], and [A_{tot}] affect the dependent variables [H^+] and [HCO_3^-] in order to maintain electrical neutrality, conserve mass, and satisfy dissociation equilibria (Stewart, 1981; 1983). Examining muscle, plasma, and erythrocytes with this physicochemical method is necessary to quantify individual

contributions of strong ions to acid-base balance during exercise (Heigenhauser et al., 1990).

The present investigation compared simultaneous changes in acid-base status of arterial and central venous blood associated with repeated sprint exercise. In addition, a comprehensive physicochemical approach to acid-base evaluation was compared to a limited conventional one to determine if one method offered advantages over the other.

Materials and Methods

Horses. Seven Arabian horses, age 4 to 5 yrs old were used. They weighed 410 kg ± 41 kg (mean ± SE), and had been previously conditioned twice weekly for 8 wks on a high-speed treadmill (Kagra Ag Mustang 2200; Switzerland) were used. Each horse had its right carotid artery relocated to a subcutaneous position at least 2 months prior to the start of conditioning. They were fed a cracked corn and molasses mix, and ad libitum grass hay to meet requirements for moderate to heavy exercise in horses (NRC, 1989). This protocol was approved by the University's animal care commitee.

Experimental protocol. Feed, but not water was witheld for at least 12 hr prior to the morning of the exercise test. The treadmill barn was climate-controlled to maintain

a temperature of 10°C and 35% relative humidity. Horses were brought into the barn at least 1 hr prior to any handling. Areas over the left jugular vein, and right carotid artery were surgically prepared. A sterile 18 g catheter (Angiocath, Becton-Dickinson) with a 5 ml extension set was introduced into the artery and kept patent with heparinized saline.

An area of skin over the midcervical region of the jugular vein was anaesthetized with 1 ml of lidocaine (2% lidocaine HCl, Butler), and a small incision was made in the skin. A 10 g needle was placed aseptically into the vein, and a sterile polyethylene catheter (PE-240, Intramedic) attached to a saline manometer was introduced into the right atrium of the heart. A 5 ml extension set was attached to the tubing and kept patent with heparinized saline. The horse was allowed to stand quietly for at least 1 hr before resting heart rate (HR) and rectal temperature (RT) were recorded just prior to a sprinting exercise test. Heart rates were monitored during the test with a commercial digital heart monitor (Polar Pacer, Polar CIC), and RT was obtained at each blood sampling period.

Sample Collection and Analysis. Prior to the sprinting exercise test (Table 1), two resting blood samples were taken simultaneously from the artery and right atrium, 10 min apart. Data from the resting samples were similar

(P>.25), and means were used as resting values. Additional samples were obtained 15 sec prior to the end of the warm-up phase, and 15 sec prior to the end of sprints 5 and 6. Similar experiments have shown that asymptotic values for metabolic variables were approached on the 5th and 6th sprints (Ferrante et al, 1994). Data from sprints 5 and 6 were similar, and means were used to estimate the maximal response to exercise. Blood samples were also drawn at 5 (Rec 5) and 30 (Rec 30) min of recovery.

All analyses were conducted on arterial and central venous samples. Blood samples (2 ml) were drawn anaerobically into heparinized syringes (300 units lithium heparin, Sigma), and stored in ice water until analyzed for $[H^+]$, pCO_2, pO_2, Hct, $[Na^+]$, and $[K^+]$ (Stat Profile 1, Nova Biomedical) within 5 - 30 min. Additional samples (35 ml) were drawn and placed in heparinized tubes (Vacutainer #6484, Becton-Dickinson). Aliquots were immediately deproteinized in cold perchloric acid (1:2), and the supernatent drawn off and stored at -70°C for determination of blood $[Lac^-]$ by a lactate dehydrogense method (Proc. #826-UV, Sigma). Additional aliquots of whole blood were stored at -5°C for determination of [Hb] by cyanmethemoglobin method (Proc. #525, Sigma), Plasma was immediately separated from the remaining samples, and stored at -5°C for determination of [Alb] by bromcresol green method (Proc. #631, Sigma), and $[Cl^-]$ by titration (Chloridometer, Buchler instruments).

Plasma $[H^+]$, pCO_2, and pO_2 were adjusted to RT, and $[HCO_3^-]$ was calculated (Sigaard-Andersen, 1963). Plasma [Alb] was used to estimate $[A_{tot}]$ (Rossing et al., 1986). SID was calculated as follows (Stewart, 1981):

$$[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [Lac^-])$$

All results are given as lsmean $\pm$ SE, unless stated otherwise. Data were analyzed by analysis of variance for repeated measures, with orthogonal contrasts (SAS, 1988).

Results

Changes in individual strong ions. There were differences between sampling sites (A vs V) in plasma $[Na^+]$ ($P = .0006$), $[K^+]$ ($P = .0186$), $[Cl^-]$ ($P = .0001$), and blood $[Lac^-]$ ($P = .015$) (Table 2). Plasma $[Na^+]_v$ increased ($P = .0001$) during exercise, and was 2% higher than $[Na^+]_a$, which was increased ($P = .0001$) at maximal exercise only. Plasma $[K^+]$ increased ($P = .0001$) during exercise at both sites, but $[K^+]_v$ was 7% higher than $[K^+]_a$ during maximal exercise. Plasma $[Cl^-]_v$ decreased ($P = .0001$), but $[Cl^-]_a$ increased ($P = .0001$) during exercise, so that $[Cl^-]_v$ was 3 - 8% lower than $[Cl^-]_a$ throughout the test. Blood $[Lac^-]$ increased ($P = .0001$) at both sites, and $[Lac^-]_a$ was 5% higher than $[Lac^-]_v$ at maximal exercise (Table 2).

Changes in independent variables: pCO_2, [SID], and [Alb]. There were differences between sampling sites (A vs V) in plasma pCO_2, and [SID] (P = .0001) (Table 3). Plasma $pvCO_2$ increased (P = .0001) during exercise, and was 13 - 43% higher than $paCO_2$, which decreased (P = .0001) throughout the test. Plasma $[SID]_v$ increased (P = .0001), but $[SID]_a$ decreased (P = .0001) during exercise, so that $[SID]_v$ was 12 and 20% higher than $[SID]_a$ during sub-maximal and maximal exercise, respectively. During recovery, $[SID]_v$ decreased, and $[SID]_a$ increased (P = .0001), so that the difference between them was reduced to 5%. Plasma [Alb] increased during exercise, with no site differences. The increase in [Alb] at maximal exercise would correspond to a decrease of 9.7% in plasma volume if the albumin content of plasma remained constant (Van Beaumont et al., 1981). This deficit was restored partially at Rec-5, and completely at Rec-30.

Changes in dependent variables: $[H^+]$, and $[HCO_3^-]$. There were differences (P = .0001) between sampling sites (A vs V) in plasma $[H^+]$ and $[HCO_3^-]$ throughout the test (Table 4). Plasma $[H^+]_v$ was 8% higher than $[H^+]_a$ at rest. During exercise, $[H^+]_v$ increased, but $[H^+]_a$ decreased, so that $[H^+]_v$ was 30% higher than $[H_+]_a$ at maximal exercise. The directions of changes were reversed during recovery, so that there was no difference between $[H^+]_v$ and $[H^+]_a$ at Rec-30. Plasma $[HCO_3^-]_v$ wasd 6% higher than $[HCO_3^-]_a$ at rest (Table

4). Venous [HCO_3^-] increased during warm-up, but decreased at maximal exercise and Rec-5. Arterial [HCO_3^-] was unchanged during warm-up, and thus was 15% less than [HCO_3^-]$_v$ at this point. It also was decreased at maximal exercise and at Rec-5. Both arterial and venous [HCO_3^-] returned to resting values at Rec-30.

Changes in Hct, [Hb], and pO_2. Changes in Hct, [Hb], and pO_2 are shown in Table 5. Changes in Hct corresponded to a decrease of 14.6% in plasma volume at maximal exercise and an increase of 24.7% in plasma volume at Rec-30. These calculations assume that the increase in Hct during the warm-up is due primarily to splenic contraction, and subsequent values for Hct are adjusted accordingly (McKeever et al., 1993). A greater hemoconcentration in arterial blood than venous blood is suggested by the higher values of [Hb]$_a$, compared to [Hb]$_v$, during warm-up and maximal exercise. The paO_2 remained at pre-exercise levels during exercise, but increased ($P = .0001$) 18% at Rec 5. The pvO_2 decreased ($P = .0001$) 46% during exercise before returning to the resting level at Rec 5.

Discussion

Individual ions. The increase in plasma [Na^+] at both sites is due primarily to the change in PV -- the loss of

free water from the vascular compartment, rather than an addition of Na^+ to the vascular compartment (Geiser et al., 1992). The greater change in $[Na^+]_v$ relative to arterial plasma could be due to a larger water loss in venous plasma. Increases in plasma $[K^+]$ at both sites may be due in part to a decrease in PV, but K^+ loss from contracting muscle also contributes to the increase in the plasma (Lindinger and Sjogaard, 1991). A loss of intramuscular K^+ contributes as much as the gain in intramuscular $[Lac^-]$ to the increase in intramuscular $[H^+]$ (Jones, 1990). The greater increase in $[K^+]_v$ may reflect shifts between the plasma and erythrocyte compartments during circulation through the lung and heart, and due to delay in measurement, resulting in the decreased $[K^+]$ in the arterial samples.

The changes in plasma $[Cl^-]$ during exercise at different sites (Figure 1) are evidence of the chloride shift, which has been implicated in acid-base balance during exercise in horses (Carlson, 1992). The CO_2 produced in working muscle diffuses into blood via a concentration gradient, with minimal hydration to $[HCO_3^-]$ in the plasma due to a low level of the enzyme, carbonic anhydrase. This enzyme is found in the erythrocyte, where the CO_2 quickly combines with water to form H^+ and HCO_3^-. The H^+ produced combine with Hb, and the HCO_3^- enters the venous plasma in exchange for Cl^-. This explains the increase in $[HCO_3^-]_v$, and the reduction in $[Cl^-]_v$ during exercise with minimal signs of

sweating. This process is reversed in the lung, with CO_2 being expired, and Hb binding to O_2. The H^+ liberated from Hb react with HCO_3^- from plasma, which enters the erythrocyte in exchange for Cl^- leaving the cell, resulting in the increase in $[Cl^-]_a$, and the decrease in $[HCO_3^-]_a$. Measurement of the distribution of Cl^- ions between plasma and erythrocytes has demonstrated that the chloride shift in fish (Cameron, 1978) and bears (Brix et al., 1990) aids in excretion of CO_2, and may directly facilitate unloading of oxygen from hemoglobin.

The increases in blood $[Lac^-]$ at both sites during exercise are due primarily to lactate efflux from contracting muscle into plasma and erythrocytes, which have been proposed as a "sink" for Lac^- during intense exercise in humans (Kowalchuk et al., 1988) and horses (Ferrante et al., 1994). The greater increase in $[Lac^-]_a$ relative to $[Lac^-]_v$ has been found previously in plasma of exercising horses (Harris and Snow, 1988), and may be due to dilutional effects from blood mixing at the venous sampling location with drainage from the relatively inactive head and neck, which would have a lower $[Lac^-]$ than blood draining the hind end. There is also evidence that heart produces and consumes Lac^- simultaneously during exercise in humans when myocardial blood flow changes, and the $[Lac^-]$ rises above resting levels (Stanley, 1991).

Effects of independent variables on $[H^+]$ and $[HCO_3^-]$.

Although site differences between pCO_2 and pO_2 were expected during exercise, the differences between the strong ions, [SID], and the effects these changes had on $[H^+]$ and $[HCO_3^-]$ at the two sites during exercise have not been previously documented in horses. An increase in [SID] alone (increase in strong basic cations) results in a reduction of $[H^+]$, and an increase in $[HCO_3^-]$ to maintain electroneutrality (Stewart, 1981). Albumin, which comprises the majority of $[A_{tot}]$ in horses, is important in acid-base balance for it's buffering ability (Rossing et al., 1986). The protein structure includes ionizable groups and side chains, such as histidine, that have dissociation constants suitable for buffering blood. Histidine dissociation influences the charge state and pH-dependent functions of proteins, and protects their integrity from increases in temperature (Nattie, 1990). Changes in [SID] may also act directly on central chemoreceptors to alter ventilation and maintain $[H^+]$ in relation to protein dissociation (Jennings, 1993). An increased $[A_{tot}]$, as estimated by [Alb], increases the amount of anion, causing an increase in $[H^+]$, and a decrease in $[HCO_3^-]$ (Rossing et al., 1986). An increased pCO_2 causes increases in both $[H^+]$ and $[HCO_3^-]$.

Increases in mixed venous [Alb] and pCO_2 contributed to the increased $[H^+]_v$, overwhelming the influence of increased $[SID]_v$, which was due to increased $[Na^+]_v$, $[K^+]_v$, and $[Lac^-]_v$,

and decreased $[Cl^-]_v$ (Figure 2). The mixed venous [SID] and pCO_2 also contributed to the increased $[HCO_3^-]_v$. The increase in [Alb], and the decrease in $[SID]_a$, due to the greater increases in $[Lac^-]_a$ and $[Cl^-]_a$ compared to the other strong ions, would contribute to a reduction in arterial pH and $[HCO_3^-]$. Although the concomitant decrease in $paCO_2$ enhanced the decline in $[HCO_3^-]_a$, it had a greater influence on increasing the pH_a.

This interpretation demonstrates how a physicochemical approach to acid-base evaluation can be more useful than the classical approach of examining only changes in pH and $[HCO_3^-]$, which are dependent upon, and will change in relation to each other. Both approaches are valid, but if there is variation in the pCO_2, then some responses may be obscured. Changes in either $[HCO_3^-]$ or base excess by themselves cannot differentiate between possible sources of non-respiratory acid-base disturbances (Fencl and Leith, 1993). It is also important to note that $[Cl^-]$, [SID], and pCO_2 were different at the two sites, and their influence upon pH and $[HCO_3^-]$ was evident at both sites. The bicarbonate buffer approach would have also revealed an arterial "metabolic acidosis" according to the $[HCO_3^-]$ alone, but this would have been confounded by the increase in pH_a, which was due to the combined effects of a decrease in both $[SID]_a$ and $paCO_2$. Interpretation involving the $[HCO_3^-]$ would have incorrectly indicated a venous metabolic

acidosis at maximal exercise, rather than the venous metabolic alkalosis as demonstrated by the increased $[SID]_v$. The changes in $[SID]_v$ were opposed by the influence of $pvCO_2$ and $[Alb]_v$, which contributed to decreasing the pH_v.

Changes in Hct, [Hb], and pO_2. The increases in blood Hct and Hb at the onset of exercise are due primarily to the mobilization of splenic erythrocytes (Persson, 1973), and a small part is due to decreases in plasma volume (McKeever et al., 1993). Mobilization of the splenic reserve can increase the oxygen carrying capacity of the blood significantly, and contributes to the horse's ability to tolerate longer periods of maximal exercise.

The high paO_2 observed at the end of sprints 5 and 6 in this study has been seen previously primarily during low intensity or moderate exercise in horses. Exercising horses typically show a slight initial increase in paO_2, which subsequently falls, and Thoroughbreds undergoing repeated sprint exercise exhibited a 45% decrease in paO_2 at the end of exercise (Butler et al., 1993). This response may be somewhat unique to the Arabian horse, as this breed seems to have an increased aerobic capacity, as evidenced by lower lactate accumulation during exercise (Wickler and Troy, 1991; McCollum et al., 1993), and a higher proportion of oxidative muscle fibers (Rivero et al., 1993).

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TABLE 1: Repeated sprint exercise test protocol

Time (min)	Treadmill Slope (%)	Treadmill Speed (m/sec)	Gait
Warm - up sequence:			
5	0	1.6	Walk
5	6	3.5	Trot
1	6	1.6	Walk
Repeated sprint sequence (6 repetitions):			
1	6	10.0	Gallop
0.5	6	3.5	Trot
3.5	6	1.6	Walk
Recovery sequence:			
30	0	1.6	Walk

TABLE 2. Strong ion concentrations in arterial (A) and central venous (V) plasma at rest, at the end of warm-up and maximal exercise, and 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (lsmeans, n = 7)

[Ion] (mEq/L):	Site:	Rest	Warm-up	Max	Rec 5	Rec 30
$[Na^+]$	A	136.5^a	136.4^{a*}	138.3^{b*}	135.6^c	136.2^a
	V	136.8^a	138.0^b	141.0^c	135.9^d	136.3^a
	SE	0.1	0.2	0.3	0.2	0.3
$[K^+]$	A	3.84^a	5.01^b	5.36^{c*}	3.93^d	3.76^a
	V	3.86^a	5.06^b	5.75^c	3.96^d	3.75^a
	SE	0.01	0.04	0.08	0.02	0.04
$[Cl^-]$	A	95.7^{a*}	96.9^{b*}	97.2^{b*}	94.8^{c*}	94.8^{c*}
	V	93.7^a	92.4^b	90.3^c	92.5^b	92.4^b
	SE	0.49	0.21	0.33	0.51	0.36
$[Lac^-]^\#$	A	0.409^a	0.365^a	5.55^{b*}	3.50^c	0.796^d
	V	0.409^a	0.381^a	5.31^b	3.36^c	0.781^d
	SE	0.006	0.021	0.08	0.04	0.01

^{a,b,c,d}Means with different superscripts in the same row are different from each other

*Sites are different; $^\#[Lac^-]$ is concentration in whole blood

TABLE 3. Concentrations of the independent variables in arterial (A) and central venous (V) plasma at rest, at the end of warm-up and maximal exercise, and at 5 (Rec 5) and 30 (Rec 30) min of a walking revovery period (lsmeans, n = 7)

		Rest	Warm-up	Max	Rec 5	Rec 30
Variable:	Site:					
pCO_2	A	42.05^{a*}	37.24^{b*}	29.12^{c*}	31.67^{d*}	40.26^{a*}
(mmHg)	V	48.14^{a}	53.21^{b}	50.72^{c}	39.78^{d}	45.51^{e}
	SE	0.6	0.5	0.8	0.3	0.6
[SID]#	A	44.24^{a*}	44.14^{a*}	40.89^{b*}	41.66^{b*}	44.37^{a*}
(mEq/L)	V	46.55^{a}	50.18^{b}	50.93^{b}	43.94^{c}	46.83^{a}
	SE	0.5	0.4	0.4	0.4	0.5
[Alb]++	A	3.12^{a}	3.26^{b}	3.45^{c}	3.30^{b}	3.11^{a}
(mg/dL)	V	3.19^{a}	3.37^{b}	3.46^{b}	3.28^{b}	3.04^{c}
	SE	0.03	0.06	0.05	0.04	0.07

^{a,b,c,d,e}Means with different superscripts in the same row are different from each other;

*Sites are different; #[SID] is strong ion difference;

++[Alb] is albumin

TABLE 4. Concentrations of the dependent variables in arterial (A) and central venous (V) plasma at rest, at the end of warm-up and maximal exercise, and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (lsmeans, n = 7)

		Rest	Warm-up	Max	Rec 5	Rec 30
Variable:	Site:					
$[H^+]$	A	37.9^{a*}	33.8^{b*}	33.5^{b*}	36.2^{c*}	36.3^{c*}
(nmol/L)	V	41.0^{a}	41.3^{a}	43.4^{b}	39.8^{c}	36.2^{d}
	SE	0.34	0.46	0.94	0.51	0.38
$[HCO_3^-]^{\#}$	A	26.48^{a*}	26.58^{a*}	21.33^{b*}	21.46^{b*}	26.51^{a*}
(mmol/L)	V	28.03^{a}	30.69^{b}	28.54^{c}	24.15^{d}	28.18^{c}
	SE	0.23	0.25	0.28	0.32	0.33

^{a,b,c,d}Means with different superscripts in the same row are different; *sites differ; $^{\#}[HCO_3^-]$ is bicarb. concentration;

TABLE 5. Arterial (A) and central venous (V) blood hematocrit (Hct) and hemoglobin concentration ([Hb]), and plasma pO_2 at rest, at the end of warm-up and maximal exercise, and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (lsmeans, n = 7)

Variable:	Site:	Rest	Warm-up	Max	Rec 5	Rec 30
Hct	A	34.46^{a}	41.37^{b}	44.87^{c}	43.89^{c}	36.54^{a*}
(%)	V	34.72^{a}	42.14^{b}	46.03^{c}	44.80^{c}	35.42^{a}
	SE	0.5	0.95	0.35	0.46	0.38
[Hb]	A	11.11^{a}	14.05^{b*}	17.27^{c*}	13.98^{b}	12.30^{d}
(g/dL)	V	10.72^{a}	13.23^{b}	14.92^{c}	13.44^{b}	10.84^{a}
	SE	0.21	0.32	0.52	0.34	0.54
pO_2	A	96.87^{a*}	101.35^{b*}	97.71^{a*}	118.29^{c*}	94.66^{a*}
(mmHg)	V	38.30^{a}	22.68^{b}	17.91^{c}	40.73^{a}	33.36^{d}
	SE	1.25	1.80	1.86	1.58	0.60

^{a,b,c,d}Means with different superscripts in the same row are different; *sites differ;

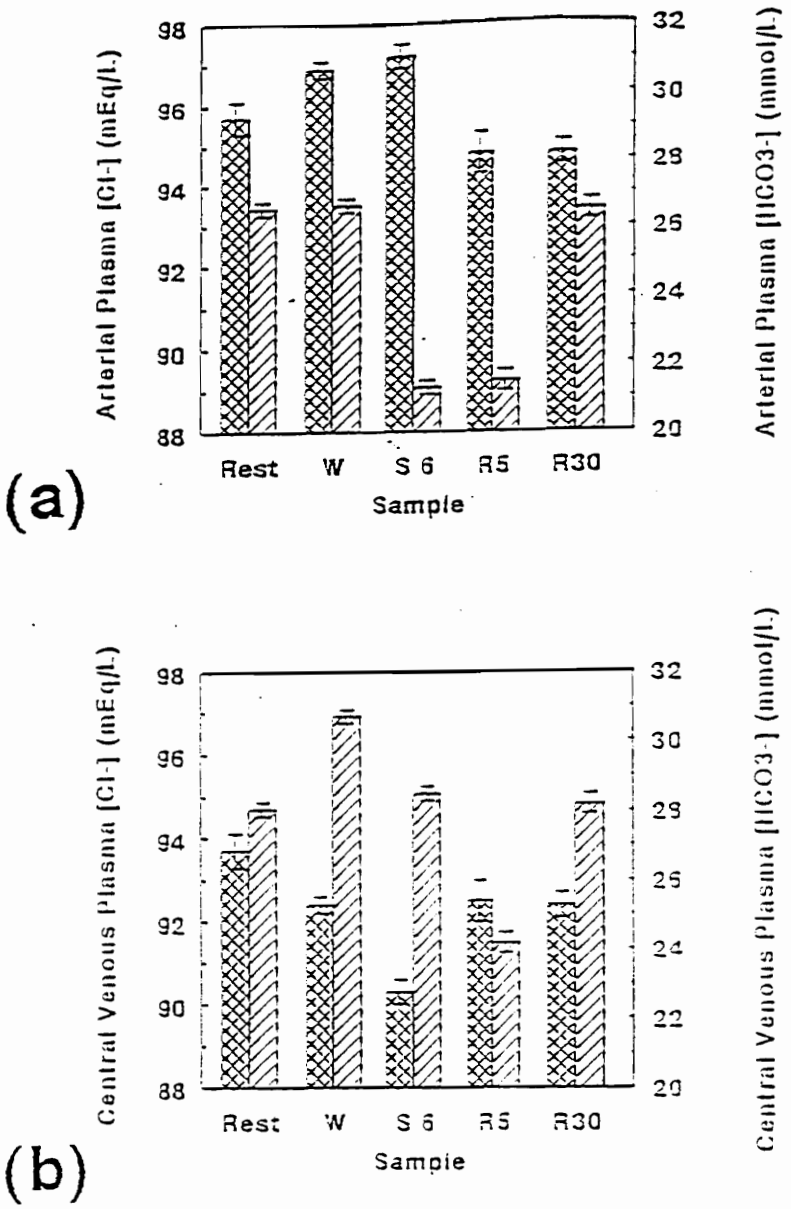

FIGURE 1. Chloride and bicarbonate concentrations in arterial (a) and central venous (b) plasma: evidence of the chloride shift during warm-up (W) and sprinting exercise (S 6), and at 5 (R 5) and 30 (R 30) min of a walking recovery period (Chloride = crossed bars; Bicarbonate = hatched bars; lsmeans, n = 7)

