SARCOPLASMIC RETICULUM RESPONSES TO REPEATED SPRINTS, CONDITIONING AND DIETARY LECITHIN IN THE HORSE

by

Judith A. Wilson

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Approved:

D.S. Kronfeld, Chairperson

L.A. Eng

L.A. Lawrence

J.W.Rankin

J.H.Williams

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(ABSTRACT)

Sarcoplasmic reticulum (SR) releases and sequesters calcium during muscle contraction and relaxation. Calcium uptake rates have been shown to respond to high intensity exercise, and we propose this response may be affected by conditioning and dietary lecithin. Ten Quarter Horses performed repeated sprints on a high speed treadmill. Muscle samples were taken before and after exercise from the middle gluteal muscle, and SR vesicles were isolated. Horses were then conditioned for 12 weeks and the experiment was repeated. Calcium uptake was determined spectrophotometrically using antipyralazo III, and Ca^{2+}-ATPase activity was determined using an enzyme-linked optical assay. Resting calcium uptake rates and Ca^{2+}-ATPase activities were greater when the horses were conditioned than when unconditioned ($p=.05$, $p=.004$, respectively). After exercise calcium uptake was slower in horses unconditioned ($p=.02$) or conditioned ($p=.002$) and this fatigue response to exercise was reduced by conditioning

(p=.01). After exercise Ca^{2+}-ATPase activity was decreased (p=.007) to 71% of resting activity in unconditioned horses, and to 82% in conditioned horses (p=.001).

The same ten horses were divided into two groups and were fed either a conventional diet or a diet with 10% added dietary fat in the form of corn oil/soy lecithin for a 12 week period. The diets were switched and the study was continued for another 12 weeks. Horses were exercised 4 days a week on a high speed treadmill throughout the study. The repeated sprinting experiments, as in the first part of the study, were performed at the end of both 12 week periods. Resting calcium uptake rates tended to be higher (p=.09) when horses were fed the fat modified diet, but decreased to a greater extent with an acute bout of exercise (p=.058). Ca^{2+}-ATPase activity was lower after exercise when horses consumed the lecithin (p=.02) and the decrease from resting values was greater (p=.05).

These results suggest that high intensity repeated sprinting fatigues SR function in horses, and that conditioning reduces the depression of SR function with exercise. Feeding a lecithin supplemented diet to conditioned horses increases the response of the SR to an acute bout of exercise.

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INTRODUCTION

The sarcoplasmic reticulum (SR) is a network of fine membranous sacs that regulates intracellular distribution of calcium in skeletal muscle. Calcium ions are released via a protein channel embedded in the SR membrane to prompt muscle contraction. The resting state subsumes when calcium is pumped into the SR through the action of an active transport system, called the Ca^{2+}-ATPase.

Alterations in SR function occur during prolonged exercise (Fitts et al., 1982; Byrd et al., 1989b; Favero et al., 1993). Decreases in calcium uptake rates, Ca^{2+}-ATPase activity and calcium release rates have been described with an single bout of exercise. High intensity work reduced both the rate of calcium uptake and Ca^{2+}-ATPase activity in horses (Byrd et al., 1989a).

Sarcoplasmic reticulum function may be affected by exercise conditioning or training. Ca^{2+}-ATPase activity of skeletal muscle from trained rats exercised to exhaustion was increased relative to trained rats at rest, and the same trend occurred in non-trained rats (Bonner et al., 1976). Studies of cardiac SR and conditioning are equivocal, some reporting a greater capacity to take up calcium with conditioning (Penpargkul et al., 1976) while others found no effect of exercise conditioning on SR function (Tate et al.,1993).

Abnormal SR responses during exercise may be a central feature in exertional rhabdomyolysis (Hodgson, 1993). This disease is common in exercising

horses, and it appears to be responsive to several dietary factors (Beech, 1994).

Membrane structure and function may be influenced by diet. The synthesis of the molecular structure of biological membranes and lipoproteins is affected by dietary lecithin, chemically known as phosphatidylcholine (PC). Because of the importance of PC as a cell membrane constituent, it is possible that dietary lecithin could alter SR responses to exercise and conditioning.

Choline and lecithin have been utilized in an attempt to enhance athletic performance. Administration of choline citrate improved running time in long-distance athletes during a 20-mile run (Sandage et al., 1992). Lecithin given to triathletes and adolescent runners prior to exercise prevented a decrease in plasma choline (Allworden et al., 1993).

The general aim of this study was to elucidate SR function in the horse because of its importance in fatigue and possibly exertional rhabdomyolysis. Specific objectives were:

1) to develop quantitative methods for analysis of SR function in horse skeletal muscle tissue;

2) to determine the effects of high intensity repeated sprints on calcium uptake rate and Ca^{2+}-ATPase activity in horses;

3) to evaluate the effects of exercise conditioning in horses on the changes in calcium uptake rate and Ca^{2+}-ATPase activity associated with an acute bout of exercise;

4) to examine the role of lecithin supplementation on calcium uptake rates and Ca^{2+}-ATPase activity in conditioned horses.

Review of Literature

INTRODUCTION

The ability of muscle to do work lies in the contractile process. Calcium ions are essential in the binding of the actin (thin) and myosin (thick) filaments during contraction. Within the sarcoplasm of skeletal muscle is an extensive endoplasmic reticulum which is called the sarcoplasmic reticulum (SR). The physiological role of skeletal muscle SR is to release and sequester calcium during the contraction-relaxation cycle. The SR of cardiac muscle is not as well developed as that of skeletal muscle and does not store enough calcium to provide for full contraction. In addition to the calcium ions released into the sarcoplasm from the SR of cardiac muscle, calcium ions also diffuse into the SR from the transverse tubules (T tubules) during cardiac contraction. Sarcoplasmic reticulum function has been implicated in fatigue in skeletal muscle (Heilmann and Pette, 1979; Byrd et al., 1989a; Favaro et al., 1993) and alterations in cardiac SR function have been associated with conditioning (Penpargkul et al., 1976).

Phospholipids are the main lipid component of the native SR membrane

with phosphatidylcholine being the main phospholipid species (65-75%). The fatty acyl composition of phosphatidylcholine and phosphatidylethanolamine is characterized by a number of highly unsaturated fatty acids, and this may be important for the SR enzyme function (Hidalgo, 1987). Phosphatidylcholine may be especially important for Ca^{2+}-ATPase functioning (Infante, 1987).

SARCOPLASMIC RETICULUM RESPONSES TO PHYSIOLOGIC ALTERATIONS

Physiology of muscle contraction

When a muscle is stimulated, an action potential travels along the sarcolemma (muscle membrane) and into the T tubules toward the interior of the muscle fiber. This in turn causes release of calcium ions by the SR, which initiates contraction. Released calcium ions diffuse to the adjacent myofibrils where they bind strongly with troponin C, and this in turn elicits muscle contraction by allowing actin-myosin interaction. Extracellular calcium ions also diffuse into the sarcoplasm from the T tubules of cardiac muscle at the time of the action potential.

Calcium sequestration by the SR is a critical determinant in muscle relaxation (Guyton, 1991). Because SR function is closely associated to contraction and relaxation of muscle, it has been suggested that an alteration in its structure and/or function may be involved in the development of skeletal

muscular fatigue (Booth and Thomason, 1991; Byrd et al., 1989a, 1989b; Fitts et al., 1982; Westerblad and Allen, 1991). Others have suggested that conditioning may alter the cardiac SR function (Levine and Kinasewitz, 1986; Pierce et al., 1989).

Skeletal muscle and acute exercise

Alterations in SR of skeletal muscle have been found after prolonged exercise (Belcastro et al., 1981; Fitts et al., 1982; Byrd et al., 1989b). The quantity of SR and the calcium uptake per unit of SR are greater in fast-twitch than slow-twitch muscles (Fitts et al., 1982). Calcium uptake capacity of the SR was reduced in type 1 (slow-twitch) and type 2a (fast-twitch oxidative) but not 2b (fast-twitch glycolytic) muscle fibers of rats following a prolonged swim to exhaustion, yet the SR adenosine triphosphatase (ATPase) activity was unchanged (Fitts et al.,1982). Because the type 1 and 2a fibers are known to be preferentially recruited during endurance activity, they suggested that the decline in the functional capacity of the SR is probably dependent on the degree of muscle contractile activity rather than to any inherent differences in the susceptibility of the SR in the three fiber types.

Contrary to Fitts et al. (1982), Belcastro et al. (1981) reported rats exhausted by treadmill running showed a significant depression in calcium activated ATPase activity in the gastrocnemius muscle (predominately fast-twitch

glycolytic) compared with the same muscle of control (rested) rats. They suggested that depressed calcium activated SR ATPase activity of fatigued fast-twitch muscle may contribute to the decreased force production at exhaustion. Calcium uptake activity of the sarcoplasmic reticulum isolated from rat fast-twitch muscle was depressed following exhaustion by treadmill running (Semrowich and Gollnick, 1977).

Exercise training and exhaustion affect calcium uptake by rat skeletal muscle mitochondria and sarcoplasmic reticulum (Bonner et al., 1976). Mitochondrial and sarcoplasmic reticular Ca^{2+}-ATPase activity and mitochondrial calcium uptake were determined in skeletal muscle from exercise trained and non-trained rats at rest or following short-term exhaustive exercise. In trained rats exercised to exhaustion, mitochondrial calcium uptake was significantly depressed as compared to non-trained rats at rest. Ca^{2+}-ATPase activity of sarcoplasmic reticulum from trained rats exercised to exhaustion was significantly increased as compared to trained rats at rest. Exercise training resulted in an adaptation of the skeletal muscle mitochondria which blocked the movement of calcium ions across the membrane. The authors proposed that this inhibition of calcium transport may be a protective mechanism to insure both adequate adenosine triphosphate (ATP) supply for continued muscle contraction as well as to maintain the function of the tricarboxylic acid (TCA) cycle. The increased calcium concentration within the mitochondria inhibits the activity of isocitrate dehydrogenase, the main regulatory

enzyme of the TCA cycle. This suggests other mechanisms than decreased TCA cycle activity caused fatigued in the trained rats. No explanation was given for the increased Ca^{2+}-ATPase activity in exhausted trained rats. One would expect training to increase enzyme activity, but the increase with exhaustive exercise seems contrary to other reports of SR function with an acute bout of exercise.

Thoroughbred horses were exercised at maximal oxygen uptake on a high speed treadmill until fatigued in order to examine the effects of acute high-intensity exercise on the rate and capacity of calcium uptake and Ca^{2+}-ATPase activity of the sarcoplasmic reticulum (Byrd et al., 1989a). Significant increases in muscle and blood lactate and reductions in muscle pH were found immediately after exercise. The muscle lactate and pH returned toward rest values by 60 min after exercise. A major finding was the 50% reduction in the calcium uptake by skeletal muscle sarcoplasmic reticulum after a single high-intensity bout of exercise. This result was paralleled by decreased activity of the Ca^{2+}-ATPase. Similar depressions in sarcoplasmic reticulum function occurred in rat heart and skeletal muscle after prolonged exhaustive running (Scherer and Deamer, 1985) and in the human quadriceps femoris after exhaustive kicking exercise (Gollnick et al., 1991).

In the Thoroughbred study (Byrd et al., 1989a), both calcium uptake (rate and capacity) and Ca^{2+}-ATPase activity returned to normal by 60 min after exercise. The data from this experiment did not identify the mechanisms that produced the sarcoplasmic reticulum dysfunction. However, several of the

changes that occur in muscle during exercise were suggested as possible mechanisms for producing the decreased function, such as 1) alterations in substrates, 2) depressions in pH, 3) elevations in temperature, 4) the production of superoxide radicals and 5) elevations in cytoplasmic calcium concentration.

Rat muscle homogenates and isolated SR vesicles were investigated after treadmill runs of 20 or 45 min or to exhaustion (approximately 140 min) (Byrd et al., 1989b). Muscle homogenates and the purified sarcoplasmic reticulum fraction of the superficial and deep fibers of the gastrocnemius and vastus muscles of rats were obtained to examine sarcoplasmic reticulum calcium uptake and calcium ATPase activity. The results of this study confirmed previously reported fiber type specific depression in the initial rate and maximum capacity of calcium uptake and altered ATPase activity after exercise (Heilmann and Pette, 1979; Fitts et al.,1982). After approximately 20 min of exercise, a depression of the Ca^{2+}-ATPase activity was evident in sarcoplasmic reticulum isolated from deep muscle fibers, which are predominately type 2a. The lowered ATPase activity was followed by a depression in the initial rate of calcium uptake in both muscle homogenates (superficial and deep) and isolated sarcoplasmic reticulum fractions after 45 min of exercise. Maximum calcium uptake capacity was lower in isolated sarcoplasmic reticulum only after exhaustive exercise. Sarcoplasmic reticulum dysfunction may contribute to the onset of muscular fatigue and result in altered calcium homeostasis, and this in turn could affect many calcium-mediated processes within the muscle cell (Byrd

et al., 1989b).

More recently the relationship between muscle fatigue and SR dysfunction has been substantiated by a number of reports from the 1992 American Physiological Society Conference. A bout of cycling to fatigue at 75% VO_{2max} in moderately trained female cyclists produced a 70% reduction in calcium uptake compared to rest (Parsons et al., 1992). Green and coworkers (1992) reported a depression in Ca^{2+}-ATPase activity by 16% with 30 minutes of cycling in untrained males. After cycling to fatigue at 72% VO_2max Ca^{2+}-ATPase activity was reduced by 30% compared to resting activity. The differences in degree of reduction in SR function between the two studies may be related to such factors as time to exhaustion, sex of the subjects and methods of analysis. Luckin and coworkers (1992) reported on post exercise recovery of SR function in rats. They found calcium uptake, Ca^{2+}-ATPase activity and calcium release to be markedly compromised after a single bout of prolonged submaximal exercise, but this depression in SR function was a reversible process, taking 2 hours for complete recovery.

Prolonged submaximal exercise in rats reduced the rate of calcium release by 20-30% from actively loaded SR vesicles without affecting the total amount released (Favero et al., 1993). Ryanodine (a plant alkaloid that specifically binds the SR release channel) binding also was depressed by 20% in SR vesicles isolated from exercised animals. Ryanodine binds with a high affinity to open

calcium channels. Therefore it was suggested that exercise reduces the number of channels in the open state or possibly reduces calcium channel conductance. Since calcium is released through a specific channel following modification to an open state configuration, release rate is determined by the number of open channels, the calcium gradient and calcium conductance across the SR membrane. The total amount of calcium released is dependent upon the volume of vesicles that contain calcium channels. The observation that equal amounts of calcium were released from vesicles isolated from rested and exercised rats indicates that exercise did not alter the total volume of vesicles that contain functional calcium channels.

Prolonged moderate-intensity exercise caused a 13% reduction in Ca^{2+}-ATPase activity from rat gastrocnemius muscle within 2 minutes after the onset of exercise (Belcastro et al., 1993). This reduction in enzyme activity progressed to 18% at 30 minutes of exercise and remained at this activity throughout 90 minutes of exercise. Calcium uptake (bidirectional) increased by approximately 30% with exercise, but there was no difference in oxalate supported calcium loading (unidirectional). These observations suggest a decrease in the conductance pathways for calcium efflux in response to prolonged exercise. The most significant finding of this paper was that changes in SR function occur early during exercise and are not exclusively associated with the end of exercise. Most of the reduced Ca^{2+}-ATPase activity occurred in the first 2 minutes of contractile activity

when fatigue is unlikely, so the SR changes may reflect an accommodation to increased contractile activity, i.e., a favorable rather than unfavorable change.

No single element may be responsible for the decrease in contractility that occurs in skeletal muscle during fatiguing exercise. More likely several changes contribute to the decrease in force produced. The changes in sarcoplasmic reticulum function are partially responsible for alterations in contractility that occur with fatiguing exercise.

Cardiac muscle and conditioning

Physical conditioning improves indexes of cardiac contractility such as increasing stroke volume and ejection fraction and enhancing relaxation rates (Schaible and Scheuer, as cited by Pierce et al., 1989). Exercise training may increase the calcium transport by cardiac SR (Penpargkul et al., 1976, 1980; Pierce et al., 1989; Levine and Kinasewitz, 1986; Laughlin et al., 1991). In one of the earlier studies, Penpargkul et al. (1976) conditioned rats by swimming. The SR vesicles from hearts of conditioned rats showed a greater capacity to take up calcium compared to SR vesicles harvested from sedentary rats.

In contrast the basal calcium uptake into the SR was similar for sedentary and conditioned rats (Levine and Kinasewitz, 1986). The addition of calmodulin (calcium binding protein) produced a significantly greater increment in calcium uptake into SR in the exercise conditioned animals. The Ca^{2+}-ATPase activities

were similar between the control and conditioned groups whether assayed in the presence or absence of calmodulin. Furthermore, the addition of calmodulin did not produce a significant increase in Ca^{2+}-ATPase activity of the SR-enriched microsomal fraction from either the conditioned or control group.

Endurance exercise training in miniature swine did not provide sufficient stress on the heart to induce changes in the calcium regulatory system (Laughlin et al., 1991). There were no differences between SR calcium-ATPase activities nor was the yield of SR protein different between control and exercised-trained animals. A program of chronic swimming training in rats did demonstrate an increase in sarcolemmal ATP-dependent calcium uptake (Pierce et al., 1989). The elevation in sarcolemmal calcium pump activity in exercised rats was suggested to help in the reduction of intracellular calcium and to augment cardiac relaxation rates.

Canine cardiac SR was found to be unaltered by endurance exercise training (Tate et al., 1993). Mongrel dogs either remained sedentary or were trained by running for 8-10 weeks. The running protocol involved daily exercise with alternating sprint and endurance training. Grade and speed were increased weekly. The trained state was confirmed by increased skeletal muscle citrate synthase activity (from the gastrocnemius) and decreases in submaximal heart rate in the exercised but not sedentary group. The properties of isolated cardiac SR were similar between groups. The variables tested were Ca^{2+}-ATPase activity,

calcium transport and calcium release.

The results of conditioning on cardiac SR function are equivocal. Differences found in the studies could be due to variations in subjects, training protocols, isolation methods and methods for measuring calcium flux. Further research is necessary to determine the extent of training that will elicit improved cardiac calcium handling by the sarcoplasmic reticulum.

Isolated single muscle fibers

Single muscle fibers from a clawed frog, *Xenopus laevis*, have been used to demonstrate associations between changes in calcium concentrations and fatigue (Allen et al., 1989; Lee et al., 1991). Calcium concentration changes during fatigue of isolated muscle fibers were associated with much of 1) the tension reduction during fatigue, 2) the slowing of relaxation and 3) the tension reduction during post-contractile depression (Allen et al., 1989). Fatigue was produced in single muscle fibers from *Xenopus laevis* lumbrical muscles by repeated intermittent tetanic stimulation continued until tension had declined to approximately 50% of the initial level. Fibers were allowed to recover by giving tetani at less frequent intervals, and a measure of calcium concentration and tension were quantitated during fatiguing stimulation and recovery. During fatiguing stimulation, tetanic tension declined steadily, but peak myoplasmic free calcium concentration first increased before declining substantially. Fibers showed

a characteristic slowing of relaxation in the fatigued state which was associated with a slowing of the rate of decline of the calcium concentration. After fatiguing stimulation, tension and calcium concentration showed a secondary decline followed by a slower recovery (post-contractile depression) in the majority of fibers.

The same group of co-workers (Lee et al., 1991) examined changes in tetanic and resting calcium concentration during fatigue and recovery in single muscle fibers from the toe muscles of *Xenopus laevis*. Fibers were fatigued by repeated tetani until developed tension had fallen to 50% of control (tension developed before fatiguing stimulation). There was a uniform decline in tetanic tension during fatiguing stimulation, whereas the tetanic myoplasmic calcium concentration first increased and then declined. These results are similar to those reported in the previous article by the same researchers (Allen et al., 1989). The rate of decline of both tension and myoplasmic calcium concentration after a tetanus slowed during fatigue. During recovery, the tension and calcium concentration recovered in parallel. Furthermore, the resting myoplasmic calcium concentration (immediately before each tetanus) increased throughout fatigue and towards the end of fatiguing stimulation it was greater than the tetanic calcium concentration in the early part of recovery, yet there was no detectible increase of resting tension during fatiguing stimulation. This observation lead the authors to suggest that the calcium sensitivity of the contractile proteins was reduced at the end of fatiguing stimulation. In conclusion it was implied that both reduced tetanic

myoplasmic calcium concentration and reduced calcium sensitivity contribute to the decline of tension during fatigue. The elevated resting calcium concentration during fatigue appears to result from either an increase in the calcium leak from the sarcoplasmic reticulum or a reduction in calcium sequestration by the sarcoplasmic reticulum.

