Effects of Reduced Muscle Glycogen on Sarcoplasmic Reticulum (SR), Muscle and Exercise Performance

by

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Dissertation submitted to the faculty of

Virginia Polytechnic Institute and State University

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

The Department of Human Nutrition, Foods, and Exercise

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April 19, 2002

Blacksburg, Virginia

Key Words: fatigue, calcium, SERCA, metabolism

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(ABSTRACT)

Fatigue during exercise is associated with reduced muscle glycogen. However, evidence linking glycogen content to fatigue is lacking. In this study we examined whether reduced muscle glycogen content limited SR function or muscle performance. Two groups of female Sprague-Dawley rats were fasted for 24 hr and exercised for 90 min to reduce muscle glycogen; rats fasted after exercise formed the low glycogen (LG) group. Rats in the high glycogen (HG) group were allowed free access to food and a 5% sucrose solution. The LG group had 42% less muscle glycogen and 90% less glycogen associated with the sarcoplasmic reticulum (SR) than the HG group. Notably, time to exhaustion during a subsequent treadmill run (21 m/min at 10% grade) was markedly lower in the LG group (35 vs. 166.75 min). Despite less glycogen, the LG group had a higher SR Ca^{2+} uptake rate (45%) and Ca^{2+}-stimulated ATPase activity (51%) possibly due to a 33% greater SERCA content. Surprisingly, in situ gastrocnemius initial twitch and tetanic forces were not different between groups although the rates of relaxation were higher in the LG group. The force

responses to fatigue-inducing stimulus trains (20 Hz for 333 ms every 1 sec for 30 min) also were similar for both groups as were twitch and tetanic forces in the fatigued state. These results suggest that despite reduction in exercise performance, reduced muscle glycogen does not limit muscle performance or SR function.

ACKNOWLEDGEMENTS

I would like to thank the following persons for their assistance with, and moral support during this study:

- My advisory committee: Drs. Jay H. Williams (Chairman), Laurence D. Moore, David M. Moore DVM, Robert Talmadge, and Chris Ward.
- Drs. Randolph Grayson and Laurence D. Moore for their financial support via the Minority Academic Opportunity Program (MAOP).
- Drs. Brian Duling and Richard Murphy for hiring me as a Cardiovascular Research Post-Doctoral Fellow while I was finishing this process.
- Drs. John S. Walker and Robert Wardel for their intellectual assistance.
- Dr. George Mandl for the use of pictures from his web page.
- Dave Gimmel and staff for their cooperation at the LAR.
- My immediate and extended family, especially Jerome and Ernestine Batts for financial and moral support.
- Dr. Ayonda E. Lanier and family for their acts of kindness and moral support throughout this process.
- My Virginia Tech family for keeping me focused during this process.
- Simon Lees, Jeff Otis, and Pete Franks for assistance with tissue handling in the lab.

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CHAPTER ONE:

INTRODUCTION

INTRODUCTION

In today's athletic community, one's ability to withstand long duration's of high intensity exercise has become an ever-important mission. An athlete's capacity to sustain a maximal effort could determine how successful he/she is. This could mean the difference between being an elite or novice athlete. At the professional level, athletes are becoming more and more interested in dietary needs as well as training and equipment. Depending on what event the athlete is training for, equipment may or may not play an important role. However, strength and diet almost always are limiting factors. Of the two factors, diet can be more easily manipulated because its effects can be seen over a shorter time period. Taking this into consideration, much research has been done to determine the optimal diet regimen for certain events. As a result of that research, it has been confirmed that the level of muscle glycogen prior to and during an event can increase or decrease one's endurance. More specifically, higher starting muscle glycogen levels are better. Endogenous muscle glycogen is the primary fuel source for contraction (Green *et al.,* 1990). The depletion of muscle glycogen has often times been linked to fatigue, which has been defined as the inability to maintain an expected power output (Hermansen *et al.,* 1967). Studies using alterations in pre-exercise muscle glycogen reserves by dietary manipulation have, in general, established a close relationship between the level of muscle glycogen and the development of fatigue. For example, Bergstrom *et al.,* (1967) have done research that strongly suggests that pre-exercise glycogen levels are in fact linked to endurance. These researchers varied beginning levels of

glycogen in individuals and tested their fatiguability. They found higher beginning glycogen levels were correlated with increased endurance. As a result of numerous similar studies, it has become a common practice in the endurance athletic community to load up with carbohydrates before an event. However, the effect of glycogen levels on initial force production is not as obvious.