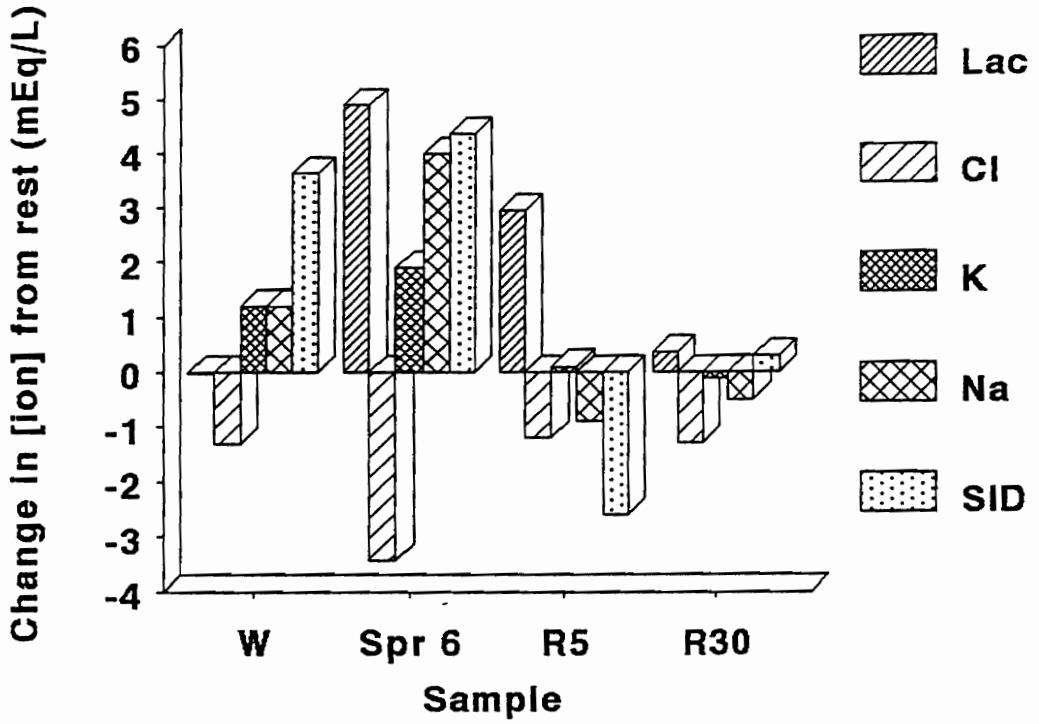

FIGURE 2. Contribution of central venous individual ions to changes from rest in [SID] during warm-up (W) and sprinting exercise (S 6), and at 5 (R 5) and 30 (R 30) min of a walking recovery period (Lac = lactate; Cl = chloride; K = potassium; Na = sodium; SID = strong ion difference, n = 7)

Journal Article 2. Effects of Sprint Training and a Lecithin/Corn Oil Diet During Intermittent Exercise in Arabian Horses I: Arterial and Central Venous Acid-base Balance

ABSTRACT

Eight Arabian horses were used in a crossover design with repeated measures to study the effects of lecithin and training on acid-base status. Blood samples were taken from arterial (A) and central venous (V) sites, and plasma $[H^+]$, pCO_2, pO_2, albumin ([Alb]) and strong ion concentrations ($[Na^+]$, $[K^+]$, $[Cl^-]$), and blood lactate ($[Lac^-]$), and hemoglobin ([Hb]) concentrations were measured. Strong ion difference ([SID]) and bicarbonate concentration ($[HCO_3^-]$) were calculated, and plasma total weak acid ($[A_{tot}]$) was calculated. Differences were found between sites for $[H^+]$, pCO_2, pO_2, $[HCO_3^-]$, $[Na^+]$, $[Cl^-]$, [SID], and [Hb]. Exercise increased plasma $[H^+]_V$, $pvCO_2$, paO_2, $[HCO_3^-]_V$, $[Cl^-]_A$, $[SID]_V$, and blood $[Lac^-]$ and [Hb], and plasma $[K^+]$, $[Na^+]$, $[A_{tot}]$, and [Alb] at both sites. Exercise decreased plasma $[H^+]_A$, $paCO_2$, pvO_2, $[HCO_3^-]_A$, $[Cl^-]_V$, and $[SID]_A$. Training increased responses during exercise for $[Hb]_A$, arterial and mixed venous $[Na^+]$, $[K^+]$, and [Alb], and decreased responses for $[Cl^-]_V$, and arterial and mixed venous $[Lac^-]$. Horses consuming the

lecithin-supplemented diet had higher $pvCO_2$, $[HCO_3^-]$, and $[Cl^-]_v$ during exercise, lower blood $[Lac^-]_v$ and $[H^+]$ during exercise, and higher plasma $[H^+]_v$ during recovery.

INDEXING KEY WORDS:

acid-base, exercise, strong ion difference, horses, lecithin, training

Introduction

Increased dietary fat combined with sprint training has been found to influence acid-base variables (Taylor et al., 1994a), increase blood lactate accumulation (Ferrante et al., 1993), and affect the skeletal muscle sarcoplasmic reticulum in horses (Wilson et al., 1995) during repeated sprints. Soy lecithins, which are predominantly phosphatidylcholine, have potential for improving mental function (Mauron and Leathwood, 1987), and affecting triglyceride digestibilty (Jenkins and Fotouhi, 1989), and responses to exercise, including muscle contraction (Conlay et al., 1992; von Allworden et al., 1993). Oral supplementation of choline and lecithin in humans has prevented the exercise-associated drop in plasma choline and may improve endurance capacity (Hirsch et al,. 1978; Sandage et al., 1992).

Many studies measurimg blood gases and strong ions during exercise in the horse have been conducted on ponies (Parks and Manohar, 1984; Forster et al., 1990), and Thoroughbreds (Littlejohn and Snow, 1988; Rose et al., 1990), but there are at present no similar studies in the Arabian horse, which is used primarily for endurance exercise. There is evidence that this particular breed differs quantitatively from others in muscle metabolism and responses to exercise (Snow and Guy, 1980; McCollum et al., 1993). Data on simultaneous changes in arterial and central venous blood during exercise and recovery

is limited, but may be important when examining acid-base balance (Carlson et al., 1992). A previous study on Arabian horses examined the effects of repeated sprints on acid-base status and strong ion difference during exercise (Taylor et al., 1994b). The present report extends the study to include the effects of sprint training and supplemental lecithin.

Materials and Methods

Animals. Eight Arabian horses, six geldings and two mares, age 5 to 7 y were used in a randomized switchback design with repeated measures. They weighed 403 ± 12 kg (mean ± SE), and had been previously sprint-trained twice weekly for 8 wk on a Mustang 2200 high speed treadmill (Kagra AG Mustang 2200, Switzerland). Each horse had its right carotid artery relocated to a sub-cutaneous position at least 2 months prior to the start of conditioning. Horses were housed and fed individually in box stalls (8.5 m^2) at night, and housed together in a dirt paddock (60 x 78 m) during the day, where water and trace-mineralized salt were available ad libitum. Horses were evaluated for lameness, as described (Taylor et al., 1994b), and exercised twice weekly on the treadmill to maintain a desirable level of fitness (**Table 1**). This protocol was approved by the University's animal care committee.

Diets. Horses were randomly assigned to one of two total mixed rations: a control diet (CON) consisting of chopped hay, corn, crimped oats, soybean meal, beet pulp, and molasses (n = 4), or an isocaloric amount of a similar diet (n = 4) containing 14% fat (CO/LEC) in the form of 50% corn oil and 50% lecithin (**Table 2**). Diets were fed initially at 2% of bodyweight, were formulated to meet or exceed moderate work in horses (NRC, 1989), and were fed in two equal portions at 0700, and 1500 hr each day. Horses were weighed weekly on an electronic scale (E-Z Weigh, Cave Creek, AZ), and feed was adjusted to maintain bodyweight. Each horse was fed its assigned diet for 10 wk (Period 1), then consumed the other diet for 10 wk (Period 2) after the first exercise test.

Standard exercise test. The standard exercise test (SET) consisted of 6 repeated sprints (**Table 3**). Feed, but not water was witheld for 12 h prior to the morning of the SET to avoid digestive and metabolic responses to a meal. The treadmill barn had an average ambient temperature of 19°C, and relative humidity of 50%. Two horses, one from each group, were tested per day, and were brought into the barn at least one hour prior to any handling. Resting heartrate (HR) and rectal temperatures were taken, and areas over the left jugular vein, and right carotid artery were surgically prepared. A sterile 18-gauge catheter with a 5-ml extension set was introduced into the artery and kept patent with

heparinized saline.

An area over the midcervical region of the jugular vein was anaesthetized with 1 ml of lidocaine, and a small incision was made in the skin with a #10 scalpel blade. A 10 gauge needle was placed aseptically into the vein, and a sterile polyethylene catheter attached to a saline manometer was introduced into the right atrium of the heart. When the position was verified, the manometer was removed, and a 5 ml extension set was attached to the tubing and kept patent with heparinized saline. The prepared horse was allowed to stand quietly for at least 1 h before the test. Heart rate was monitored during the test (**Figure 1**) with with a commercial digital heart monitor (Polar CIC, Clifton, NJ), and rectal temperature was taken at each sampling time.

Sample collection and assays. Prior to the start of the test, resting blood samples were taken simultaneously from the artery and right atrium. Additional samples were obtained 15 s prior to the end of the warm-up (W), 15 s prior to the end of sprint 6 (Spr 6), and at 5 (R 5) and 30 (R 30) min of recovery. All analyses and calculations were conducted on A and V blood. Blood samples (2 ml) were drawn anaerobically into heparinized syringes, and stored in an ice water bath until analyzed for plasma hydrogen ion concentration ($[H^+]$), partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2), and plasma concentrations of sodium ($[Na^+]$), and potassium

($[K^+]$) within 5 - 30 min using selective electrodes (Stat Profile 1, Nova Biomedical). Additional samples (35 ml) were drawn and placed in heparinized tubes. Aliquots were immediately deproteinized in cold perchloric acid (1:2), and the supernatent drawn off and stored at -70°C for determination of blood lactate concentration ($[Lac^-]$) by lactate dehydrogenase method (Sigma Diagnostics, Proc. #826-A). Additional aliquots of whole blood were stored at -5°C for determination of hemoglobin concentration ([Hb]) by cyanmethemoglobin method (Sigma Diagnostics, Proc. #525). Plasma was immediately harvested from the remaining samples, and stored at -5°C for determination of albumin concentration ([Alb]) by bromcresol green method (Sigma Diagnostics, Proc. #631), and chloride concentration ($[Cl^-]$) by titration with silver ion (Chloridometer, Buchler Instruments). Plasma $[H^+]$, pCO_2, and pO_2, were adjusted to rectal temperature, bicarbonate concentration ($[HCO_3^-]$) was calculated, total weak acid ($[A_{tot}]$) was calculated according to Stewart (1983), and strong ion difference ([SID]) was calculated as follows: $[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [Lac^-])$ according to Stewart (1983).

Statistical Analysis. Statistical significance of data collected was tested by site by the GLM procedure for repeated measures (SAS, 1990) with horse, SET, and diet as the independent variables. Orthogonal polynomial contrasts were used to compare changes in exercise variables to the resting

values, and Dunnett's t-tests were used to compare differences between means. Significance was set at $P < 0.05$. Results are expressed as lsmeans $\pm$ 1 SEM unless otherwise stated.

Results

Feed intake and bodyweight. All horses became accustomed to consuming the total mixed ration within 3 - 4 d, and there were no problems with palatability. Horses consuming the CON diet ate significantly less feed than horses on the CO/LEC diet, but there were no differences in bodyweight between diets or periods (**Table 4**).

Effects of Exercise. Mean heartrate (beats/min) increased from 34.3 $\pm$ 0.8 at rest to 201.4 $\pm$ 3.1 at the end of Spr 6, and had returned to 65.6 $\pm$ 2.5 at Rec 30. Mean rectal temperature (°C) was 37.9 $\pm$ 0.3 at rest, rose to 40.2 $\pm$ 0.6 at the end of Spr 6, and was 39.3 $\pm$ 0.5 at Rec 30.

The changes in plasma A and V strong ions and [SID] are summarized in Table 5. One horse " Blazer ", was determined to be an outlier (z-test; 4 - 10 SD from the mean of 7 horses) for $[H^+]$, $[HCO_3^-]$, pO_2, $[Lac^-]$, and [SID], so all acid-base results are presented for 7 seven horses due to the relationship between these variables, with data for Blazer presented separately. The means for [Alb] and [Hb] are for

all 8 horses.

Both A and V $[Na^+]$ and $[K^+]$ increased during exercise before returning to resting values at recovery; the $[Na^+]_V$ increase was greater then $[Na^+]_A$, resulting in a site difference at W. The $[Cl^-]_A$ increased, and $[Cl_-]_V$ decreased during exercise, resulting in a site difference at W, Spr 6, and Rec 5. Blood $[Lac^-]$ increased consistently at both sites during exercise, and remained above the resting values at R 30. The $[SID]_A$ was decreased and $[SID]_V$ increased at Spr 6, resulting in a site difference at all sampling times.

Changes in plasma A and V $[A_{tot}]$, pCO_2, and pO_2 are summarized in Table 6. The $[A_{tot}]$ increased at both A and V during Spr 6, and returned to pre-exercise levels by Rec 30, with no site differences. The $paCO_2$ decreased, and $pvCO_2$ increased during exercise, resulting in a site difference at all sampling times. The paO_2 showed an increasing trend at W, decreased at Spr 6, and increased at Rec 5 before returning to pre-exercise levels at Rec 30. The pvO_2 was decreased during exercise, but had returned to resting levels at Rec 5.

The changes in plasma $[H^+]$, $[HCO_3^-]$, [Alb], and [Hb] are summarized in Table 7. The $[H^+]_A$ was lower than $[H^+]_V$ at rest, and decreased at W before returning to resting values at Spr 6. The $[H^+]_V$ increased at Spr 6 before returning to pre-exercise levels at Rec 5. There was a site difference for $[H^+]$ at all sample times except Rec 30. The $[HCO_3^-]_A$ decreased, and $[HCO_3^-]_V$ increased during exercise, resulting in

a site difference at W, Spr 6, and Rec 5. Plasma [Alb] was increased at W and Spr 6, with no site differences. Whole blood [Hb] increased with exercise before returning to resting levels at Rec 30, with no site differences.

Effects of Training. Training resulted in a decreased heart rate (beats/min) at sprints 1 - 3, and at 10, 20, and 30 min of recovery (Figure 1). The changes associated with training in A and V plasma are summarized in Table 8 and Table 9. Training increased plasma $[Na^+]$ at both sites at W, Rec 5, and Rec 30, and resting $[Na^+]_V$ was increased at SET 2. At Rec 5, $[K^+]$ was increased at both sites during SET 2, but training decreased resting $[K^+]_A$. Training increased plasma $[Cl^-]_V$ at all sampling times at SET 2, but had no effect on $[Cl^-]_A$. Blood $[Lac^-]_V$ showed a decreasing trend ($P = 0.159$) after training at Spr 6 and Rec 5 during SET 2. There was an increased $[SID]_A$ after training at W, and Rec 5 (Figure 2), but there were no effects on $[SID]_V$. Training increased $[HCO_3^-]_V$ and decreased $[H^+]$ at both sites at Spr 6 (Figures 3 and 4, respectively), and increased A and V plasma [Alb] at all sampling times (Figure 5).

Effects of Diet. The CO/LEC diet increased plasma $[Cl^-]_V$, and decreased blood $[Lac^-]_V$ during the warm-up period (Table 10). The CO/LEC diet also affected the change from resting values in $[SID]_V$ during exercise and recovery (Figure 6);

horses had greater increases in [SID] at Spr 6 and Rec 30 when consuming the CO/LEC diet.

Discussion

The acceptance and palatability of the CO/LEC diet is in agreement with other studies from this laboratory using the same diet (Holland et al., 1995). The previous study showed that horses preferred a corn oil supplemented diet first, but the CO/LEC mixture was the next preferred, compared to two other lecithin-containing diets. Lecithin and soybean oil have been shown to improve nutrient digestibility in pigs (Jones et al., 1992), and the CO/LEC diet increased ether extract digestibilty, but decreased fiber and protein digestibility in horses (Holland et. al., 1995).

Exercise. The changes in A and V strong ions and blood gas measurements during exercise followed a pattern similar to earlier findings from this laboratory (Taylor et al., 1994b). It is important to note that the present study used rectal temperature instead of blood temperature for the adjustment of $[H^+]$ and blood gases during exercise. This will underestimate these variables (Taylor et al., 1994c), but does not change the absolute site differences. Horses in the present study would have had higher $[H^+]$, pO_2 and pCO_2 values than reported if these variables were adjusted to blood temperature during

exercise.

The increase in plasma [Na^+] is probably due to the loss of water from the vascular compartment. Increases in plasma [K^+], and blood [Lac^-] are due to movement from contracting muscle. Blood [Lac^-] for Blazer was more than twice the mean of the other 7 horses at SET 1 and SET 2 (Figure 7).

The chloride shift proposed to occur in exercising horses by Carlson (1991) was demonstrated in the first part of this study, and confirmed here. The CO_2 from contracting muscle moves through the plasma into the red cells in the V blood, where most of it is hydrated to H_2CO_3, which dissociates to [HCO_3^-] and [H^+]. The [H^+] is buffered by Hb, and the [HCO_3^-] moves into the venous plasma in exchange for [Cl^-]. The process is reversed in the A blood, causing a difference between sites (Figure 8). The differences for [Na^+] and [Cl^-] resulted in a site difference for [SID] in the present study. The A and V [SID] was lower in Blazer when compared to the mean of the other horses at Spr 6 and Rec 5 (Figure 9), resulting in an increased [H^+] (Figure 10), and decresaed [HCO_3^-] (Figure 11) in this horse by comparison.

Changes in the dependent variables, [H^+] and [HCO_3^-], may be explained in terms of the changes in the independent variables, [A_{tot}], pCO_2, and [SID] (Stewart, 1981). In arterial blood during warm-up exercise, the decrease in $paCO_2$ coupled with no change in [SID] caused the decreases in [H^+] and [HCO_3^-]. The [SID] then decreased, and exerted the primary

influence at Spr 6, causing the $[H^+]$ to return to resting values, overwhelming the influence of the $paCO_2$. The decreased [SID] and $paCO_2$, and increased $[A_{tot}]$ had a combined effect to further decreased the $[HCO_3^-]$ at Spr 6. The further decrease in [SID] and the increased $paCO_2$ at Rec 5 resulted in a slight increase in $[H^+]$.

In venous blood during warm-up exercise, the increased $pvCO_2$ overwhelmed the increased [SID], causing an increased $[H^+]$ and $[HCO_3^-]$. The decreased $[HCO_3^-]$ at Spr 6 was due to the drop in $pvCO_2$, but the slight decrease in [SID] contributed to the continuing rise in $[H^+]$. If these data were interpreted according to the conventional bicarbonate buffer system, then the decrease in $[HCO_3^-]_A$ during exercise would have indicated an arterial acidosis, which was not observed (Table 7). Also, the increase at W, and the decrease at Rec 5 in $[HCO_3^-]_V$ were not directly opposed by the $[H^+]$. This physicochemical approach to acid-base balance can show the separate influence of the independent variables during exercise in horses (Ferrante and Kronfeld, 1994), and emphasizes the differences between sites in the present study.

The changes seen in blood gases, particularly the relatively small (5%) decrease in paO_2 and the lack of a large increase in $pvCO_2$ during exercise may be unique to the Arabian horse (Taylor et al., 1994b, c). Thoroughbred horses undergoing similar repeated sprint exercise had a 45% decrease in paO_2, and a 50% increase in $pvCO_2$ (Butler et al., 1993). Evidence

seems to show that the Arabian breed has a higher aerobic capacity than other breeds, as shown by lower [Lac^-] during exercise (Wickler and Troy, 1991; McCollum et al., 1993), and a high proportion of oxidative (Type I and IIa) muscle fibers (Snow and Guy, 1980; Rivero et al., 1993a). Higher proportions of these muscle fiber types have recently been directly related to superior performance during endurance competition (Rivero et al., 1993b). The maintenance of a high paO_2, and a low A and V pCO_2 even at relatively high workloads (HR > 200 beats/min) may aid in avoiding the exercise-associated acidosis seen in other breeds of horses, and may reflect breed differences in muscle metabolism, control of breathing, and ventilation and perfusion.

Training. The lower heart rates observed in SET 2 (Figure 1) are evidence that the horses continued to improve their fitness level, even though the initial goal of the 2 d/wk of training was to maintain a constant level of fitness throughout the study. There is evidence that the Arabian breed has a greater capacity for improved fitness with training when compared to other breeds, and that this level of fitness can be maintained for months with little or no training (Rivero and Serrano, 1995).

The increased [Na^+] at both sites with training (Table 8) may be due to changes in plasma volume, as there was an increase in plasma [Alb] as well, which has been seen

previously in horses (McKeever et al, 1987). Plasma [K^+] was increased at both sites after training during the recovery, and this may be due in part to the repeated sprint training. Sprint trained men and women also had higher plasma [K^+] during recovery when compared to endurance trained subjects (Medbo and Sejersted, 1994). The sprint trained individuals were able to clear [K^+] from the plasma faster than the endurance trained subjects, resulting in a lower [K^+] later in recovery. Sprint training may have caused changes in the Na-K pump content or activity, which resulted in a difference in [K^+] regulation between the groups.

The was a trend for a training-induced decrease in A and V [Lac^-] at Rec 5 and Rec 30 (Table 5). It may be due in part to an enhanced rate of clearance by oxidation, or a decreased rate of muscle [Lac^-] production. Lower blood and plasma [Lac^-] during a sub-maximal treadmill test has recently been associated with superior performace at the racetrack in Thoroughbred horses (Evans et al., 1994).