Fatigue which was produced in single mouse muscle fibers by repeated tetani generally occurred in three phases (Westerblad and Allen, 1991). Initially, tension declined rapidly to approximately 90% of the original tension and during this period tetanic intracellular concentration of calcium increased significantly (phase 1). This was followed by a lengthy period of almost stable tension production and tetanic calcium concentration (phase 2). Finally, both the tetanic calcium concentration and tension fell relatively quickly (phase 3). The resting calcium concentration (between tetani) rose continuously throughout the stimulation period. During phases two and three, 10 s pauses were interspersed during fatiguing stimulation. During phase three this resulted in a significant increase of both tetanic calcium concentration and tension, whereas during phase two it did not have any marked effect. Application of caffeine (a calcium releasing agent) under control conditions (tetani elicited before fatiguing stimulation in a fiber) and early in phase two resulted in a substantial increase of the tetanic calcium concentration but no marked tension increase, whereas caffeine applied at the end of fatiguing stimulation (tension depressed to approximately 30% of the

original tension) gave a marked increase in both tetanic calcium concentration and tension. The tetanic calcium concentration for a given tension was generally higher during fatiguing stimulation than under control conditions. According to these researchers, these results indicated that fatigue produced by repeated tetani is caused by a combination of reduced maximum tension-generating capacity, reduced myofibrillar calcium sensitivity, and reduced calcium released from the sarcoplasmic reticulum. The depression of maximum tension-generating capacity develops early during fatiguing stimulation and it is of greatest importance for the force decline at early stages of fatigue. As fatigue gets more severe, reduced calcium release becomes quantitatively more important for the tension decline.

Calcium uptake rate by the SR of frog is reduced and that calcium sensitivity is increased and caffeine sensitivity is decreased in skinned muscle fibers that have been subjected to fatiguing stimulation (Williams et al., 1993). The addition of caffeine after calcium loading of the SR for periods of 5-30 seconds evoked smaller contractions in fatigued fibers compared to rested fibers. When the calcium loading period lasted 60-240 seconds, force developed following caffeine application was similar for both conditions, suggesting that the rate but not the maximal capacity of calcium loading by the SR is reduced by fatigue. After calcium loading, significantly more caffeine was required to evoke a contracture in fatigued fibers compared to controls. The concentration of calcium required to elicit 50% of maximal Ca^{2+}-activated force was lower in fatigued fibers. These

results indicate the SR of fatigued muscle displays depressed calcium exchange kinetics and the contractile apparatus exhibits increased calcium sensitivity.

pH

The inability of the sarcoplasmic reticulum to regulate calcium may contribute to the changes in contractility that occur during experimentally induced acidosis (Fabiato and Fabiato, 1978) and exercise (Fitts et al., 1982). The effects of lowering of pH may be important in altering skeletal muscle contractility because it increases calcium binding and decreases calcium release by skeletal muscle sarcoplasmic reticulum (Nakamaru and Schwartz, 1972). Changes in sarcoplasmic reticulum function after high-intensity exercise may be induced but not sustained by local changes in muscle pH and/or temperature (Byrd et al., 1989a).

The effects of pH on sarcoplasmic reticulum function have been investigated (Nakamaru and Schwartz, 1972; Fabiato and Fabiato, 1978; Rousseau and Pinkos, 1990). Calcium release and binding produced by alterations in pH were investigated in isolated sarcoplasmic reticulum from dog skeletal muscle (Nakamaru and Schwartz, 1972). Abrupt increases and decreases in pH were associated with increases and decreases in calcium released from the sarcoplasmic reticulum. Therefore the calcium release process was suggested to be a function of pH change. A decrease in pH also increased calcium binding activity of the sarcoplasmic reticulum. In another study (Fabiato and Fabiato,

1978) decreasing pH diminished maximal calcium activated force and calcium sensitivity of both skeletal and cardiac fibers. Similar results were reported by Godt and Nosek (1989) who also suggested that the effect on calcium sensitivity arises from competition between hydrogen ions and calcium ions at the thin filament. The proton concentration may also be important in modulating the sarcoplasmic reticulum release channel activity (Rousseau and Pinkos, 1990). Sarcoplasmic reticulum vesicles were isolated from rabbit skeletal muscle, and the pH was independently varied on either side of the channel (cytoplasmic face and luminal face). Acidification of the cytoplasmic side induced a modification of the channel gating behavior, resulting in a decrease of the open conformation. This effect was completely reversible. When the proton concentration was increased on the luminal face of the sarcoplasmic reticulum calcium channel, there was a modification of calcium conductance rather than just a conformational change.

Decreased pH is reported to reduce SR Ca^{2+}-ATPase activity and to inhibit calcium uptake by the SR (Fabiato and Fabiato 1978; Inesi and Hill, 1983). Increased pH is known to stimulate calcium release from the SR (Shoshan et al., 1981). Reduced pH has been reported to decrease (Fabiato and Fabiato, 1978) and to increase (Allen et al., 1989) calcium release. Williams and Ward (1991) reported a reduced CICR (calcium-induced calcium-release) from skeletal muscle SR at a low pH. Using chemically skinned frog semitendinosus muscles, they found rate constants for CICR to be significantly greater at pH 7.0 than at pH 6.5,

indicating that reduced pH depresses calcium release from the SR.

Phospholipid alterations

Phospholipid alterations have also been suggested as playing a role in sarcoplasmic reticulum dysfunction in cardiac muscle (Franson et al., 1986). Myocardial ischemia resulted in a breakdown of the excitation-contraction coupling system of cardiac muscle associated with lysosomal activation (Hess et al., 1981, as cited by Franson et al., 1986). The hypothesis that myocardial ischemia results in the activation of lysosomal phospholipase C and disruption of calcium transport in sarcoplasmic reticulum mediated by oxygen free radicals was tested by Franson et al. (1986). A significant depression of calcium uptake rates and calcium stimulated ATPase activity with acidotic conditions (pH of 7.0 or 6.4) was demonstrated in isolated sarcoplasmic reticulum of cardiac muscle from dogs. This depression of sarcoplasmic reticulum function was significantly inhibited in hearts pretreated with superoxide dismutase plus catalase. In general, the investigators found that, in preparations of sarcoplasmic reticulum from normal and ischemic myocardium, increased activity resulted in membrane dysfunction and phospholipid alterations that are mediated by an acid active lysosomal phospholipase C. Also, these functional and structural alterations can be attenuated by free radical scavengers.

Prolonged activation of skeletal muscle leads to a decline of force

production known as fatigue, which is accompanied by a number of ionic and metabolic changes in muscle (Klug and Tibbitts, 1988; Booth and Thomason, 1991; Westerblad et al., 1991). Westerblad et al. (1991) suggest that the causes of force decline can be divided into three groupings: 1) reduced calcium release from the sarcoplasmic reticulum, 2) reduced myofibrillar calcium sensitivity and 3) reduced maximum calcium activated tension. Reduced calcium release can be due to impaired action potential propagation in the T tubules, and this is a major cause of the tension decline with continuous tetanic stimulation. Changes in intracellular metabolites, particularly increased concentrations of inorganic phosphate and reduced pH, lead to reduced calcium sensitivity and reduced maximum tension, which make an important contribution to the force decline, especially with repeated tetanic stimulation.

Clearly the sarcoplasmic reticulum of skeletal muscle is involved in the development of fatigue. It is probable that reductions in calcium output and uptake by the sarcoplasmic reticulum represent a fundamental site of force decline in prolonged exercise as well as short-term high-intensity work. The studies pertaining to SR adaptations of myocardium with exercise training may suggest that SR function of skeletal muscle may improve from conditioning. Future studies are likely to explore such issues.

DIETARY LIPIDS

Fat supplemented diets for athletes

Muscle contraction requires the conversion of chemical energy to mechanical energy. This is accomplished by the release of energy from adenosine triphosphate (ATP) in high energy phosphate bonds during hydrolysis to adenosine diphosphate (ADP) and inorganic phosphate (Sahlin, 1986). The ATP utilized during muscular contraction must be resynthesized in order to maintain activity. There are four biochemical pathways to achieve this: 1) the transfer of high energy phosphate bonds from creatine phosphate to ADP; 2) glycolysis, which is the breakdown of glycogen to lactic acid; 3) oxidation of glucose; and 4) oxidation of fatty acids. The amount and rate of energy provided differs among the processes, and the relative contribution of these energy sources varies with the intensity and duration of exercise (Sahlin, 1986).

Fatty acids from triglycerides either stored within the muscle or adipose tissue can be utilized for energy production during exercise. Long-chain fatty acids are oxidized to produce acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle and subsequently forms ATP via oxidative phosphorylation.

Within the past 20 years, researchers have attempted to improve athletic performance in horses by supplementing their diets with the addition of fat (Slade et al., 1975; Hintz et al., 1978). Including fat in the diet increases energy density

of a ration without greatly increasing the amount of feed needed to meet energy requirements for work. Supplementing equine rations with fat has been shown to be beneficial for supplying energy for maintenance and growth without increasing carbohydrate intake (Rich et al., 1981; McCann et al., 1987; Scott et al., 1989).

Studies in rodents demonstrated benefits of fat supplementation on endurance capacity (Conlee et al.,1990; Simi et al., 1991). Rats adapted to a high fat diet for approximately 5 weeks ran as long (Conlee et al., 1990) or longer (Miller et al., 1984) than carbohydrate-fed rats with lower pre-exercise muscle and liver glycogen concentrations. Fat-adapted rats also had a decreased rate of muscle (Miller et al., 1984) and liver (Miller et al., 1984; Simi et al., 1991) glycogen utilization and decreased blood lactate levels during exercise. Muscle activity of 3-hydroxyacyl CoA dehydrogenase (3-HAD) and citrate synthase were increased after fat adaptation (Miller et al., 1984; Simi et al., 1991). These changes are indicative of amplification of lipid metabolism. Furthermore Conlee and coworkers (1990) reported an increase in muscle triglyceride stores in the rats fed a high fat diet. Triglycerides can be used as a fuel source either to spare glycogen or in the absence of adequate glycogen supply.

Attempts at improving athletic performance of horses by feeding fat supplemented diets have been equivocal. Some studies demonstrate feeding fat may maintain plasma glucose during endurance type exercise (Hambleton et al., 1980; Webb et al., 1987). Resting muscle glycogen concentration in horses fed

fat supplemented diets were similar (Hambleton et al., 1980), increased (Oldam et al.,1990; Scott et al., 1992) or decreased (Greiwe et al., 1989; Pagan et al., 1987) compared to controls. A glycogen sparing effect was found during prolonged, submaximal exercise with fat supplementation (Greiwe et al., 1989), but Hambleton et al. (1980) demonstrated no effect of fat on glycogen utilization with prolonged exercise. Pagan and coworkers (1987) found no difference in glycogen utilization in a stepwise test. Increased muscle glycogen utilization during high intensity exercise was reported in fat supplemented horses (Oldham et al., 1990), while muscle glycogen utilization was decreased in fat supplemented horses working during a high speed exercise test (Pagan et al., 1987).

Recent work at Virginia Tech demonstrated the acceptability and digestibility of dietary fat, particularly corn oil and lecithin (Holland, 1993; 1995). Full metabolic adaptation to a corn oil supplemented diet was achieved in 11 weeks and this diet increased the lactate threshold of horses undergoing an incremental exercise test (Custalow et al., 1993). When fed the corn oil diet, horses had an increased blood lactate concentration, indicating an altered metabolism in which either lactate production is increased or clearance is decreased (Taylor et al., 1993). Inclusion of lecithin in the diet reversed the effects of exercise on blood lactate, possibly by facilitating fatty acid transport and oxidation in muscle cells, thereby reducing the rate of glycolysis (Taylor et al., 1995).

Although there is inconsistency in fat supplementing equine diets in relation

to performance, substituting fat for carbohydrate as an energy source can benefit the horse by decreasing the bulk in the stomach and large intestine (Ferrante and Kronfeld, 1992). Large amounts of soluble carbohydrates have been associated with digestive disturbances that are painful and sometimes fatal. High carbohydrate diets can contribute to laminitis (Baxter, 1992), exertional rhabdomyolysis (Turner, 1992) and developmental orthopedic disease (Williams, 1992). As research continues to unravel fat supplementation in athletic horses, it appears fat adaptation facilitates metabolic regulation to achieve specific power needs of the exercising horse (Kronfeld et al., 1994). Further research is required to assess different types of fats and potential effects on specific cellular functions.

Dietary lecithin

The food processing industry defines lecithin as a mixture of polar and neutral lipids with a polar-lipid content of at least 60% and contains phosphatidylcholine and other phosphatides (Wurtman, 1979). In biochemistry, the term lecithin refers to phosphatidylcholine, which contains about 13% choline by weight (Canty and Zeisel, 1994). Other bases in lecithin are inositol, ethanolamine and serine. The most common dietary sources of lecithin are found in egg yolks, organ meats, nuts and wheat germ (Bonacker, 1988; Canty and Zeisel, 1994).

Soybeans, rapeseed and corn are the three primary sources of commercial lecithins. Soybeans are the preferred source of lecithin due to the high

concentration of phosphatidylcholine and the high levels of mono- and polyunsaturated fatty acids (Bonacker, 1988).

Lecithin supplementation decreased plasma cholesterol concentration (Ishida et al., 1988; Jiminez et al.,1990), liver triglycerides (Ishida et al., 1988) and plasma triglycerides, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) (Jiminez et al., 1990) in rats. High density lipoprotein (HDL) was increased in rats (Jiminez et al., 1990) and guinea pigs (O'Brien and Corrigan, 1988) fed lecithin.

Lecithin feeding in livestock has produced mixed results. Feed efficiency and average daily gain (ADG) were not affected by inclusion of lecithin in the diets of growing-finishing swine (Van Wormer and Pollman, 1985; Overland et al., 1993). Slight increases in daily feed intake and ADG were found in sheep supplemented with lecithin (Lough et al., 1990) and small improvements in carcass characteristics are associated with feeding lecithin to cattle (Zinn, 1989) and lambs (Lough et al., 1992).

Lecithin also is important because it is the primary source of choline in the diet. The choline molecule is a quaternary ammonium base which has basically three functions in the body. It is a precursor for the biosynthesis of phospholipds (phosphatidylcholine, sphingomyelin and choline plasmalogens), which are important components of biological membranes. It is a precursor for the biosynthesis of the neurotransmitter acetylcholine, which is necessary for muscle

activation; and it functions as a methyl group donator in transmethylation reactions (Allworden et al., 1993).

Recently choline and lecithin have been supplemented in an attempt to enhance athletic performance. Participants in the 1986 Boston marathon had decreased concentrations of plasma choline associated with intense physical stress (Conlay et al., 1986). After the race the mean reduction of the runners concentration of plasma choline was 40%. Administration of choline citrate improved mean running time in long-distance runners during a 20-mile run (Sandage et al., 1992). Choline citrate supplementation prevented the fall in plasma choline concentration during the post-run period. Lecithin given to top level triathletes and adolescent runners prior to hard physical exercise prevented a decrease in plasma choline. Lecithin given without exercise led to a significant increase in plasma choline concentrations (Allworden et al., 1993).

Lecithin supplementation also may affect membrane fluidity because of the importance of phosphatidylcholine in biological membranes. Further research is needed to determine the effects of feeding lecithin on membranes and subsequent changes in cellular function such as cell transport, cell metabolism, generating second messengers and enzymatic function.

MEMBRANE LIPID COMPOSITION AND ENZYMATIC FUNCTION

Biological membranes

Mammalian cell membranes consist of a lipid bilayer composed primarily of phospholipids and cholesterol. The physicochemical properties of the membranes are largely governed by the nature of the fatty acid components. Proteins that have important cellular functions, such as receptors, transporters and enzymes are embedded in the lipid bilayer and are highly sensitive to the lipid environment. The critical features of the fatty acids which govern the physicochemical properties are the chain length and the position and number of *cis*-double bonds. All of the major unsaturated n-6 and n-3 fatty acids are considered to be essential in that a dietary source is required (Stubbs and Smith, 1990). Therefore a great deal of effort has been directed toward understanding the relationship between these fatty acid properties and the physicochemical environment and in turn its effect on the protein function.

Membrane Lipid Composition and Protein Function

Fluidity allows the various membrane proteins to function optimally, which is the primary function of fatty acyl unsaturation in the lipid bipolar phase. Membrane proteins are surrounded by the fatty acyl constituents of membrane phospholipids. The function of these proteins is affected by the chain length and

extent of unsaturation of fatty acids to a degree that varies from protein to protein. For example, Ca^{2+}-ATPase activity is affected by chain length but not by unsaturation (East et al., 1984; Stubbs and Kisielewski, 1990). The activity of Ca^{2+}-ATPase was maximal with a chain length of 18 and decreased substantially with longer or shorter chains (Cornea and Thomas, 1994). Chain length shorter or longer than the optimal 18 resulted in a significantly decreased mobility of the Ca^{2+}-ATPase. By contrast, Na^{+} K^{+}-ATPase does appear to be sensitive to unsaturation (Kimelberg and Papahadjopoulos, 1974; Sutherland et al., 1988).

The activity and properties of a number of membrane-bound enzymes have been examined following lipid modification in cultured cells. Adenylate cyclase activity is affected by changes in the composition of the phospholipid fatty acyl chains and polar head groups, as well as by changes in the cholesterol content of the plasma membrane (Klein et al., 1978). Acyl-CoA synthetase and Acyl-CoA transferase activities have been reported to be dependent on phospholipid order of rat liver microsomes (Koshlukova et al., 1992). The incorporation of phosphatidylglycerol, phosphatidylserine and phosphatidylethanolamine induced a marked activation of these enzymes. Similarly, Momchilova and coworkers (1991) described the influences of phospholipid modification on acyl-CoA transferase in rat liver plasma membranes. The incorporation of phospholipids that induced membrane fluidization was accompanied by an elevated enzyme activity, whereas the phospholipids causing augmentation of membrane rigidity induced a

decrease of this activity.

Na^+,K^+-ATPase Enzyme Activity

Membrane fluidity also affects the Na^+,K^+-ATPase pump which is found in virtually all tissues. This pump operates as an antiport, actively pumping sodium out of the cell and potassium into the cell against their concentration gradients. The sodium and potassium gradients maintained by this pump are responsible not only for the cell's membrane potential, but also for controlling cell volume and for driving the active transport of sugars and amino acids. Because of the magnitude of importance of this system, a number of researchers have been interested in its physicochemical properties. In one of the earlier studies, Kimelberg and Papahadjopoulos (1973) reported the effects of membrane fluidity on Na^+,K^+-ATPase activity obtained from rabbit kidney. The fluidity of the hydrocarbon region of the phospholipid bilayer was altered by the addition of cholesterol to the isolated kidney microsomes. Cholesterol, which is known to reduce fluidity of phospholipid fatty acyl chains, inhibited the Na^+,K^+-ATPase activity. Chong et al. (1985) found a direct correlation between the rate of Na^+,K^+-ATPase turnover and the fluidity of the membrane from dog kidney. They proposed that decreased membrane fluidity hinders the conformational transitions associated with the rate-limiting steps of the Na^+,K^+-ATPase reaction.

This same enzyme was investigated in a somatic cell mutant (CRI) of the

Chinese hamster ovary cell line which is defective in the regulation of cholesterol biosynthesis (Sinensky et al., 1979). It can be grown under conditions in which plasma membranes from these cells display various cholesterol contents and acyl chain order parameters. This group concluded that the rate of catalysis by the Na^+,K^+-ATPase is determined by the order parameter and not cholesterol content. In other words, enzyme activity changes with differences in fatty acyl chain properties. The activity of the enzyme increased as the degree of unsaturation increased in the fatty acyl chains of the cell membranes.

The localization of Na^+,K^+-ATPase in hepatocytes has been controversial (Sutherland et al., 1989). Activity of this enzyme has been seen in the basolateral membrane (Poupon and Evans, 1979; Meier et al., 1984) and it has been located in the bile canalicular (or apical) surface (Schenk and Leffert, 1980). Sutherland et al. (1989) reported that hepatic Na^+,K^+-ATPase is distributed in both surface membranes of rat livers. Furthermore they suggest that the enzyme functions more efficiently and, perhaps, specifically in the sinusoidal (basolateral) membranes because of the higher bulk lipid fluidity of that membrane surface.

Ca^{2+}-ATPase Activity

Phospholipids are the main lipid component of the native SR membrane with phosphatidylcholine being the main phospholipid species (65-75%). There are 80 to 100 molecules of phospholipid associated with one molecule of Ca^{2+}-ATPase

enzyme in the native SR membrane. The fatty acyl composition of phosphatidylcholine and phosphatidylethanolamine (15-20%) is characterized by a number of highly unsaturated fatty acids, and this may be important for the SR enzyme function (Hidalgo, 1987).

The structural characteristics of the SR Ca^{2+}-ATPase have been extensively studied. Infante (1987) suggested that phosphatidylcholine may be especially important for Ca^{2+}-ATPase functioning and that high content of polyunsaturated fatty acids in SR membranes may play a role in calcium transport. An optimal lipid environment has been suggested for the Ca^{2+}-ATPase which is provided by 16:0 - 18:1 phosphatidylcholine and 16:0-18:2 phosphatidylcholine, the two most common lipids of the SR (Matthews et al., 1993).

Supplementing the diet of rats with safflower oil resulted in marked changes in the fatty acid composition of the skeletal muscle SR but did not affect such properties of the sarcoplasmic reticulum as the rate of calcium uptake, the total amount of calcium taken up, the rate of calcium release or the ATPase activity (Tume et al., 1973). Supplementing also resulted in large increases in the proportion of linoleic acid in the SR, but the reason for this was unknown.