Despite evidence that suggests glycogen depletion is linked to fatigue the actual mechanism has yet to be determined. Glycogen plays a very specific role in the contraction process. It provides energy for the sequestering of Ca^{2+} by the sarcoplasmic reticulum (SR) and force generation by the contractile apparatus. During contraction, SR Ca^{2+} release causes the myofilaments to interact and generate force. There is a sigmoidal relationship between the myoplasmic [Ca^{2+}] and force generation. That is, the higher levels of [Ca^{2+}] in the cytoplasm the more force will be generated. It has been shown that after exhaustive exercise Ca^{2+} release and [Ca^{2+}] is reduced and as a result, force generation is also reduced. [ATP] influences both Ca^{2+} release and sequestration. Since muscle glycogen is the chief substrate for ATP production in the muscle, its decrease could inhibit the functioning of the Ca^{2+} pump (Ca^{2+} ATPase) as well as the Ca^{2+} release channel. Xu *et al.,* (1995) have demonstrated that SR vesicles have glycolytic enzymes such as aldolase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglyceromutase, enolase, and pyruvate kinase that are bound to the membrane that are just as, if not more effective, in producing ATP to support Ca^{2+} uptake than are cytosolic ATP sources. This being the case, it is possible

that the Ca^{2+} ATPase activity and Ca^{2+} release channel associated with the SR membrane are more dependent on ATP that is generated from glycolysis than from free ATP within the myoplasm. Hence, low levels of substrate (glycogen) could vastly affect this system which fuels glycolysis and provide ATP for Ca^{2+} uptake.

JUSTIFICATION FOR RESEARCH

There is no published direct evidence suggesting that glycogen depletion has detrimental effects on SR function or muscle performance. However, experiments by Xu *et al.,* (1995) Xu *et al.,* (1995) imply that a depletion of substrate could inhibit normal functioning of the SR.

The purpose of this current study was to examine the effects of decreased glycogen in rested muscle on SR function, exercise and muscle performance. The first aim of this study was to determine if the proposed exercise protocol caused an adequate decrease in total muscle and SR glycogen. The second and most important aim of this study was to determine the effect of glycogen depletion on SR, muscle and exercise performance. Two groups of female Sprague-Dawley rats were fasted for 24 hr and exercised for 90 min (21 m/min at 10% grade) to reduce muscle glycogen; rats fasted after exercise formed the low glycogen (LG) group. Rats in the high glycogen (HG) group were allowed free access to food and a 5% sucrose solution. After glycogen depletion was determined muscle performance was assessed using an in situ stimulation protocol outlined in chapter 3. Then SR function was evaluated by monitoring

Ca^{2+} uptake in isolated SR vesicles. Finally, endurance performance was tested. The first aim of this study was to determine if the proposed exercise protocol caused an adequate decrease in total muscle and SR glycogen. The second and most important aim of this study was to determine the effect of glycogen depletion on SR, muscle and exercise performance.

RESEARCH HYPOTHESIS

The following null hypotheses were tested during this investigation:

H_0 The glycogen depletion protocol would not alter whole muscle glycogen levels.

H_0: The glycogen depletion protocol would not alter SR glycogen levels.

H_0: The glycogen depletion protocol would not alter Ca^{2+} uptake rates.

H_0: The glycogen depletion protocol would not alter Ca^{2+} ATPase activity.

H_0: The glycogen depletion protocol would not alter muscle performance.

H_0: The glycogen depletion protocol would not alter exercise performance.

BASIC ASSUMPTIONS

The investigator, prior to and during this experiment, made the following basic assumptions:

1. All animals started with the same glycogen levels.
2. The SR prep yielded viable vesicles.
3. All proteins and enzymes associated with SR were present after isolation.

4. The glycogen assay indicated correct measurements.
5. Animals were free of disease.

LIMITATIONS

The results of this investigation are limited to:

1. Female, Sprague Dawley rats.
2. A single glycogen depletion protocol
3. Measurements of Ca^{2+} uptake and ATPase activity as indicators of SR function.
4. In situ stimulation as muscle function measures.

SUMMARY

There is a positive correlation between beginning muscle glycogen levels and endurance. There is no direct evidence that glycogen depletion has detrimental effects on SR function and muscle performance. This study investigated the effects of decreased glycogen in rested muscle on SR function and muscle performance. It was hypothesized that a muscle depleted of glycogen would have decreased Ca^{2+} uptake thus, contributing to a loss in force (i.e. fatigue). This finding would substantiate the link between glycogen and muscle performance.