The training-induced increase in plasma $[Cl^-]_V$ seen at all sampling times may indicate changes in ion exchange to balance the changes in [Na^+], and [Lac^-]. The changes in strong ions resulted in a significant effect of training on $[SID]_A$ (Figure 2). The $[SID]_A$ was increased at W, and Rec 5 during SET 2, which caused a decrease in $[H^+]_A$ (Figure 4), and an increase in $[HCO_3^-]_V$ (Figure 3) at Spr 6. Both of these effects would be beneficial to the exercising horse. Sprint training may

alter the movement of strong ions during exercise compared to other types of training.

Diet. Consumption of the CO/LEC diet decreased blood [Lac^-] and increased plasma $[Cl^-]_V$ at W, but there were no differences by Spr 6. Horses consuming the CO/LEC diet had a greater increase in $[SID]_V$ from rest to Spr 6 and to Rec 30 (Figure 6). An increase in $[SID]_V$ could cause a decrease in $[H^+]$ and an increase in $[HCO_3^-]$, which would be beneficial to the horse during exercise. A central venous alkalosis could potentially enhance $[H^+]$ efflux from muscle tissue, protecting the animal from fatigue and muscle damage. Changing the degree of saturation and the amounts of dietary fatty acids is reflected in cell membrane composition and fluidity (Croset et al., 1989), and may alter ion transport. Supplementation with dietary phospholipid changed the Na^+/K^+ ATPase activity in erythrocyte membranes in rats (Bordoni et al., 1992). A recent study demonstrated that feeding a lecithin supplemented diet combined with sprint training altered calcium transport in skeletal muscle during exercise in Quarter Horses (Wilson et al., 1995).

There was a decreased blood [Lac^-] during the warm-up in horses on the CO/LEC diet (Figure 12). This is in contrast to a previous study which found higher blood and plasma [Lac^-] during repeated sprints in horses fed corn oil supplemented diets (Ferrante et al., 1993). The effects on [Lac^-] seen in

the previous study from adaptation to the corn oil supplemented diet may have been altered by the inclusion of lecithin. Facilitating free fatty acid transport and oxidation would reduce the rate of glycolysis in muscle cells, producing less [Lac^-] during SET 2. This effect may have been masked at SET 3 by the additive effects of training and diet, which are synergistic in promoting aerobic capacity (Simi et al., 1991).

This study suggests that horses may adapt to fat utilization at different rates, depending on the type of fat included in the diet, with the CO/LEC diet promoting early increases in aerobic capacity. The CO/LEC diet is high in phospholipid content, and may alter the production and transport of strong ions during exercise. The lecithin diet protected against the decrease in [SID], which increased [HCO_3^-], and decreased [H^+] during sprinting.

Finally, the Arabian horse may be superior to other breeds in their capacity for fat adaptation, the combination of a high fat diet and training. The horses in this study responded differently to high-intensity exercise than Thoroughbreds, maintaining a relatively high paO_2, with relatively little build-up of $paCO_2$.

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TABLE 1

Bi-weekly treadmill sprint training protocol

Time (min)	Slope (%)	Speed (m/s)	Gait
0 - 2:30	0%	1.5	walk
2:30 - 5	6%	1.5	walk
5 - 9	6%	3.5	trot
9 - 11	6%	7.0	gallop
11 - 15	6%	4.5	trot
15 - 17	6%	8.0	gallop
17 - 21	6%	4.5	trot
21 - 23	6%	9.0	gallop
23 - 27	6%	3.5	trot
27 - 30	0%	1.5	walk

TABLE 2

Composition of diets

Ingredient (%)	Control diet	CO/LEC diet
Alfalfa/Orchardgrass hay mix	42.0	56.0
Soybean meal	5.0	10.0
Cracked corn	18.5	3.3
Crimped oats	18.5	3.3
Beet pulp	5.0	11.1
Wet molasses	10.0	-
Dry molasses	-	5.0
Vitamin pre-mix[1]	1.0	1.4
Corn oil/soy lecithin[2]	-	10.0

[1] Hoffman-LaRoche Inc., Nutley, NJ

[2] Lucas-Meyer, Decatur, IL; Composition: corn oil, 50%; soybean oil, 18%; phosphatides, 32%; Provided per 100 g: phosphatidylcholine, 7.8 g; phosphatidylethanolamine, 6.3 g; phosphatidylinositol, 5.0 g; other phosphatides, 12.4 g; Fatty acid profile (%): linoleic, 57.4; oleic, 21.3; palmitic, 13.4; stearic, 3.4; linolenic, 1.9.

TABLE 3

Treadmill standard exercise test (SET) protocol

Time (min)	Slope (%)	Speed (m/s)	Gait
Warm-up sequence:			
5	0	1.5	walk
5	6	3.5	trot
Repeated sprint sequence (6 repetitions):			
1	6	10.0	gallop
0.5	6	3.5	trot
3.5	6	1.5	walk
Recovery sequence:			
30	0	1.5	walk

TABLE 4

Chemical composition of diets[1], feed intake, and bodyweights

	Control diet	CO/LEC[2]
Dry Matter, %	88.90	90.80
Crude protein, %	12.10	13.00
Acid detergent fiber, %	24.80	24.70
Ether extract, %	2.60	11.60
Feed consumption, kg DM/d	7.73 ± 0.42*	6.68 ± 0.34
Energy Intake, Mcal DE/d[3]	20.70	20.00
Energy Density, Mcal DE/kg DM	2.68	2.99
Bodyweight, kg		
SET 1:	400 ± 15	398 ± 12
SET 2:	407 ± 10	404 ± 13

[1] As fed basis

[2] CO/LEC = corn oil/lecithin-supplemented diet

[3] Calculated from Holland et al. (1994)

* Differs from CO/LEC (P = 0.042)

TABLE 5

Effects of sprint exercise on plasma arterial (A) and central venous (V) strong ions and strong ion difference ([SID]) (n = 7, values are ls means ± 1 SE; SET 1 and 2 have been combined)

(meq/L)		Rest	Warm-up	Spr 6	Rec 5	Rec 30
$[Na^+]$	A	138.20	138.86#	141.58*	138.34	138.55
	V	138.45	140.60*	143.40*	139.00	139.15
	SE	0.30	0.50	0.50	0.40	0.40
$[K^+]$	A	3.57	4.84*	5.42*	3.73*	3.50
	V	3.55	4.89*	5.51*	3.75*	3.52
	SE	0.04	0.04	0.07	0.04	0.05
$[Cl^-]$	A	96.29	97.05#	98.45#*	95.88#	95.44
	V	94.50	93.08	91.45*	92.50	93.15
	SE	0.40	0.30	0.50	0.60	0.90
$[Lac^-]$[1]	A	0.36	0.30	4.98*	3.44*	0.76*
	V	0.35	0.29	5.50*	3.57*	0.69*
	SE	0.02	0.02	0.56	0.64	0.11
[SID][2]	A	45.10#	45.86#	43.54#*	42.72#*	45.82#
	V	47.10	52.10*	51.93*	46.62	48.75*
	SE	0.32	0.44	0.80	0.45	0.45

[1] $[Lac^-]$ = whole blood lactate concentration, n = 7

[2] [SID] = n = 7; # sites are different ($P < 0.05$)

* different from resting value ($P < 0.05$)

TABLE 6

Effects of sprint exercise on plasma arterial (A) and central venous (V) total weak acid ($[A_{tot}]$), pCO_2, and pO_2 (n = 7, values are ls means ± 1 SE; SET 1 and 2 have been combined)

Variable	Rest	Warm-up	Spr 6	Rec 5	Rec 30
$[A_{tot}]$, meq/L					
A	20.32	22.31	24.51*	22.68*	20.64
V	20.08	22.98	26.19*	24.61*	21.38
SE	0.63	1.25	1.60	0.70	0.65
pCO_2, Torr					
A	38.49#	34.67#*	29.70#*	32.07#*	37.57#
V	45.56	52.98*	50.07*	39.37*	44.31
SE	1.83	1.49	1.05	0.84	0.82
pO_2, Torr					
A	98.54#	101.31#	93.48#*	115.15#*	99.55#
V	37.92	21.78*	17.50*	40.00	32.99
SE	1.13	0.66	0.42	0.66	0.91

sites are different ($P < 0.05$)

* different from resting value ($P < 0.05$)

TABLE 7

Effects of sprint exercise on plasma arterial (A) and central venous (V) $[H^+]$, $[HCO_3^-]$ (n = 7), albumin ([Alb]), and blood hemoglobin ([Hb]) concentrations (n = 8); Values are ls means ± 1 SE; SET 1 and 2 have been combined

Variable		Rest	Warm-up	Spr 6	Rec 5	Rec 30
$[H^+]$	A	36.92	34.66#*	35.49#	36.80#	35.49
(neq/L)	V	40.12	42.78	44.43*	40.75	38.44
	SE	0.98	0.99	0.98	0.98	0.98
$[HCO_3^-]$	A	25.62	24.58#	20.32#*	21.07#*	25.85
(mmol/L)	V	27.91	30.40*	27.25	23.30*	25.15
	SE	1.01	1.03	0.65	0.58	0.58
[Alb]	A	3.31	3.52*	3.50*	3.30	3.22
(g/dL)	V	3.34	3.46*	3.64*	3.38	3.32
	SE	0.08	0.09	0.07	0.10	0.10
[Hb]	A	10.50	13.80*	15.50*	14.80*	11.60
(g/dL)	V	10.10	12.90*	14.90*	13.50*	10.90
	SE	0.30	0.30	0.40	0.30	0.20

sites are different ($P < 0.05$)

* different from resting value ($P < 0.05$)

TABLE 8

Effects of sprint training on arterial (A) and central venous (V) plasma sodium and potassium concentrations (n = 7, values are ls means ± 1 SE)

(meq/L)		Rest	Warm-up	Spr 6	Rec 5	Rec 30
$[Na^+]_A$	SET 1	137.62	138.09*	141.31	137.29*	137.63*
	SET 2	138.56	139.43	141.76	139.06	139.21
	SE	0.29	0.36	0.55	0.47	0.24
$[Na^+]_V$	SET 1	137.89*	139.81*	143.14	138.29*	138.33*
	SET 2	139.01	141.39	143.66	139.70	139.97
	SE	0.17	0.47	0.65	0.41	0.11
$[K^+]_A$	SET 1	3.59	4.88	5.46	3.64*	3.43
	SET 2	3.55	4.82	5.38	3.80	3.54
	SE	0.03	0.05	0.06	0.02	0.03
$[K^+]_V$	SET 1	3.59	4.90	5.46	3.67*	3.46
	SET 2	3.50	4.86	5.55	3.82	3.57
	SE	0.04	0.04	0.07	0.03	0.04

* SET 1 and 2 are different within site ($P < 0.05$)

TABLE 9

Effects of sprint training on arterial (A) and central venous (V) plasma chloride and blood lactate ($[Lac^-]$) (n = 7, values are ls means ± 1 SE)

(meq/L)		Rest	Warm-up	Spr 6	Rec 5	Rec 30
$[Cl^-]_A$	SET 1	96.10	96.90	98.61	96.04	94.66
	SET 2	96.54	97.28	98.40	95.80	95.96
	SE	0.81	0.87	0.77	0.69	0.61
$[Cl^-]_V$	SET 1	93.98*	92.75	90.85*	91.60*	91.76*
	SET 2	95.09	93.40	92.05	93.43	94.63
	SE	0.29	0.27	0.44	0.40	0.59
$[Lac^-]_A$	SET 1	0.358	0.299	5.07	4.10	1.01
	SET 2	0.358	0.290	4.93	3.01	0.61
	SE	0.01	0.02	0.50	0.58	0.21
$[Lac^-]_V$	SET 1	0.335	0.292	6.05	4.06	0.758
	SET 2	0.365	0.282	4.96	3.09	0.622
	SE	0.02	0.02	0.47	0.35	0.11

* SET 1 and 2 are different within site ($\underline{P} < 0.05$)

TABLE 10

Effects of a corn oil/lecithin diet (CO/LEC) on central venous (V) blood [Lac^-], and plasma chloride [Cl^-] concentration during the warm-up step of a sprinting standard exercise test (SET) (n = 7), results are ls means ± 1 SE; SET 1 and 2 have been combined)

Sample	Diet	[Lac^-] (mEq/L)	$[Cl^-]_V$ (meq/L)
Warm-up			
	Control	0.330	92.50
	CO/LEC	0.245	93.41
	SE	0.01	0.19
	p value	0.009	0.008

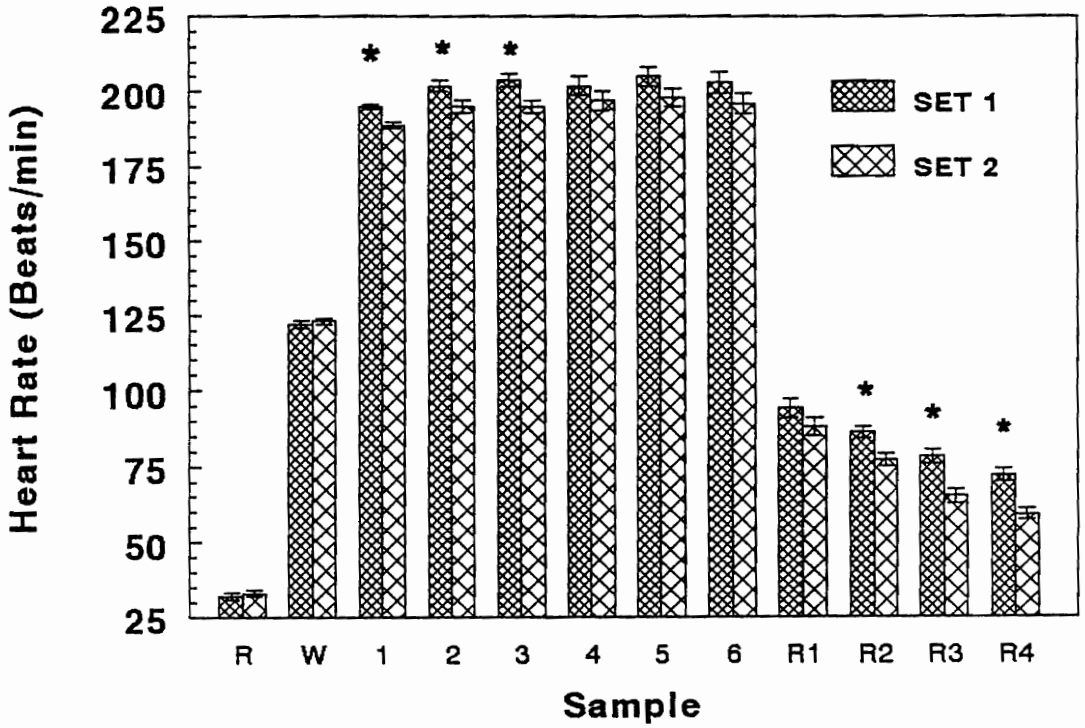

FIGURE 1 Effect of sprint training on heart rate during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$).

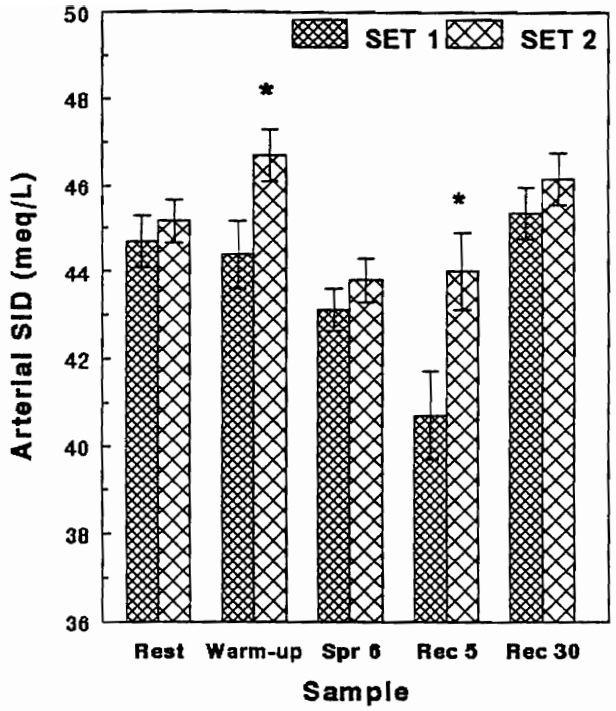

FIGURE 2 Effect of sprint training on arterial strong ion difference ([SID]) during a standard exercise test (SET). Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$).

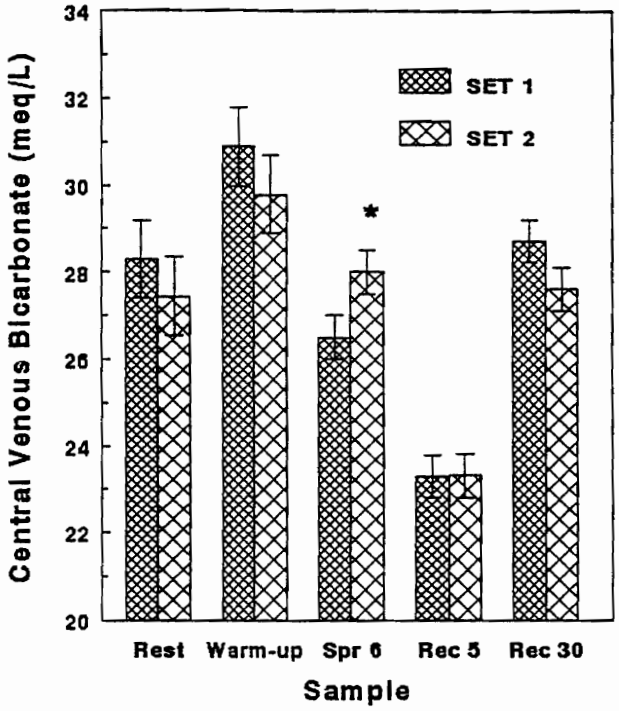

FIGURE 3 Effect of sprint training on central venous plasma bicarbonate ($[HCO_3^-]$) concentration. Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$).

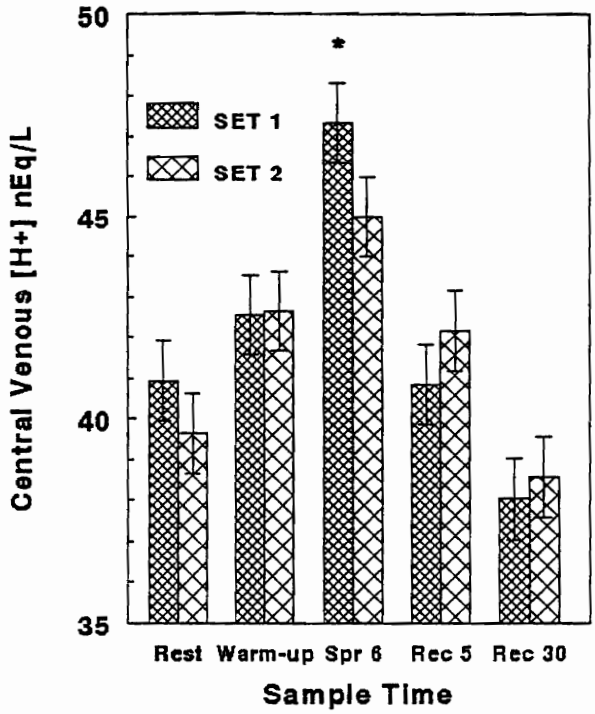

FIGURE 4 Effect of sprint training on central venous plasma $[H^+]$ during a standard exercise test (SET). Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$). Responses were similar in arterial plasma.

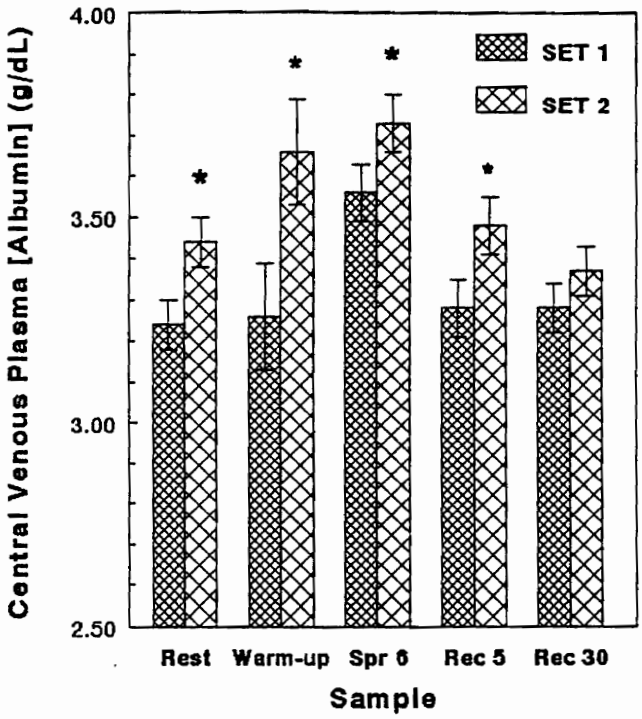

FIGURE 5 Effect of sprint training on central venous plasma albumin [Alb] concentration during a standard exercise test (SET). Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans ± 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$). Responses were similar in arterial plasma.

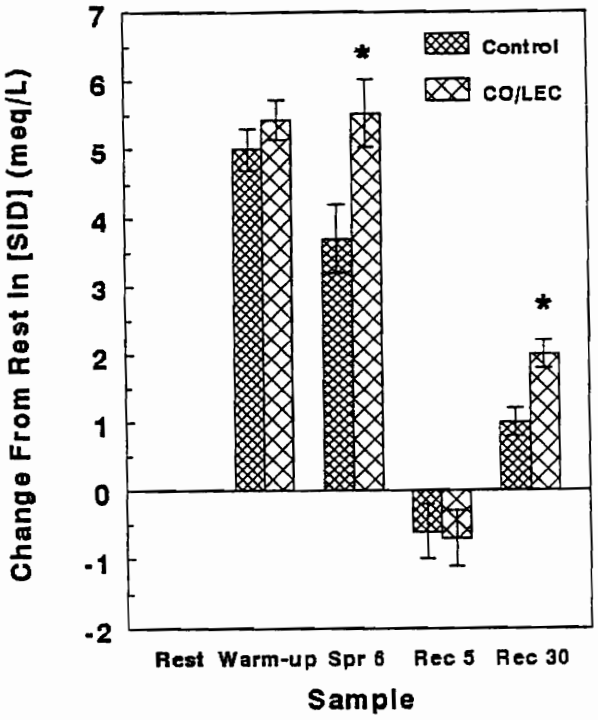

FIGURE 6 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on the change from resting value in central venous [SID] during a standard exercise test (SET). Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * Diets are significantly different at that sampling time ($P < 0.05$).