Mice supplemented with dietary menhaden oil for two weeks were found to have significantly higher levels of n-3 and lower levels in n-6 cardiac sarcoplasmic reticulum phopholipids than did mice supplemented with either corn oil or safflower oil (Swanson et al., 1988). These changes in fatty acid composition resulted in a

significant decrease in the value of the n-6/n-3 fatty acid ratio of cardiac SR phospholipids. This reduced ratio was associated with a lower relative activity of Ca^{2+}-ATPase, and a lower initial rate of calcium transport and maximal calcium uptake in SR vesicles from mice fed menhaden oil rather than olive or corn oils. The authors indicate that modification of SR by n-3 fatty acids, particularly by 22:6(n-3), may change the lipid bilayer structure resulting in alterations of the calcium transport properties of cardiac SR vesicles. Furthermore they maintain that reduction of calcium flux across cardiac SR following fish oil consumption also may reduce the susceptibility of myocytes to rapid changes in calcium concentrations which may occur during ischemia and reperfusion.

Dietary n-3 and n-6 polyunsaturated fatty acids affect the fatty acid composition of phospholipid, Ca^{2+}-ATPase and calcium transport activities of mouse cardiac SR (Croset et al., 1989). Although feeding n-6 polyunsaturated fatty acid induced little modifications of the phospholipid SR fatty acid composition, feeding n-3 polyunsaturated fatty acid altered it markedly, causing an accumulation of 22:6(n-3). There was a decrease in the n-6/n-3 polyunsaturated fatty acid ratio and a decrease in the ratio of 20 carbon to 22 carbon fatty acids esterified in the phospholipid sarcoplasmic reticulum. These changes were associated with a decrease in calcium uptake by n-3 polyunsaturated fatty acid enriched SR vesicles as compared with n-6 fatty acid and control diet SR vesicles, similar to the findings of Swanson et al. (1989). However, neither the affinity for calcium nor the maximal

velocity of ATP hydrolysis activity of Ca^{2+}-ATPase were altered by the different diets.

Dietary fish oil and corn oil were compared in regards to the lipid dynamics and Ca^{2+}-ATPase activity of rat skeletal SR (Stubbs and Kisielewski; 1990). Fish oil supplementation led to a marked increase in the level of all the n-3 fatty acids, especially 22:6. The Ca^{2+}-ATPase was found to be unaffected by supplementation which the authors feel is consistent with the observed modest changes in lipid order and suggest that the enzyme is relatively insensitive to the level of unsaturation. They argued that if large increases in fatty acyl polyunsaturation in mammalian cell membranes would lead to marked alterations in "bulk membrane lipid motional properties", this may not be in the interest of preserving physiological function. Additionally they suggested that the complex mixture of phospholipid molecular species present in natural membranes may buffer against this by a type of passive adaptation, without the expenditure of metabolic energy, thus providing a homeoviscous environment able to optimally support membrane protein function.

Dietary manipulation can induce changes in fatty acid composition and phospholipid order in the sarcoplasmic reticulum. How altering the membrane fluidity will affect the calcium transporting enzyme and therefore calcium transport is controversial. Recently it has been reported that ADR (arrested development of righting reflex) mice have an abnormal fatty acid composition in sarcolemma and sarcoplasmic reticulum and this may affect the function of the ion channels

affiliated with muscle (Birkle et al., 1993), adding strength to the implication that membrane lipid composition does affect calcium transport in the sarcoplasmic reticulum.

In my view, most researchers have undertaken investigations into altering membrane fluidity with the hope of increasing the functioning of the Ca^{2+}-ATPase enzyme in skeletal muscle. Results, however, have been equivocal. There has been no change or an actual decrease in Ca^{2+}-ATPase associated with increased lipid motional parameters. All of the studies have been carried out using sedentary subjects. It is possible that no increase in activity was observed because the enzyme was not undergoing any stressful stimulus that would promote a needed increase. More practical studies should incorporate exercise and dietary manipulation in assessing alterations in SR function.

Membrane fluidity of plasma membranes has generated a plethora of studies since the dynamic state of the lipids in the bilayer was described in 1972 by Singer and Nicolson. From the standpoint of cellular physiology and metabolic regulation, the critical questions that have been asked are whether changes in membrane lipid composition actually can occur in a living cell and if so, whether the changes are of sufficient magnitude to affect membrane function. Modifications to cell membranes have been detailed pertaining to such factors as age, dietary status of an animal and dietary manipulation. The modifications can be extensive enough to alter membrane fluidity and affect a number of cellular functions. At this time

evidence abounds that membrane lipids can influence the function of certain membrane proteins. However, the effects of lipid modification on cellular function are very complex and vary from cell to cell. It is difficult to make any generalizations or to predict how a given system may respond to a particular type of lipid modification. It is inevitable that future research will continue to unravel the complexities of membrane fluidity changes and associated alterations in cellular function.

Literature Cited

Allen, D.G., J.A. Lee and H. Westerblad. 1989. Intracellular calcium and tension during fatigue in isolated single muscle fibres from *Xenopus laevis*. J. Physiol. 415:433.

Allworden, H.N., S. Horn, J. Kahl and W. Feldheim. 1993. The influence of lecithin on plasma choline concentrations in triathletes and adolescent runners during exercise. Eur. J. Appl. Physiol. 67:87.

Baxter, G.M. 1992. Laminitis. In: N.E. Robinson (Ed.). Current Therapy in Equine Medicine 3. W.B. Saunders Co., Philadelphia.

Beech, J. 1994. Treating and preventing chronic intermittent rhabdomyolysis. Eq. Prac. 5:458.

Belcastro, A.N., M. Rossiter, M.P. Low and M.M. Sopper. 1981. Calcium activation of sarcoplasmic reticulum ATPase following strenuous activity. Can. J. Physiol. Pharmacol. 59:1214.

Belcastro A.N., J.S. Gilchrist and J. Scrubb. 1993. Function of skeletal muscle sarcoplasmic reticulum vesicles with exercise. J. Appl. Physiol. 75:2412.

Birkle,D. L., V. Shahamat, J. Lucci, E. A. Johnson, J. E. Riggs and A.J. Azzaro. 1993. Abnormal fatty acid composition in sarcolemma and sarcoplasmic reticulum from myotonic ADR mouse muscle. Biochim. Biophys. Acta. 1146:236.

Bonacker, U. 1988. The significance of phosphatidylcholine for the lipid metabolism. Pub. No. 6, Lucas Meyer Corp. Hamburg, Germany.

Bonner, H.W., S.W. Leslie, A.B. Combs and C.A. Tate. 1976. Effects of exercise training and exhaustion on ^{45}Ca uptake by rat skeletal muscle mitochondria and sarcoplasmic reticulum. Res. Commun. Chem Pathol. Pharmacol. 14:767.

Booth, F.W. and D.B. Thomason. 1991. Molecular and cellular adaptations of muscle in response to exercise: Perspectives of various models. Physiol. Rev. 71:541.

Byrd, S.K., L.J. McCutcheon, D.R. Hodgson and P.D. Gollnick. 1989a. Altered sarcoplasmic reticulum function after high-intensity exercise. J. Appl. Physiol. 67:2072.

Byrd, S.K., A.K. Bode and G.A. Klug. 1989b. Effects of exercise of varying duration on sarcoplasmic reticulum function. J. Appl. Physiol. 66:1383.

Canty, D.J. and S.H. Zeisel. 1994. Lecithin and choline in human health and disease. Nutrit. Reviews. 52:327.

Chong, P. L., P. A. Fortes and D. M. Jameson. 1985. Mechanisms of inhibition of (Na,K)-ATPase by hydrostatic pressure studied with Fluorescent probes. J. Biol. Chem. 260:14484.

Conlay, L.A., R.J. Wurtman, K. Blusztajn, I.J. Coviella, T.J. Maher and G.E. Evoniuk. 1986. Decreased plasma choline concentrations in marathon runners (letter). New Engl. J. Med. 175:892.

Conlee, R.K., R.L. Hammer, W.W. Winder, M.L. Bracken, A.G. Nelson and D.W. Barnett. 1990. Glycogen repletion and exercise endurance in rats adapted to a high fat diet. Metabolism. 39:289.

Cornea, R.L. and D.D. Thomas. 1994. Effects of membrane thickness on the molecular dynamics and enzymatic activity of reconstituted Ca-ATPase. Biochemistry. 33:2912.

Croset, M., J. M. Black, J. E. Swanson and J. E. Kinsella. 1989. Effect of dietary n-3 polyunsaturated fatty acids on phospholipid composition and calcium transport in mouse cardiac sarcoplasmic reticulum. Lipids. 24:278.

Custalow, S.E., P.L. Ferrante, L.E.Taylor, H.D. Moll, T.N. Meacham, D.S. Kronfeld and W. Tiegs. 1993. Lactate and glucose responses to exercise in the horse are affected by training and dietary fat. Proc. 13th Equine Nutr. Physiol. Symp. 179. Gainsville, FL.

East, J. M., O. T. Jones, A. C. Simmonds and A. G. Lee. 1984. Membrane Fluidity is not an important physiological regulator of the (Ca^{2+}-Mg^{2+})-dependent ATPase of sarcoplasmic reticulum. J. Biol. Chem. 259:8070.

Fabiato, A. and F. Fabiato. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. J. Physiol. 276:233.

Favero, T.G., I.N. Pessah and G.A. Klug. 1993. Prolonged exercise reduces Ca^{2+} release in rat skeletal muscle sarcoplasmic reticulum. Pflugers Arch. 422:472.

Ferrante, P.L. and D.S. Kronfeld. 1992. Ergogenic diets and nutrients. In: N.E. Robinson (Ed.) Current Therapy in Equine Medicine 3. p 809. W.B. Saunders Co., Philadelphia.

Fitts, R.H., J.B. Courtright, D.H. Kim and F.A. Witzmann. 1982. Muscle fatigue with prolonged exercise:contractile and biochemical alterations. Am. J. Physiol. 242:C65.

Franson, R., D. Gamache, W. Blackwell D. Eisen and M.L. Hess. 1986. Sarcoplasmic reticulum dysfunction: phospholipid alterations induced by lysosomal phospholipase C. Am. J. Physiol. 251:H1017.

Godt, R.E. and T.M. Nosek. 1989. Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. J. Physiol. 412:155.

Gollnick, P.D., P. Korge, J. Karpakka and B. Saltin. 1991. Elongation of skeletal muscle relaxation during exercise is linked to reduced calcium uptake by the sarcoplasmic reticulum in man. Acta. Physiol. Scand. 142:135.

Green, H., F. Grange, E. Chin, C. Goreham, D. Ranney and R.J. Xu. 1992. Alterations in sarcoplasmic reticulum Ca^{2+}-ATPase activity in human skeletal muscle with prolonged exercise. The Physiologist. 35:215.

Greiwe, K.M., T.N. Meacham, G.F.Fregin and J.L. Walberg. 1989. Effect of added dietary fat on exercising horses. Proc. 11th Equine Nutrit. and Physiol. Symp. p 101. Stillwater, OK.

Guyton, A.C. 1991. Textbook of medical physiology. pp 67-78,98-109. W. B. Saunders Co., Philadelphia.

Hambleton, P.L., L.M. Slade, D.W. Hamar, E.W. Kienholtz and L.D. Lewis. 1980. Dietary fat and exercise conditioning effect on metabolic parameters in the horse. J. Anim. Sci. 51:1330.

Heilmann, C. and D. Pette. 1979. Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation. J. Biochem. 93:437.

Hess, M. L., E. Okabe and H.A. Kontos. 1981. Proton and free radical interaction with the calcium transport system of cardiac sarcoplasmic reticulum. J. Mol. Cell. Cardiol. 13:767.

Hidalgo, C. 1987. Lipid-protein interactions and the function of the Ca^{2+}-ATPase of sarcoplasmic reticulum. C. R. C. Critical Reviews in Biochemistry. 21:319.

Hintz, H.F., M.W. Ross and F.R. Lesser, P.F. Leids, K.K. White, J.E. Lowe, C.E. Short and H.F. Schryver. 1978. The value of dietary fat for working horses. I. Biochemical and hematological evaluations. J. Equine Med. Surg. 2:483.

Holland, J.A., T.N. Meacham, D.S. Kronfeld and W. Tiegs. 1993. Acceptance of lecithin containing diets by horses. Proc. 13th Equine Nutr. Physiol. Symp. p. 64. Gainsville, FL.

Holland, J.A., T.N. Meacham and D.S. Kronfeld. 1995. Digestibility of lecithin containing diets by horses. Proc. 14th Equine Nutr. Physiol. Symp. p. 80. Ontario, CA.

Inesi, G. and T.L. Hill. 1983. Calcium and proton dependence of sarcoplasmic reticulum ATPase. Biophys. J. 44:271.

Infante, J. P. 1987. Docosahexanote-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in Ca^{2+}-ATPase of sarcoplasmic reticulum. Mol. Cell. Biochem. 74:111.

Ishida, T., K. Koba, M. Sugano, K. Imaizumi, S. Watanbe and R. Minoshima. 1988. Cholesterol levels and eicosanoid production in rats fed phosphatidylinositol or soybean lecithin. J. Nutr. Sci. Vitaminol. 34:237.

Jimenez, M.A., M.L. Scarino, F. Vignolini and E. Mengheri. 1990. Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. J. Nutr. 120:659.

Kimelberg, H. K. and D. Papahadjopoulos. 1974. Effects of phospholipid acyl chain fluidity, phase transitions, and cholesterol on (Na^++K^+)-stimulated adenosine triphosphatase. J. Biol. Chem. 249:1071.

Klein, I., L. Moore and I. Pastan. 1978. Effect of liposomes containing cholesterol on adenylate cyclase activity of cultured mammalian fibroblasts. Biochim. Biophys. Acta. 506:42.

Klug, G.A. and G.F. Tibbits. 1988. The effect of activity on calcium-mediated events in striated muscle. In Exerc. and Sports Sci Rev (K. B. Pandolf, Ed), vol 16. pp 1-59.

Koshlukova, S. E., A. B. Momchilova-Pankova, T. T. Markovska and K. S. Koumanov. 1992. Phospholipid dependence of rat liver microsomal acyl:CoA synthetase and acyl-CoA:1-acyl-*sn*-glycero-3-phosphocholine O-acyltransferase. J. Membrane Biol. 127:113.

Kronfeld, D.S., P.L. Ferrante and D. Grandjean. 1994. Optimal nutrition for athletic performance, with emphasis on fat adaptation in dogs and horses. J. Nutr. 124:2745S.

Laughlin, M.H., C.C. Hale, L. Novela, D. Gute, N. Hamilton and C.D. Ianuzzo. 1991. Biochemical characterization of exercise-trained porcine myocardium. J. Appl. Physiol. 71:229.

Lee, J.A., H. Westerblad and D.G. Allen. 1991. Changes in tetanic and resting Ca^{2+} during fatigue and recovery of single muscle fibres from *Xenopus laevis*. J. Physiol. 433:307.

Levine, S.N. and G.T. Kinasewitz. 1986. Exercise conditioning increases rat myocardial calcium uptake. J. Appl. Physiol. 60:1673.

Lough, D.S., M.B. Solomon, T.S. Rumsey. T.H. Elsasser, L.L. Slyter, S. Kahl and G.P. Lynch. 1990. Effects of dietary canola seed and soy lecithin in high-forage diets on performance, serum lipids, and carcass characteristics of growing ram lambs. J. Anim. Sci. 69:3292.

Lough, D.S., M.B. Soloman, T.S. Rumsey, T.H. Elsasser, L.L. Slyter, S. Kahl and G.P. Lynch. 1992. Effects of dietary canola seed and soy lecithin in high-forage diets on cholesterol content and fatty acid composition of carcass tissues of growing ram lambs. J. Anim. Sci. 70:1153.

Luckin, K.A., S.B. Smulovitz and G.A. Klug. 1992. Post exercise recovery of sarcoplasmic reticulum function. The Physiologist. 35:200.

Matthews, P.L., E. Bartlett, V.S. Ananthanarayanan and K.M. Keough. 1993. Reconstitution of rabbit sarcoplasmic reticulum calcium ATPase in a series of phosphatidylcholines containing a saturated and unsaturated chain: suggestion of an optimal lipid environment. Biochem. Cell. Biol. 71:381.

McCann, J.S., T.N. Meacham and J.P. Fontenot. 1987. Energy utilization and blood traits of ponies fed fat-supplemented diets. J. Anim. Sci. 65:1019.

Meier, P. J., E. S. Sztul, A. Reuben and J. L. Boyer. 1984. Structural and functional polarity of canalicular and basolateral plasma membrane vesicles isolated in high yield from rat liver. J. Cell Biol. 98:991.

Miller, W.C., G.R. Bryce and R. K. Conlee. 1984. Adaptations to a high-fat diet that increases exercise endurance in male rats. J. Appl. Physiol. 56:78.

Momchilova, A. B., T. T. Markovska, S. E. Koshlukovan, K. S. Koumanov and R. G. Pankov. 1991. Phospholipid modification influences acyl-CoA:1-acyl-glycero-3-phosphocholine *O*-acyltransferase in rat liver plasma membranes. Biochem. Cell Biol. 69:643.

Nakamaru, Y. and A. Schwartz. 1972. The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J. Gen. Physiol. 59:22.

O'Brien, B.C. and S.M. Corrigan. 1988. Influence of dietary soybean and egg lecithins on lipid responses in cholesterol-fed guinea pigs. Lipids. 23:647.

Oldham, S.L., G.D. Potter, J.W. Evans, S.B. Smith, T. S. Taylor and W.S. Barnes. 1990. Storage and mobilization of muscle glycogen in exercising horses fed a fat supplemented diet. Equine Vet. Sci. 10:353.

Overland, M., M.D. Tokach, S.G. Cornelius, J.E. Pettigrew and M.E. Wilson. 1993. Lecithin in swine diets:II. Growing-finishing pigs. J. Anim. Sci. 71:1194.

Pagan, J.D., B. Essen-Gustavsson, A. Lindholm and J. Thornton. 1987. The effect of dietary energy source on exercise performance in Standardbred horses. In: J.R. Gillespie, and N.E. Robinson (Ed.). Equine Exercise Physiology 2. p 686. ICEEP Publications, Davis, CA.

Parsons, D., G. Tritchler, A. Dickinson and S. Byrd. 1992. Effects of fatigue on sarcoplasmic reticulum calcium uptake in female cyclists. The Physiologist. 35:215.

Penpargkul, S., D.I. Repke, A.M. Katz and J. Scheuer. 1976. Effect of physical training on calcium transport by rat cardiac sarcoplasmic reticulum. Circ. Res. 40:134.

Penpargkul, S., A. Malhorta, T. Schaible and J. Scheuer. 1980. Cardiac contractile proteins and sarcoplasmic reticulum in hearts of rats trained by running. J. Appl. Physiol. 48:409.

Pierce, G.N., P.S. Sekhon, H.P. Meng and T.G. Maddaford. 1989. Effects of chronic swimming training on cardiac sarcolemmal function and composition. J. Appl. Physiol. 66:1715.

Poupon, R. E. and W. H. Evans. 1979. Biochemical evidence that Na^+,K^+-ATPase is located at the lateral region of the hepatocyte surface membrane. FEBS lett. 108:374.

Rich, G.A., J.P. Fontenot and T.N. Meacham. 1981. Digestibility of animal, vegetable, and blended fats by equine. Proc. 7th Equine Nutr. Physiol. Symp. p. 30. Warrenton, VA.

Rousseau, E. and J. Pinkos. 1990. pH modulates conducting and gating behavior of single calcium release channels. Eur. J. Physiol. 415:645.

Sahlin, K. 1986. Metabolic changes limiting muscle performance. In: B. Sahlin (Ed.) Biochemistry of Exercise, Vol. 16. Human Kinetics Publishers, Champaign, Il.

Sandage, B. W., L. Sabounjain, R. White and R.J. Wurtman. 1992. Choline citrate may enhance athletic performance. The Physiologist. 25:4.

Schaible, T.F. and J. Scheuer. 1979. Effects of physical training by running or swimming on ventricular performance of rat hearts. J. Appl. Physiol. 46:854.

Schenk,D. B. and H. L. Leffert. 1983. Monoclonal antibodies to rat Na^+,K^+-ATPase block enzymatic activity. Biochemistry. 80:5281.

Scherer, N.M. and D.W. Deamer. 1986. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca^{2+}-ATPase. Arch. Biochem. Biophys. 246:589.

Scott, B.D., G.D. Potter, J.W. Evans, J.G.Reagor, G.W. Webb and S.P. Webb. 1989. Growth and feed utilization by yearling horses fed added dietary fat. Equine Vet. Sci. 9:210.

Scott, B.D., G.D. Potter, L.W. Greene, P.S. Hargis and J.G. Anderson. 1992. Efficacy of a fat-supplemented diet on muscle glycogen concentrations in Thoroughbred horses maintained in varying body conditions. Equine Vet. Sci. 12:109.

Sembrowich, W.L., and P.D. Gollnick. 1977. Calcium uptake by heart and skeletal muscle sarcoplasmic reticulum from exercised rats (Abstract). Med. Sci. Sports. 91:64.

Shoshan, V., D.H. MacLennan and D.S. Wood. 1981. A proton gradient controls a calcium-release channel in sarcoplasmic reticulum. Proc. Natl. Acad. Sci. U. S. A. 78:4828.

Simi, B., B. Sempore, M.H. Mayet and R.J. Favier. 1991. Additive effects of training and high-fat diet on energy metabolism during exercise. J. Appl. Physiol. 71:197.