CHAPTER TWO:

LITERATURE REVIEW

INTRODUCTION

One important factor in achieving peak performance during endurance events is determined by the athlete's ability to avoid fatigue. Marathoners and triathletes depend almost solely on their ability to have more stamina and be less fatigable than their opponent. Traditional training programs that would normally be focused on physical training have been transformed into highly specialized programs that incorporate the importance of nutrition and exercise. In an effort to maximize their workouts and event performance most endurance athletes rely heavily on the nutritional aspects of their training as well as physical training. Glycogen loading is a common practice in the athletic community and involves depleting glycogen 2-3 days prior to the event and repleting muscle glycogen with a large quantity of carbohydrates the day before the event. This practice coupled with consuming carbohydrate beverages or snacks during the event delays or even prevents the onset of fatigue by providing an adequate source of fuel for exercise. Fatigability during the exercise bout ultimately determines an endurance athlete's success. The inability of a muscle to maintain a prescribed force output is termed "muscle fatigue", and is likely to be affected by numerous factors including diet (Hermansen *et al.,* 1967). The cause of the loss in force remains to be somewhat of a mystery. However, there are three theories that have been postulated that attempt to describe why fatigue may occur they are energy depletion, metabolite accumulation, and sarcoplasmic reticulum (SR) dysfunction. A number of groups argue that the loss of muscle glycogen, a key substrate for energy production plays a very important role in exercise

performance. It is widely accepted that glycogen is the limiting substrate when determining endurance performance however, the relationship between glycogen and muscle force production has yet to be made. As a consequence, this project examined the idea that glycogen depletion and SR dysfunction are closely related and as a result cause decreased force production and exercise performance in rested muscles with different glycogen levels.

BACKGROUND

MUSCLE DEVELOPMENT

The following is a brief summary of muscle development. For a more detailed description, see Lieber *et al.,* (1992), Bailey *et al.,* (2001) and Buckingham *et al.,* (2001). There are primarily 4 phases in muscle development that occur simultaneously. These phases are myogenesis, axonal outgrowth, synaptogenesis, and synapse elimination. All muscle fibers are derived from myoblasts that enter myogenesis. Myogenesis is further divided into proliferation, alignment, and fusion. The myoblast will undergo two phases of proliferation, the first phase of proliferation gives rise to the 1° myotube and the second the 2° myotube. After the first phase of proliferation is over, the cells align to form rows, which eventually fuse to form the 1° myotube. Then the second phase of proliferation starts, these cells align beside the 1° myotube and fuse to become 2° myotubes. The 2° myotube eventually splits from the 1° myotube. After this they both develop membranes and are called muscle fibers.

At about the same time as myogenesis is occurring, axonal outgrowth is occurring. Developing cells within the spinal cord become α-motor neurons and their axons begin to migrate to different areas of the body where myogenesis is occurring. When they reach the developed muscle fiber the neuromuscular junction (NMJ) is formed, this process is called synaptogenesis. This process is aided by a neural cell adhesion molecule (NCAM), which is found on the entire newly developed muscle fiber. NCAM is a chemoattractant to motor neurons that allows a NMJ to be formed at any point on the fiber. Another protein present on the entire surface of the fiber is the acetylcholine receptor (Ach) (will eventually bind Ach from α-motor neuron). Since the NCAM is non-specific in how many neurons are attracted, several neurons converge at the fiber and make several NMJ's. The Ach receptors then begin to migrate to the newly formed NMJ's. In order to have a functioning fiber all but one of the NMJ's is eliminated (synapse elimination). Therefore, you end up with one motor neuron/fiber. Synapse elimination is most likely determined by the activity of the neuron. Thus, the most active neuron will remain and the others will have to look for another home. However, the α-motor neuron has many axon branches that will innervate other fibers forming a motor unit (fast or slow). Fast motor units innervate fast fibers and slow motor units innervate slow fibers. Fast or slow refers to the activity and frequency of the neuron. A fast unit has a high frequency but low activity and a slow unit is just the opposite.

At this point, there is a functional NMJ and it is ready to be differentiated. Muscles can be divided into 2 primary categories "fast" and "slow." "Fast" fibers

can be divided into 3 major classes (IIa, IIx, and IIb). It should be noted that between each of these fiber types is a transition type (IIa/IIx and IIx/IIb). Slow fibers are noted as type I. The type of myosin heavy chain that is present determines fiber types. What determines the type of myosin heavy chain that is present depends on the type of myotube it is and what type of motor unit innervates it, as noted above. Primary myotubes become “slow” fibers and 2° myotubes become “fast” fibers.

SKELETAL MUSCLE STRUCTURE

There are numerous texts that provide in depth descriptions on skeletal muscle structure, the following is a summary taken from various texts and journals (i.e. Lieber *et al*., (1992), Berne *et al*., (1998), Aidley (1971), Peachey *et al*., (1983), Ahern *et al*., (2001), Gorgon *et al*., (2000), Squire *et al*., (1998), and Gordon *et al*., (2001)). There are three types of muscle in the body: smooth, cardiac, and skeletal. These muscles can further be divided into either voluntary or involuntary or striated or non-striated. Cardiac and smooth muscle are both under involuntary control, meaning that there is no conscious control as opposed to skeletal muscle, which is under voluntary control. The other characteristic is based on the appearance of bands (striations). Smooth muscle does not have striations as compared to cardiac and skeletal, which do. It is this distinctive banding pattern that gives skeletal muscle some of its unique properties and therefore will be the focus of the discussion.