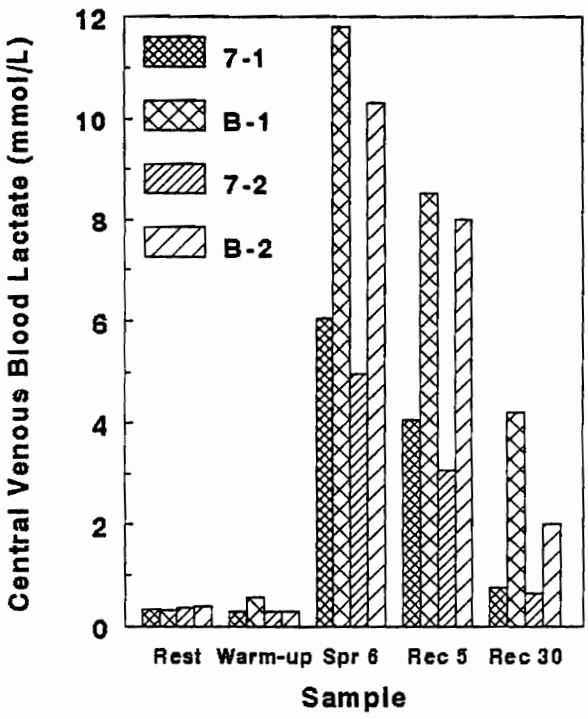

FIGURE 7 Comparison of central venous blood lactate concentration at SET 1 and SET 2 in Blazer and the mean of the other 7 horses. Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period. B-1, B-2, and 7-1, 7-2 are the values for Blazer, and the means of the other 7 horses at SETs 1 and 2, repectively.

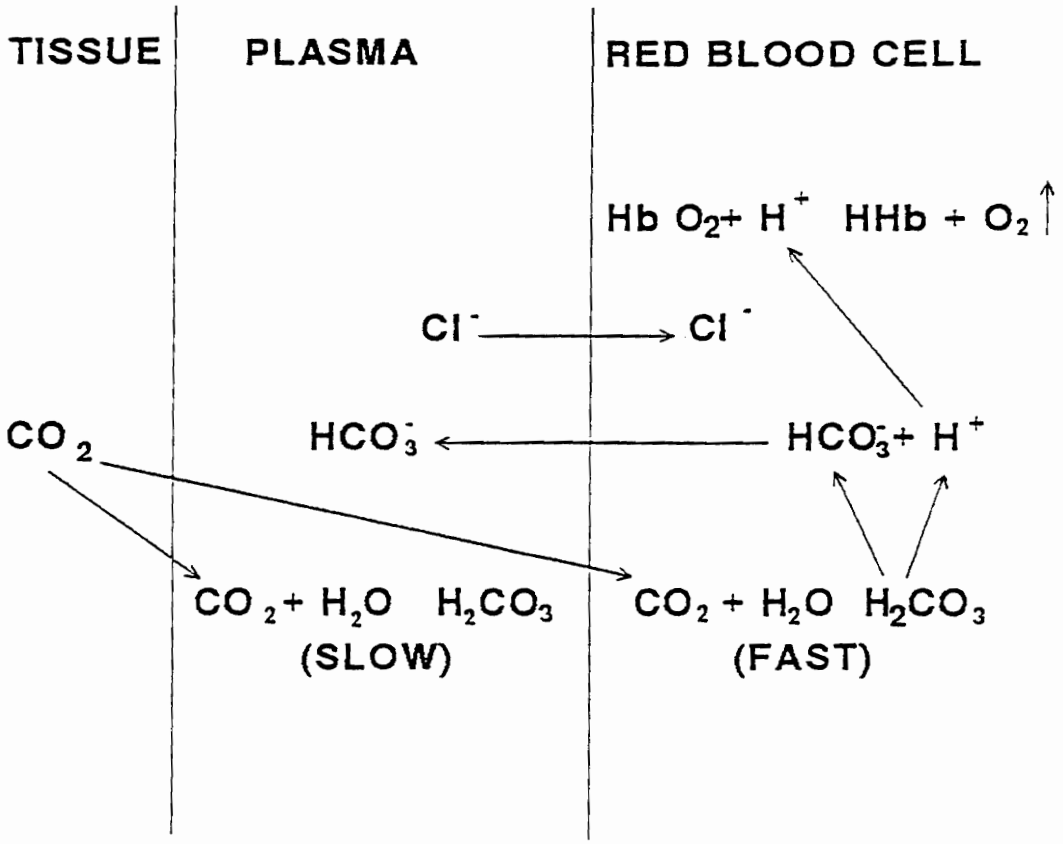

FIGURE 8 Representation of the chloride shift. From E. E. Selkurt, Ed. Physiology, 4th Edition. Little, Brown, and Co., Boston, MA, 1976, pp. 466.

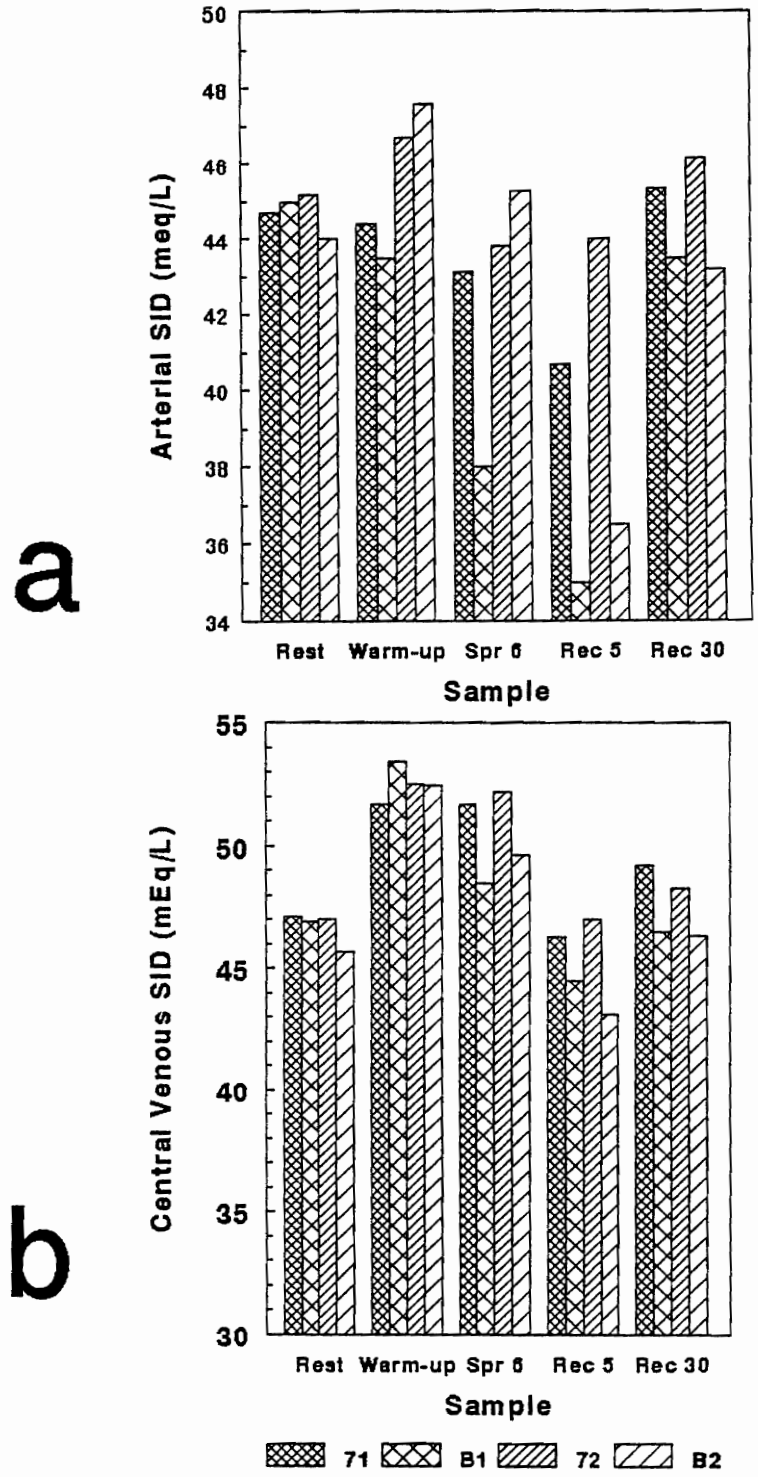

FIGURE 9 Comparison of arterial (a) and central venous (b) strong ion difference in Blazer and the mean of the other 7 horses. Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period. B-1, B-2, and 7-1, 7-2 are the values for Blazer, and the means of the other 7 horses at SETs 1 and 2, respectively.

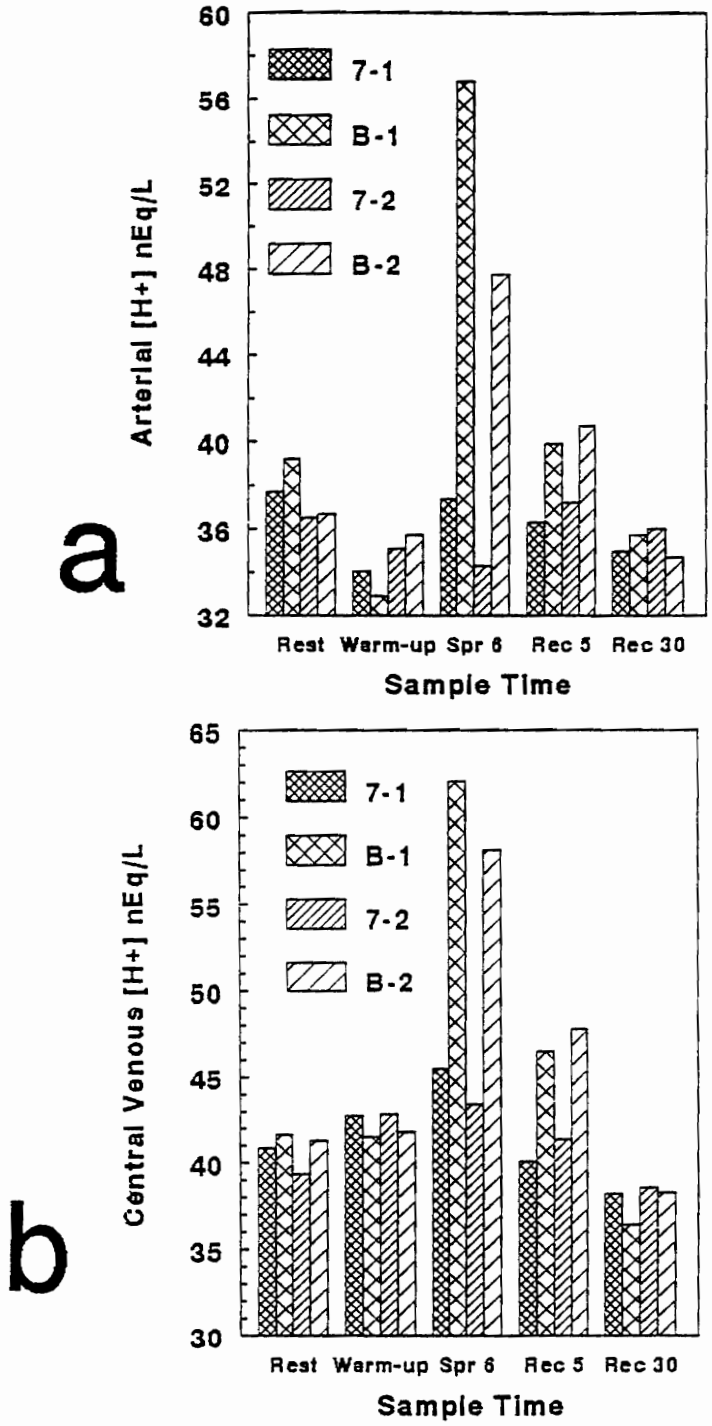

FIGURE 10 Comparison of arterial (a) and central venous (b) [H^+] in Blazer and the mean of the other 7 horses. Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period. B-1, B-2, and 7-1, 7-2 are the values for Blazer, and the means of the other 7 horses at SETs 1 and 2, respectively.

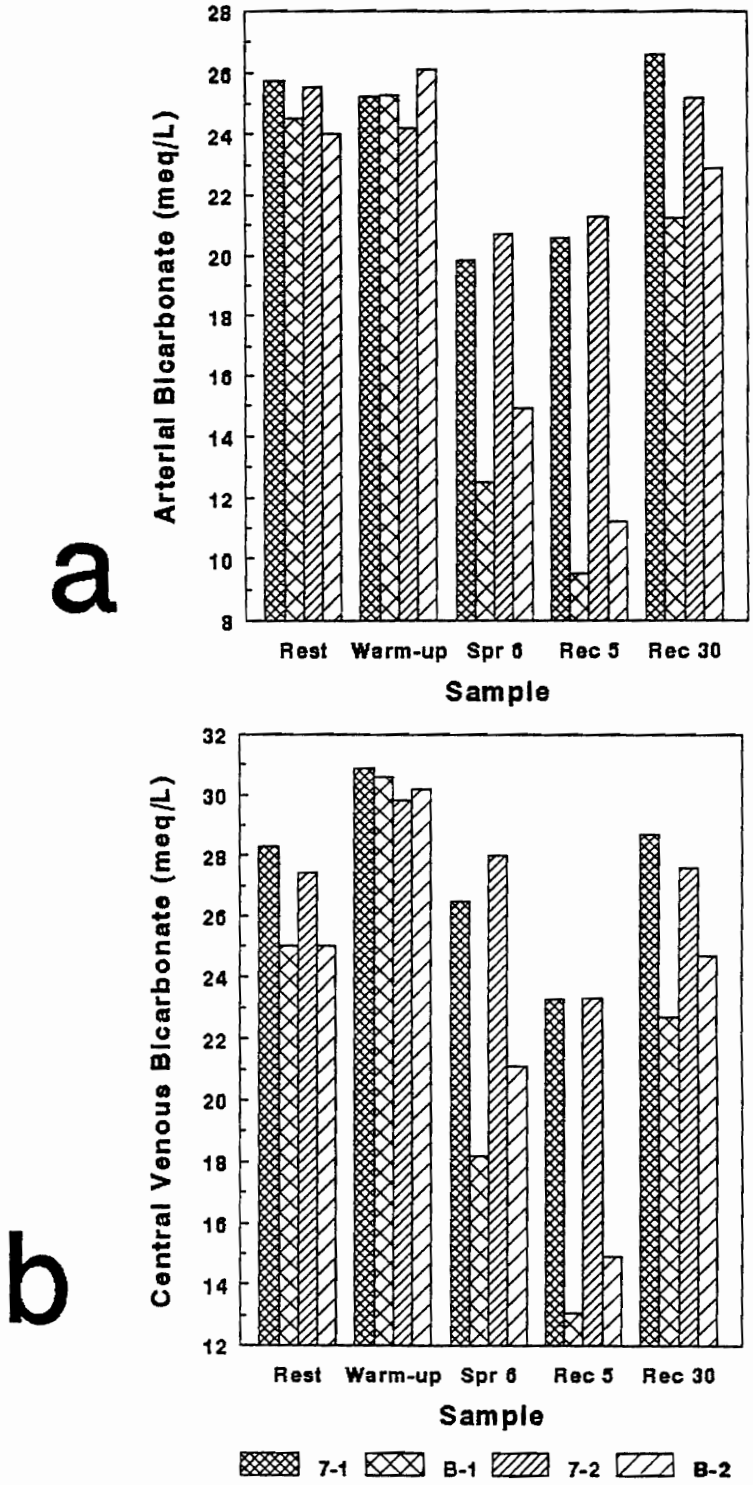

FIGURE 11 Comparison of arterial (a) and central venous (b) [HCO_3^-] in Blazer and the mean of the other 7 horses. Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period. B-1, B-2, and 7-1, 7-2 are the values for Blazer, and the means of the other 7 horses at SETs 1 and 2, respectively.

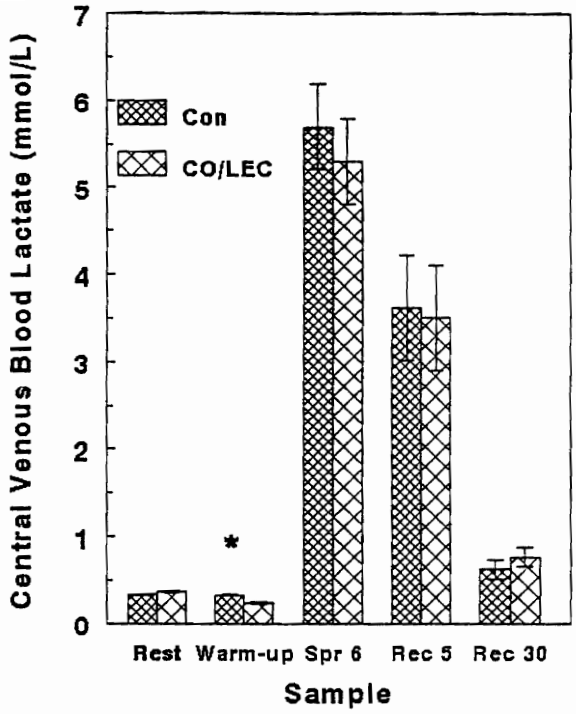

FIGURE 12 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous blood [Lac^-] during a standard exercise test (SET). Samples were obtained at rest, at the end of warm-up, at the end of sprint 6 (Spr 6), and at 5 (Rec 5) and 30 (Rec 30) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * Diets are significantly different at warm-up (P = 0.009).

Journal Article 3. Effects of sprint training and a lecithin/corn oil diet during intermittent exercise in Arabian Horses II: Plasma free fatty acid, triglyceride, beta-hydroxybutyrate, cholesterol, and glucose concentrations

ABSTRACT

Eight Arabian horses were used in a crossover design to study the effects of exercise, training, and dietary lecithin on blood metabolites. Blood samples were taken from the right heart (central venous, V), and assayed for plasma free fatty acids, triglycerides, beta-hydroxybutyrate, cholesterol, and glucose concentrations. Exercise decreased plasma free fatty acids and beta-hydroxybutyrate, and increased plasma triglycerides and blood glucose. Plasma cholesterol did not change during exercise. Training increased beta-hydroxybutyrate responses, and decreased cholesterol responses during exercise. The lecithin-supplemented diet lowered triglycerides at rest and during exercise, raised cholesterol at rest, exercise, and recovery, and raised glucose at the beginning of exercise, and at the end of recovery.

KEY WORDS: exercise, horses, lecithin, training, glucose, free fatty acids

Introduction

Fat supplemented diets for horses offer advantages over conventional diets because they contain more energy in less bulk, can enhance endurance capacity, alter muscle glycogen content and availability during exercise, and may affect behavior (Frape, 1994). The source of fat and dietary level have been widely variable, and the results inconsistent, especially regarding fat adaptation (training combined with a fat supplemented diet) for enhanced exercise performance (Potter et al., 1992).

Soy lecithins are a mixture of phospholipids which have been found to affect the metabolism of lipids and lipoproteins (Wong et al., 1980), and to play an important role in triglyceride transport out of the intestinal mucosa (O'Doherty et al., 1973). Phosphatidylcholine, a predominant class of phospholipid in soy lecithin, affects responses to exercise, including muscle contraction (Conlay et al., 1992; von Allworden et al., 1993). Oral supplementation of choline and lecithin in humans prevents the exercise-associated drop in plasma choline, and may improve endurance capacity (Hirsch et al,. 1978; Sandage et al., 1992).

Previous studies with horses have shown that mixtures of corn oil and lecithins are palatable and highly digestible in complete rations, despite a slighlty lowered crude protein and fiber digestibilty (Holland et al., 1995). The present report

examines the effects of a supplemental lecithin and corn oil diet combined with sprint training on plasma free fatty acids, triglycerides, beta-hydroxybutyrate, cholesterol and glucose concentrations.

Materials and Methods

Animals. Eight Arabian horses, six geldings and two mares, age 5 to 7 years were used in a randomized switchback design with repeated measures. They weighed 403 ± 12 kg (mean ± SE), and had been previously sprint-trained twice weekly for 8 wk on a Mustang 2200 high speed treadmill (Kagra Ag Mustang 2200, Switzerland). Horses were housed and fed individually in box stalls (8.5 m^2) at night, and housed together in a dirt paddock (60 x 78 m) during the day, where water and trace-mineralized salt were available ad libitum. Horses were evaluated for lameness, described previously (Taylor et al., 1994b), and exercised twice weekly on the treadmill to maintain a desirable level of fitness (**Table 1**). This protocol was approved by the University's animal care committee.

Diets. Horses were randomly assigned to one of two total mixed rations: a control diet (CON) consisting of chopped hay, corn, crimped oats, soybean meal, beet pulp, and molasses (n = 4), or an isocaloric amount of a similar diet (n = 4)

containing 14% fat (CO/LEC) in the form of 50% corn oil and 50% lecithin (**Table 2**). Diets were fed initially at 2% of bodyweight, were formulated to meet or exceed moderate work in horses (NRC, 1989), and were fed in two equal portions at 0700, and 1500 hr each day. Horses were weighed weekly on a large animal scale (E-Z Weigh, Cave Creek, AZ), and feed was adjusted to maintain bodyweight. Each horses was fed its assigned diet for 10 wk (Period 1), then consumed the other diet for 10 wk (Period 2).

Standard exercise test (SET) procedures. Feed, but not water was witheld for 12 h prior to the morning of the sprinting exercise test (**Table 3**) to avoid the acute influence of a meal, and possible digestive disturbances. The treadmill barn had an average ambient temperature of 19°C, with 50% relative humidity. Two horses, one from each group, were tested per day, and were brought into the barn at least one hour prior to any handling. Resting heartrate (HR) and rectal temperatures were taken, and an area over the left jugular vein was surgically prepared.

An area over the midcervical region of the jugular vein was anaesthetized with 1 ml of lidocaine, and a small incision was made in the skin with a #10 scalpel blade. A 10 gauge needle was placed aseptically into the vein, and a sterile polyethylene catheter attached to a saline manometer was introduced into the right atrium of the heart. When the

position was verified, the manometer was removed, and a 5 ml extension set was attached to the tubing and kept patent with heparinized saline. The horse was allowed to stand quietly for at least 1 h before the test. Heart rate was monitored during the test with with a commercial digital heart monitor (Polar CIC, Clifton, NJ), and rectal temperature was taken at each sampling time.

Sample collection and assays. Prior to the start of the test, resting blood samples were taken from the right atrium. Additional samples were obtained 15 s prior to the end of the warm-up (W), 15 s prior to the end of sprints 1 - 6 (S 1 - 6), and at 5 (R 1), 10 (R 2), 20 (R 3), and 30 (R 4) min of recovery. Blood samples (35 ml) were drawn and placed in heparinized tubes, and plasma was immediately harvested from the samples, and stored at -5°C for determination of free fatty acids by enzymatic method (Wako Chemical, Dallas, TX), cholesterol and triglycerides by enzymatic methods, (Sigma Chemical, Proc. # 352 and # 337, respectively), and beta-hydroxybutyrate by a dehydrogenase method (Sigma Chemical, Proc. # 310-A). An additional blood sample (3 ml) was drawn and placed in tubes containing sodium flouride for determination of plasma glucose by a hexokinase method (Sigma Chemical, Proc. # 16).

Statistical Analysis. The data were examined by analysis of

variance with repeated measures using the GLM procedure of SAS (SAS, 1990), with horse, SET, and diet as the independent variables. Dunnett's t-tests were used to compare changes in exercise variables to the resting values. Significance was set at $P < 0.05$. Results are expressed as lsmeans $\pm$ 1 SEM unless otherwise stated. One horse exhibited very high concentrations of glucose and triglycerides (" Blazer "), 10 and 8 standard deviations higher than the mean for the other 7 horses, respectively. The glucose and triglyceride data will be presented as the mean for 7 horses, with data for Blazer presented separately for comparison.