Sinensky, M., F. Pinkerton, E. Sutherland and F.R. Simon. 1979. Rate limitation of $(Na^+ + K^+)$-stimulated adenosinetriphosphatase by membrane acyl chain ordering. Proc. Natl. Acad. Sci. 76:4893.

Singer, S.J. and G.L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. Science. 175:720.

Slade, L.M., L.D. Lewis and C.R. Quinn. 1975. Nutritional adaptation of horses for endurance type performance. Proc. 4th Equine Nutr. and Physiol. Symp. p. 114. Pomona, CA.

Stubbs, C.D. and A.E. Kisielewski. 1990. Effect of increasing the level of ◆-3 fatty acids on rat skeletal muscle sarcoplasmic reticulum. Lipids. 25:553.

Stubbs, C.D. and A.D. Smith. 1991. Essential fatty acids in membrane:physical properties and function. Biochem. Soc. Trans. 18:779.

Sutherland, E., B.S. Dixon, H. L. Leffert, H. Skally, L. Zaccaro and F.R. Simon. 1988. Biochemical localization of hepatic surface-membrane Na^+,K^+-ATPase activity depends on membrane fluidity. Proc. Natl. Acad. Sci. 85:8673.

Swanson, J.E., B.R. Lokesh and J.E. Kinsella. 1989. Ca^{2+}-Mg^{2+} ATPase of mouse cardiac sarcoplasmic reticulum is affected by membrane n-6 and n-3 polyunsaturated fatty acid content. J. Nutr. 119:364.

Tate, C., M. Hamra, G. Shin, G. Taffet, P. McBride and M. Entman. 1993. Canine cardiac sarcoplasmic reticulum is not altered with endurance exercise training. Med. Sci. Sports Exerc. 25:1246.

Taylor, L.E., P.L. Ferrante, T.N. Meacham, D.S. Kronfeld and W. Tiegs. 1993. Acid-base responses to exercise in horses trained on a diet containing added fat. Proc. 13th Equine Nutr. Physiol. Symp. p. 185. Gainsville, FL.

Taylor, L.E., P.L. Ferrante, J.A. Wilson and D.S. Kronfeld. 1995. Acid-base status and blood variables during repeated sprint exercise in horses fed lecithin. Proc. 14th Equine Nutr. Physiol. Symp. p. 94. Ontario, CA.

Tume, R.K., R.P. Newbold and D.J. Horgan. 1973. Changes in the fatty acid composition of sarcoplasmic reticulum lipids and calcium uptake activity. Arch. Biochem. Biophys. 157:485.

Turner, T.A. 1992. Muscular disorders. In: N.E. Robinson (Ed.). Current Therapy in Equine Medicine 3. W.B. Saunders Co., Philadelphia.

Van Wormer, D.M. and D.S. Pollman. 1985. Effect of lecithin addition to starter pig diets with and without added fat and dried whey. Nutr. Reports Int. 33:801.

Westerblad, H., J.A. Lee, J. Lannergren and D.G. Allen. 1991. Cellular mechanisms of fatigue in skeletal muscle. Am. J. Physiol. 261:C195.

Westerblad, H. and D.G. Allen. 1991. Changes in myoplasmic calcium concentration during fatigue in single mouse muscle fibers. J. Gen. Physiol. 98:615.

Williams, J.H. and C.W. Ward. 1991. Reduced Ca^{2+}-induced Ca^{2+} release from skeletal muscle sarcoplasmic reticulum at low pH. Can. J. Physiol. Pharmacol. 70:926.

Williams, J.H., C.W. Ward and G.A. Klug. 1993. Fatigued-induced alterations in Ca^{2+} and caffeine sensitivities of skinned muscle fibers. J. Appl. Physiol. 75:586.

Williams, M.A. 1992. Risk factors associated with developmental orthopedic disease. In: N.E. Robinson (Ed.). Current Therapy in Equine Medicine 3. W.B. Saunders Co., Philadelphia.

Webb, S.P., G.D. Potter and J. W. Evans. 1987. Physiologic and metabolic response of race and cutting horses to added dietary fat. Proc. 10th Equine Nutrit. and Physiol. Soc. Symp. p115. Fort Collins, CO.

Wurtman, J.J. 1979. Sources of choline and lecithin in the diet. In: A. Barbeau, J.H. Growdon and R.J. Wurtman (Ed.). Nutrition and the Brain, Vol. 5. Raven Press, New York.

Zinn, R.A. 1989. Influence of level and source of dietary fat on its comparative feeding value in finishing diets for steers: feedlot cattle growth and performance. J. Anim. Sci. 67:1029.

Journal Paper 1. Isolating equine sarcoplasmic reticulum and evaluating its function with observations on high intensity repeated sprints.

Abstract

A method has been developed for isolating equine sarcoplasmic reticulum (SR) and measuring calcium uptake by modifying methods previously established in rat and rabbit muscle tissue. Excisional biopsies were used to obtain muscle samples from the gluteal muscles of 10 Quarter Horses. Sarcoplasmic reticulum vesicles were isolated through homogenization and differential centrifugation. Calcium uptake into the vesicles was monitored spectrophotometrically using a calcium probe, antipyrylazo III. An enzyme-linked optical assay was employed to assess Mg^{2+}-stimulated, Ca^{2+}-stimulated and total ATPase activity in the isolated SR vesicles. The present results provide a system for obtaining SR microsomes and measuring calcium uptake in the isolated vesicles in a relatively short period of time. High intensity sprinting decreased calcium uptake and Ca-ATPase activity of SR vesicles in horses.

Introduction

The sarcoplasmic reticulum (SR) has been implicated in muscle fatigue and exertional rhabdomyolysis in horses (Byrd et al. 1989b; Hodgson 1993). It is a specialized endomembrane system within skeletal muscle fibers which regulates myoplasmic calcium concentration (Hidalgo 1987). During the contraction-relaxation cycling of muscle, calcium ions are released from the SR via calcium release channels to elicit contraction of muscle. Relaxation occurs when calcium is pumped back into the SR by a calcium ATPase pump.

Various methods have been employed to isolate SR from a number of species in order to investigate the function of this organelle (Meissner 1975; Saito et al. 1984; Volpe et al. 1988). Calcium uptake rates for SR have been reported for several species including horses (Byrd et al. 1989b), rats (Byrd et al. 1989a), and rabbits (Heilmann and Pette 1979). Furthermore Byrd et al (1989b) reported a decrease in calcium uptake and ATPase activity in horses with a bout of short-term high intensity exercise. Release of calcium from SR has been studied in frogs (Volpe et al. 1988), rats (Favero et al. 1993) rabbits (Palade 1987) and swine and horses (Fletcher et al. 1990).

Byrd and coworkers (1989b) used a percutaneous needle biopsy to harvest muscle samples and a calcium mini-electrode to measure calcium uptake in isolated SR vesicles to investigate the effects of high intensity exercise on SR

function. We sought to develop methods for isolation of SR vesicles and for spectrophotometric analysis of calcium transport in isolated SR, because we are interested in determining the effects of repeated sprints, conditioning and added dietary fat on SR function. This paper will describe the development of methods and observations on repeated sprints on SR function.

Material and Methods

Subjects. Two Sprague-Dawley rats (approximately 200 g) and one New Zealand white rabbit (3 kg) were initially used to develop this method. Ten Quarter Horses (2-4 yr old; 7 mares and 3 geldings) were used.

Collection of muscle samples. The rats were killed by decapitation and the rabbit by overdose of pentobarbital in an ear vein. Muscles of the right hind limb were exposed, dissected free from surrounding muscles, and excised. For horses, biopsy samples were obtained from the middle gluteal muscle by standard surgical procedures. Muscle samples were collected using either a large bore needle (6 mm) designed for extraction of large samples (relative to the Bergstrom needle) as described by Byrd et al. (1989b), or by an excisional biopsy technique. About 4 g of muscle was accumulated using the 6 mm needle by taking several small samples of about .1-.2 g each. For the excisional biopsy, a site on the skin was

locally anesthetized with 20 ml lidocaine, and semi-circular incisions were made in a caudal-ventral direction through the skin and fascia, approximately 3 cm in length and 2.5 cm in width. About 4 g of muscle was obtained by grasping small strips with Rochester-Carmalt forceps and excising with curved Metzenbaum scissors. A horizontal mattress suture closed the skin.

Isolation of SR vesicles. The original method employed to isolate SR vesicles in rats has been used in horses and swine (Fletcher et al. 1990). We were unable to obtain viable vesicles with this method in rats. Heilmann and Pette (1979) have described a method for isolating SR vesicles that subsequently has been applied successfully in rats and horses (Byrd et al. 1989a; 1989b). We attempted to use this method but were unsuccessful in producing uptake rates and ATPase activities that were similar to published values. Using the buffer system described by Heilmann and Pette (1979) and the centrifugation process described by Volpe et al. (1988) for frog tissue, we were able to obtain viable vesicles and repeatable calcium uptake rates and ATPase activities in rat, rabbit and horse tissue.

Muscle biopsy samples were immediately washed in ice-cold homogenization buffer. Samples were homogenized in 1 g increments, as soon as the muscle was obtained. The tissue was minced with scissors before homogenization. A Tempest Virtishear was used to grind the samples, and for

each 1 g of tissue, 3 ml of homogenization buffer was used. Each sample was subjected to three 5 sec bursts at a setting of 40 on the homogenizer. Samples were kept on ice throughout the process. After each homogenization of a gram of tissue, the shaft was rinsed with 3 ml of buffer. This process was continued until 4 g of tissue was homogenized. The homogenates were transferred to a centrifuge tube, and the final volume of buffer was brought up to 40 ml.

The homogenate was centrifuged in a Sorvall SS-34 rotor for 20 min at 10,000 rpm. The pellet was rehomogenized in 40 ml of homogenization buffer, and centrifuged as before at 10,000 rpm for 20 min. The supernatant was saved on ice. The pellet from the second centrifugation was discarded, and the supernatant was pooled with the first. This supernatant was centrifuged in a Beckman 70 Ti rotor for 90 min at 40,000 rpm. The final pellet was gently removed from the centrifuge tube and homogenized in 300 µl homogenization buffer using a hand-held glass homogenizer. Protein concentrations of the final SR fraction were determined using the Bio-Rad procedure (Bio-Rad, Richmond, CA). The total yield of SR protein for each sample was approximately 6000 µg.

Ca^{2+}-Mg^{2+}-ATPase activity. Ca^{2+}-independent (basal or Mg^{2+}-stimulated) and total (Ca^{2+}-dependent + Ca^{2+}-independent) activities of the Ca^{2+}-Mg^{2+}-ATPase were determined in an enzyme-linked optical assay at 37°C according to the methods of Weidekamm and Brdiczka (1975). Ca^{2+}-dependent activity was calculated as

total minus basal activity. The amount of SR used in this assay was approximately 50 µg protein. Three replicates of each sample were performed for each analysis.

Calcium uptake. Calcium uptake was determined spectrophotometrically using the calcium probe antipyrylazo III in a system similar to the one described for measurement of calcium release (Palade 1987). The following conditions and procedures were based on a method used for rabbit SR with modifications to quantitate an average uptake by addition of a bolus of calcium rather than preloading the vesicles with .5 µl increments to stimulate release. A 3 x 6-mm magnetic stir bar and 100 mM KCl, 20 mM KMOPS and 250 µM antipyrylazo III, pH 7.0, were added to a 3 ml cuvette. From separate stock solutions were added creatine phosphokinase at 2 mg/ml, 100 mM MgATP, and 250 mM phosphocreatine. Ten µl of SR protein was added to the cuvette (200-280 µg SR protein/10µl). Final volume in the cuvette was 1.0 ml.

The cuvette was placed in a Varian DMS 100 spectrophotometer and allowed to equilibrate at 35°C for 90 sec. The mixture was stirred continuously using a Hellma Cuv-O-Stir, Model 333. After the equilibration period, 10 µl $CaCl_2$ was added from a 10 mM stock solution. Calcium concentration changes were monitored by measuring the absorbance at 710 nm until completion of calcium uptake into SR vesicles. This was determined by the return of absorbance to baseline. Three replicates of each sample were performed for each analysis.

Standard Exercise Test. A standard exercise test of repeated sprint-type work was performed by five of the Quarter Horses (Table 1) to assess fatigue of the SR.

Statistical Analysis. Data are summarized as means and standard errors. Paired-t tests were performed on data before and after exercise.

Results

Sampling Method. The excisional biopsy technique was found to be superior to the bore needle biopsy to obtain 4 g samples. Single excision samples can be obtained in a much shorter period of time than multiple needle samples (5 min vs 30 min), which is critical immediately following an exercise bout (if one is interested in fatigue and recovery of the SR). The samples also were much more uniform when considering the fiber type procured because primarily superficial (fast twitch) muscle is taken, whereas using the bore needle both fast and slow twitch fibers are acquired. Furthermore, using the excisional biopsy was much less stressful and traumatic to the animals than repeated needle biopsies. The incisions were treated daily and healed within 2 weeks.

Ca^{2+}-Mg^{2+}-ATPase activity. The Ca^{2+}-ATPase, Mg^{2+}-ATPase and total

activities of the rodents and horses are given in Table 2.

Calcium uptake. A typical calcium uptake curve is shown in Figure 1. In the 10 horses, uptakes rates ranged from .10-.29 μmol Ca/mg protein/min (Table 2).

Standard Exercise Test. Comparisons of calcium ATPase activity and calcium uptake before and after exercise are shown in Figures 2 and 3. Calcium uptake and ATPase activity were depressed to 50% and 65% of resting values following high intensity repeated sprints ($p=.04$, $p=.01$, respectively).

Discussion

This study revealed an advantage of sampling muscle by use of a single surgical biopsy rather than by repeated needle biopsies in order to accumulate a similar amount of tissue. Surgical biopsies are less traumatic and uniform fiber types can be obtained. Furthermore, determining calcium uptake into SR vesicles spectrophotometrically was an efficient, quick and repeatable system for equine muscle.

The methods developed by this investigation can be useful in assessing such aspects of muscular function as conditioning/exercise and nutritional manipulation on SR behavior. Additionally, this procedure may be useful in

researching such disorders as malignant hyperthermia and extertional rhabdomyolysis, both of which have been previously linked to the sarcoplasmic reticulum (Fletcher et al. 1990; Hodgson 1993).

The values reported in this paper for rats and rabbits tended to be lower than previously published data (Byrd et al. 1989a; Heilmann and Pette 1979). This could be due to the large amount of protein used in our assay and our presentation of average rates rather than initial and total rates of calcium uptake. The values for our horse data were lower than those for the rodents, which has been reported previously (Byrd et al. 1989b).

With repeated sprints to exhaustion, the decreases from resting values for calcium uptake and ATPase activity in our Quarter Horses were 50% and 65%, respectively. After a run to exhaustion, calcium uptake and calcium ATPase activity of Thoroughbred horses decreased to approximately 50% of resting values (Byrd et al. 1989b). The resting rate of calcium uptake in the Thoroughbred horses was .55 µmol/mg/min, which is approximately double the uptake rates found in our study. The total ATPase activity was .73 µmol/mg/min for their Thoroughbred horses, which is 2 to 4 times the activity in our unconditioned horses. The differences could be due to such factors as variations in isolation techniques, method of measuring calcium uptake, breed, muscle fiber type and amount of SR protein used in the assay. Additionally the fitness of the animals may contribute to differences in absolute values.

Acknowledgment We thank Christine Palade for her helpful advice in applying these methods to horses.

Literature Cited

Byrd, S.K., A.K. Bode and G.A. Klug. 1989a. Effects of exercise of varying duration on sarcoplasmic reticulum function. J. Appl. Physiol. 66:1383.

Byrd, S.K., L.J. McCutcheon, D.R. Hodgson, and P.D. Gollnick. 1989b. Altered sarcoplasmic reticulum function after high-intensity exercise. J. Appl. Physiol. 67:2072.

Favero, T.G., I.N. Pessah and G.A. Klug. 1993. Prolonged exercise reduces Ca^{2+} release in rat skeletal muscle sarcoplasmic reticulum. Pflügers Arch. 422: 472.

Fletcher, J.E., L. Tripolitis, K. Erwin, S. Hanson, H. Rosenburg, P.A. Conti and J. Beech. 1990. Fatty acids modulate calcium-induced calcium release from skeletal muscle heavy sarcoplasmic reticulum fractions: implications for malignant hyperthermia. Biochem. Cell Biol. 68:1195.

Heilmann, C. and D. Pette. 1979. Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation. J. Biochem. 93:437.

Hidalgo, C. 1987. Lipid-protein interactions and the function of the Ca^{2+}-ATPase of sarcoplasmic reticulum. Crit. Revs. Biochem. 21:319.

Hodgson, D.R. 1993. Exercise-associated myopathy: is calcium the culprit? Equine Vet. J. 25:1.

Meissner, G. 1975. Isolation and characterization of two types of sarcoplasmic reticulum vesicles. Biochim. Biophys. Acta 389:51.

Palade, P. 1987. Drug-induced Ca^{2+} release from isolated sarcoplasmic reticulum. J. Biol. Chem. 262:6135.

Saito, A., S. Seiler, A. Chu, and S. Fleischer. 1984. Preparation and morphology of sarcoplasmic reticulum terminal cisternae from rabbit skeletal muscle. J. Cell Biol. 96:875.

Volpe, P., M. Bravin, F. Zorzato, and A. Margreth. 1988. Isolation of terminal cisternae of frog skeletal muscle. J. Biol. Chem. 263:9901.

Weidekamm, E. and D. Brdiczka. 1975. Extraction and localization of a (Ca^{2+} and Mg^{2+})-stimulated ATPase in human erythrocyte spectrin. Biochim. Biophys. Acta. 401:51.

Table 1. Exercise protocol of SET

TIME(min)	SPEED (m/s)	SLOPE (%)
0-2:30	1.2	0
2:30-5	1.2	6
5-9	3.2	6
9-11	7	6
11-13	3.2	6
13-15	8	6
15-17	3.2	6
17-19	9	6
19-21	3.2	6
21-23	10	6

TABLE 2. Ca^{2+}-Mg^{2+}-ATPase activity and Ca^{2+} uptake by SR (mean ± SE for equine SR)

	rat (n=2)	rabbit (n=1)	horse (n=10)
ATPase activity			
µmol/mg protein/min			
Ca^{2+}	1.32	2.27	.16 ±.02
Mg^{2+}	.193	0.21	.13 ±.02
total	1.52	2.48	.30 ±.04
Ca^{2+} uptake	1.61	2.27	.19 ±.02
µmol/mg protein/min			

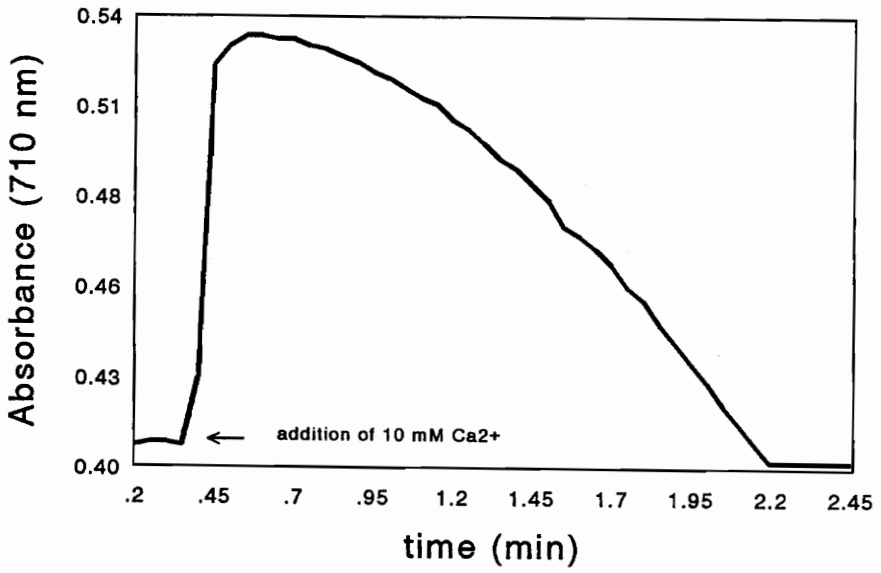


Figure 1. A sample tracing of Ca^{2+} uptake. Uptake was initiated by the addition of .1 **µ**M Ca^{2+} and allowed to continue until absorbance returned to baseline, indicating loading of SR vesicles. The rate of uptake was calculated from the amount of time to load the SR vesicles with Ca^{2+}, reported as **µ**mol Ca^{2+}/mg SR protein/min.