Skeletal muscle is comprised of muscle fibers, which are made of myofibrils, which are further divided into myofilaments, which are ultimately broken down into structural proteins (Fig. 1). The entire muscle is covered by connective tissue called epimysium. Within the epimysium are bundles of muscle fibers called fascicles. Each of the fascicles is covered by a perimysium. Inside each fascicle are myofibrils, which are enclosed in an endomysium. A sarcolemma, basil lamina, and endomysium cover each myofibril. The sarcolemma serves as the inner most membrane of the myofibril and is ultimately where the contraction process begins. The sarcolemma is periodically invaginated along the entire length of the myofibril. These invaginations are called transverse-tubules (TT), and are used to convey signals from the surface of the sarcolemma to the interior of the myofibril. The t-tubules are in direct contact with a membranous network called the sarcoplasmic reticulum (SR). The SR is the storage area for Ca^{2+} ions that activate the contractile apparatus. Within each myofibril are myofilaments (thin-actin and thick-myosin), which can be broken down to individual proteins.

The striation pattern typically associated with skeletal muscle can be visualized by phase-contrast microscopy and the pattern is typically described as bands. Skeletal muscle has an alternating light and dark pattern, which is based on how polarized light interacts with the myofilaments. Myofilaments that reflect polarized light uniformly make up the isotropic band (I-Band). Areas of the myofibril that scatter polarized light unevenly comprise the anisotropic band (A-

band). When stained, the I-band appears light in color and the A-band dark, hence the alternating banding pattern (Fig. 2).

Each myofibril is comprised of sarcomeres in series, which span the entire length of the fiber. A sarcomere is the functional unit of muscle contraction and is comprised of myofilaments (contractile apparatus) and other structural proteins. The distance between two z-lines (comprised primarily of actinin) determines a sarcomere. Within a sarcomere is where the distinctive banding occurs. The banding is due to the interdigitation of actin (comprised of troponin and tropomyosin) and myosin filaments. Other structural proteins found in the myofibril are desmin (connects z-lines of adjacent sarcomeres), nebulin (runs parallel to actin and keeps it in register via the z-line), titin (connects myosin to z-line), and m-line protein (keeps adjacent myosin filaments in register) (Fig 3).

There are two types of myofilaments, thin and thick. More specifically, they are called actin and myosin. The thin filament is comprised of two actin filaments in a helical array (Fig. 4). Each actin filament is made up of many smaller subunits that are linked together, i.e. globular actin or G-actin. Once the G-actin molecules are bound together they form a filamentous or fibrous actin strand (F-actin). Each F-actin is comprised of ≈300 G-actin subunits that are 5-6 nanometers in diameter. The thin filament also contains two major structural proteins, tropomyosin and troponin. Theses two structural proteins are often referred to as regulatory proteins because they regulate thin and thick filament interaction. In skeletal muscle, the three proteins are present in a ratio of 7:1:1.

The point where the two F-actin filaments meet form a groove that allows tropomyosin to bind. Tropomyosin is a double stranded helical molecule (≈ 40 nanometers), which runs along the two grooves formed by the two strands of F-actin. In this arrangement, one molecule of tropomyosin extends over seven G-actin monomers (Fig 4).

The third regulatory protein of the thin filament is troponin and is composed of three subunits: troponin-I (Tn-I), troponin-T (Tn-T), and troponin-C (Tn-C). Each troponin subunit has a specific function, i.e. Tn-T binds the complex to tropomyosin, Tn-I binds to and blocks the activation site for actomyosin interaction, and Tn-C is the portion that binds Ca^{2+} and links TnT and Tn-I and ultimately anchors tropomyosin to actin. In a relaxed muscle (no Ca^{2+}), the troponin complex inhibits cross-bridge formation between actin and myosin by preventing globobular myosin heads from attaching to actin.

The thick filament (Fig. 5) consists of about 400 myosin molecules. Each molecule is a dimer composed of two tightly intertwined "heavy" polypeptide chains - the body or tail - consisting of L-meromyosin (LMM). Each of the two polypeptide chains, in turn, ends in a globular head which is attached to its main long chain via a short elastic stalk. Both head and stalk are made up of H-meromyosin (HMM). The HMM, in turn, consists of two sub-types: S-1, which forms the globular head, and S-2, which forms the short stalk (see also Fig. 7B-a). A pair of "light" polypeptide chains (L1 and L2) are associated with each of the two globular heads, resulting in four light chains being associated with a terminal "double head" projecting laterally from one end of each molecule. The

heads contain specific binding sites for actin, an ATP receptor site, and a catalytic site capable of hydrolyzing ATP.