Results and Discussion

Feed intake and bodyweight. All horses became accustomed to consuming the total mixed ration within 3 - 4 d, with no palatability problems. Horses consuming the CON diet ate less feed than horses on the CO/LEC diet, with no differences in bodyweight between diets or periods (**Table 4**). This is in agreement with other studies showing a good acceptibility and utilization of fat-supplemented diets by horses (Hintz, 1994).

Heartrate and temperature. Mean heartrate (beats/min) increased from 34.3 $\pm$ 0.8 at rest to 201.4 $\pm$ 3.1 at the end of Spr 6, and had returned to 65.6 $\pm$ 2.5 at Rec 30. Training resulted in a decreased heart rate (beats/min) at sprints 1 -

3, and at 10, 20, and 30 min of recovery (Figure 1). Mean rectal temperature (°C) was 37.9 ± 0.3 at rest, rose to 40.2 ± 0.6 at the end of Spr 6, and was 39.3 ± 0.5 at Rec 30, and was not significantly affected by training.

Effects of Exercise. Plasma concentrations reflect changes in both entry and removal (Figure 2). Changes in plasma metabolites are summarized in Table 5. Plasma free fatty acid concentration ([FFA]) initially decreased at Spr 1 and 2, but by Spr 4 values were not different from rest (Table 5). The initial decrease was probably due mainly to an increased rate of utilization by muscle, and the subsequent increase during exercise probably reflected an increase in FFA release from adipose tissue (Figure 2). The further increase during recovery could be attributed to an increased rate of FFA entry into plasma, augmented by an abrupt decrease in utilization by muscle.

The increase in [FFA] during exercise has been seen previously during 50 min of incremental exercise in horses (Zimmerman et al., 1992), during 1 hr of steady exercise in sled dogs (Reynolds et al., 1994), and in humans exercising for 4 hr at 40% VO_2max (Wolfe et al., 1990). The increase in [FFA] during exercise is due to both a decrease in reesterification, and an increase in triglyceride hydrolysis, making FFA available for oxidation (Wolfe et al., 1990). Plasma epinephrine, which stimulates lipolysis, increases with

exercise, and can be 12 times greater during exercise in the horse (heart rate 200 beats/min) than in humans at maximal effort (Snow et al., 1992). Feed was witheld prior to exercise for 10 - 12 hr in the present study, which may have contributed slightly to the increase in [FFA] via adipose tissue lipolysis (Watson and Love, 1994).

Plasma glycerol is also liberated at hydrolysis, and has been shown to increase during exercise in horses (Duren et al., 1986). The subsequent increase in [FFA] back to pre-exercise levels at Spr 4 in the present study may indicate that preferential utilization of FFA occurs early in exercise. However, it is possible that FFA was still being used as fuel for work during the last sprints, as there was a sudden 30% increase in [FFA] immediately after exercise (Rec 1).

The plasma triglyceride concentration ([TG]) increased at Spr 2, and returned to resting values by Rec 3 (Table 5). This agrees with previous findings during different types of exercise in horses (Duren et al., 1986; Harkins et al., 1992; McCollum et al., 1993; Pagen et al., 1995) but the response in humans is inconsistent (Pronk, 1993). The increase found in the present study may be due in part to increases in FFa to TG-rich VLDL secretion during exercise by the liver (Figure 2), which remained greater than any increase in the rate of utilization by muscle until at least Rec 2.

The beta-hydroxybutyrate concentration ([BHB]) decreased immediately at W, probably due to an increased utilization

combined with little compensatory increased production by liver (Figure 2). The [BHB] remained below pre-exercise levels throughout exercise and recovery, which is in contrast to previous findings in the horse (Harkins et al., 1992). Studies in rats and humans have shown an increase in the production rate of ketone bodies by the liver during exercise (Fukuda et al., 1991). However, the decreased [BHB] in the present study is inversely proportional to the increased plasma TG, which is consistent with a partitioning effect in the liver towards FFA esterification (Zammit, 1983).

Cholesterol concentration ([CHOL]) increased throughout exercise before returning to pre-exercise levels at Rec 1, probably due to an increased production, combined with no increase in the rate of utilization (Figure 2). The decrease at Rec 3 and 4 is most likely due to a subsequent decreased production. This effect has been seen inconsistently during aerobic exercise in humans subjects (Baker et al., 1986; Dufaux et al., 1986), and during exercise in horses (Hambleton et al., 1980). The rise in total [CHOL] during exercise in humans has been associated primarily with the rise in the high-density lipoprotein (HDL) fraction (Ekstedt et al., 1991). Kurcz and coworkers (1993) reported no change in the HDL fraction of cholesterol, and an increase in the very low density lipoprotein (VLDL) fraction at rest in trained horses, but measurements were not obtained during exercise.

Plasma glucose concentration ([GLU]) increased at Spr 2, and

remained elevated throughout exercise and recovery, which probably reflects an increase in production that was greater than any increase in the rate of utilization (Figure 2). This response agrees with previous studies in horses during different exercise intensities (Duren et al., 1986; Essen-Gustavsson et al., 1991; Harkins et al., 1992; Custalow et al., 1993; McCollum et al., 1993). Circulating blood GLU originates primarily from the liver, and is derived either from glycogen stores or is newly synthesized, with its production stimulated by catecholamines (Stanley and Connett, 1991). The sustained rise in plasma [GLU] in the present study may indicate either an increased mobilization from glycogenolysis, or, with time, an increase in gluconeogenesis. When a large mass of muscle is active in humans, plasma catecholamines increase quickly, and the mobilization of glucose exceeds peripheral uptake (Kjaer et al., 1991).

Effects of Training. Although the protocol was aimed at maintaining a steady plane of fitness between periods, the 2 d/wk of exercise resulted in training effects. The changes associated with training in plasma and blood variables are summarized in Table 6. Training increased plasma [FFA] (Figure 3), which has been demonstrated previously during exercise in horses after 9 weeks of conditioning (Hambleton et al., 1980). In addition to increasing the availabilty of FFA at rest and during exercise, there was a larger decrement in

[FFA] at the onset of exercise after training, which may indicate an enhanced utilization.

The elevated [FFA] in the present study could be due to a training-induced increase in lipolysis of muscle TG, but the delivery of FFA to working tissues by equine albumin may exceed uptake. Plasma albumin increased in Arabian horses during similar exercise tests, and showed a greater response after sprint training (Taylor et al., 1995). In vitro experiments have shown that canines possess "aerobic" albumin, which may improve fatty acid transport by 50% as compared to a more sedentary species (goat) (McClellend et al., 1994). Horses are similar to dogs in many aspects associated with exercise, and both have been called "elite athletes" (Snow, 1985). Arabian horses seem to be particularly adapted to superior aerobic capacity due to a greater percentage of Type I and IIa muscle fibers (Snow and Guy, 1980), and a low lactate production compared to other breeds (Mc Collum et al., 1993).

Training increased plasma [BHB] (Figure 4) at rest, exercise and recovery, which may indicate an increased availability of this ketone for oxidation. Training increased oxidation of ketone bodies for fuel during exercise in rats (Askew et al., 1975). Effects of training on changes in ketone bodies have not been widely reported in the horse, but ketone body formation by the liver via acetyl CoA units may be enhanced by the training-induced increase in plasma [FFA] seen in the

present study.

Training decreased plasma [CHOL] at rest, exercise, and recovery (Figure 5). This is in contrast to previous findings in horses, which demonstrated increased resting serum cholesterol after training (Goodman et al., 1973). Although findings are not consistent, responses in horses and humans could be similar, as regular training may decrease serum VLDL and LDL cholesterol, and increase HDL cholesterol (Ekstedt et al., 1991).

Effects of Diet. The CO/LEC diet increased plasma cholesterol (mg/dL) by a large and consistent margin at rest, exercise and recovery (overall lsmeans $\pm$ 1 SE: control = 88.13 $\pm$ 3.3, CO/LEC = 120.83 $\pm$ 2.9). This is in agreement with previous studies in horses (Rich et al., 1981), and there may be a direct positive relationship between amount of fat in the diet and serum cholesterol level (Hambleton et al., 1980). The other diet effects were not as consistent, and are summarized in Table 7.

Plasma TG was higher in the CON group at rest, W, Spr 1 and 2, and Rec 1 and 2 (Figure 6), and is in agreement with previous findings in horses (Duren et al., 1986). Horses on the CON diet may have absorbed significant dietary energy as glucose, which enters the liver for conversion to glycogen, or VLDL for storage in adipose tissue. The higher plasma TG in CON horses may be due to increased concentrations of

circulating TG-rich VLDL. The [TG] for Blazer on the CON diet was 3 times higher than the mean for the other 7 horses (Figure 7).

Although there were no differences at rest or during sprinting, the plasma [BHB] was higher in the CON group at W, and Rec 1 - 3 (Figure 8). This surprising result has been seen previously in Thoroughbred horses at rest and at 8 and 16 min after a 1600 m race (Harkins et al., 1992). The lack of differences during sprinting exercise may be the result of an increased utilization of BHB by the horses on the CO/LEC diet only during warm-up and recovery. The [BHB] decreased abruptly with the onset of exercise regardless of diet, but horses consuming the CO/LEC diet may have continued to oxidize plasma BHB during recovery, as [BHB] in control horses began to increase by Rec 4.

Plasma [GLU] was higher in the CO/LEC group at W, Spr 1, 5, and 6, and Rec 1 - 4 (Figure 9), with Blazer having [GLU] 20-60 mg/dL higher than the mean of the other horses at all times during exercise and recovery (Figure 10). The glucose-sparing effect of the fat supplemented diet has been seen previously in horses, despite differences between type of dietary fat, breed of horse, and exercise intensity and duration (Hambleton et al., 1980; Duren et al., 1986; Harkins et al., 1992). Increased plasma [FFA] availability will decrease GLU utilization (Nolte et al., 1994), but the CO/LEC diet did not significantly affect plasma [FFA] in the present study. It is

possible that this effect was masked by the combination of diet and sprint training.

The Arabian breed of horse possesses a larger proportion of Type I and IIa oxidative muscle fibers, and training improves intrmuscular TG storage and mitochondrial density in these fiber types in humans (Hagerman, 1992). This pre-disposition for increased aerobic capacity may have led to some FFA utilization in both groups during exercise.

In conclusion, repeated sprinting exercise caused decreases in plasma [FFA] and [BHB], increases in plasma [CHOL] and [TG], and increased blood [GLU] in Arabian horses. Sprint training 2d/wk was sufficient to elicit training effects on heart rate, and plasma [FFA], [BHB], and CHOL. Addition of a corn oil/lecithin diet resulted in increased plasma [CHOL], decreased [TG] and [BHB], and a glucose-sparing effect in plasma. Horses may be different in some responses to exercise and fat oxidation than humans and rats, and the Arabian breed may be superior in their response to fat adaptation as compared to other breeds.

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TABLE 1

Bi-weekly treadmill sprint training protocol

Time (min)	Slope (%)	Speed (m/s)	Gait
0 - 2:30	0%	1.5	walk
2:30 - 5	6%	1.5	walk
5 - 9	6%	3.5	trot
9 - 11	6%	7.0	gallop
11 - 15	6%	4.5	trot
15 - 17	6%	8.0	gallop
17 - 21	6%	4.5	trot
21 - 23	6%	9.0	gallop
23 - 27	6%	3.5	trot
27 - 30	0%	1.5	walk

TABLE 2

Composition of diets

Ingredient (%)	Control diet	CO/LEC diet
Alfalfa/Orchardgrass hay mix	42.0	56.0
Soybean meal	5.0	10.0
Cracked corn	18.5	3.3
Crimped oats	18.5	3.3
Beet pulp	5.0	11.1
Wet molasses	10.0	-
Dry molasses	-	5.0
Vitamin pre-mix[1]	1.0	1.4
Corn oil/soy lecithin[2]	-	10.0

[1] Hoffman-LaRoche Inc., Nutley, NJ

[2] Lucas-Meyer, Decatur, IL; analysis as previous (pp. 96)

TABLE 3

Treadmill standard exercise test (SET) protocol

Time (in min)	Slope (%)	Speed (m/s)	Gait
Warm-up sequence:			
5	0	1.5	walk
5	6	3.5	trot
Repeated sprint sequence (6 repetitions):			
1	6	10.0	gallop
0.5	6	3.5	trot
3.5	6	1.5	walk
Recovery sequence:			
30	0	1.5	walk

TABLE 4

Chemical composition of diets[1], feed intake, and bodyweights

	Control diet	CO/LEC[2]
Dry Matter, %	88.90	90.80
Crude protein, %	12.10	13.00
Acid detergent fiber, %	24.80	24.70
Ether extract, %	2.60	11.60
Energy, Mcal DE/d[3]	20.70	20.00
Feed consumption, kg/d	7.73 ± 0.42*	6.68 ± 0.34
Bodyweights, kg		
SET 1:	400 ± 15	398 ± 12
SET 2:	407 ± 10	404 ± 13

[1] As fed basis

[2] CO/LEC = corn oil/lecithin-supplemented diet

[3] Direct calculation (Holland, 1995)

* differs from CO/LEC (P = 0.042)

TABLE 5

Effects of sprint exercise on central venous (V) plasma free fatty acids (FFA), cholesterol (CHOL), triglycerides (TG), beta-hydroxybutyrate (BHB), and glucose (GLU) concentrations (n = 8, values for SET 1 and 2 have been combined)

	[FFA] (meq/L)	CHOL (mg/dL)	TG (mg/dL)	[BHB] (mg/dL)	[GLC] (mg/dL)
Rest	0.75^{a}	99.0^{a}	13.1^{a}	4.50^{a}	86.9^{a}
SE	0.10	1.90	1.80	0.14	0.82
Warm[1]	$0.52^{a,b}$	105.2^{b}	14.8^{a}	3.21^{b}	84.6^{a}
SE	0.08	2.73	2.22	0.14	1.70
Spr 1[2]	0.46^{b}	105.5^{b}	14.6^{a}	3.24^{b}	87.8^{a}
SE	0.07	2.99	2.31	0.17	0.76
Spr 2	0.47^{b}	105.3^{b}	21.0^{b}	3.38^{b}	101.5^{b}
SE	0.07	3.34	2.44	0.23	3.26
Spr 3	0.52^{b}	104.8^{b}	23.4^{b}	3.45^{b}	101.6^{b}
SE	0.08	3.16	3.34	0.22	3.64
Spr 4	$0.57^{a,b}$	104.5^{b}	24.0^{b}	3.52^{b}	103.7^{b}
SE	0.09	2.90	3.57	0.19	5.39
Spr 5	$0.56^{a,b}$	104.7^{b}	24.4^{b}	3.69^{b}	104.5^{b}
SE	0.09	3.17	3.22	0.23	4.95
Spr 6	$0.54^{a,b}$	103.5^{b}	25.0^{b}	3.64^{b}	107.1^{b}
SE	0.09	2.59	3.46	0.13	5.97
Rec 1[3]	0.75^{a}	$99.5^{a,b}$	26.2^{b}	3.73^{b}	109.3^{b}
SE	0.09	2.54	3.25	0.12	5.22
Rec 2	0.77^{a}	$98.5^{a,b}$	21.5^{b}	3.67^{b}	106.4^{b}
SE	0.09	2.43	3.64	0.09	5.21
Rec 3	0.72^{a}	$96.0^{a,b}$	12.8^{a}	3.47^{b}	104.3^{b}
SE	0.05	2.64	2.35	0.11	5.01
Rec 4	$0.64^{a,b}$	$96.0^{a,b}$	9.5^{a}	3.51^{b}	100.3^{b}
SE	0.09	4.18	2.35	0.42	5.93

[a,b] means sharing superscript letters in the same column are not different ($P < 0.05$)

[1,2,3] Warm = warm-up sample; Spr = sprint #; Rec = recovery sample: 1 = 5 min; 2 = 10 min; 3 = 20 min; 4 = 30 min

TABLE 6

Effects of sprint training on central venous (V) plasma free fatty acid ([FFA]), cholesterol ([CHOL]), and beta-hydroxybutyrate ([BHB]) concentrations. SET 1 differs from SET 2 at all sample times (P < 0.05; n = 8)

	[FFA], (meq/L)		CHOL, (mg/dL)		[BHB], (mg/dL)	
	SET 1	SET 2	SET 1	SET 2	SET 1	SET 2
Rest	0.57	0.93	101.8	94.1	3.95	5.10
SE	0.10	0.10	3.00	3.07	0.12	0.14
Warm[1]	0.42	0.61	109.4	100.9	2.48	3.96
SE	0.05	0.06	2.73	3.15	0.12	0.14
Spr 1[2]	0.40	0.53	110.3	101.4	2.49	4.00
SE	0.04	0.05	2.99	3.45	0.17	0.20
Spr 2	0.39	0.55	110.0	100.5	2.68	4.08
SE	0.05	0.06	3.34	3.86	0.23	0.26
Spr 3	0.43	0.63	109.1	100.5	2.74	4.16
SE	0.06	0.07	3.16	3.65	0.19	0.22
Spr 4	0.47	0.67	108.7	100.3	2.82	4.23
SE	0.08	0.08	2.90	3.35	0.19	0.17
Spr 5	0.46	0.65	108.8	100.6	2.94	4.43
SE	0.07	0.08	3.17	3.40	0.23	0.24
Spr 6	0.45	0.62	109.1	98.4	2.82	4.45
SE	0.06	0.08	2.59	2.99	0.13	0.15
Rec 1[3]	0.66	0.89	104.5	95.9	2.99	4.47
SE	0.08	0.09	2.40	2.80	0.13	0.15
Rec 2	0.67	0.87	104.1	93.6	2.76	4.57
SE	0.08	0.09	2.43	2.81	0.09	0.11
Rec 3	0.59	0.85	100.8	92.5	2.52	4.41
SE	0.05	0.06	2.64	3.04	0.11	0.13
Rec 4	0.59	0.78	103.7	89.2	2.77	4.24
SE	0.05	0.09	4.18	4.83	0.24	0.28

[1,2,3] Warm = warm-up sample; Spr = sprint #; Rec = recovery sample: 1 = 5 min; 2 = 10 min; 3 = 20 min; 4 = 30 min

TABLE 7

Effects of a corn oil/lecithin (CO/LEC) diet on central venous plasma triglyceride ([TG], n = 7), beta-hydroxybutyrate ([BHB], n = 8), and glucose ([GLU], n = 7) concentrations

	TG, (mg/dL)		[BHB], (mg/dL)		[GLC], (mg/dL)	
	CON	CO/LEC	CON	CO/LEC	CON	CO/LEC
Rest	16.1*	10.0	4.57	4.53	83.9	87.0
SE	1.72	1.85	0.14	0.12	2.16	2.10
Warm[1]	17.2*	12.3	3.51*	3.20	82.3*	87.0
SE	1.99	2.02	0.07	0.09	1.73	1.70
Spr 1[2]	17.0*	12.2	3.34	3.14	84.0*	91.5
SE	2.04	2.11	0.14	0.17	0.79	0.76
Spr 2	24.2*	17.8	3.35	3.41	100.8	102.1
SE	2.21	2.44	0.21	0.23	3.26	3.73
Spr 3	25.9	20.8	3.60	3.30	99.3	103.9
SE	3.21	3.34	0.20	0.22	3.17	3.64
Spr 4	26.3	21.6	3.61	3.44	99.1	108.4
SE	3.24	3.52	0.17	0.19	4.39	5.17
Spr 5	27.2	21.5	3.73	3.65	98.7*	110.3
SE	3.19	3.22	0.11	0.13	3.90	4.22
Spr 6	27.3	22.7	3.77	3.51	100.6*	113.6
SE	3.31	3.46	0.10	0.13	3.93	4.15
Rec 1[3]	29.3*	23.0	3.86*	3.59	103.4*	115.3
SE	2.24	2.30	0.08	0.09	3.22	3.58
Rec 2	24.7*	18.2	3.81*	3.52	100.6*	112.2
SE	1.98	2.12	0.07	0.08	3.21	3.63
Rec 3	15.0	10.6	3.65*	3.28	96.8*	111.8
SE	2.30	2.35	0.09	0.11	3.01	3.37
Rec 4	10.4	7.9	3.86	3.15	91.6*	109.1
SE	1.37	1.46	0.10	0.11	3.90	3.79

[1,2,3] Warm = warm-up sample; Spr = sprint #; Rec = recovery sample: 1 = 5 min; 2 = 10 min; 3 = 20 min; 4 = 30 min;
* diets are different within that sampling time ($P < 0.05$)

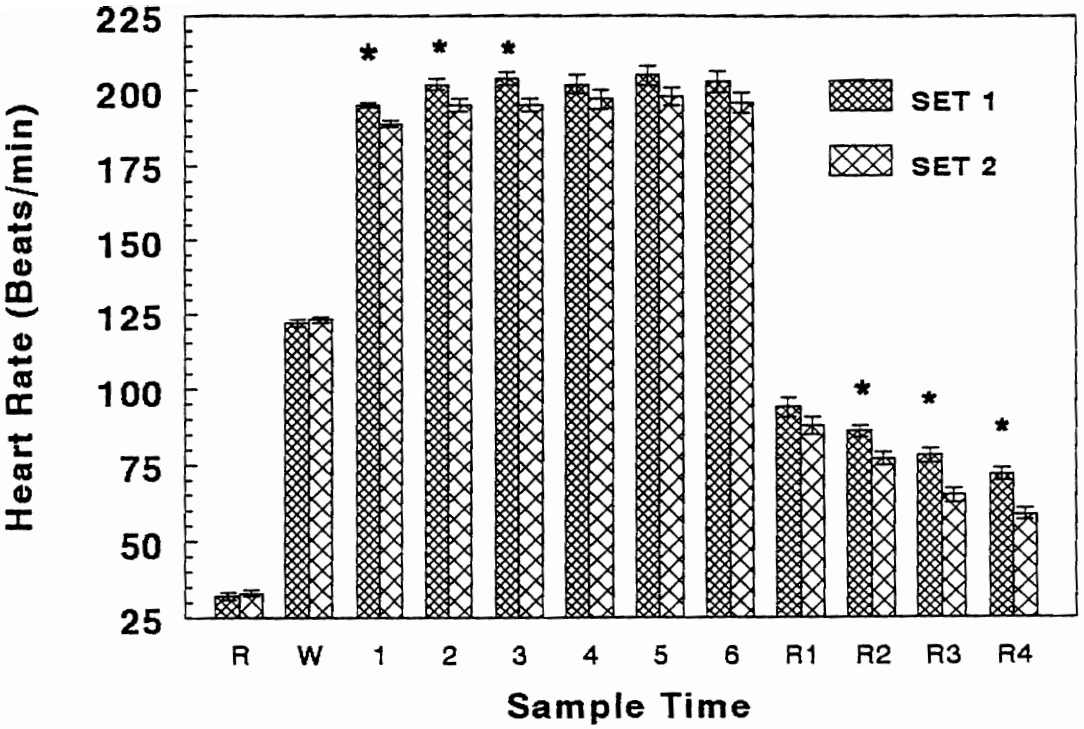

FIGURE 1 Effect of sprint training on heart rate during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 8). Values are lsmeans ± 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$).