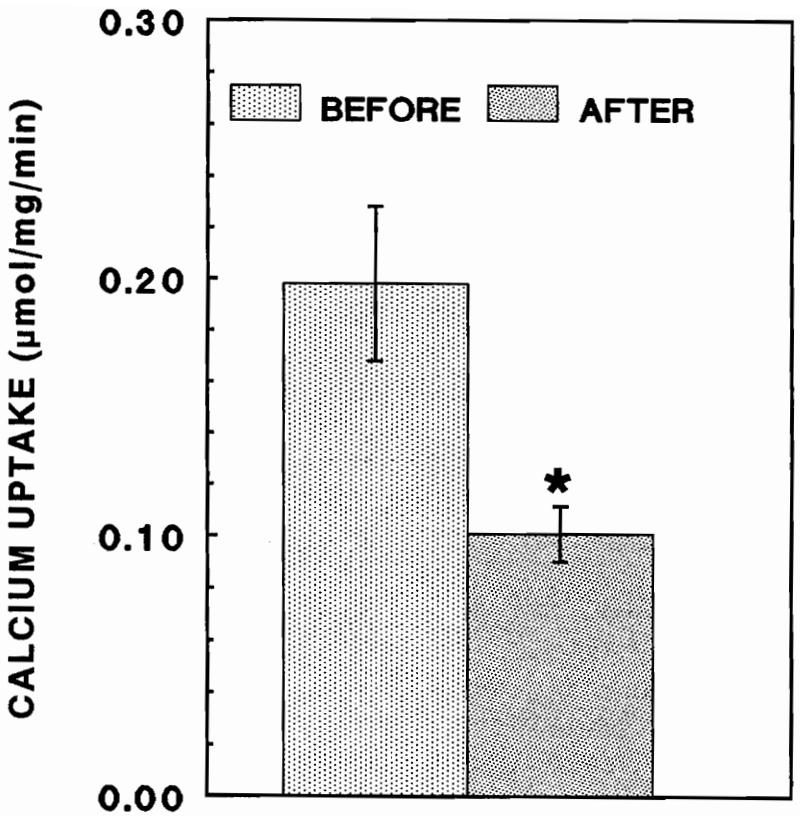


Figure 2. Calcium uptake in isolated sarcoplasmic reticulum (SR) at rest and after repeated sprints to exhaustion (*p=.04, n=5).

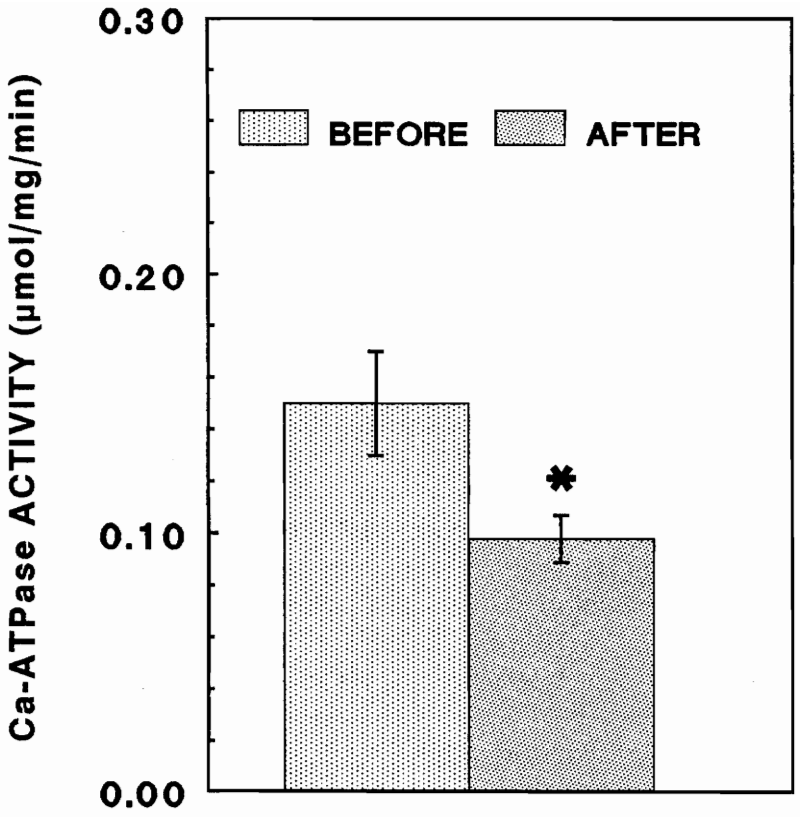


Figure 3. Calcium-stimulated ATPase activity of isolated sarcoplasmic reticulum at rest and after repeated sprints to exhaustion (*p=.01, n=5).

Journal Paper 2. Sarcoplasmic Reticulum Responses to High Intensity Repeated Sprints and Conditioning in Horses.

Abstract

Sarcoplasmic reticulum (SR) releases and sequesters calcium during muscle contraction and relaxation. Calcium uptakes have been shown to respond to high intensity exercise, and we propose this response may be affected by conditioning. Ten Quarter horses performed repeated sprints on a high speed treadmill. Muscle samples were taken before and after exercise from the middle gluteal muscle, and SR vesicles were isolated. Horses then were conditioned for 12 weeks and the experiment repeated. Calcium uptake was determined spectrophotometrically using antipyrylazo III, and Ca^{2+}-ATPase activity was determined using an enzyme-linked optical assay. Data were analyzed using a paired t-test. Resting calcium uptake rates and Ca^{2+}-ATPase activities were greater when the horses were conditioned than when unconditioned ($p=.054$, $p=.0037$, respectively). After exercise calcium uptake was slower in horses unconditioned ($p=.020$) or conditioned ($p=.0018$). This fatigue response to exercise was reduced by

conditioning (p=.013). After exercise Ca^{2+}-ATPase activity was decreased (p=.0074) to 71% of resting activity in unconditioned horses, and by 82% in conditioned horses (p=.0012). Muscle temperature increased 4.9° C with exercise when the horses were unconditioned (P<.00001) and 4.0° C when conditioned P<.00001). These results suggest that high intensity exercise fatigues SR function in equine gluteal muscles, and that exercise conditioning reduces the depression of SR function with exercise.

Introduction

Skeletal muscle SR regulates intracellular free Ca^{2+} in myoplasm. Contraction is initiated by release of Ca^{2+} from the SR. Relaxation occurs when myoplasmic Ca^{2+} is returned to the SR by active transport via a membrane-bound pump protein driven by the hydrolysis of ATP. Alterations in SR structure and function may be involved in the development of muscular fatigue and damage, such as exertional rhabdomyolysis (Hodgson, 1993). Several studies reported SR function to be affected by prolonged acute exercise in rats (Byrd et al., 1989a; Belcastro et al., 1993; Favero et al., 1992; Fitts et al., 1982) and by intense exercise in horses (Byrd et al., 1989b).

There is a paucity of information concerning high-intensity, short-term exercise on SR function. Byrd and coworkers (1989b) demonstrated reduced

calcium uptake and Ca^{2+}-ATPase activity from equine SR after a run to exhaustion. The only other study of the effect of high-intensity exercise is for rats running on a treadmill in which changes in Ca^{2+}-ATPase were reported but not calcium uptake rates. Ca^{2+}-ATPase activity for SR from trained rats run to exhaustion was significantly increased as compared to trained rats at rest. This increase with exhaustive exercise is contrary to other reports of decreased Ca^{2+}-ATPase with an acute bout of exercise, but their results do indicate a training effect in SR function.

No other information is available concerning conditioning of skeletal muscle and SR function. The results of conditioning on cardiac muscle SR are ambiguous. Endurance exercise training produced no improvement in calcium handling by the SR in dogs (Tate et al., 1993) or miniature swine (Laughlin et al., 1991), but SR from rats conditioned by swimming showed a greater capacity to take up calcium compared to SR from sedentary rats (Penpargkul et al., 1976). Further investigation is necessary to determine the extent and type of conditioning that will produce improvement in calcium handling by the SR.

The present investigation was undertaken to determine the effects of high-intensity repeated sprints on the capacity of Ca^{2+} uptake and Ca^{2+}-ATPase activity of SR. In addition to the effects of high-intensity acute exercise on SR function, we propose that conditioning also may affect SR function. This study was undertaken to investigate the effects of short-term, high intensity sprint-type work and conditioning on SR function in Quarter Horses.

Materials and Methods

Experimental animals and exercise procedures. Ten Quarter Horses (7 mares and 3 geldings), aged 3-5 yr, average weight 464.6 kg, were used in this study. These animals were acclimated to run on a high speed treadmill before the experiments and were unconditioned at the beginning of the study. On the day of the experiment, the horses performed an Standard Exercise Test (SET) that consisted of 5 min of walking followed by 4 min of trotting as an initial warm-up. The slope of the treadmill was inclined to 6% at 2.5 min. After the warm-up period, horses ran a series of repeated sprints (Fig.1). Heart rates increased from 43 beats/min at rest to 219 beats/min at the end of the final sprint. After the first SET, horses were conditioned for 12 wk by running 5 d/wk on a high speed treadmill. Horses underwent 3 bouts of repeated sprints per week (Fig.1), initially starting out with 1 sprint for the first 2 wk. Weeks 3 and 4 incorporated the second sprint, a third sprint was added at wk 5, and at wk 7 horses were completing 4 sprints. The alternate 2 d consisted of a bout of trotting at 3.5 m/s, 6% slope after 2.5 min at 1.2 m/s, 0% slope followed by 2.5 min at 1.2 m/s, 6% slope. After the 12 wk conditioning period, horses underwent a second SET identical to the first SET. Heart rates increased from 40 beats/min to 222 beats/min.

Diet. The animals were fed a corn mix concentrate consisting of cracked

corn, soybean meal (SBM), cane molasses and limestone twice daily and orchardgrass hay free choice to approximate 1989 NRC recommendations for moderate/high intensity work and to maintain weight throughout the study. Horses had free access to water and a mineral/vitamin supplement (Southern States SSC-317805) and were housed in a 7080 m^2 dry lot.

Collection of muscle samples. The animals were prepared for the collection of biopsy samples from the middle gluteal muscle by using standard surgical procedures. Two sites on the skin were anesthetized with lidocaine and small semi-circular incisions were made in a caudal-ventral direction through the skin and fascia, approximately 3 cm in length and 2.5 cm in width. Samples were obtained from the contralateral gluteal muscle at the time of the second SET.

Muscle samples were collected before exercise and immediately following the final sprint. About 5 g of muscle were obtained by grasping small strips with Rochester-Carmalt forceps and excising with curved Metzenbaum scissors. A horizontal mattress suture closed the skin. A portion of each muscle sample (1 g) was immediately quenched in liquid N_2 and stored at -80° C for electrolyte and metabolite analysis. The remaining portion of the sample was placed in ice cold HEPES-sucrose homogenization buffer (pH 7.4).

SR preparation methods. Muscle samples were immediately washed in ice-

cold buffer. Samples were homogenized in 1 g increments, as soon as the muscle was obtained. The tissue was minced with scissors before homogenization and treated as described by Wilson et al.(1995).

Calcium uptake. Calcium uptake was determined spectrophotometrically using the calcium probe antipyrylazo III described earlier (Wilson et al.,1994). Briefly, a 3 x 6-mm magnetic stir bar and 100mM KCL, 20 mM KMOPS and 250 µm antipyrylazo III, pH 7.0, were added to a 3 ml cuvette. From separate stock solutions were added creatine phosphokinase at 2 mg/ml, 100 mM MgATP, and 250 mM phosphocreatine. Ten µl of SR protein was added to the cuvette (200-280 µg SR protein/10 µl). Final volume in the cuvette was 1.0 ml.

The cuvette was placed in a Varian DMS 100 spectrophotometer and allowed to equilibrate at 35°C for 90 sec. The mixture was stirred continuously and after the equilibration period, 10 µl $CaCl_2$ was added from a 10 mM stock solution. Calcium concentration changes were monitored by measuring the absorbance at 710 nm until completion of calcium uptake into SR vesicles.

Ca^{2+}-Mg^{2+}-ATPase activity. Ca^{2+}-independent (basal or Mg^{2+}-stimulated) and total (Ca^{2+}-dependent + Ca^{2+}-independent) activities of the Ca^{2+}-Mg^{2+}- ATPase were determined in an enzyme-linked optical assay at 37° C according to the methods of Weidekamm and Brdiczka (1975). Ca^{2+}-dependent activity was

calculated as total minus basal activity. The amount of SR used in this assay was approximately 50 µg protein.

Muscle temperature (Tm). A skin site between the 2 biopsy incisions on the middle gluteal muscle was prepared and anesthetized according to the biopsy procedure described above. Tm was measured at the time of each biopsy by inserting a Copper/constantan thermocouple into the muscle.

Muscle ions. Muscle inorganic ions (Na^+, K^+, Cl^-, Mg^{2+}, and Ca^{2+}) were measured by instrumental neutron activation analysis (Lindinger and Heigenhauser, 1987). This technique measures both bound and free muscle ions; however, only the total ion concentrations are reported in this study (no distinction is made between bound and free ions). Because plasma was not obtained and extracellular fluid volume was not measured in this study, average ion content for the intracellular fluid compartment was not determined and total muscle ion content is reported as µmol/g dry mass.

Muscle glycogen. Muscle glycogen concentration was determined by the method of Lo et al. (1970). This method has been used previously to determine glycogen concentration of equine gluteal muscles (Miller and Lawrence, 1986).

Statistics. For statistical analysis all measurements were compared before and after exercise as well as before and after conditioning using a paired t-test.

Results

Ca^{2+} uptake. The rate of calcium uptake into isolated SR vesicles decreased with exercise in unconditioned (p=.020) and conditioned (p=.0018) horses when compared to resting values (Fig. 2). The depression in calcium uptake with an acute bout of exercise was greater when the horses were unconditioned (p=.013). Resting uptake rates (p=.054) and after exercise uptake rates (p=.043) were both higher in conditioned horses compared to when the animals were unconditioned.

Ca^{2+}-ATPase activity. The Ca^{2+}-stimulated activity was decreased with exercise in unconditioned (p=.0074) and conditioned (p=.0013) horses (Fig. 3). Resting (p=.0037) and after exercise (p=.0002) values were higher in conditioned horses.

Muscle temperature (Tm). Muscle temperature increased with exercise in unconditioned (p<.00001) and conditioned (p<.00001) horses (Fig. 4). The increase in Tm was higher when horses were unconditioned (p=.0051).

Muscle ions. There was a slight increase in K^+ with acute exercise when the horses were unconditioned (p=.080). No differences were found for other ions at SET 1 (Table 1). When the horses were fit, muscle ion concentrations did not change with exercise (Table 2). Resting K^+ was greater when the horses were conditioned (p=.036) and Ca^{2+} tended to be lower in conditioned horses (p=.075). The concentrations of Mg^{2+} (p=.054) and K^+ (p=.082) after exercise tended to be higher when the horses were fit.

Muscle glycogen. Muscle glycogen concentration decreased with exercise when the horses were unconditioned (p=.0009) and conditioned (p=.0013). There were no differences in resting glycogen concentration between fit and unfit horses nor were there differences in the degree of depletion of glycogen with exercise between conditioned and unconditioned animals (Figure 6).

Discussion

The major findings of this study were 1) high-intensity repeated sprinting exercise fatigues SR and 2) conditioning reduces the SR response to an acute bout of high intensity exercise (Fig. 5). This was paralleled by changes in Ca^{2+}-stimulated ATPase activity. Similar depressions in SR function occurred after a

run to exhaustion in Thoroughbred horses (Byrd et al., 1989b). In that study, calcium uptake and calcium ATPase activity decreased to approximately 50% of resting values after a bout of high intensity exercise. With repeated sprints to exhaustion, the decrease from resting values for calcium uptake and ATPase activity in our unfit Quarter Horses were 61% and 71%, respectively. However, resting uptake rates in the Thoroughbred horses were approximately double the uptake rates found in our study. This difference could be due to such factors as variations in isolation techniques, method of measuring calcium uptake, species and muscle fiber type.

This is the first report of the effects of conditioning on skeletal SR function in horses. As far as we know, these effects are unknown in other species. The data from this experiment do not identify the mechanisms that produced the changes in SR function observed with conditioning. There was an increased amount of SR protein/gram of muscle from fit animals compared to when they were unfit. This most likely contributed to the enhanced function of the SR. The increase in uptake and ATPase activity could be due to an increased number of ATPase protein pumps as well as an increased sensitivity of the enzyme to pump calcium into the SR because measurements of uptake rates and ATPase activity are reported on a per mg SR protein basis.

The Tm increased 4.9°C with a bout of exercise during the first SET and 4.0°C at the second SET. Isolated SR vesicles incubated at high temperatures

resulted in depressed function (Inesi et al., 1973). This could be due to changes in membrane fluidity associated with increased temperature, which may reduce calcium uptake by the SR. Changes in muscle temperature with exercise could have an adverse affect on SR function, but the muscle is washed in ice cold buffer and the assays for uptake and ATPase activity are carried out at 35° and 37°, respectively, and these facts diminish the potential for increased temperature as a primary cause for the exercise-induced changes in the SR seen with exercise.

Depression in muscle pH has been suggested to inhibit SR function (Byrd et al., 1989a; Fabiato and Fabiato, 1978; Godt and Nosek, 1989; Williams and Ward, 1992). Decreased pH reduces SR Ca^{2+}-ATPase activity and inhibits calcium uptake by the SR (Fabiato and Fabiato, 1978; Inesi and Hill, 1983). Furthermore decreases in calcium release by the SR arise from decreased pH (Nakamaru and Schwartz, 1972) and elevation in proton concentration can alter calcium conductance and conformation of the release channel (Rousseau and Pinkos, 1990). Levitsky and Benevolensky (1986) suggested H^+ and Ca^{2+} may compete for the Ca^{2+} binding sites on the ATPase pump. We did not measure pH in this study, but with the type of exercise performed there most likely was a depression in muscle pH with a concomitant increased muscle lactate concentration. Changes in pH may have contributed to altered SR function in vivo with repeated sprints, but as with temperature, the assay is run at physiologic pH.

Substrate depletion could affect the activity of the Ca^{2+}-ATPase pump.

Glycogen phosphorolysis has been reported to form a metabolic shuttle to support SR calcium uptake in skeletal muscle (Cuenda et al., 1993). Similar decreases in muscle glycogen were seen in unconditioned and conditioned horses, yet there was an attenuation in the exercise-induced depression of SR function with conditioning. This implies that the role of muscle glycogen in maintaining calcium uptake is small. ATP depletion occurred following intermittent maximal exercise in Thoroughbred horses (Harris and Snow, 1984), but not in Thoroughbred horses run to exhaustion at VO_{2max} (Byrd et al., 1989a). Although ATP concentration was not measured it is unlikely that changes in its concentration are responsible for SR dysfunction because ATP is supplied in excess in the in vitro assay. Phosphocreatine (Pcr) and creatine kinase (CK, CPK) have been implicated as an important ATP regeneration system for Ca^{2+}-ATPase (Korge et al., 1993). Both the substrate and enzyme were in sufficient quantity in the assay nullifying this ATP regeneration system as a major cause of SR dysfunction.

Evidence indicates that alterations in SR function are associated with exercise-induced muscle damage. The depression in calcium uptake after high intensity exercise occurs in parallel with observed ultrastructural deformities in the SR (Byrd et al., 1989a; McCutcheon et al., 1992). Gross focal dilation of the SR after strenuous exercise is accompanied by depressed Ca^{2+} uptake and release as well as an increase in the intracellular free calcium concentration (Byrd, 1992). A single bout of prolonged exercise induced structural changes in the Ca^{2+}-ATPase

protein of rat skeletal muscle which was not a direct result of increased muscle temperature or gross lipid alterations (Luckin et al., 1991). It is probable that a combination of structural and metabolic changes with exercise affect SR function. These changes disrupt normal calcium handling by the SR which can lead to increased intracellular calcium concentration and eventually result in fiber degradation. Conditioning diminishes these exercised-induced alterations and sustains SR function more closely to pre-exercise levels.

Ionic changes in contracting muscles have been implicated in muscle fatigue (Lindinger and Heigenhauser, 1988; 1990). With exercise, plasma K^+ increases as K^+ is lost from the muscle (Medbo and Sejersted, 1994; Vollestad, 1994) as the muscle membrane is depolarized. In this study muscle K^+ increased after exercise in unfit horses. We were unable to differentiate between intra- and extracellular muscle ion content and this may have contributed to the increases. Furthermore, K^+ reuptake by muscle occurs the moment exercise ceases and the rate of reuptake is linearly related to power output (Vollestad, 1994) with sprinters clearing post-exercise plasma K^+ faster than endurance athletes (Medbo and Sejersted, 1994). It took approximately 5 minutes to obtain the muscle sample for electrolytes, and rapid reuptake of K^+ could be occurring. Medbo and Sejersted (1994) and Busse and Maasen (1989) reported a decline in plasma K^+ below pre-exercise concentrations during recovery; the greater the peak plasma K^+, the greater the subsequent recovery undershot (Medbo and Sejersted, 1994).

Decreasing plasma K^+ indicates reuptake into muscle as well as distribution to other tissues. By 5 minutes post-exercise, the rate of Na^+/K^+-ATPase could have been sufficient to cause an undershot in plasma K^+ and a subsequent "overshot" in muscle K^+ .

With training, the changes in muscle K^+ with an acute bout of exercise were abolished in this study. Training from 1-7 weeks decreases the rise in plasma K^+ associated with exercise (Green et al., 1993; McKenna et al., 1993; Madsen et al., 1994; Medbo and Sejersted, 1994). This is due, at least in part, to an increased concentration of Na^+/K^+-ATPase pumps (Green et al., 1993; McKenna et al., 1993; Madsen et al., 1994) and increased Na^+/K^+-ATPase activity (Green et al., 1993). Up regulation of Na^+/K^+-ATPase pumps most likely occurred with 12 weeks of training and contributed to the post-exercise muscle K^+ concentration. Little information is available concerning training and changes in resting muscle ions. The changes in resting K^+ and Ca^{2+} seen with conditioning may have been the result of enhanced Na^+/K^+ -ATPase and Ca^{2+}-ATPase function.