The thick filament is composed of myosin molecules arranged in an anti-parallel linear bundle about 1.6 microns long. Along this bundle, there are terminal heads that projecting from - either of the two opposing end regions. The tails are aligned parallel along the bare center portion, where they are bound together by additional structural proteins in the region of the sarcomere's M-line.

EXCITATION-CONTRACTION COUPLING (ECC)

This summary of ECC was taken from various texts and journals (i.e. Lieber *et al*., (1992), Berne *et al*., (1998), Aidley (1971), Peachey *et al*., (1983), Ahern *et al*., (2001), Gorgon *et al*., (2000), Squire *et al*., (1998), Brau *et al*., (1997), Franch *et al*., (1999), Cuenda *et al*., (1993), and Gordon *et al*., (2001)). ECC starts with the depolarization of the α-motor neuron. Depolarization involves a change in the polarity of the inside of the cell in reference to the outside. The inside of the axon is relatively negative to the outside (positive). The ions that maintain this gradient are Na^+ and K^+. Na^+ is higher outside the cell and K^+ is higher inside. The natural diffusion rate involves both ions migrating opposite of each other, and they do so because of diffusion. To maintain this polarity there is a Na^+/ K^+ pump that pumps Na^+ out of the cell and K^+ into the cell. When stimulated the cell becomes highly permeable to Na^+ and the net charge across the membrane changes thus depolarizing the axon. This is called an action potential, and it will propagate all the way to the axon terminus where it

triggers voltage gated Ca^{2+} channels. These channels allow Ca^{2+} to enter the terminus and bind to synaptic vesicles (contain neurotransmitter-Acetylcholine (Ach)) causing them to migrate to the presynaptic membrane and fuse, thus releasing Ach into the cleft.

The Ach binds to Ach receptors on the post-synaptic membrane of the muscle fiber, which causes Na^{+} channels to open and continue the depolarization process. The excess Ach in the cleft is degraded by Ach-esterase, which stops further depolarization. Next, the action potential travels across the sarcolemma and down the TT. The action potential eventually reaches the dihydropyridine receptor (DHPR- a voltage-gated channel – Fig. 6), which is physically linked to the ryanodine receptor (RyR- Fig. 6). The DHPR is comprised of several subunits, one of which is in direct contact with the RyR (α_{1s}) and has been shown to promote a conformational change that opens the RyR when the sarcolemma is depolarized. Another subunit of the DHPR is the α_2-δ, which is highly glycosylated has been shown to increase the amount of charge movement across the membrane, which facilitates depolarization. From the previous information it can be deduced that the α_2-δ subunit is closest to the t-tubule and the α_{1s} subunit is facing the RyR, which is located in the SR membrane (membrane bound vesicle that store Ca^{2+}). Associated with the RyR is a protein called FK506 binding protein (FKBP) and has been shown to stabilize the RyR in the closed or open state and to be coupled with the DHPR. However, when this protein is removed Ca^{2+} release is decreased due to loss of coordinated channel opening. Another protein associated with the RyR is calmodulin (CaM). CaM is a

Ca^{2+} binding protein that has binding sites on the RyR and has been shown to increase Ca^{2+} release at nmolar concentrations and inhibit at umolar concentrations. There are two states that CaM has been found on the RyR, one is the Ca^{2+} free state (nmolar [Ca^{2+}]) and the other is the Ca^{2+} bound state (μmolar [Ca^{2+}]). In the free state CaM enhances Ca^{2+} release from the RyR but in the bound state it inhibits release.

Ca^{2+} is released from the SR after the DHPR and RyR interact. Next, Ca^{2+} binds to troponin C (Tn-C) on the tropomyosin filament, which is attached to the actin filament. There are two other troponin molecules present on tropomyosin they are Tn-I and Tn-T. Tn-T achors the subunit to tropomyosin and TnI inhibits actin-myosin binding by holding tropomyosin over myosin binding sites on actin. Once Ca^{2+} binds to Tn-C there is a conformational change in TnI and TnT which causes tropomyosin to uncover the myosin binding site on actin. A summary of ECC can be found in Figure 7.

Before Ca^{2+} arrives the cross bridges are in a weak-binding state where ADP and Pi are bound to the myosin head. After Ca^{2+} binding and exposure of the binding site on actin, the myosin head binds to actin (strong-binding state) and at the same time the ADP and Pi are released, causing the myosin head to ratchet 45° (sliding the actin filament across the myosin- shortening the sarcomere) (Fig. 8). After that, ATP binds to the myosin head again and the cross-bridge is detached. Myosin is finally reactivated after ATP is hydrolyzed back to ADP and Pi as before in the weak binding state. This process continues as long as the [Ca^{2+}] is high enough (Fig. 9).