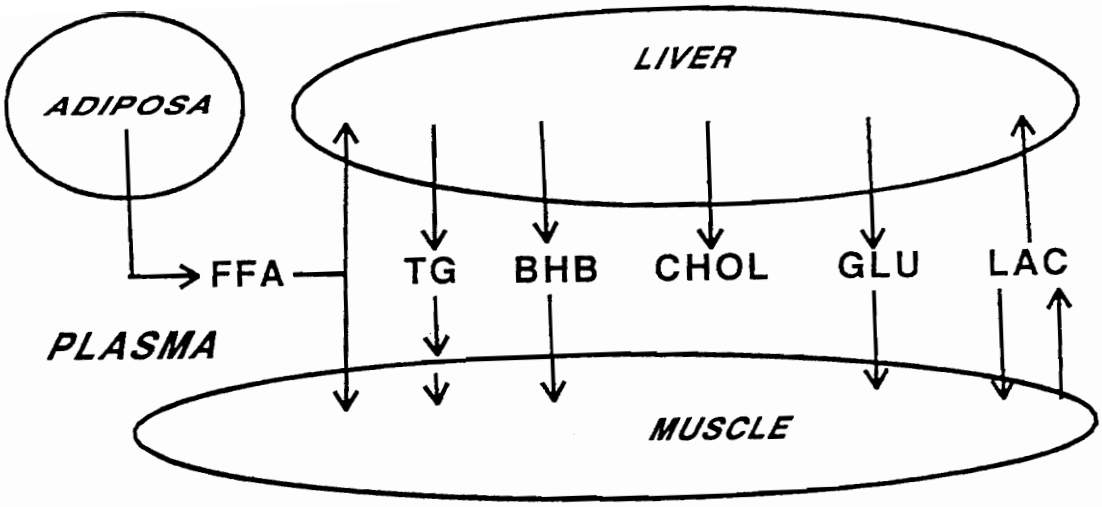

FIGURE 2 Representation of the relationship between the entry and clearance of various metabolites in plasma, and adipose, liver, and muscle tissue.

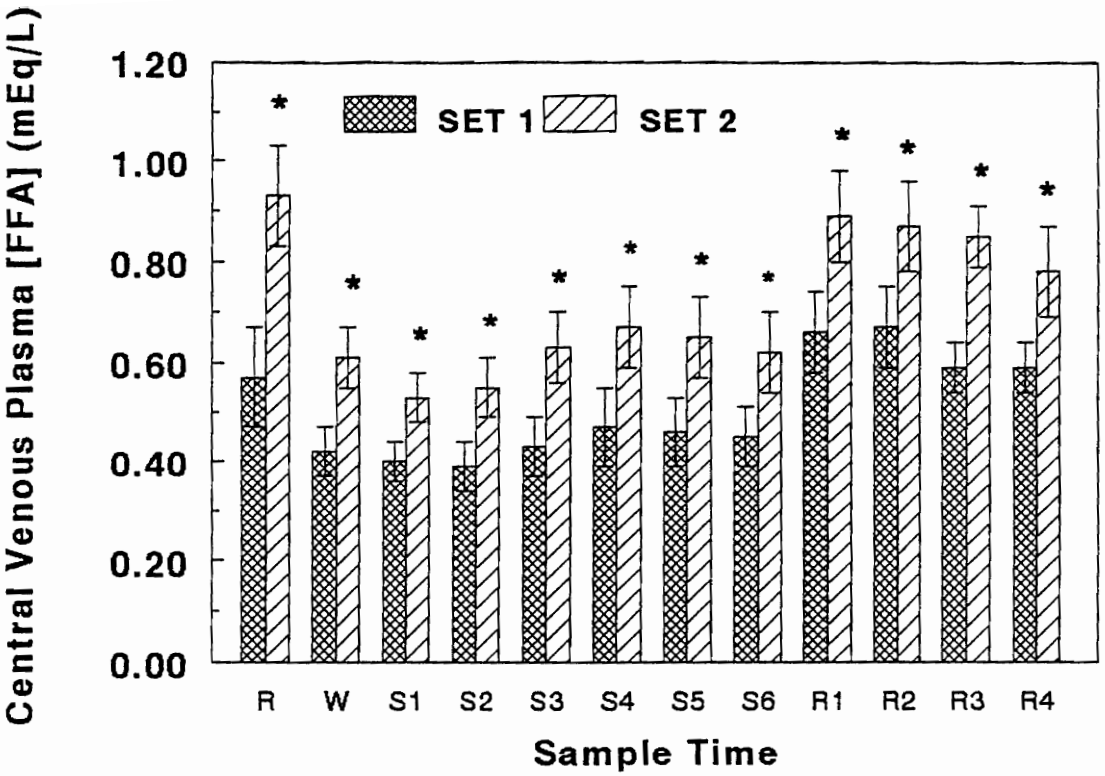

FIGURE 3 Effect of sprint training on central venous plasma free fatty acid concentration ([FFA]) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 8). Values are lsmeans ± 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$). See also Table 6.

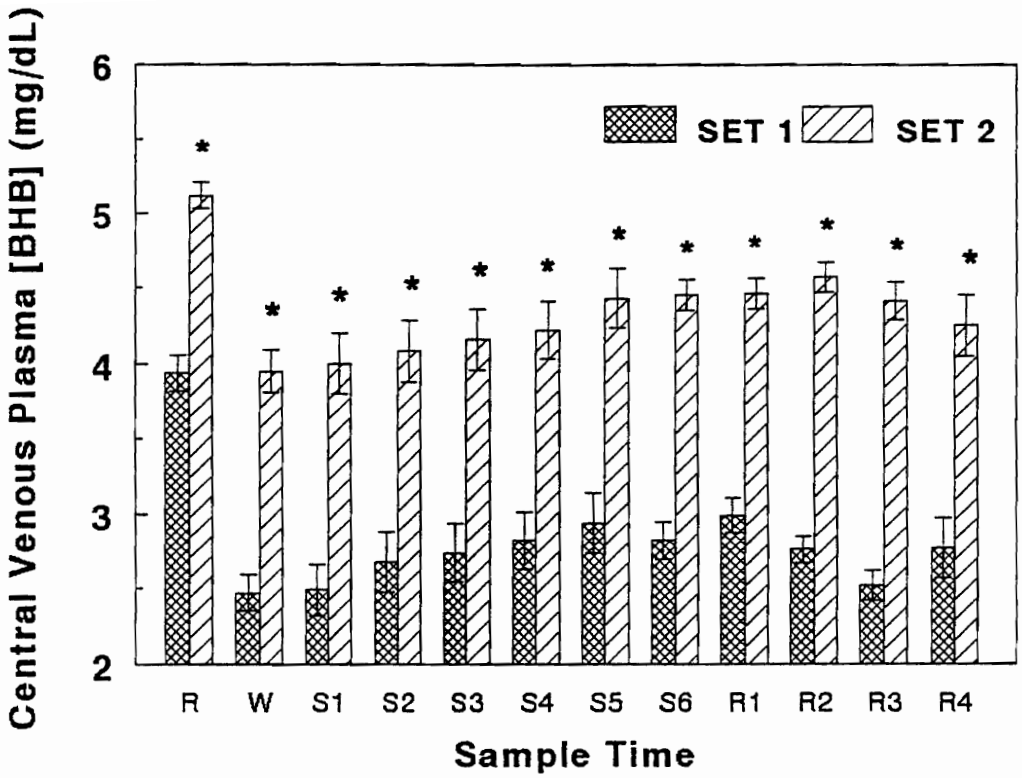

FIGURE 4 Effect of sprint training on central venous plasma beta-hydroxybutyrate concentration ([BHB]) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 8). Values are lsmeans $\pm$ 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$). See also Table 6.

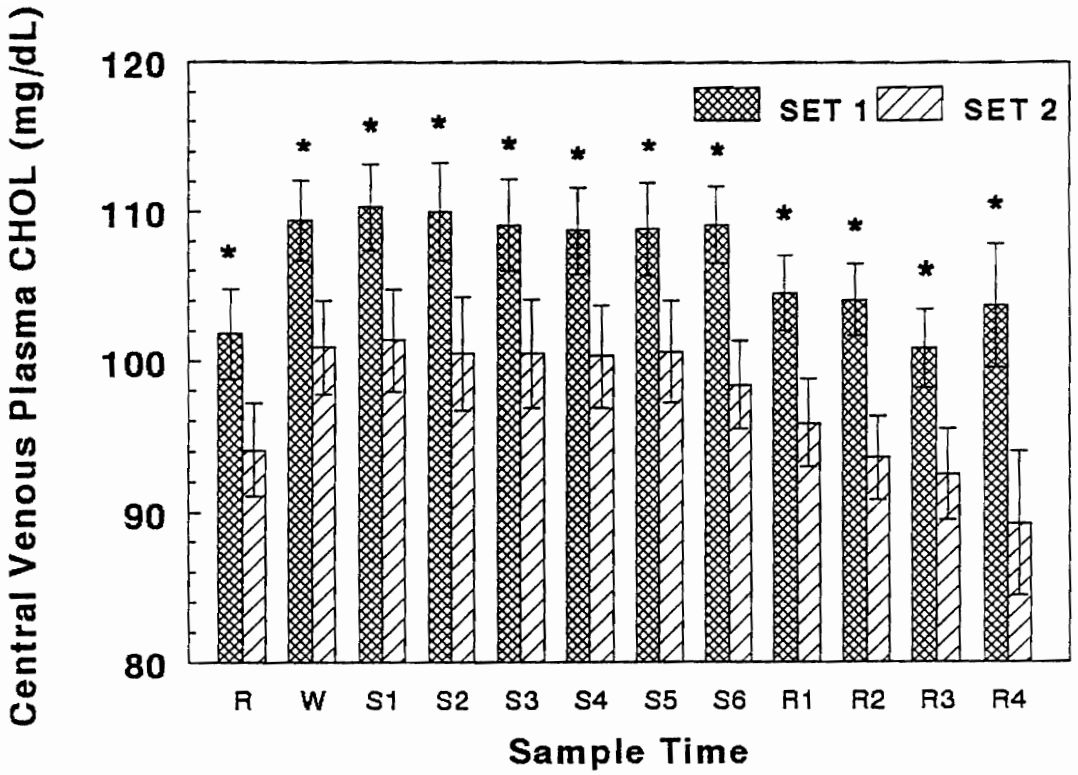

FIGURE 5 Effect of sprint training on central venous plasma cholesterol concentration ([CHOL]) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 8). Values are lsmeans ± 1 SEM. * SETs are significantly different at that sampling time ($P < 0.05$). See also Table 6.

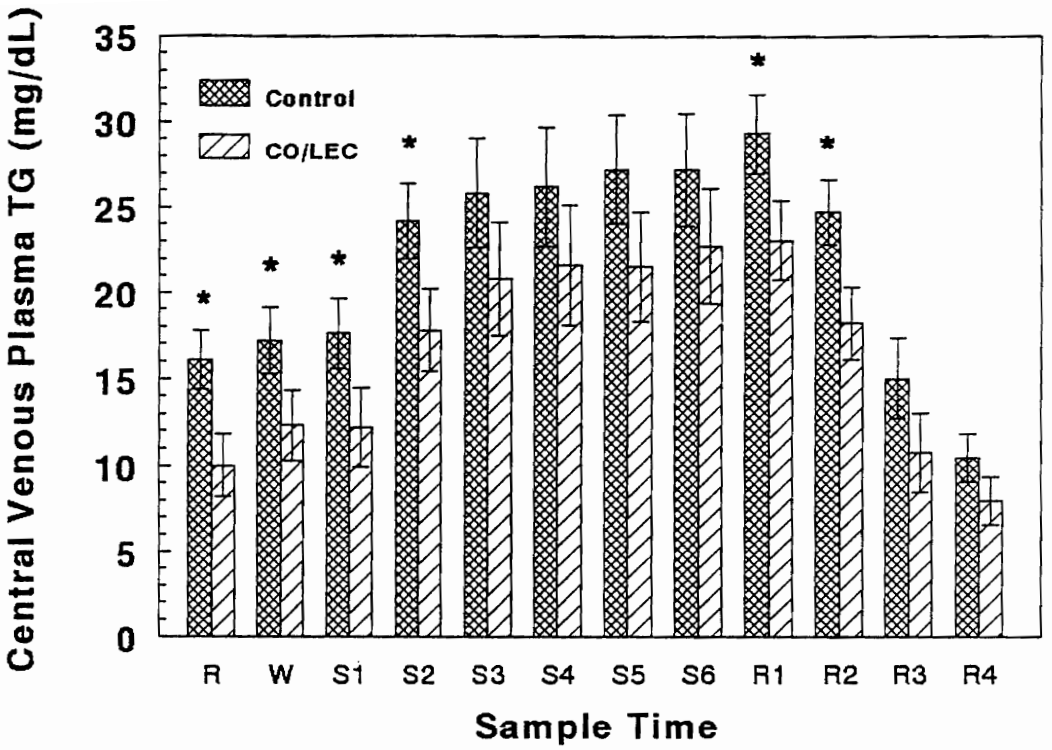

FIGURE 6 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous plasma triglycerides (TG) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * Diets are significantly different at that sampling time ($P < 0.05$).

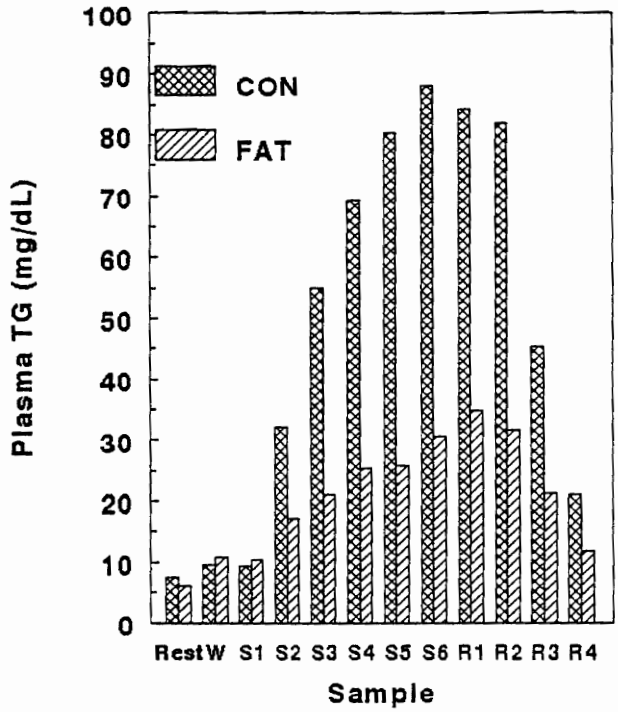

FIGURE 7 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous plasma triglycerides (TG) in Blazer during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period.

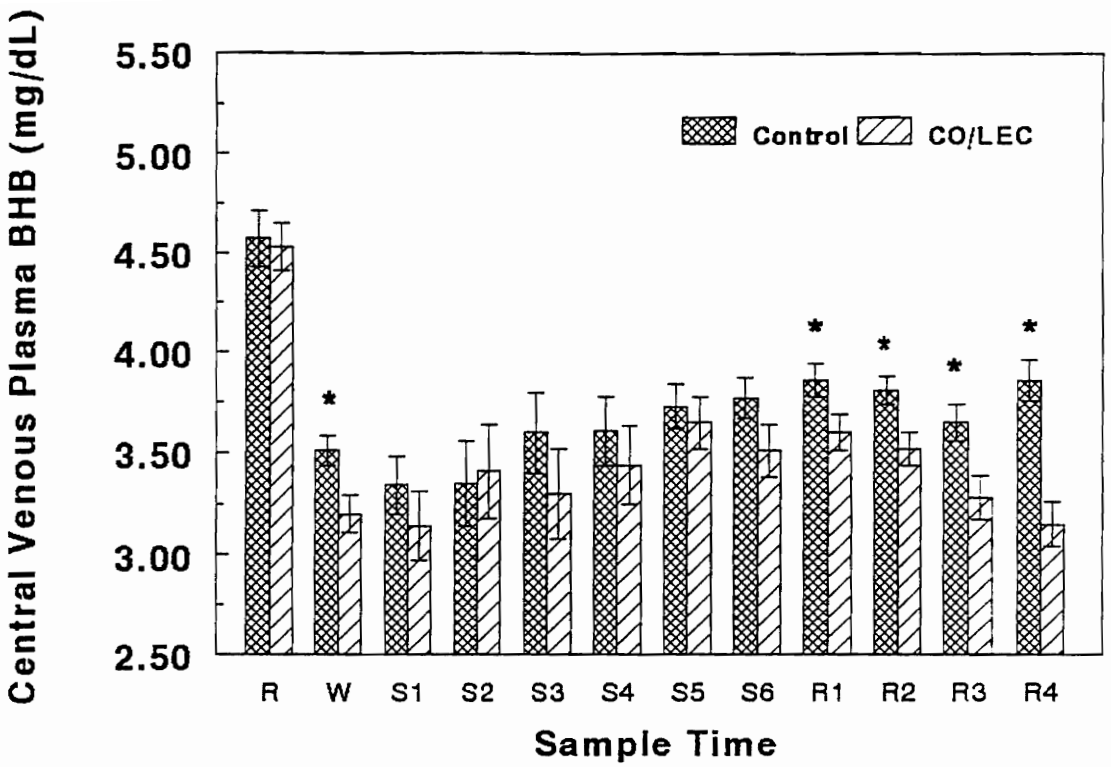

FIGURE 8 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous plasma beta-hydroxybutyrate concentration ([BHB]) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 8). Values are lsmeans $\pm$ 1 SEM. * Diets are significantly different at that sampling time ($P < 0.05$).

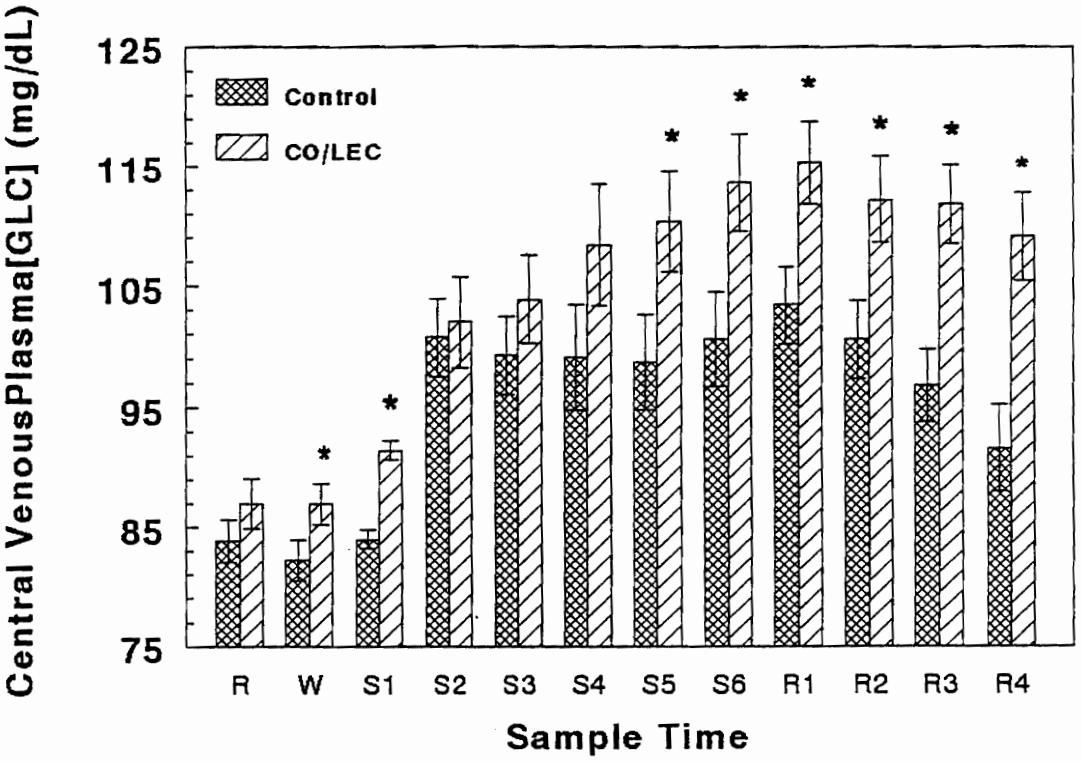

FIGURE 9 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous plasma glucose concentration ([GLU]) during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period (n = 7). Values are lsmeans $\pm$ 1 SEM. * Diets are significantly different at that sampling time ($P < 0.05$).

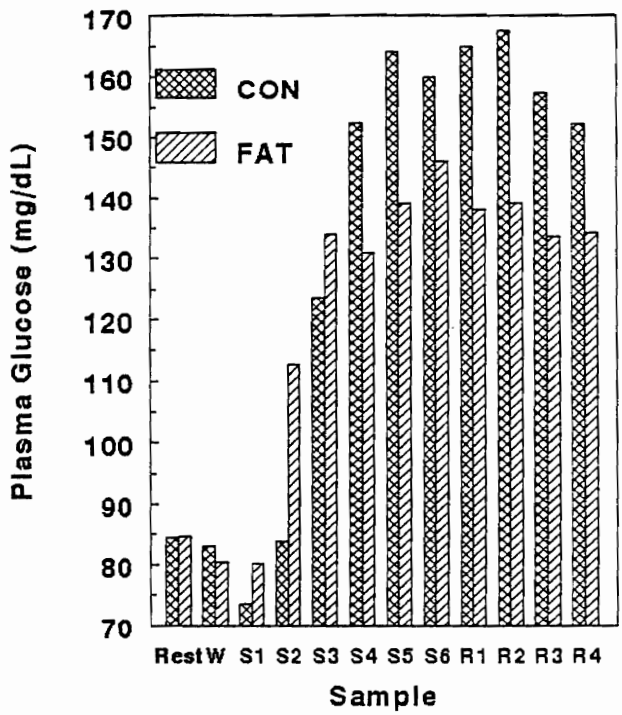

FIGURE 10 Effect of a corn oil/lecithin supplemented diet (CO/LEC) on central venous plasma glucose concentration ([GLU]) in Blazer during a standard exercise test (SET). Measurements were obtained at rest (R), at the end of warm-up (W), at the end of sprints 1 - 6, and at 5 (R1), 10 (R2), 20 (R3), and 30 (R4) min of a walking recovery period.

Journal Article 4. Blood Gas Measurements During Incremental Exercise: Comparison of Temperatures at Four Sites

Abstract

Rectal temperature is often used to adjust measurements of blood gases but may not approximate temperatures during exercise at the main sites of gas exchange, muscle and lung. To evaluate differences between sites, horses completed an incremental exercise test on a treadmill. Temperature (T) was measured with thermocouples in the rectum (R), blood (B), muscle (M), and on the skin (S). Blood samples were drawn from the carotid artery and right atrium (central venous) 10 sec prior to each increase in speed, and during recovery. Blood gases and pH were measured at standard temperature, bicarbonate concentration was calculated, and all variables were adjusted to RT, BT, and MT. The three sites were significantly different from each other for the adjusted variables in blood during exercise and recovery. Linear and polynomial equations predicted BT and MT from RT and ST during exercise, and from ST during recovery. Interpretation of blood gas data based on rectal or blood temperature may not be exactly relevant to metabolism in contracting muscle, or gas exchanges in muscle and lung.

temperature; prediction equations; exercise; horses

Introduction

Responses of blood gases, pH, and bicarbonate concentration to exercise are used to help assess fitness. Adjustment of blood gas data for changes in temperature during moderate and strenuous exercise is important for proper interpretation, as measures of theses variables are dependent on dissociation constants that vary with temperature (Jones et al., 1986; Fedde, 1991). Rectal, blood, and muscle temperature have all been utilized previously in horses for adjustment factors during exercise (Pan et al., 1986; Bayly et al., 1989), but rectal temperature may underestimate temperatures in the working muscles and the lung, and lead to an overestimation of pH, and an underestimation of pO_2, pCO_2, and $[HCO_3^-]$. Previous studies in horses have shown differences between rectal, blood, and muscle temperatures during periods of short, strenuous exercise (Hodgson et al., 1993), and adjusted blood gas data has been shown to be different at the three sites (Jones et al., 1989). No studies have been found in the literature concerning temperature changes during submaximal exercise in fit Arabian horses, which have certain characteristic responses compared to other breeds (Wickler and Troy, 1991; McCollum et al., 1993; Rivero et al., 1993b).