These findings indicate that a single bout of repeated sprinting exercise alters Ca^{2+}-stimulated ATPase activity and Ca^{2+} uptake and that conditioning attenuates the depression in SR function associated with acute exercise. An elevated cytosolic free Ca^{2+} can alter muscle protein synthesis and degradation (McGrath and Goldspink, 1980) and may affect glycolytic enzymes (Schudt and Pette, 1978). Therefore, depression of calcium regulation by the SR could affect

many Ca^{2+}-sensitive processes in the muscle cell and could contribute to metabolic disturbances associated with exercise. Accordingly the SR is a potential site for intervention in fatigue and tying up, a major muscle disorder of horses.

Literature Cited

Belcastro, A.N., J.S. Gilcrest, and J. Scrubb. 1993. Function of skeletal muscle sarcoplasmic reticulum vesicles with exercise. J. Appl. Physiol. 75: 2412.

Busse, M.W. and N. Maassen. 1989. Effect of consecutive exercise bouts on plasma potassium concentration during exercise and recovery. Med. Sci. Sports Exerc. 21:489.

Byrd, S.K. 1992. Alterations in the sarcoplasmic reticulum: a possible link to exercise-induced muscle damage. Med. Sci. Sports Exerc. 24:531.

Byrd, S.K., A.K. Bode and G.A. Klug. 1989a. Effects of varying duration on sarcoplasmic reticulum function. J. Appl. Physiol. 66:1383.

Byrd, S.K., McCutcheon, L.J., Hodgson, D.R. and P.D. Gollnick. 1989b. Altered sarcoplasmic reticulum function after high-intensity exercise. J. Appl. Physiol. 67: 2072.

Cuenda, A., M. Nogues, C. Gutierrez-Merino and L. de Meis. 1993. Glycogen phosphorolysis can form a metabolic shuttle to support Ca^{2+} uptake by sarcoplasmic reticulum membranes in skeletal muscle. Biochem. Biophys. Res. Comm. 196:1127.

Fabiato, A., and F. Fabiato. 1978. Effect of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. J. Physiol. Lond. 276: 233.

Favero, T.G., I.N. Pessah, and G.A. Klug. 1993. Prolonged exercise reduces Ca^{2+} release in rat skeletal muscle sarcoplasmic reticulum. Pflugers Arch. 422: 472.

Fitts, R.H., B. Courtright, D.H. Kim, and F.A. Witzmann. 1982. Muscle fatigue with prolonged exercise:contractile and biochemical alterations. Am. J. Physiol. 242: C65.

Godt, R.E. and T.M. Nosek. 1989. Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. J. Physiol. 412;155.

Green, H.J., E.R. Chin, M. Ball-Burnett and D. Ranney. 1993. Increases in human skeletal muscle Na^+ -K^+-ATPase concentration with short-term training. Am. J. Physiol. 33:C1538.

Harris, R.C. and D.H. Snow. 1984. ATP depletion following intermittent maximal exercise in the horse. J. Physiol. 353:58P.

Hodgson, D.R. 1993. Exercise-associated myopathy: is calcium the culprit? Equine Vet. J. 25:1.

Inesi, G. and T.L. Hill. 1983. Calcium and proton dependence of sarcoplasmic ATPase. Biophys. J. 44:271.

Inesi, G., M. Millman, and S. Eletr. 1973. Temperature-induced transitions of function and structure in sarcoplasmic reticulum membranes. J. Mol. Biol. 81: 483.

Korge, P., S.K. Byrd and K.B. Campbell. 1993. Functional coupling between sarcoplasmic-reticulum-bound creatine kinase and Ca^{2+}-ATPase. Eur. J. Biochem. 213:973.

Laughlin, M.H., C.C. Hale, L. Novela, D. Gute, N. Hamilton and C.D. Ianuzzo. 1991. Biochemical characterization of exercise-trained porcine myocardium. J. Appl. Physiol. 71:229.

Lindinger, M.I. and G.J.F. Heigenhauser. 1987. Intracellular ion content of skeletal muscle measured by instrumental neutron activation analysis. J. Appl. Physiol. 63:426.

Lindinger, M.I. and G.J.F. Heigenhauser. 1988. Ion fluxes during tetanic stimulation in isolated perfused rat hindlimb. Am. J. Physiol. 254:R117.

Lindinger, M.I. and G.J.F. Heigenhauser. 1990. The roles of ion fluxes in skeletal muscle fatigue. Can. J. Physiol. Pharmacol. 69:246.

Levitsky, D.O. and D.S. Benevolensky. 1986. Effects of changing Ca^{2+}-to H^{+} ratio on Ca^{2+} uptake by cardiac sarcoplasmic reticulum. Am. J. Physiol. 250:H360.

Lo, S., J.G. Russell and A.W. Taylor. 1970. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28:234.

Luckin, K.A., T.G. Favero and G.A. Klug. 1991. Prolonged exercise induces structural changes in SR Ca^{2+}-ATPase of rat muscle. Biochem. Med. Met. Biol. 46:391.

Madsen, K., J. Franch and T. Clausen. 1994. Effects of intensified endurance training on the concentration of Na,K-ATPase and Ca-ATPase in human skeletal muscle. Acta Physiol. Scand. 150:251.

McCutcheon, L.J., S.K. Byrd and D. R. Hodgson. 1992. Ultrastructural changes in skeletal muscle after fatiguing exercise. J. Appl. Physiol. 72:1111.

McGrath, J.A. and D.F. Goldspink. 1980. The effects of glucocorticoids on protein turnover and growth of different striated muscles in the rat. Biochem. Soc. Trans. 8: 448.

McKenna, M.J., T.A. Schmidt, M. Hargreaves, L. Cameron, S.L. Skinner and K. Kjeldsen. 1993. Sprint training increases human skeletal muscle Na^+-K^+-ATPase concentration and improves K^+ regulation. J. Appl. Physiol. 75:173.

Medbo, J.I. and O.M. Sejersted. 1994. Plasma changes during intense exercise in endurance-trained and sprint-trained subjects. Acta Physiol. Scand. 151:363.

Miller, P.A. and L.M. Lawrence. 1986. Changes in equine metabolic characteristics due to exercise fatigue. Am. J. Vet. Res. 47:2184.

Nakamaru, Y., and A. Schwartz. 1972. The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J. Gen. Physiol. 59: 22.

NRC. Nutrient requirements of horses. (5^{th} Ed.) National Academy of Sciences-National Research Council, Washington, DC. 1989.

Penpargkul, S., D.I. Repke, A.M. Katz and J. Scheuer. 1976. Effect of physical training on calcium transport by rat cardiac sarcoplasmic reticulum. Circ. Res. 40:134.

Rousseau, E. and J. Pinkous. 1990. pH modulates conducting and gating behavior of single calcium release channels. Eur. J. Physiol. 415:645.

Schudt, C., and D. Pette. 1978. Ca^{2+} ions as coupling agents in enzymatic differentiation and carbohydrate metabolism of cultured muscle cells. Adv. Enzyme Regul. 16: 121.

Tate, C., M. Hamra, G. Shin, G. Taffet, P. McBride and M. Entman. 1993. Canine cardiac sarcoplasmic reticulum is not altered with endurance exercise training. Med. Sci. Sports Exerc. 25:1246.

Vollestad, N.K., J. Hallen and O. M. Sejersted. 1994. Effect of exercise intensity on potassium balance in muscle and blood of man. J. Physiol. 475:359.

Weidekamm, E. and D. Brdiczka. 1975. Extraction and localization of a (Ca^{2+} and Mg^{2+})-stimulated ATPase in human erythrocyte spectrin. Biochim. Biophsy. Acta. 401: 51.

Williams, J.H. and C.W. Ward. 1992. Reduced Ca^{2+}-induced Ca^{2+}-release from skeletal muscle sarcoplasmic reticulum at low pH. Can. J. Physiol. Pharmacol. 70:926.

Wilson, J., D. Kronfeld, L. Gay, and T. Wilson. Isolating equine sarcoplasmic reticulum and evaluating its function with observations on high intensity repeated sprints. *Equine Vet. J.* In Press, 1995.

TABLE 1. Muscle ions before and after intense exercise at SET 1.

ION	BEFORE	AFTER
Ca	11.13 ± 2.14	8.61 ± 1.70
Cl	86.51 ± 12.59	93.65 ± 6.15
K	187.79 ± 12.88	221.93 ± 15.42*
Mg	21.04 ± 2.27	24.27 ± 1.79
Na	129.95 ± 20.42	144.56 ± 11.76

Values are means ± SE (μmol/g dry mass).

*Indicates before mean different from after (p=.08)

TABLE 2. Muscle ions before and after intense exercise at SET 2.

ION	BEFORE	AFTER
Ca	8.12 ± .98	8.45 ± .63
Cl	85.24 ± 4.98	86.32 ± 4.16
K	226.6 ± 9.46	246.45 ± 22.38
Mg	25.74 ± 2.45	27.79 ± 2.8
Na	129.56 ± 8.83	119.87 ± 13.16

Values are means ± SE (μmol/g dry mass).

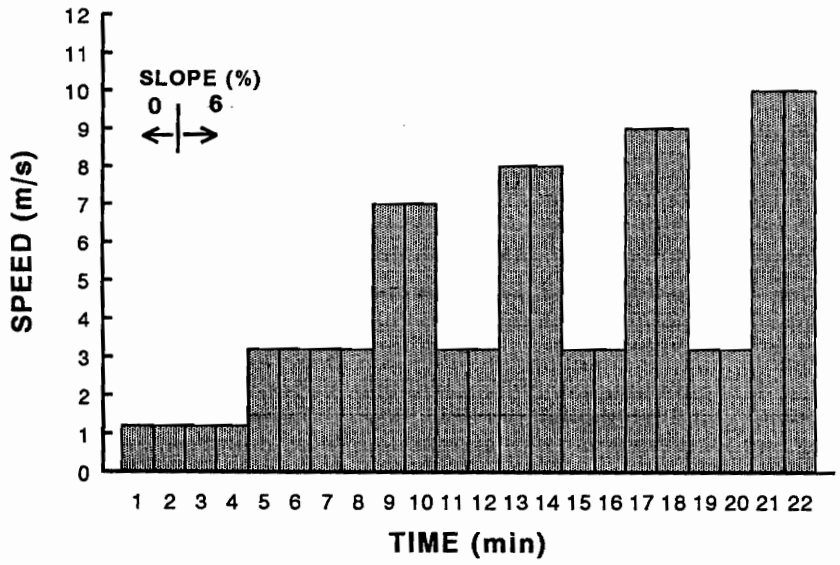


Figure 1. Standard exercise test (SET) used for unconditioned and conditioned horses.

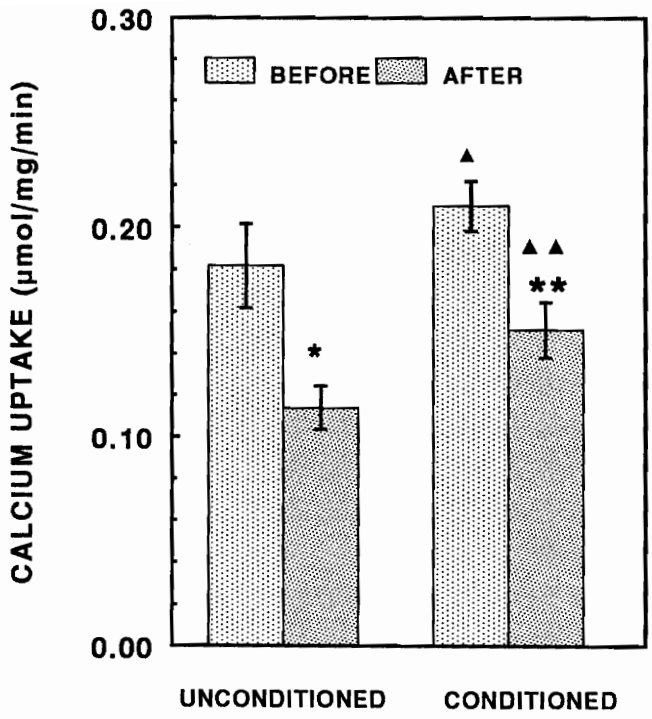


Figure 2. Calcium uptake in isolated sarcoplasmic reticulum (SR) at rest and after repeated sprints when the horses were unconditioned and conditioned (n=10). Differences were found with acute exercise in unconditioned (*p=.02) and conditioned (**p=.002) horses and with conditioning at rest (▲p=.05) and after exercise (▲▲p=.04). The depression in the rate of uptake associated with acute exercise was greater in unconditioned horses (p=.01).

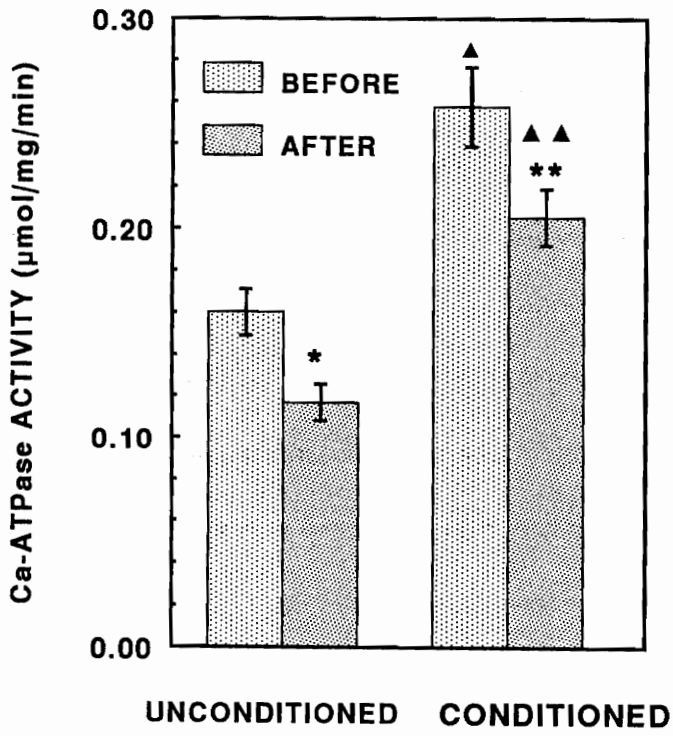


Figure 3. Calcium-stimulated ATPase activity of isolated sarcoplasmic reticulum (SR) at rest and after repeated sprints when the horses were unconditioned and conditioned (n=10). Differences were found with acute exercise in unconditioned (*p=.007) and conditioned (**p=.001) horses and with conditioning at rest (▲p=.004) and after exercise (▲▲p=.0002).

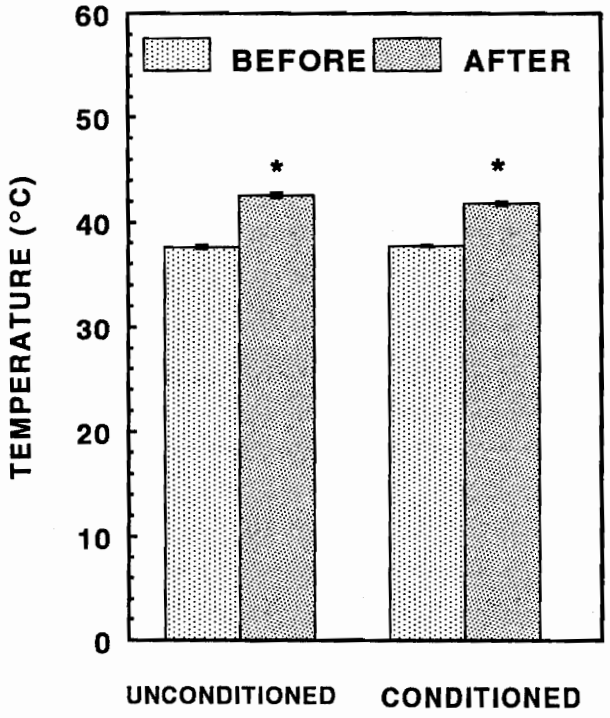


Figure 4. Temperature of middle gluteal muscle at rest and after repeated sprints in unconditioned and conditioned horses (n=10, *p=.00001). Temperature increased 4.9° C in unconditioned horses and 4.0° C in conditioned horses (p=.005).

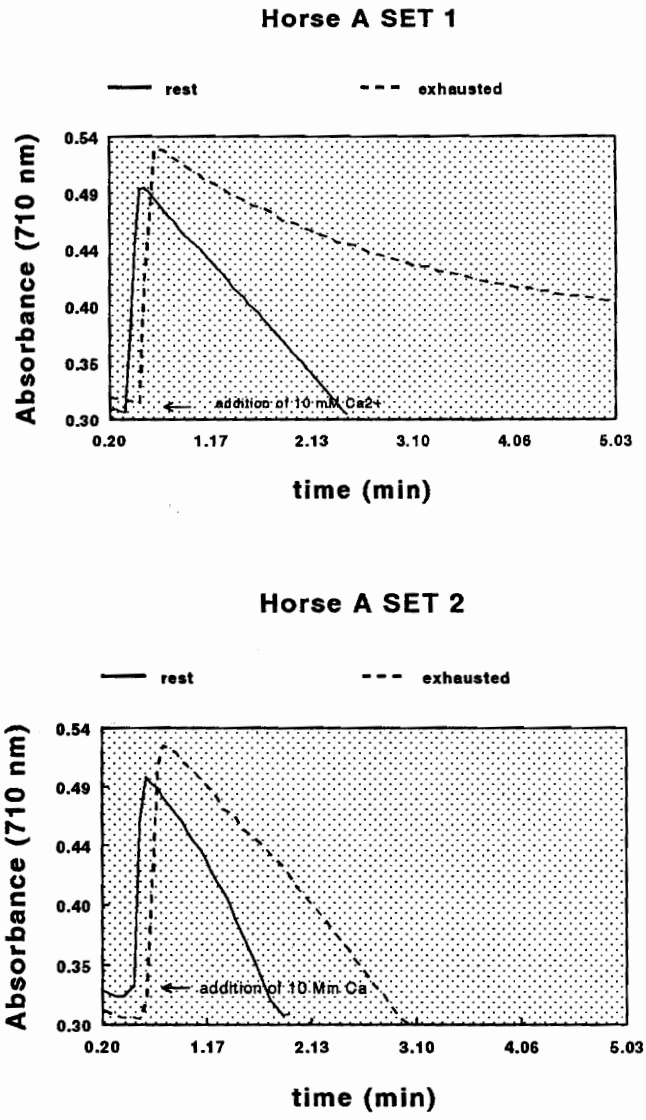


Figure 5. Comparison of rate of calcium uptake in a horse before and after exercise when the horse was unfit and after 12 weeks of conditioning.

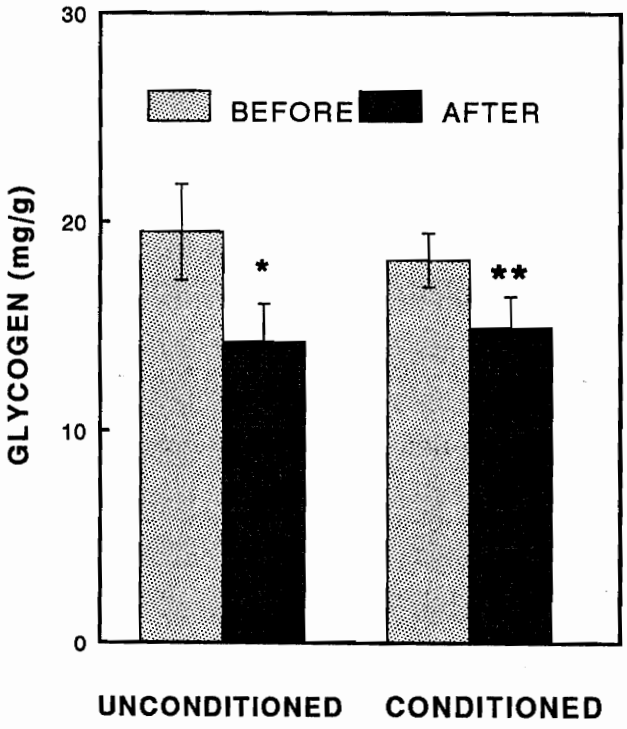


Figure 6. Muscle glycogen concentration of the middle gluteal muscle at rest and after repeated sprints in unconditioned and conditioned horses (n=10). Decreased muscle glycogen concentration was found with acute exercise in unconditioned (*p=.0009) and conditioned (**p=.001) horses.

Journal Paper 3. Sarcoplasmic Reticulum Responses to Dietary Lecithin in Conditioned Horses.

Abstract

Dietary fat intakes can alter the plasma membrane of the sarcoplasmic reticulum (SR) and therefore may affect its function. Previous research in our lab has shown high intensity repeated sprints decreases calcium uptake and Ca^{2+}-ATPase activity in equine SR, and that conditioning reduces this depression in SR function. This study was undertaken to determine the effects of feeding a lecithin modified diet on SR responses to repeated sprints in fit horses. Ten Quarter Horses were fed either a conventional diet or a diet with 10% added dietary fat in the form of corn oil/soy lecithin for a 12 week period. The diets were switched and the study was continued for another 12 weeks. Horses were exercised 4 days/week on a high speed treadmill throughout the study to maintain level of fitness. Standard exercise tests (SETs), consisting of 4 repeated sprints after a warm-up walk and trot, were performed at the end of both 12 week periods. Muscle samples were taken before and after exercise, and SR vesicles were isolated. Calcium uptake was determined spectrophotometrically using antipyrylazo III, and Ca^{2+}-ATPase

activity was determined using an enzyme-linked optical assay. Data were analyzed using a one-way ANOVA to determine differences between diets. Resting calcium uptake rates tended to be higher (p=.092) when the horses were on the fat modified diet, but decreased to a greater extent with an acute bout of exercise (p=.058). Ca^{2+}-ATPase activity was lower after exercise when the horses were fed added fat (p=.017) and the decrease from resting values was greater (p=.048). Resting muscle temperature was lower in horses when on the fat supplemented diet (p=.001). These results suggest feeding a lecithin modified diet to conditioned horses increases the depression in SR function with an acute bout of exercise and therefore may be contraindicated in horses that suffer from tying up.