To ensure that [Ca^{2+}] doesn't stay too high or in the myoplasm too long, Ca^{2+} is shuttled back to the longitudinal portion of the SR via parvalbumin to expedite Ca^{2+} sequestering. Since the concentration of Ca^{2+} is higher inside the SR a pumping mechanism is needed to shuttle Ca^{2+} back into the SR. The protein used for this process is called the sarco-endoplasmicreticulum calcium ATPase (SERCA) (Fig. 10). This process requires ATP because it's going against a concentration gradient. The ATP is regenerated via creatine phosphate (PCr). It is thought that glycolytic enzymes found in the SR membrane produce the ATP that is need for this process. An important enzyme present on the SR membrane is glycogen phosphorylase (GP). GP is an enzyme that is involved in glycogen breakdown and is associated with the SR. GP is activated during exercise via adenyl cyclase activation. The activation of adenylyl cyclase stimulates the production of cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate activates protein kinase-A, which activates phosphorylase kinase by phosphorylating it. Phosphorylase kinase then activates GP, which in turn stimulates glycogen breakdown for energy production in the exercising muscle. At the same time kinase-A inactivates glycogen synthase by phosphorylation, which inactivates it, thus shutting off glycogen synthesis. The same signal that activates glycogen breakdown also inactivates protein phosphatase–I, which would normally dephosphorylate everything that was activated. It does this by activating phosphatase inhibitor protein, which binds to active phoshatase-I, renedering it inactive. It has been implied that GP may be bound to the SR via glycogen. Cuenda et al (1993) have

shown that SR glycogenolysis may cause GP to be released into the myoplasm. This loss in GP content should also manifest itself as a decreased GP activity, as was proven by Cuenda *et al.,* (1993). A decrease in GP activity would ultimately decrease energy production for ATPase activity. Another protein found in the SR membrane is phospholamban (Fig. 10). When phosporylated, this protein enhances Ca^{2+} uptake. Once the Ca^{2+} is back in the SR it is bound to calsequestrian (protein that loosely binds Ca^{2+}) to inhibit leakage. It is unbound when the RyR is opened again.

EXCITATION-CONTRACTION FAILURE AND GLYCOGEN DEPLETION

There are several sites between the TT and up to SR Ca^{2+} release that can cause EC coupling failure as a result of glycogen depletion. As stated earlier, the DHPR is a voltage gated ion sensor for ECC. This receptor is comprised of several subunits, one of which is in direct contact with the RyR (α_{1s}) and has been shown to promote a conformational change that opens the RyR (Ahern *et al*., 2001). According to Ahern *et al*., (2001), another subunit of the DHPR is the α_2-δ, which is highly glycosylated and has been shown to increase the amount of charge movement across the membrane (sarcolemma), which aids in depolarization. From the previous information it can be deduced that the α_2-δ subunit is closest to the t-tubule and the α_{1s} subunit is facing the RyR. If during glycogen depletion the α_2-δ subunit is deglycosylated then it is likely that the ability of the DHPR to respond to depolarization could be adversely affected. This could cause a slower depolarization or maybe even no depolarization.

Either way the ability of the α_{1s} subunit to interact with the RyR to release Ca^{2+} will be decreased if not inhibited completely, thus a decrease in Ca^{2+} release, which translates into decreased force.

Another component in the ECC process that could be adversely affected by glycogen depletion is the RyR. There has been no evidence linking glycogen to the RyR, however there is glycogen that is associated with the SR membrane that could possibly, when depleted or lowered, alter function by either a biochemical or structural change in the RyR. This change would ultimately be manifested as a decrease in Ca^{2+} release, which translates into decreased force. Another possible adverse affect of glycogen depletion could be less evident directly at the RyR but the outcome would be the same. Unpublished data has been shown that glycogen depletion inhibits Ca^{2+} uptake via decreased SR glycogen. This being the case [Ca^{2+}] would increase to μmolar levels, which causes a negative feedback on Ca^{2+} release via a Ca^{2+}-calmodulin (CaM) interaction at the RyR (to be discussed next). Net result, decrease in Ca^{2+} release, which translates into decreased force.

If glycogen depletion does not directly affect the function of the RyR a negative feedback mechanism may ultimately decrease Ca^{2+} release via increased [Ca^{2+}]. The following is a compilation of information taken from various journals describing the mechanism of CaM and Ca^{2+} (i.e. Moore *et al*., (1999), Fruen *et al*., (2000), Yamaguchi *et al*., (2001), and Hamilton *et al*., (2000)). CaM is a protein that has binding sites on the RyR and has been shown to increase Ca^{2+} release at nmolar concentrations and inhibit at μmolar concentrations.