The present investigation had three purposes: 1) to determine simultaneously the temperature in the muscle (T^M),

central venous blood (T^B), rectum (T^R), and on the skin surface (T^S) during incremental exercise in fit Arabian horses; 2) to compare the differences in central venous (V) and arterial (A) blood gases when adjusted to T^R, T^B, and T^S; and 3) to derive regression equations for prediction of T^M and T^B from T^R and T^S during exercise, and from T^S during recovery.

Materials and Methods

Experimental animals. Seven Arabian horses (4-5 yr) weighing 410 ± 41 kg (mean ± SE) were conditioned previously for 20 wk on a treadmill (Kagra Ag Mustang 2200; Switzerland). Each horse had its right carotid surgically relocated to a subcutaneous position at least 6 months prior to the study. Horses were fed a cracked corn and oat mix, and grass hay to meet requirements for moderate exercise in horses (NRC, 1989). All procedures were approved by the University's animal care committee.

Experimental protocol. Feed, but not water, was withheld overnight for at least 12 hr prior to the morning of the exercise test. The climate-controlled barn which housed the treadmill had an average ambient temperature of 12°C with 40% relative humidity. Horses were brought to the barn at least 1 hr prior to any handling. Areas over the left jugular vein, right carotid artery, and left and right middle gluteal muscles were surgically prepared. A sterile 18 g catheter

(Angiocath, Becton-Dickinson) with a 5 ml extension set was introduced into the carotid artery and kept patent with heparinized saline.

An area of skin over the midcervical region of the jugular vein was anaesthetized with 1 ml of lidocaine (2% lidocaine HCl, Butler), and a small incision was made in the skin. A 10 g needle was placed aseptically into the vein, and a sterile polyethylene catheter (PE-240, Intramedic) attached to a saline manometer was introduced into the right atrium of the heart, and kept patent with heparinized saline. Copper-constantan thermocouples (Physitemp Instruments, Clifton, NJ) were used to measure all temperatures: one was passed through the tubing into the right atrium for measurement of T^B (model IT-18EXLNG). The T^M was measured 8 cm deep in the belly of the left gluteal muscle (model MT 23/8), T^R was measured 10 cm deep in the rectum (model ESO-1), and T^S was measured on the dried surface of the skin over the right gluteal muscle (model SST-1). Heart rates were monitored throughout the test with a commercial digital heart monitor (Polar Pacer, Polar CIC).

Sampling protocol. Exercise consisted of an incremental test, with moderate increases in speed at each step to elicit steady changes in temperature (Table 1). Simultaneous resting A and V samples were taken before, during, and after the test. Samples were drawn every 4 min, just prior to each speed increase, and rectal, blood, and skin temperatures were taken during blood sampling. The horse was stopped for 10 sec prior

to each speed increase after blood sampling to allow measurement of T^M. Samples were also taken every 4 min for 16 min during the walking recovery period.

The A and V samples (2 mL) were drawn anaerobically into heparinized syringes (300 units lithium heparin, Sigma) and stored in an ice water bath until analyzed for pH, pCO_2, and pO_2 at standard temperature (Stat Profile 1, Nova Biomedical) within 30 min. Bicarbonate concentration was calculated (Sigaard-Andersen, 1963). All measurements were adjusted to T^R, T^B, and T^M (NCCLS, 1982), taking into account the changes in the dissociation constant of carbonic acid in plasma with varying pH and temperature (Severinghaus, 1971).

Data are summarized as least squares means $\pm$ SEM, and examined by analysis of variance for repeated measures (SAS, 1988). Dunnett's t-test was used for comparison of pre-exercise means with means during exercise and recovery. Differences of $P < 0.01$ were considered significant. Predictive equations were based on mean values for the seven horses at each step, and were obtained with a curve fitting program (SlideWrite Plus, 1990).

Results

Temperatures and heart rate. Temperature increased at all four sites during exercise (Figure 1), and all sites were different from each other by 20 min of exercise (3.5 m/s,

medium trot). Temperature (°C) increases were from 33.5 ± 0.06 to 37.9 ± 0.1, 37.8 ± 0.05 to 40.4 ± 0.1, 37.4 ± 0.05 to 41.03, and 37.8 ± 0.05 to 42.0 ± 0.1 for the skin, rectum, blood, and muscle, respectively. The T^R continued to rise for 5 min during the walking recovery period, but T^S, T^B, and T^M began to decrease immediately, T^M at a relatively slower rate. Only T^B returned to the pre-exercise value by the completion of the recovery period.

Heart rate (beats/min) was 33 ± 0.7 at rest, and increased linearly during exercise to 173 ± 2.3 at the last step (52 min, 7.5 m/s) of the exercise test, and returned to 80 ± 4.3 at 16 min of recovery.

pH. Changes in pH_A and pH_V are summarized in Figure 2. The pH_A values were between 7.41 and 7.42 at rest, and increased during exercise, reaching peaks at 30 min (5.0 m/s) before returning to near resting values at the end of exercise. By 20 min, the difference in temperatures resulted in a difference between pH values when adjusted to the three sites. The pH_A^R was highest, followed by the pH_A^B, with the pH_A^M being the lowest. The rapid decline in T^B resulted in the pH_A^B being the highest of the three during recovery, with the pH_A^M being the lowest.

The pH_V values were between 7.38 and 7.39 at rest (Figure 2), and showed a steady decline after 30 min of exercise. However, the values exhibited a pattern similar to the pH_A in terms of the relationship between T^R, T^B, and T^M.

$\underline{pCO_2}$. Changes in $paCO_2$ and $pvCO_2$ are summarized in Figure 3. The $paCO_2$ values were between 41 and 42 Torr at rest. The increased muscle temperature resulted in a difference in pCO_2^M in both the A and V blood at the first sampling time. The $paCO_2$ at the three sites declined steadily during exercise, with differences between the three adjusted values by 20 min of exercise. None of the values had returned to pre-exercise levels by the end of recovery. The rapid decline in T^B during recovery resulted in the $paCO_2^B$ being the lowest; $paCO_2^M$ was the highest (Figure 3a). A similar pattern was found for the $[HCO_3^-]$, and total CO_2 measurements.

The $pvCO_2$ values were between 47 and 48 Torr at rest (Figure 3), decreased by 20 Torr during the first 4 min of recovery, and were not different from the $paCO_2$ values at the end of recovery. The $pvCO_2^M$ was highest throughout exercise, followed by the $pvCO_2^B$ and the $pvCO_2^R$.

$\underline{[HCO_3^-]}$. Changes in $[HCO_3^-]$ are summarized in Figure 4. The $[HCO_3^-]_A$ values were 25.5 to 26.0 mmol/L at rest, increased at a walk, then decreased steadily during exercise. The $[HCO_3^-]_A^M$ was always the highest value, followed by the $[HCO_3^-]_A^B$ and $[HCO_3^-]_A^R$. All values increased during recovery and approached pre-exercise levels by 16 min of recovery.

The $[HCO_3^-]_V$ values were 27.5 to 28 mmol/L at rest, and remained higher than in $[HCO_3^-]_A$ blood at all times, reaching a peak value of 33 mmol/L at 16 min (3 m/s) before returning to pre-exercise levels by the end of exercise (Figure 4b).

Adjusted values at all three sites were different at 20 min of exercise. The $[HCO_3^-]_V^M$ was higher then the other values at the first sampling, and all values increased during recovery, but failed to reach pre-exercise levels.

pO_2. Changes in paO_2 and pvO_2 are summarized in Figure 5. The paO_2 values were between 90 and 94 Torr at rest, and all increased gradually during exercise; they were different from each other at 20 min of exercise. However, the paO_2^M was different from the other values at the first sampling during exercise (4 min, at 1.5 m/s) when the horse was still walking. Also, the paO_2^B was lower than at the other sites at rest, partly due to the resting blood temperature being the lowest value. During exercise, the paO_2^M was always the highest, followed by the paO_2^B, and paO_2^R, respectively. All three values declined during recovery, but still had not returned to pre-exercise values by 16 min of recovery.

The pvO_2 values were between 37 and 38 Torr at rest (Figure 5), and remained lower than the paO_2 values at rest, exercise, and recovery. All three values decreased to between 20 and 25 Torr by 20 min, and remained low until the end of exercise. The pvO_2^M was different at the first sampling, and all three values were different by 20 min of exercise. The pvO_2^M was highest, followed by the pvO_2^B, and pvO_2^R, respectively.

Prediction Equations. Quadratic equations were slightly better than linear regression in predicting muscle and blood temperature from rectal or skin temperature during exercise

(Figures 6 and 7). Predictions of muscle and blood temperature from skin temperature fit the data during the recovery period (Figure 8).

To test the applicability of these equations for use in our laboratory with other exercise intensities, a regression equation was developed comparing the T^R in this study, with T^R values obtained during previous experiments using repeated sprint tests (Taylor et al, 1994). Four pairs of values at rest, and at min 5 (3.5 m/s), 30 (5^{th} 1 min sprint at 10 m/s), and 35 (6^{th} 1 min sprint at 10 m/s) were used in the regression (Figure 9). The T^R values from the two experiments were highly correlated (R^2 = 0.98), indicating that the equations from the present study could be used to predict blood or muscle temperature during exercise in other studies involving moderate exercise (heart rate 150 - 180 beats/min) from this laboratory. These derived values could then be applied for more appropriate adjustment of pH, and blood gas data.

Discussion

This study amplifies previous findings that moderate exercise, even in fit horses results in significant site differences for rectal, blood, and muscle temperatures (Sexton et al, 1983), and that these differences are reflected in adjusted parameters, such as pH, and blood gases (Jones et al,

1989). These adjustments need to be considered with interpretation of metabolism and gas exchange during exercise at different locations in the body. Linear and quadratic equations described the relationship between skin, rectal, blood, and muscle temperatures during exercise and recovery, so blood and muscle temperatures can be predicted from rectal and skin temperatures. A high correlation was found between T^R in this incremental test and T^R during repeated sprint tests (Taylor et al., 1994). Application of the equations obtained from the present investigation may be able to be used to predict T^B or T^M at similar exercise intensity from other investigators.

Temperature. The pattern of responses at the different measurement sites has been seen previously in different breeds of horses at various exercise intensities (Jones et al., 1989; Hodgson et al., 1993). The T^M was always highest during exercise, followed by T^B, T^R, and T^S, respectively. The T^R has a characteristic lag period during early recovery as it continues to rise before gradually decreasing. The T^B, and T^S declined rapidly during recovery, with T^M decreasing at a slower rate. At lower exercise intensities (5 - 15 min, 1.5 - 3.0 m/s), the T^B and T^R were not different, and this has been seen previously in horses (Forster et al, 1985). It is important to note that little cooling of the blood occurs across the lung, and slight differences in temperature, about 0.4°C, between the pulmonary and carotid arteries have been

found during exercise in horses (Hodgson et al, 1993). Increasing the exercise intensity in an incremental fashion in the present study resulted in a separation of temperatures after 20 min (3.5 m/s). The highest mean temperature recorded during this study was 42.02°C, which was in the muscle at the end of exercise. The horses used in this study had been in training for 5 months, and training attenuates the rise in T^M during exercise (Sexton et al, 1985; Wilson et al, 1994).

pH. The pattern of the pH response to incremental exercise has been observed previously in ponies and Thoroughbred horses during moderate exercise (Parks and Manohar, 1984; Rose et al., 1991). It is important to note that the difference in T^M at the first sampling time resulted in A and V pH values both being different from the other two sites. Near the end of exercise in both pH_A and pH_V, there was a difference between the pH^B and pH^M of 0.02, ($[H^+]$ = 1.8 neq/L) which may be physiologically important, considering the narrow range in which pH is maintained. During exercise, the pH in the working muscle was actually 0.02 - 0.03 lower than would have been estimated with T^R and T^B, which is important during interpretation of muscle metabolism.

pCO2. The use of T^R or T^B after exercise at 3.5 m/s would have underestimated A and V pCO_2 during exercise and recovery. The overall response of pCO_2 to exercise has been seen previously in other breeds of horses exercising at similar intensities (Hodgson et al., 1990; Wagner et al., 1989), but

the duration of exercise time in those studies (8 - 15 min) was shorter than in the present study. The steady decrease in all three $paCO_2$ values throughout the full 52 min of incremental exercise has not been seen previously, and clearly demonstrates the Arabian horse's ability to hyperventilate, hence to avoid the exercise-induced hypercapnia and acidosis that has been observed during high intensity exercise in other breeds.

The $pvCO_2$ increased slightly at the onset of exercise, but never changed more than 5 Torr in either direction during exercise. The lack of a large, or steady increase in CO_2 in the central venous blood may be due to the submaximal exercise intensity, but also in part to the A-V difference. At rest, this value averaged -6 Torr, increased to -20 Torr after 28 min of exercise, and was -25 Torr at the end of exercise. The steady decline in the $paCO_2$ helped maintain the $pvCO_2$ by presenting the working muscle with arterial blood that had a relatively low pCO_2.

[HCO3-]. Differences in plasma [HCO_3^-] between the three sites were significant after 20 min of exercise, and the values adjusted to muscle temperature were different from the start of exercise. The A and V [HCO_3^-] and total CO_2 both increased at the beginning of exercise, before decreasing steadily throughout the remainder of exercise.

pO2. Differences in paO_2 at the three sites during exercise and recovery were the most dramatic of all the measured

variables. At 40 min of exercise, there was an 11 Torr difference between the $paCO_2^R$ and the $paCO_2^M$, and an 8 Torr difference between the $paCO_2^B$ and the $paCO_2^M$. These differences could lead to substantial error when interpreting oxygen extraction by the working muscle, or alveolar diffusion in the lung. They are also important when calculating variables from paO_2, such as oxygen saturation.

A small, transient increase in paO_2 at the onset of exercise, and a concomitant decrease in pvO_2 during exercise has been seen previously in ponies and Thoroughbred horses exercising at moderate intensities (Parks and Manohar, 1984; Rose et al, 1991). However, the magnitude of the steady increase in the paO_2 throughout exercise has not previously been reported. The blood gases reveal that the Arabian horses were able to maintain a high level of alveolar ventilation in addition to avoiding acidosis. This may represent a breed characteristic; the Arabian horse is very well suited for long distance aerobic exercise, as demonstrated by the predominance of slow-twitch, high-oxidative muscle fibers, and by the high activity of aerobic enzymes (Rivero et al., 1993a).

Prediction equations for temperature during exercise and recovery have sufficient impact on pH and blood gases to warrant estimation of muscle and central venous blood temperature. These equations can predict muscle and blood temperature from rectal and skin temperature, but exercise intensity and duration are modifying factors in the

applicability of which site to use. Rectal temperature will not accurately predict recovery temperature during maximal, or prolonged exercise due to the continued rise in this variable after the cessation of exercise. Further studies are needed to establish equations for application during different types of exercise.

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TABLE 1. Incremental exercise test protocol

Time (min)	Speed (m/sec)	Slope (%)
0 - 4	1.5	0
4 - 8	2.0	6
8 - 12	2.5	6
12 - 16	3.0	6
16 - 20	3.5	6
20 - 24	4.0	6
24 - 28	4.5	6
28 - 32	5.0	6
32 - 36	5.5	6
36 - 40	6.0	6
40 - 44	6.5	6
44 - 48	7.0	6
48 - 52	7.5	6
52 - 68	1.5	0

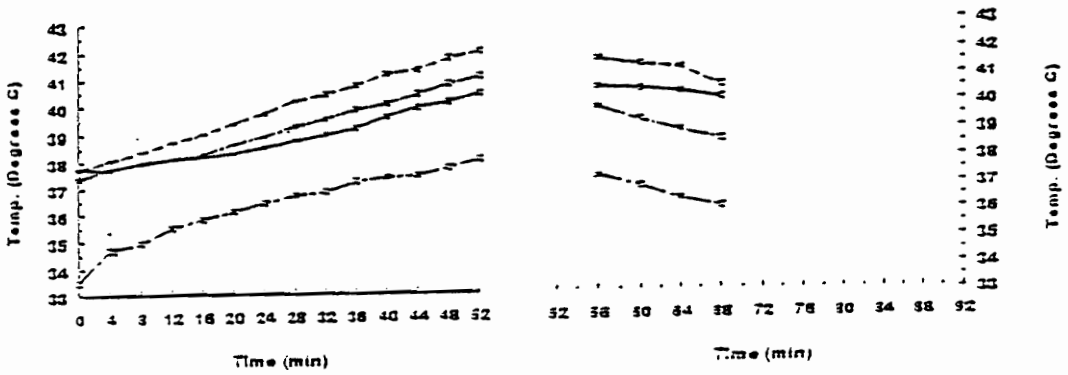


FIGURE 1 Temperature in the rectum (—), blood (·-·), muscle (--), and on the skin (··-··) in 7 horses during incremental exercise and recovery. Values are least squares means ± SE.

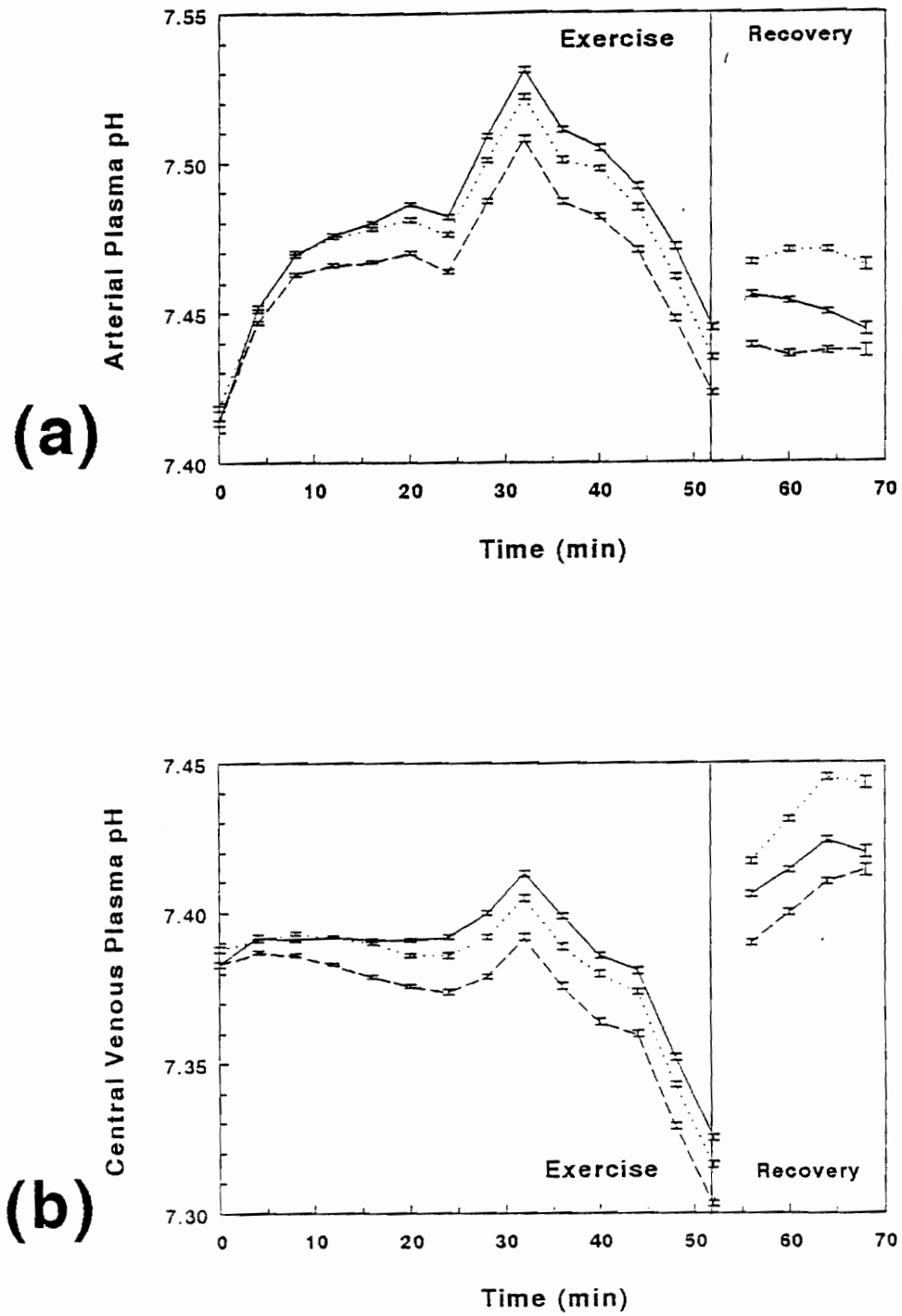


FIGURE 2 pH in arterial and mixed venous blood in 7 horses adjusted to rectal (—), blood (···), and muscle (--) temperatures during incremental exercise and recovery. Values are least squares means ± SE.