Introduction

The primary function of fatty acyl unsaturation is to provide a fluid lipid bilayer phase which will allow various membrane proteins to function optimally. Membrane proteins are surrounded by the fatty acyl constituents of membrane phospholipids. Chain length and phospholipid order may also play a role in membrane protein function. For this reason it important to understand the consequences of feeding added dietary fat on membrane lipid composition and function and subsequent effect on enzymatic function.

There is strong evidence that Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) is affected more by chain length and is unsensitive to the degree of unsaturation (East et al., 1984; Stubbs and Kisielewski, 1990). Cornea and Thomas (1994) found that the enzyme activity of Ca^{2+}-ATPase was maximal with a chain length of 18 and decreased substantially with longer or shorter chains. Chain lengths shorter or longer than the optimal 18 resulted in a significantly decreased mobility of the Ca^{2+}-ATPase. An optimal lipid environment has been suggested for the Ca^{2+}-ATPase which is provided by 16:0-18:1 phosphatidylcholine and 16:0-18:2 phosphatidylcholine, the two most common lipids of the SR (Matthews et al., 1993).

Phospholipds are the main lipid component of the native SR membrane with phosphatidylcholine being the main phospholipid species (65-75%). There are 80 to 100 molecules of phospholipid associated with one molecule of Ca^{2+}-ATPase enzyme in the native SR membrane (Hidalgo, 1987). Infante (1987) suggested that phosphatidylcholine may be especially important for Ca^{2+}-ATPase functioning. Feeding lecithin (phosphatidylcholine) could potentially alter the lipid environment of the SR membrane resulting in changes to Ca^{2+}-ATPase function.

Previous research has demonstrated dietary manipulation of the SR lipid environment. Feeding fish oil to rats increased the level of n-3 fatty acids in skeletal muscle SR with little change in lipid order and no affect on Ca^{2+}-ATPase (Stubbs and Kisielewski, 1990). They propose the high degree of unsaturation in

the native SR membrane and the complex mixture of phospholipid species can act as a passive buffer to marked alterations in bulk membrane lipid motional properties. This passive adaptation may preserve physiological membrane protein function. Safflower oil supplementation in rats led to marked changes in the fatty acid composition of skeletal muscle SR, but did not affect SR function (Tume et al., 1973). Cardiac SR function responds to changes in membrane lipid environment, contrary to the findings in skeletal muscle. Menhaden oil supplementation increases the ratio of n-3/n-6 fatty acids and decreases calcium uptake and Ca^{2+}-ATPase relative to corn oil supplementation (Swanson et al.1988; Taffet et al., 1993). We propose there may differences in the effects of feeding fat to sedentary animals compared to exercising animals in regards to skeletal muscle SR function.

Materials and Methods

Experimental animals and exercise procedures. Ten Quarter Horses (7 mares and 3 geldings), aged 3-5 yr, average weight 492.5 kg, were used in this study. These animals had been conditioned on a high speed treadmill for 12 wk in a study of repeated sprinting and conditioning effects on skeletal muscle SR function. During that 12 wk period, horses underwent a series of 4 repeated sprints 3 d/wk and moderate trotting exercise 2 d/wk.

For the current experiment, horses were assigned to one of two diets; either a conventional diet or a diet supplemented with 10% added dietary fat. Horses remained on these diets for 12 wk and then the diets were switched. The study continued for another 12 wk. Horses were exercised 4 d/wk on a high speed treadmill throughout the study to maintain level of fitness. Two days a week horses underwent repeated sprints (Fig.1). The alternate 2 d consisted of a bout of trotting at 3.5 m/s, 6% slope after 2.5 min at 1.2 m/s, 0% slope followed by 2.5 min at 1.2 m/s, 6% slope. Standard exercise tests (SETs), consisting of 4 repeated sprints (Fig. 1), were performed at the end of both 12 wk periods.

Diets. Horses were assigned to 2 diet groups (Table 1) for the first 12 wk period and then the diets were switched for the second 12 wk period. The basal diet consisted of alfalfa, cracked corn, molasses, soybean meal (SBM), limestone and a mineral/vitamin supplement in a total mixed ration, except the mineral/vitamin supplement (2:1 Ca:P ratio) (Southern States SSC-317805) was offered free choice. The fat supplemented group was fed a similar diet with corn oil/soy lecithin substituted for part of the energy requirement, which resulted in a final ration of 10% oil by weight. The diets were isocaloric and horses were fed to meet estimated energy requirements for horses in moderate/high intensity work and nutrient requirements as suggested by the NRC (1989). The diets were split into 2 equal daily portions and horse were fed in stalls at 7:00 and 16:00 hr.

When on the basal diet, horses consumed 10 kg feed/d and 9.09 kg feed/d was consumed when horses were fed the fat supplemented diet. Horses were housed in a 7080 m^2 dry lot and had ad libitum access to water. Water also was provided in the stalls at the time of feeding.

Collection of muscle samples. The horses were prepared for the collection of biopsy samples from the middle gluteal muscle by using standard surgical procedures. Two sites on the skin were anesthetized with lidocaine and small semi-circular incisions were made in a caudal-ventral direction through the skin and fascia, approximately 3 cm in length and 2.5 cm in width. Samples were obtained from the contralateral gluteal muscle at the time of the second SET.

Muscle samples were collected before exercise and immediately following the final sprint. About 5 g of muscle were obtained by grasping small strips with Rochester-Carmalt forceps and excising with curved Metzenbaum scissors. A horizontal mattress suture closed the skin. A portion of each muscle sample (1 g) was immediately quenched in liquid N_2 and stored at -80° C for electrolyte and metabolite analysis. The remaining portion of the sample was placed in ice cold HEPES-sucrose homogenization buffer (pH 7.4).

SR preparation methods. Muscle samples were immediately washed in ice-cold buffer. Samples were homogenized in 1 g increments, as soon as the muscle

was obtained. The tissue was minced with scissors before homogenization and treated as described by Wilson et al. (1995).

Calcium uptake. Calcium uptake was determined spectrophotometrically using the calcium probe antipyrylazo III described earlier (Wilson et al.,1995). Briefly, a 3 x 6-mm magnetic stir bar and 100mM KCL, 20 mM KMOPS and 250 µm antipyrylazo III, pH 7.0, were added to a 3 ml cuvette. From separate stock solutions were added creatine phosphokinase at 2 mg/ml, 100 mM MgATP, and 250 mM phosphocreatine. Ten µl of SR protein was added to the cuvette (200-280 µg SR protein/10 µl). Final volume in the cuvette was 1.0 ml.

The cuvette was placed in a Varian DMS 100 spectrophotometer and allowed to equilibrate at 35 °C for 90 sec. The mixture was stirred continuously and after the equilibration period, 10 µl $CaCl_2$ was added form a 10 mM stock solution. Calcium concentration changes were monitored by measuring the absorbance at 710 nm until completion of calcium uptake into SR vesicles.

Ca^{2+}-Mg^{2+}-ATPase activity. Ca^{2+}-independent (basal or Mg^{2+}-stimulated) and total (Ca^{2+}-dependent + Ca^{2+}-independent) activities of the Ca^{2+}-Mg^{2+}- ATPase were determined in an enzyme-linked optical assay at 37 ° C according to the methods of Weidekamm and Brdiczka (1975). Ca^{2+}-dependent activity was calculated as total minus basal activity. The amount of SR used in this assay was

approximately 50 µg protein.

Muscle temperature (Tm). A skin site between the 2 biopsy incisions on the middle gluteal muscle was prepared and anesthetized according to the biopsy procedure described above. Tm was measured at the time of each biopsy by inserting a Copper/constantan thermocouple into the muscle.

Muscle ions. Muscle inorganic ions (Na^+, K^+, Cl^-, Mg^{2+}, and Ca^{2+}) were measured by instrumental neutron activation analysis (Lindinger and Heigenhauser, 1987). This technique measures both bound and free muscle ions; however, only the total ion concentrations are reported in this study (no distinction is made between bound and free ions). Because plasma was not obtained and extracellular fluid volume was not measured in this study, average ion content for the intracellular fluid compartment was not determined and total muscle ion content is reported as µmol/g dry mass.

Muscle glycogen. Muscle glycogen concentration was determined by the method of Lo et al. (1970). This method has been used previously to determine glycogen concentration of equine gluteal muscles (Miller and Lawrence, 1986).

Statistics. Data were tested for significance by analysis of variance, using

the GLM procedure in the SAS program (SAS, 1988).

Results

Ca^{2+} uptake. Resting calcium uptake rates tended to be higher (p=.092) when the horse were on the lecithin supplemented diet, but decreased to a greater extent with an acute bout of exercise (p=.058) (Fig. 2).

Ca^{2+}-ATPase activity. The Ca^{2+}-stimulated ATPase activity was lower after exercise when horses were fed added dietary fat (p=.017) and the decrease from resting values was greater (p=.048) (Fig. 3).

Muscle temperature (Tm). Resting muscle Tm was lower in horses fed lecithin (p=.001), but there were no differences in post-exercise Tm (Fig. 4).

Muscle ions. Muscle calcium concentration (µmol/g dry mass) was higher after exercise when horses were fed dietary lecithin (p=.0065) and the difference between resting and post-exercise calcium was greater in fat fed horses (p=.0049) (Table 2). Muscle magnesium concentration after an acute bout of exercise was lower when horses were fed lecithin (p=.036) (Table 2).

Muscle glycogen. There were no differences in muscle glycogen due to diet at rest or after exercise (Fig. 5).

Discussion

Although researchers have been feeding fat-modified diets to horses for the past 15 years in an attempt to improve performance or change metabolic regulation, this is the first report of dietary lecithin effects on SR function. As far as we know, it is the first report of changes in SR function associated with dietary fat intake in exercising animals. Previous research has utilized sedentary animals as subjects.

The major finding of this study was a decrease in calcium uptake and Ca^{2+}-ATPase with an acute bout of repeated sprinting exercise when horses were fed a diet supplemented with corn oil/ soy lecithin (10% by weight) compared to horses fed a basal diet. This accounts for an approximately six-fold increase in the amount of dietary fat in the diet (2% vs 12%). After 12 wk supplementation of this magnitude it is likely alterations in the lipid environment of the SR occurred. Unfortunately at this time we do not have the results of the muscle lipid profile and therefore must speculate on potential lipid changes. However, feeding trials of much shorter length (10 d to 8 wk) have produced marked changes in the lipid environment of skeletal and cardiac SR (Tume et al., 1973; Croset et al., 1989;

Swanson et al., 1989; Stubbs and Kisielewski, 1990; Taffet et al., 1993). Generally these studies incorporated increased n-3 lipids in the diet to increase the degree of unsaturation of the fatty acids.

Cardiac SR function appears to respond to dietary manipulation more so than skeletal SR function. Feeding n-3 polyunsaturated acid to mice caused an accumulation of 22:6(n-3) and a decrease in the n-6/n-3 polyunsaturated fatty acid ratio. (Croset et al., 1989). These changes were associated with decreases in calcium uptake by n-3 polyunsaturated fatty acid enriched SR vesicles, but neither the affinity for calcium nor Ca^{2+}-ATPase activity were affected. Swanson and coworkers (1989) also increased n-3 polyunsaturated fatty acids in cardiac SR phospholipids in mice by feeding menhaden oil. The changes in fatty acid composition were associated with a lower activity of Ca^{2+}-ATPase, a lower initial rate of calcium transport and maximal calcium uptake into SR vesicles. Similar results were reported for rats fed n-3 polyunsaturated fatty acids (Taffet et al., 1993).

The lipid environment of skeletal muscle SR has responded to dietary manipulation without concomitant changes in SR function (Tume et al., 1973; Soler et al., 1988; Stubbs and Kisielewski, 1990). Stubbs and Kisielewski (1990) suggest the complex mixture of phospholipids present in natural skeletal SR membranes buffers against major changes in bulk membrane lipid motional properties and therefore provides an environment to support optimal membrane

protein function. If this were absolute we would not have seen changes in Ca^{2+}-ATPase activity and calcium uptake rates with fat supplementation. It should be recognized that horses typically have a low dietary fat content, and increasing it six-fold might induce cellular changes not seen in animals that have a high fat intake relative to the horse. Furthermore the fat supplement in this study incorporated soy lecithin composed of phospholipids, primarily phosphatidylcholine (Table 3). Phosphatidylcholine is the main phospholipid species in native SR membranes (65-75%) with phosphatidylethanolamine second in abundance (15-20%) (Hidalgo, 1987). Phosphatidylcholine may be especially important for Ca^{2+}-ATPase functioning (Infante, 1987) and it is symmetrically distributed across the SR membrane (McGill et al., 1981). If this were true, dietary supplementation of phospholipids for a 12 wk period could be sufficient to disrupt the normal lipid environment of the SR by altering phospholipid order to the extent that Ca^{2+}-ATPase activity is reduced when stressed by exercise.

Cornea and Thomas (1994) found that the activity of Ca^{2+}-ATPase was maximal with a chain length of 18 and decreased substantially with longer or shorter chains. Chain length shorter or longer than the optimal 18 resulted in a significantly decreased mobility of the Ca^{2+}-ATPase. An optimal lipid environment has been suggested for the Ca^{2+}-ATPase which is provided by 16:0-18:1 phosphatidylcholine and 16:0-18:2 phosphatidylcholine, the two most common lipids of the SR (Matthews et al., 1993). The primary fatty acids of the supplement

used in this study are oleic (18:1) and linoleic (18:2) which should enhance the optimal environment for the Ca^{2+}-ATPase protein, leading to the implication that the phosphatides in the supplement were the main impact of changes in SR function.

It should be noted that although there was a depression in SR function with exercise in conjunction with fat supplementation, there was no difference in performance due to diet. Horses performed the same SET regardless of diet and there were no differences in perceived fatigue at the end of the SETs or during weekly exercise throughout the duration of the study. Although the SR function was depressed after exercise when horses consumed lecithin, the depression apparently was not sufficient to impair performance. All horses were highly conditioned from a previous experiment. Perhaps if we had run horses to exhaustion rather than ceasing exercise after the fourth sprint we would have seen a difference in time to fatigue associated with diet. It could be possible that the corn oil/ soy lecithin supplementation as fed in this study was sufficient to depress SR function with exercise but simply not to the extent that exercise capacity would be compromised.

Depression in calcium uptake by the SR with exercise leads to increased myoplasmic calcium (Byrd, 1992) which is associated with muscle damage (McCutcheon et al., 1992). An elevated cytosolic free calcium can alter muscle protein synthesis and degradation (McGrath and Goldspink, 1980). These facts

indicate that anything contributing to an elevated myoplasmic calcium could potentially be detrimental to muscle integrity. Feeding a lecithin modified diet therefore may be contraindicated in horses that suffer from exercise/stress-induced myopathies such as tying-up, which has been linked to SR dysfunction (Hodgson, 1993).

It is interesting to note that muscle calcium concentration was higher after exercise and that the change from resting values was greater when horses were fed fat. This parallels the depression in calcium uptake by the SR. However, the INAA muscle electrolyte analysis (Lindinger and Heigenhauser, 1987) measures all myoplasmic ion content, including ions within organelles, free and bound. Therefore the increased calcium concentration would include calcium ions in the SR as well as the myoplasm and other organelles. The elevated calcium must be due to either increased influx of calcium from extracellular fluid, or decreased efflux from the muscle. This suggests the dietary fat fed in this study affected the sarcolemma, perhaps at a calcium channel such as the dihydropyridine receptor, altering calcium conductance.

Attempts at improving performance of horses by feeding fat supplemented diets have not been conclusive. Corn oil/soy lecithin supplementation did not affect resting muscle glycogen or glycogen utilization during exercise in this study nor did it improve performance. Resting muscle glycogen concentration in horses fed fat supplemented diets were similar (Hambleton et al., 1980), increased

(Oldham et al., 1990; Scott et al., 1992) or decreased (Greiwe et al., 1989; Pagan et al., 1987) compared to controls. A glycogen sparing effect was found during prolonged, submaximal exercise with fat supplementation (Greiwe et al., 1989), but Hambleton et al. (1980) demonstrated no effect of fat on glycogen utilization with prolonged exercise. Pagan and coworkers (1987) found no difference in glycogen utilization in a stepwise test. Increased muscle glycogen utilization during high intensity exercise was reported in fat supplemented horses (Oldham et al., 1990), while muscle glycogen utilization was decreased in fat supplemented horses working during a high speed exercise test (Pagan et al., 1987). Because these studies vary in type and amount of fat fed, length of the trial, level of fitness of the animals, type of exercise utilized and breed of horse there is no definitive answer to the question of feeding fat and its subsequent muscle glycogen sparing effect. Highly conditioned Quarter Horses supplemented with 10% by weight corn oil/soy lecithin do not show a muscle glycogen sparing effect with repeated sprinting exercise. One potential benefit of substituting fat for carbohydrate as an energy source is the decrease in bulk in the stomach and large intestine (Ferrante and Kronfeld, 1992).

In conclusion, there was no apparent benefit of supplementing corn oil/ soy lecithin to highly conditioned horses undergoing repeated sprints. In fact we observed a potentially detrimental effect of this supplementation on SR function as it decreased Ca^{2+}-ATPase activity and calcium uptake after exercise. No

differences were observed in performance associated with diet, but this depression in SR function could be damaging to horses that suffer from tying up.

Literature Cited

Byrd, S.K. 1992. Alterations in the sarcoplasmic reticulum: a possible link to exercise-induced muscle damage. Med. Sci. Sports Exerc. 24:531.

Cornea, R.L. and D.D. Thomas. 1994. Effects of membrane thickness on the molecular dynamics and enzymatic activity of reconstituted Ca-ATPase. Biochemistry. 33:2912.

Croset, M., J.M Black, J.E. Swanson and J.E. Kinsella. 1989. Effect of dietary n-3 polyunsaturated fatty acids on phospholipid composition and calcium transport in mouse cardiac sarcoplasmic reticulum. Lipids. 24:278.

East, J.M., O.T. Jones, A.C. Simmonds and A.G. Lee. 1984. Membrane fluidity is not an important physiologic regulator of the (Ca^{2+}-Mg^{2+})-dependent ATPase of sarcoplasmic reticulum. J. Biol. Chem. 259:8070.

Ferrante, P.L. and D.S. Kronfeld. 1992. Ergogenic diets and nutrients. In: N.E. Robinson (Ed.) Current Therapy in Equine Medicine 3. p 809. W.B. Saunders Co., Philadelphia.

Greiwe, K.M., T.M. Meacham, G.F. Fregin and J.L. Walberg. 1989. Effect of added dietary fat on exercising horses. Proc. 11th Equine Nutrit. and Physiol. Symp. p 101. Stillwater, OK.

Hambleton, P.L., L.M. Slade, D.W. Hamer, E.W. Kienholtz and L.D. Lewis. 1980. Dietary fat and exercise conditioning on metabolic parameters in the horse. J. Anim. Sci. 51:1330.

Hidalgo, C. 1987. Lipid-protein interactions and the function of the Ca^{2+}-ATPase of sarcoplasmic reticulum. C.R.C. Critical Reviews in Biochemistry. 21:319.

Hodgson, D.R. 1993. Exercise-associated myopathy:is calcium the culprit? Equine Vet. J. 25:1.

Infante, J.P. 1987. Docosahexanote-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in Ca^{2+}-ATPase of sarcoplasmic reticulum. Mol. Cell. Biochem. 74:111.

Lindinger, M. I. and G.J.F. Heigenhauser. 1987. Intracellular ion content of skeletal muscle measured by instrumental neutron activation analysis. J. Appl. Physiol. 63:426.

Lo, S., J.G. Russell and A.W. Taylor. 1970. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28:234.

Matthews, P.L., E. Bartlett, V.S. Ananthanarayanan and K. M. Keough. 1993. Reconstitution of rabbit sarcoplasmic reticulum calcium ATPase in a series of phosphatidylcholines containing a saturated and unsaturated chain:suggestion of an optimal lipid environment. Biochem. Cell. Biol. 71:381.

McCutcheon, L.J., S.K. Byrd and D.R. Hodgson. 1992. Ultrastructural changes in skeletal muscle after fatiguing exercise. J. Appl. Physiol. 72:1111.

McGill, K.A., J.P. Bennett, G.A. Smith, R.W. Plumb and G.B. Warren. 1981. Transbilayer distribution of lipids in a population of sarcoplasmic-reticulum vesicles sealed with their cytoplasmic side outwards. Biochem J. 195:287.

McGrath, J.A. and D. F. Goldspink. 1980. The effects of glucocorticoids on protein turnover and growth of different striated muscles in the rat. Biochem. Soc. Trans. 8:448.