There are two states that CaM can been found on the RyR, one is the Ca^{2+} free state (nmolar [Ca^{2+}]) and the other is the Ca^{2+} bound state (μmolar [Ca^{2+}]). In the free state CaM enhances Ca^{2+} release from the RyR but in the bound state it inhibits release. Glycogen depletion has been shown to compromise Ca^{2+} uptake, thus [Ca^{2+}] will increase, shifting CaM to its bound form, which decreases Ca^{2+} release.

FKBP is an integral protein of the RyR that has been shown to stabilize the RyR in the closed or open state and to be coupled with the DHPR (Gaburjakova *et al*., (2001), Carmody *et al*., (2001), Wagenknecht *et al*., (1997), and Ogunbunmi *et al*., (1998)). However, when this protein is removed (rapamycin) Ca^{2+} release is decreased due to loss of coordinated channel opening. Glycogen depletion may cause the FKBP to dissociate from the RyR, thus causing Ca^{2+} release to be decreased.

ISOLATED SR FRACTIONATION AND PERFORMANCE

Utilizing an isolated SR preparation to determine responses to contractile activity is a widely accepted practice. However, there are several factors that have to be assumed when interpreting results. One major assumption is that all necessary components remain intact and are functional. If this is not the case, any disruption in function of the SR could be attributed to missing proteins or enzymes (Chin *et al*., (1996). It has been shown by Xu *et al*., (1995) that the SR has glycolytic enzymes associated with the membrane that are used to produce and regenerate (PCr) ATP for various functions (e.g. Ca^{2+} uptake). This ATP that

is formed from endogenous sources is more efficient at supporting Ca^{2+}-uptake than exogenous ATP. If during the isolation process these enzymes are lost or reduced, the ATPase activity will be lower, thus Ca^{2+} will stay in the myoplasm longer and possibly disrupt Ca^{2+} release. Causing a decrease in force production.

If CaM were washed away the activation of the RyR would be affected causing a decrease Ca^{2+}release rate thus, producing less force (Moore *et al*., (1999), Fruen *et al*., (2000), Yamaguchi *et al*., (2001), and Hamilton *et al*., (2000)). Also, CaM activated kinases (phoshorylase kinase – activates glycogen breakdown and myosin regulatory light chain kinase-likely to be responsible for the ratcheting of the myosin head) will display decreased or no activity. If FKBP is lost during isolation Ca^{2+} release will be decreased.

Parvalbumin is a protein that is involved with shuttling Ca^{2+} to the Ca^{2+} uptake pump by binding to it in the myoplasm. If this protein is lost during isolation, Ca^{2+} could stay around too long and inhibit release from the RyR, as stated earlier. Causing a decrease in force production.

Phospholamban is another integral protein found within the SR membrane that plays an important role in Ca^{2+} uptake. When it is phosphorylated it enhances uptake and when it is dephosphorylated it inhibits uptake (Stokes (1997), Asahi *et al*., (2000), Kadambi *et al*., (1997), Bhogal *et al*., (1998), and Colyer (1998). This is another protein that will be affected by the loss of kinases (PKC, PKA-also important in mobilizing G-1-P for energy production, PKG, CaM-

kinase). If this protein is simply lost during isolation or preparation of the sample, uptake will be decreased causing a decrease in force.

FACTORS DETERMINING FORCE PRODUCTION

The following section was summarized from various sources. For more detailed descriptions see Lieber *et al*., (1992), Berne *et al*., (1998), Aidley (1971), and Peachey *et al*., (1983). When determining force production by a muscle there are several factors involved. The major variable in force production is the size of the muscle. The larger the muscle is (cross-sectional area) the more force it will produce. Another factor is the type of muscle it is (fiber type). Fast muscles produce more force than slow muscles. Innervation ratio (number fibers/motor unit) determines force output as well. The higher the innervation ratio the more force. Motor unit type also determines force. Fast fatigue motor units (FF) produce the most force the fatigue resistant (FR) followed by slow (S) (FF> FR >S). The number of motor units recruited determines force. The more motor units recruited the more force. Size of axon determines force as well (Henneman Size Principle). Motor units with larger axons produce the most force. Coincidentally, these larger axons correlate with FF motor units and the small axons with S. The frequency at which the muscle is stimulated (Force-Frequency curve) determines force. The higher the frequency the more force (fused tetani). The length that the muscle contracts at (Length-Tension curve) determines force. At shorter lengths force is low due to either actin filaments bumping into z-discs or actin-actin bumping. At over-stretched lengths force is

also low because there is little or no actin-myosin interaction. The most force is developed when actin-myosin interaction is optimum, which is at a length of about 2.0 μm. The amount of Ca^{2+} released from the SR determines force production also (Force pCa curve). The more Ca^{2+} released the more force, up to a point then there is no change in force. The speed at which a muscle contracts determines the force also (Force-Velocity curve). At fast speeds force production is low. As the speed slows down, force increases. This curve goes from eccentric contractions (negative velocity & lengthening of the muscle) to isometric contraction (zero velocity & no length change in muscle) to concentric contractions (fast velocity & shortening of the muscle).