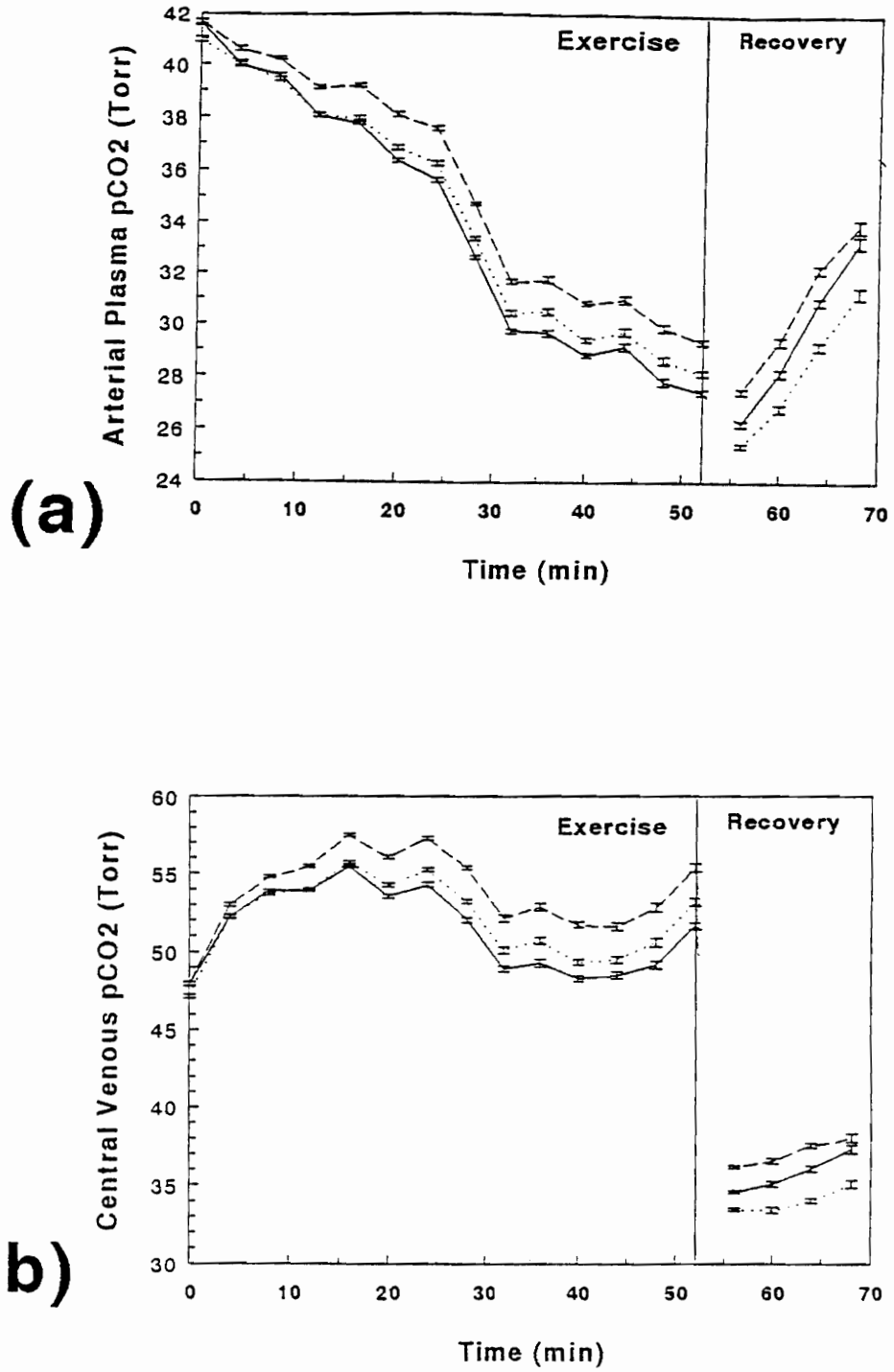
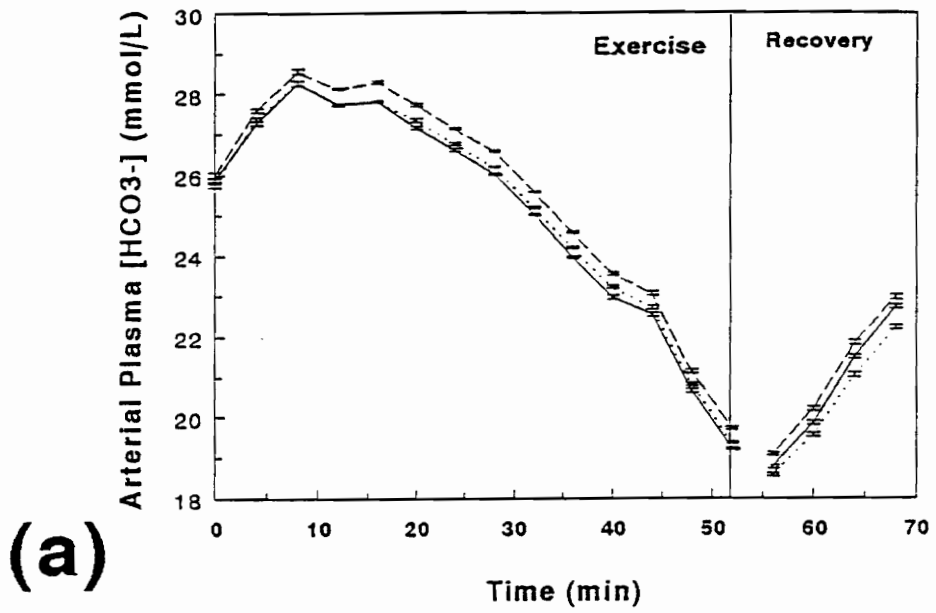


FIGURE 3 pCO_2 in arterial and mixed venous blood in 7 horses adjusted to rectal (—), blood (··), and muscle (--) temperatures during incremental exercise and recovery. Values are least squares means ± SE.



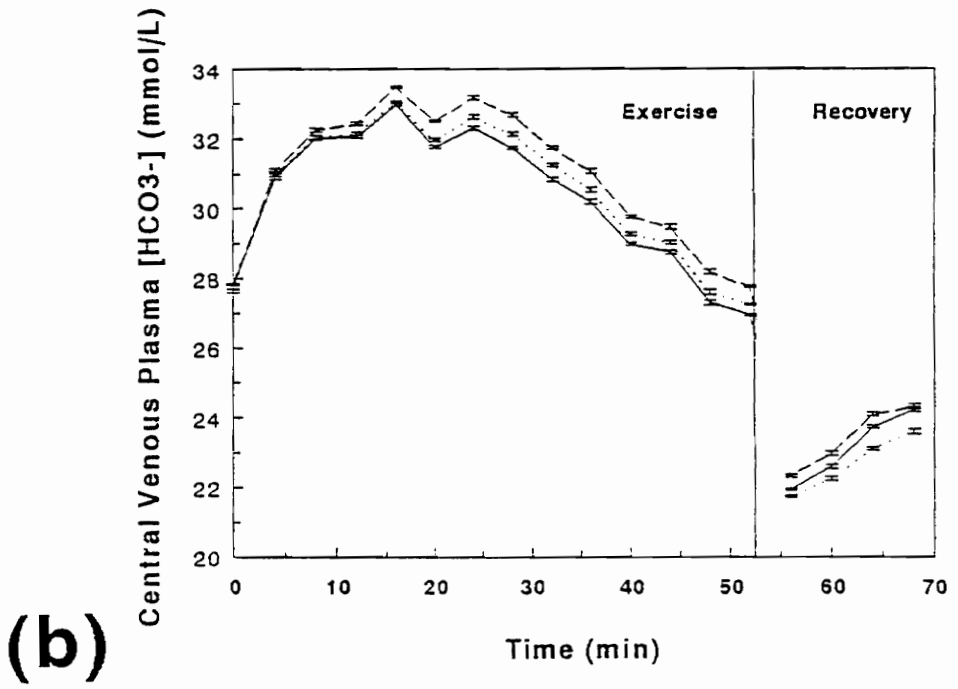
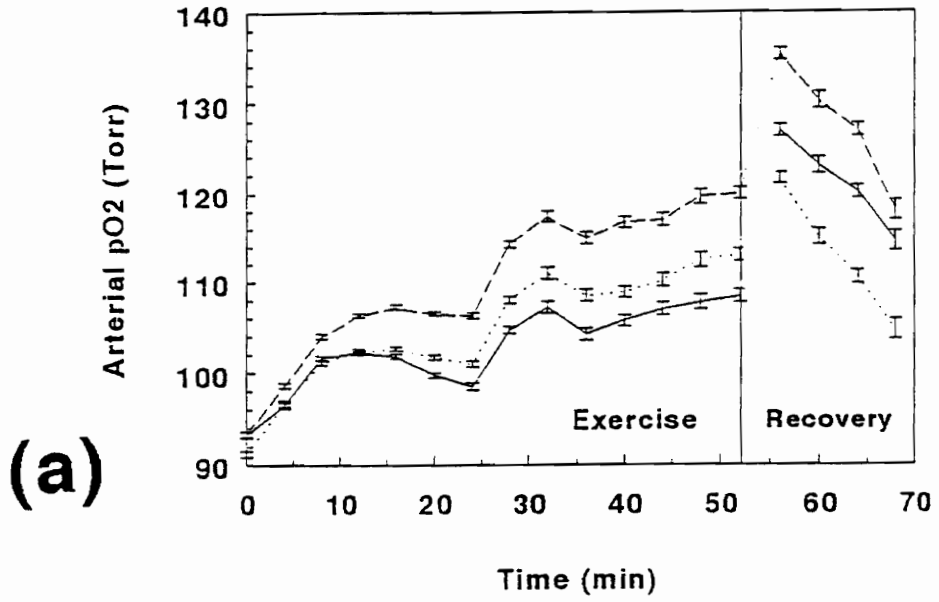


FIGURE 4 [HCO_3^-] in arterial and mixed venous blood in 7 horses adjusted to rectal (—), blood (··), and muscle (--) temperatures during incremental exercise and recovery. Values are least squares means ± SE.



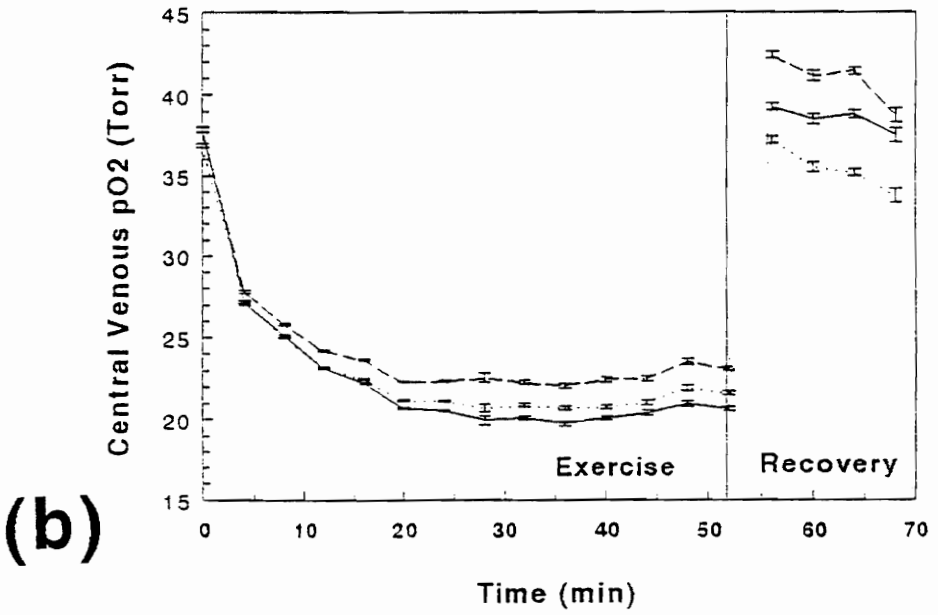


FIGURE 5 pO_2 in arterial and mixed venous blood in 7 horses adjusted to rectal (—), blood (··), and muscle (--) temperatures during incremental exercise and recovery. Values are least squares means ± SE.

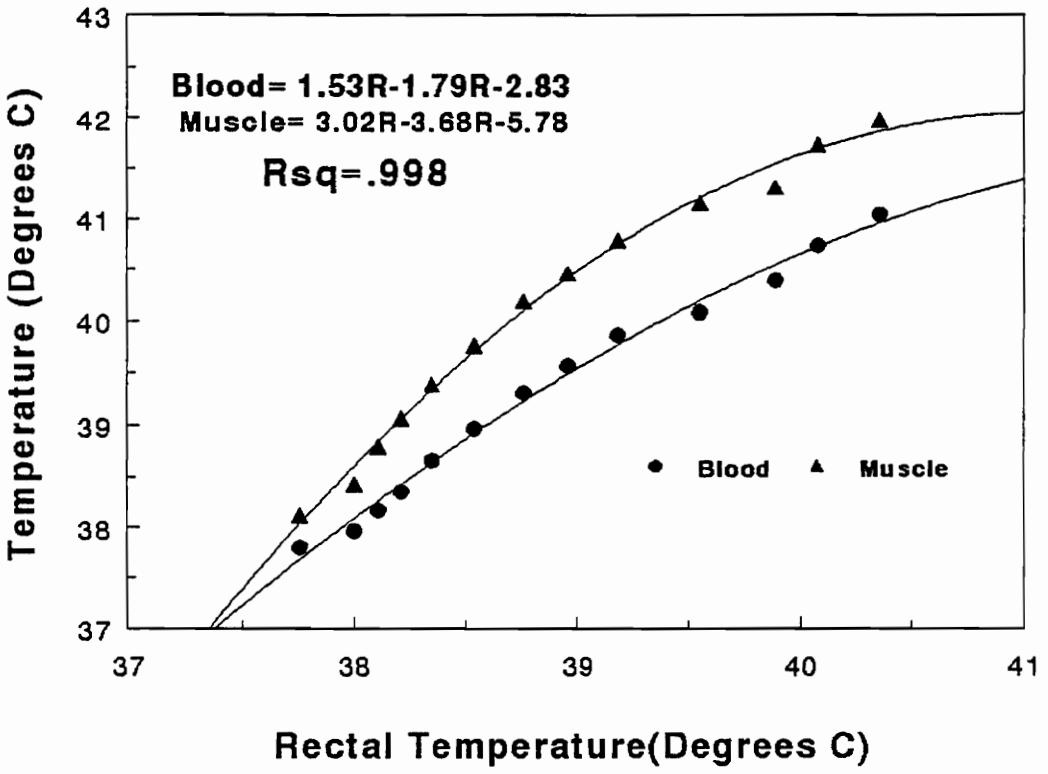


FIGURE 6 Prediction of muscle and blood temperatures during incremental exercise from rectal temperature in 7 horses. Values are least squares means ± SE.

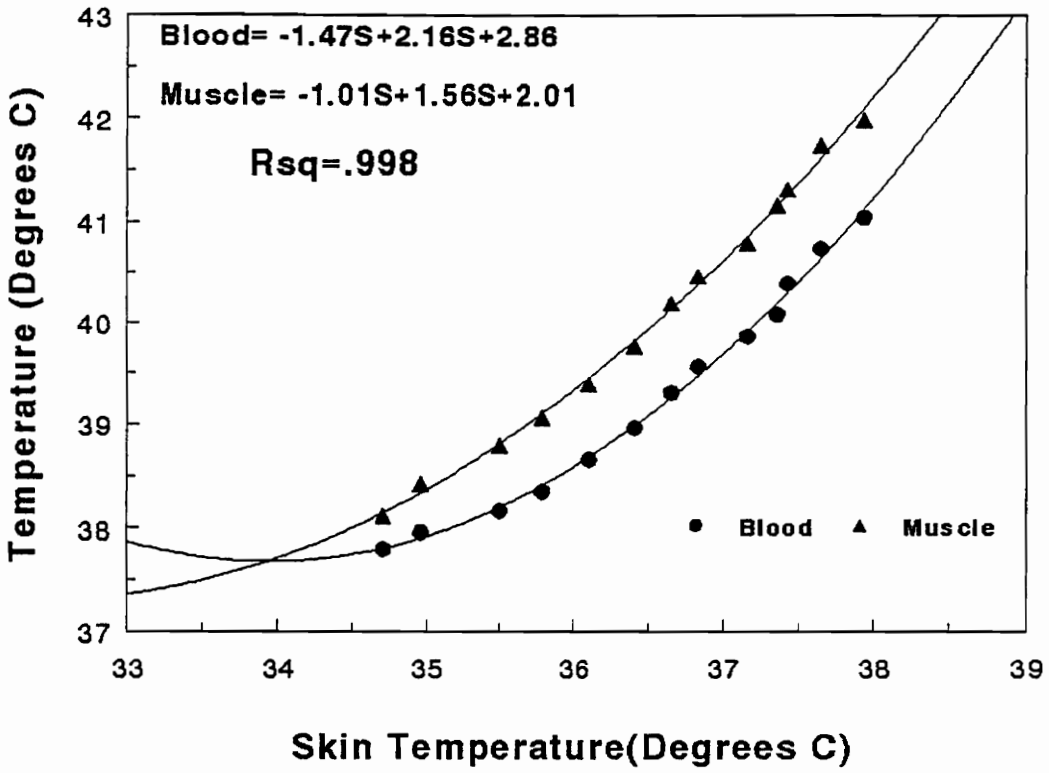


FIGURE 7 Prediction of muscle and blood temperatures during incremental exercise from skin temperature in 7 horses. Values are least squares means ± SE.

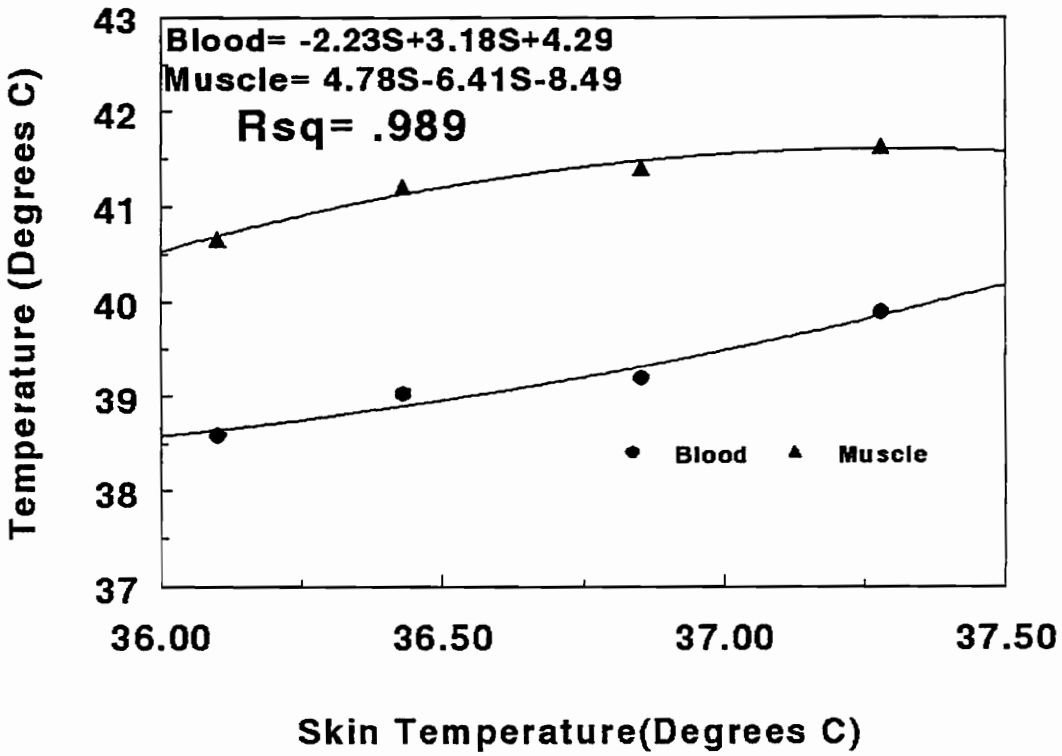


FIGURE 8 Prediction of muscle and blood temperatures during walking recovery from skin temperature in 7 horses. Values are least squares means ± SE.

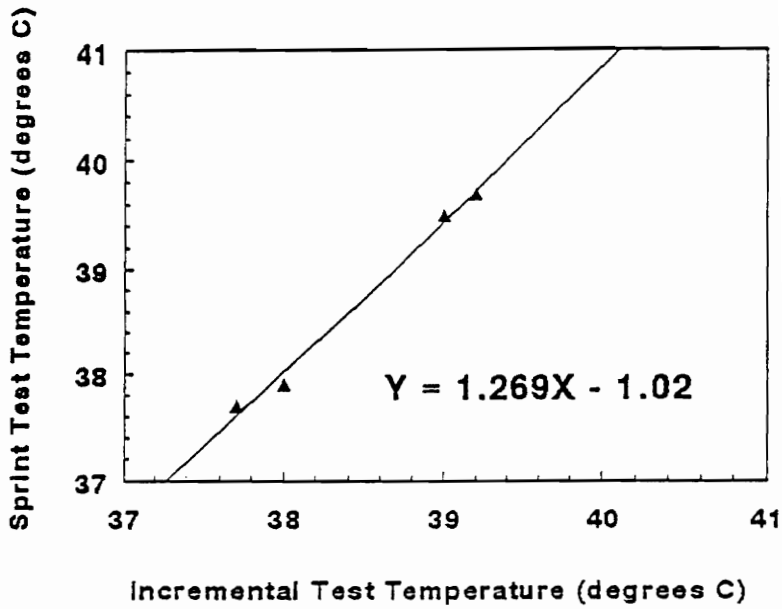


FIGURE 9 Comparison of rectal temperatures during incremental and sprint exercise in 7 horses. Values are least squares means ± SE.

Summary and Implications

These studies revealed five major findings:

1) The results of this study confirm the importance of the site chosen for blood collection in acid-base evaluation during exercise in the horse. Arterial blood is required for interpretation of ventilation, but central venous blood is necessary for the interpretation of changes associated with metabolism during exercise. The independent variables at the two sites were different with respect to their influence on dependent variables during sub-maximal and maximal exercise. There were site differences for blood gases and strong ions, as well as $[H^+]$ and $[HCO_3^-]$.

The concurrent arterial alkalosis and central venous acidosis during submaximal exercise were mainly determined by $paCO_2$ and $pvCO_2$. The paO_2 was maintained by increased ventilation sufficient to lower the $paCO_2$, and thus the $[H^+]$. This may help to explain the metabolic alkalosis exhibited in equine exercise studies in the literature.

2) There was evidence supporting the role of the chloride shift during exercise in the horse for the first time. This effect was seen during three separate exercise tests, and may have been observed because of the repeated sprinting protocol.

3) The corn oil/lecithin supplemented diet affected blood gases, strong ions, $[H^+]$, $[HCO_3^-]$, cholesterol, triglycerides, and glucose. The decreased lactate and $[H^+]$, and increased $[HCO_3^-]$ may offer advantages during exercise by maintaining a slight metabolic alkalosis during sprinting exercise. The lower blood lactate observed during sub-maximal exercise in horses fed the corn oil/lecithin diet was in contrast to previous findings in horses fed corn oil. It is possible that lecithins affected muscle cell membranes, and increased the rate of fatty acid transport, hence power output, by fat oxidation. The glucose-sparing effect observed has not been seen previously during sprinting exercise in the horse, but may have a significant role during short, high-intensity exercise, in addition to endurance work.

4) During an incremental exercise test, there were differences between skin, rectal, blood, and muscle temperatures during exercise and a walking recovery. The pH and blood gases adjusted to rectal, blood, and muscle temperatures were also different, emphasizing the importance of changes in the equilibrium constants that accompany changes in body temperature. The most pronounced effects were seen in the paO_2, and improper adjustment of these variables during exercise could lead to an overestimation of pH, and an underestimation of pO_2, pCO_2, and $[HCO_3^-]$.

Prediction equations were derived for blood and muscle temperature from rectal and skin temperature during exercise, and from skin temperature during recovery. The rectal temperature was not a good indicator of recovery blood or muscle temperature because of the continued rise of this variable after exercise was completed.

5) The two days/week of training employed here were intended to maintain the horses on a constant plane of fitness, but even this light amount of work affected many of the variables studied. There were changes in heartrate, plasma albumin, strong ions, $[H^+]$, free fatty acids, beta-hydroxybutyrate, and cholesterol. The Arabian breed may require less training, in both duration and intensity, to maintain thier level of fitness. This breed may be somewhat unique in its ability to maintain paO_2 during high intensity exercise, with no significant increases in $paCO_2$. This has not been seen previously, and may reflect an increased aerobic capacity in these horses.

Vita

Lynn Elizabeth Taylor, daughter of Don and Elizabeth deLambert of Brookfield, CT was born at Eglin Air Foirce Base in Valparaiso, Florida on January 5, 1966. She received her Bachelor of Science in Animal Science at The University of Connecticut in 1988, and a Master of Science in Animal Science at The Virginia Polytechnic Institute and State University in 1991, where she studied acid-base balance and dietary fat in exercising horses. She completed the requirements for a Doctor of Philosophy at Virginia Polytechnic Institute and State University in February, 1995.