Miller, P.A. and L.M. Lawrence. 1986. Changes in equine metabolic characteristics due to exercise fatigue. Am. J. Vet. Res. 47:2184.

NRC. Nutrient requirements of horses. (5th Ed.) National Academy of Sciences-National Research Council, Washington, DC. 1989.

Oldham, S.L., G.D. Potter, J.W. Evans, S.B. Smith, T.S. Taylor and W.S. Barnes. 1990. Storage and mobilization of muscle glycogen in exercising horses fed a fat supplemented diet. Equine Vet. Sci. 10:353.

Pagan, J.D., B. Essen-Gustavsson, A. Lindholm and J. Thorton. 1987. The effect of dietary energy source on exercise performance in Standardbred horses. In:J.R. Gillespie and N.E. Robinson (Ed.). Equine Exercise Physiology 2. p 686. ICEEP Publications, Davis, CA.

SAS. 1988. SAS Users Guide:Statistics. SAS Inst., Inc., Cary, N.C.

Scott, B.D., G.D. Potter, L.W. Greene, P.S. Hargis and J.G. Anderson. 1992. Efficacy of a fat-supplemented diet on muscle glycogen concentrations in Thoroughbred horses maintained in varying body conditions. Equine Vet. Sci. 12:109.

Soler, F., M.J. Alejandre, M. Garcia-Gonzalez, J. L. Segovia-Parra F. Fernandez-Belda and J.C. Gomez-Fernandez. 1988. Influence of dietary lipids on microsomal membranes from chick breast muscle. Comp. Biochem. Physiol. 90:767.

Stubbs, C.D. and A.E. Kisielewski. 1990. Effect of increasing the level of n-3 fatty acids on rat skeletal muscle sarcoplasmic reticulum. Lipids. 25:553.

Swanson. J.E., B.R. Lokesh and J.E. Kinsella. 1989. Ca^{2+}-Mg^{2+} ATPase of mouse cardiac sarcoplasmic reticulum is affected by membrane n-6 and n-3 polyunsaturated fatty acid content. J. Nutr. 119:364.

Taffet, G.E., T.T. Pham, D.L.M. Bick. M.L. Entman. H.J. Pownall and R.J. Bick. 1993. The calcium uptake of the rat heart sarcoplasmic reticulum is altered by dietary lipid. J. Mem. Biol. 134:35.

Tume, R.K., R.P. Newbold and D.J. Horgan. 1973. Changes in the fatty acid composition of sarcoplasmic reticulum lipids and calcium uptake activity. Arch. Biochem. Biophys. 157:485.

Weidekamm, E. and D. Brdiczka. 1975. Extraction and localization of a (Ca^{2+} and Mg^{2+})-stimulated ATPase in human erythrocyte spectrin. Biochim. Biophys. Acta. 401:51.

Wilson, J.A., D.S. Kronfeld. L. Gay and T.M. Wilson. 1995. Isolating equine sarcoplasmic reticulum:its function during high intensity repeated sprints. Equine Vet J. In Press.

Table 1. Ingredient and chemical composition of control and fat supplemented diets.

	Basal Diet	Fat Diet
Ingredients (% as fed basis):		
Alfalfa	50.0	64.0
Cracked corn	37.6	17.0
Corn oil/soy lecithin[a]	0.0	10.0
Molasses	7.2	6.4
Soybean meal (44%)	3.5	2.0
Limestone	0.2	0.1
Mineral/vitamin (estimated)[b]	0.5	0.5
Feed Analysis (dry matter basis):		
Crude protein, %	13.2	13.0
Ether extract, %	2.4	13.9
Acid detergent fiber, %	22.3	27.1
Digestible energy, Mcal/kg (estimated)	2.65	2.92

[a]Lucas Meyer, Decatur, Il.

[b] Southern States, Richmond, Va.

TABLE 2. Muscle ions before and after intense exercise when horses were fed a basal or lecithin modified diet.

ION	DIET	BEFORE	AFTER
Ca	basal	8.46 ± .66	5.65 ± 1.15
	lecithin	7.34 ± .66	11.38 ± 1.15*
Cl	basal	122.43 ± 9.26	118.22 ± 15.33
	lecithin	106.49 ± 9.26	143.92 ± 15.33
K	basal	308.18 ± 12.09	332.99 ± 9.8
	lecithin	336.06 ± 12.09	320.21 ± 9.8
Mg	basal	38.92 ± 1.8	40.03 ± 1.16
	lecithin	39.23 ± 1.8	35.99 ± 1.16
Na	basal	176.56 ± 10.61	159.83 ± 18.52
	lecithin	145.09 ± 10.61	199.35 ± 18.52

Values are lsmeans ± SE (μmol/g dry mass).
*Indicates basal mean different from lecithin (p=.0065)

TABLE 3. Nutritional profile of blend of corn oil/soy lecithin supplement.

Phosphatides (gm /100gms product)		
Total phosphatides		31.5
phosphatidylcholine		7.8
phosphatidylethanolamine		6.3
phosphatidylinositol		5.0
other phosphatides(including phosphatidic acid)		12.4
Soybean oil		18.0
Corn oil		50.0
Fatty Acids (relative composition)		
palmitic	(16:0)	13.4
palmitoleic	(16:1)	0.1
stearic	(18:0)	3.4
oleic	(18:1)	21.3
linoleic	(18:2)	57.4
linolenic	(18:3)	1.9
arachidic	(20:0)	0.1

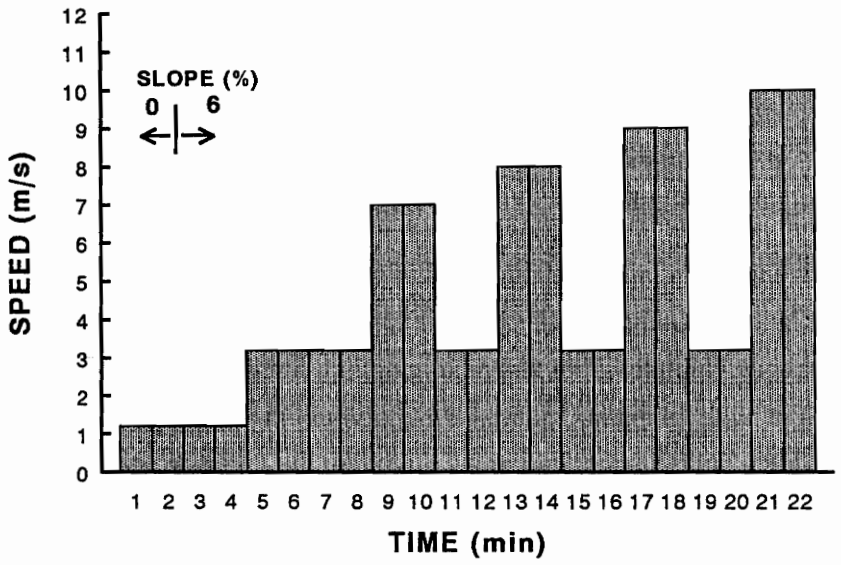


Figure 1. Standard exercise test (SET) used for determination of differences in SR function associated with diet.

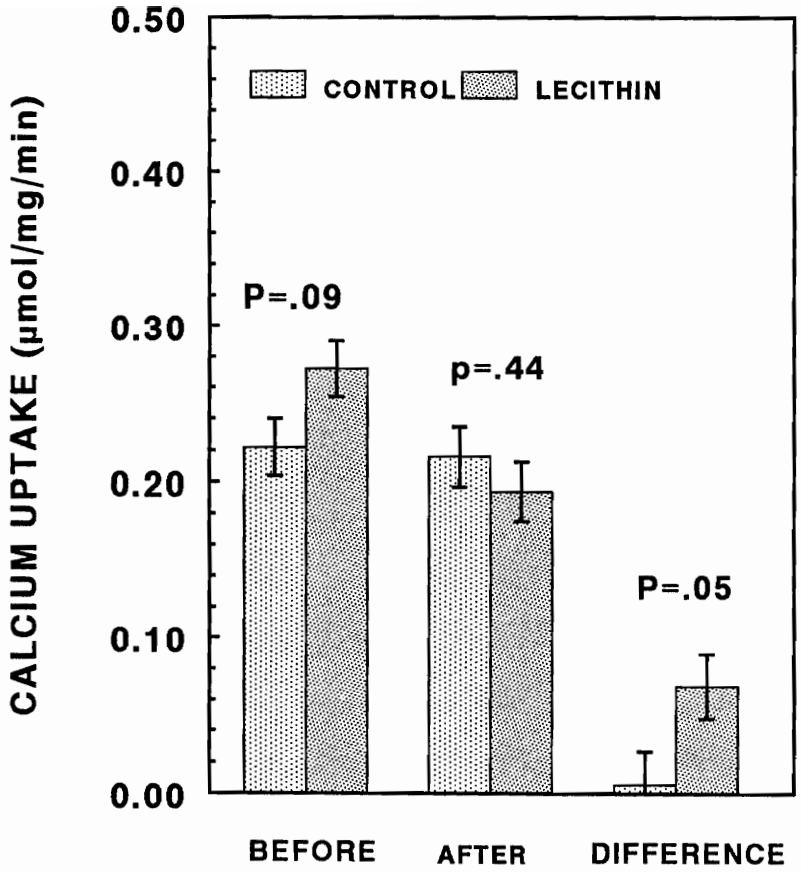


Figure 2. Calcium uptake in isolated sarcoplasmic reticulum (SR) at rest and after repeated sprints when horses were fed a basal and lecithin supplemented diet (n=10).

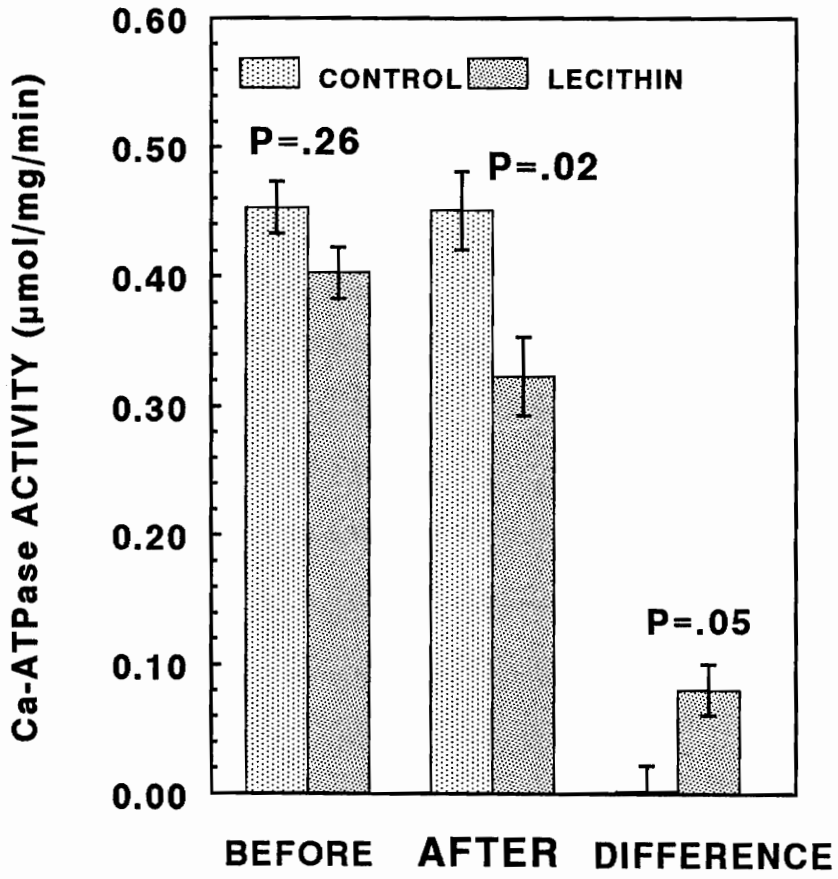


Figure 3. Calcium-stimulated ATPase activity of isolated sarcoplasmic reticulum (SR) at rest and after repeated sprints when horses were fed a basal and lecithin supplemented diet (n=10).

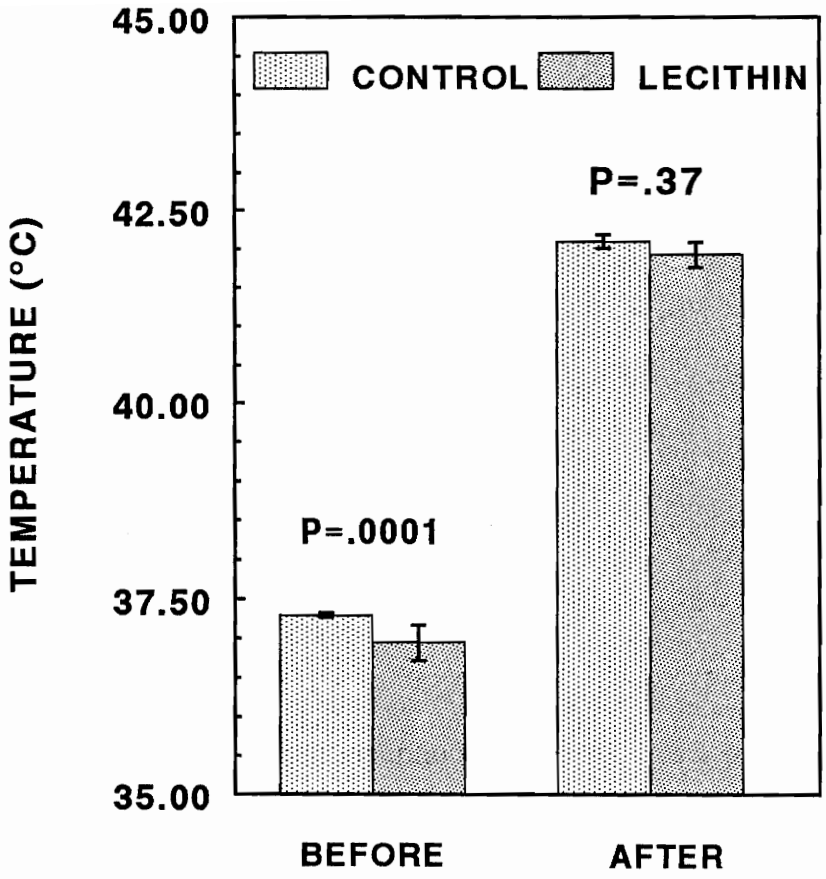


Figure 4. Temperature of middle gluteal muscle at rest and after repeated sprints when horses were fed a basal and lecithin supplemented diet (n=10).

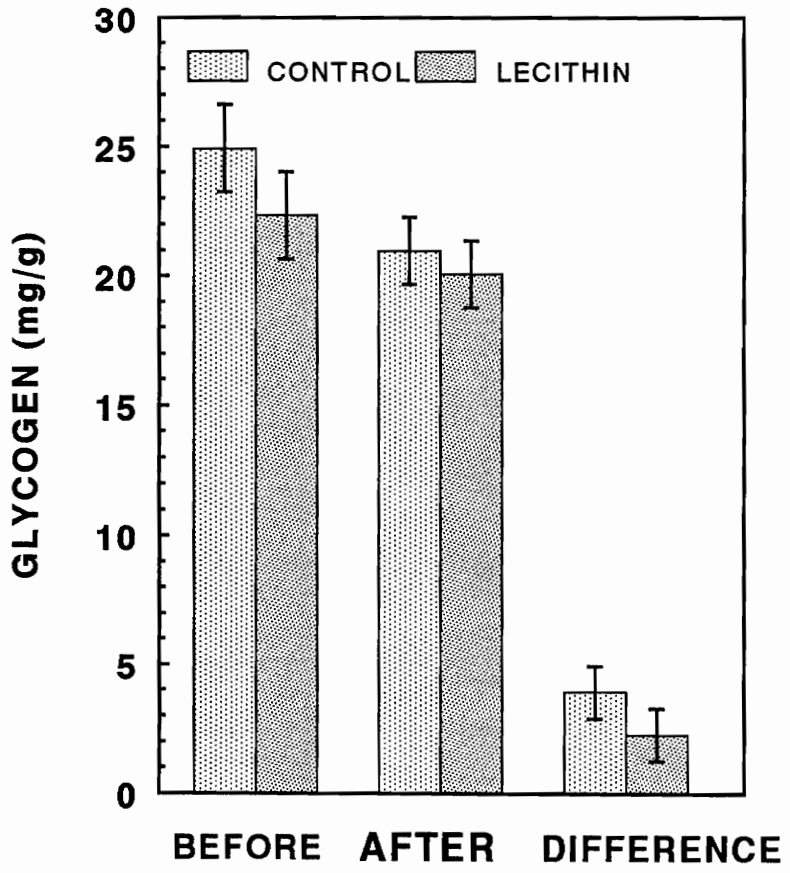


Figure 5. Muscle glycogen concentration of the middle gluteal muscle at rest and after repeated sprints when horses were fed a basal and lecithin supplemented diet (n=10).

Summary and Implications

The sarcoplasmic reticulum (SR) is a network of membranous sacs that regulates intracellular calcium concentration in skeletal muscle. Calcium ions are released via a protein channel embedded in the SR membrane to prompt muscle contraction. The resting state is achieved when calcium is pumped into the SR through the action of an active transport system called the Ca^{2+}-ATPase. Muscle fatigue has been associated with reduced SR function in a number of species including the horse. It also has been linked to myopathies common to horses referred to as tying-up or exertional rhabdomyolysis.

A set of methods was developed in equine muscle to measure SR function, specifically the rates of calcium uptake and Ca^{2+}-ATPase activity, by isolating viable SR vesicles from middle gluteal muscle of horses. This system was used in subsequent experiments to determine the effects of repeated sprints, conditioning and dietary corn oil/ soy lecithin on SR function.

Ten Quarter Horses performed repeated sprints on a high speed treadmill. Muscle samples were taken before exercise and following the final sprint from the middle gluteal, and SR vesicles were isolated. Horses were then conditioned for 12 weeks and the experiment was repeated. Calcium uptake was determined spectrophotometrically using antipyralazo III and Ca^{2+}-ATPase activity was

determined using an enzyme-linked optical assay.

Resting calcium uptake rates and Ca^{2+}-ATPase activity were greater when the horses were conditioned than when unconditioned (p=.05, p=.004, respectively). After exercise calcium uptake was slower in unfit horses (p=.02) or fit horses (p=.002) compared to rest. This fatigue response to exercise was reduced by conditioning (p=.01). After exercise Ca^{2+}-ATPase activity was decreased in unconditioned (p=.007) and conditioned horses (p=.001) relative to resting activity, with a greater depression in unconditioned horses.

After the conditioning phase of the experiment, the horses were divided into two groups and were fed either a basal diet or a diet supplemented with 10% dietary fat in the form of corn oil/soy lecithin for a 12 week period. The diets were switched and the study continued for another 12 weeks. Horses were exercised 4 days a week on a high speed treadmill to maintain fitness. The repeated sprinting experiments, as in the first part of the study, were performed at the end of both 12 week periods. Resting calcium uptake rates tended to be higher (p=.09) when horses were consuming lecithin, but decreased to a greater extent with an acute bout of exercise (p=.058). The Ca^{2+}-ATPase activity was lower after exercise in fat supplemented horses (p=.02) and the decrease from resting values was greater (p=.05).

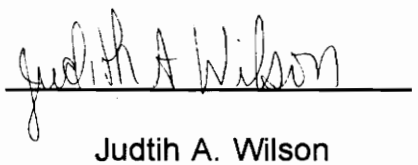
These results demonstrate that high intensity repeated sprinting fatigues SR function in horses, and that 12 weeks of conditioning reduces the depression of

SR function seen with high intensity exercise. Feeding a corn oil/soy lecithin diet to fit horses increases the response of the SR to an acute bout of exercise. This fat supplement increased the depression in calcium uptake rates and Ca^{2+}-ATPase activity associated with acute exercise without effecting exercise performance in the horses used in this study. It could, however, have deleterious effects on horses that suffer from exercise related myopathies such as tying-up. If tying-up can be identified with reduced muscle calcium regulation, the depression in calcium uptake seen after exercise with feeding corn oil/soy lecithin would exasperate this condition. Increased myoplasmic calcium concentration can stimulate proteases and phospholipases which leads to muscle degradation and reduces force production as well as increasing time to relaxation. These conditions are indicative of tying-up and therefore further research is required concerning potential damaging effects of feeding lecithin to horses suffering from myopathies.

These experiments confirm the association of diminished SR function with muscle fatigue and reveal the relationship of exercise conditioning with improved calcium uptake and Ca^{2+}-ATPase activity of skeletal muscle SR. Feeding a lecithin supplemented diet to conditioned horses proved to have no benefit on calcium regulation by the SR. Future research of the sarcoplasmic reticulum of the horse will likely examine a possible genetic link to an abnormal calcium release channel and/or Ca^{2+}-ATPase pump protein and potential intervention in tying-up.

VITA

Judith Ann Wilson, daughter of Thomas and Marilyn Warren, was born July 29, 1961, in Manchester, New Hampshire. She received a Bachelor of Science in Animal Science from the University of New Hampshire in May, 1988 and a Masters of Science in Animal Science from Kansas State University in July, 1991 where she studied equine exercise physiology. She completed the requirements for a Doctor of Philosophy in Animal Science at Virginia Polytechnic Institute and State University in April 1995.



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