SKELETAL MUSCLE FATIGUE

Central Fatigue

Figure 11 provides a summary of the possible sites of muscular fatigue. Voluntary contractions begin at the level of the central nervous system and terminate with force production via actin-myosin interaction inside the muscle fiber. As can be seen, there are several sites where fatigue can occur before the signal reaches the muscle. These types of fatigue are often described as central fatigue. They are characterized by a lack of motivation on the subject's part, which translates into less motor unit recruitment that is necessary for a maximal contraction.

The concept of central fatigue as a reason for lack of force production indicates that the muscle itself has not undergone a change but the individual

simply isn't capable of putting forth a maximal effort. This lack of motivation can be attributed to decreased low blood glucose levels, which is caused in part by liver glycogen depletion. If blood glucose levels are low, the pancreas releases glucagon, which stimulates the liver to break down glycogen stores for immediate use throughout the body, thus increasing blood glucose levels. If however the liver is depleted of glycogen the normal response to low blood glucose will be impaired, causing the lack of motivation. Muscle glycogen stores are not available to provide glucose to systemic circulation because once it (glucose) enters the muscle it is phosphorylated (glucose-6-phosphate) by a kinase, which prevents it from getting out.

Ikai *et al.,* (1961) have shown that a simple shout during exertion could increase force. Ikai *et al.,* (1969) did more research that supported their previous findings in which electrical stimulation of a muscle that had been voluntarily fatigued increased force. Asmussen *et al.,* (1978) have shown that when either a physical diversion, consisting of the contraction of non-fatigued muscles or mental diversion (i.e. mental arithmetic), was used between fatigue bouts, work output was greater than when nothing was done between bouts. These studies suggest that the upper limit of voluntary strength is limited by the central nervous system.

Peripheral Fatigue (Fig.11)

When considering the cascade of events in the contraction process of the fibers (Fig. 7), there are a number of alterations that may occur resulting in a decrease in force. The first is impaired neuromuscular transmission. Merton

(1954) has shown that the action potential still reaches the neuromuscular junction despite fatigue, which suggests that there may be a depletion of acetylcholine or reduced excitability of the motor end plate. On the other hand Bigland-Ritchie (1981) has shown that electrical activity at the neuromuscular junction is the same as in the muscle fiber, which indicates that the signal has not been decreased. If this is the case then the breakdown that is responsible for the lack of force during fatigue occurs in the muscle hardware (i.e. sarcoplasmic reticulum (SR) and actin-myosin). Since it has been shown that the SR and actin and myosin are dependent on ATP for proper muscle function it is likely that this is where the problem occurs. Actin and myosin depend on ATP both for the activation and dissociation of the cross-bridge cycle. The SR depends on ATP for the sequestering of Ca^{2+}. Jones (1981), Roberts *et al*., (1989), and Sahlin *et al*., (1992) all agree that one sign of fatigue in isometric contraction is a longer relaxation time. This longer relaxation time could be due to a slower cycling of the cross-bridges or to Ca^{2+} not being pumped back to the SR fast enough. Based on this information, fatigue can be viewed as the result of an imbalance between the ATP requirements of the muscle and the ATP generating capacity of the muscle. It is at this point (ATP availability) where most of the research has been done to determine the causes of fatigue.

GLYCOGEN AND ENDURANCE PERFORMANCE

The amount of muscle glycogen stored by an individual is controlled by their level of activity, training status, and the dietary content of carbohydrates

(CHO) (Costill *et al*., (1988)). Untrained subjects, who are rested and well nourished, have been found to possess muscle glycogen levels ranging from 70-110 mmol glucosyl units/kg (Blom *et al*., (1986)). Endurance trained athletes, on the other hand, have muscle glycogen levels ranging from 140 to over 230 mmol /kg (Costill *et al*., (1980)). These initial muscle glycogen values are directly related to ones ability to sustain an exercise bout for times longer than 1 hour above 70% VO_2max. The higher the initial values are the longer the subject will be able to exercise at that intensity.

Bergstrom *et al.,* (1967) have shown that individuals with an initial glycogen content of about 100 mmol /kg could tolerate a 75% VO_2max workload for 115 minutes. Subjects that were fed a diet low in CHO's had an initial glycogen level of 35 mmol/kg and were only able to tolerate a 75% VO_2max workload for 60 minutes. On the other hand, individuals that were fed a diet rich in CHO's for 3 days had initial glycogen values of 200 mmol/kg and were able to tolerate the same exercise bout to exhaustion for 170 minutes. This study suggests that the level of muscle glycogen strongly influence the fatigue process.

DEPLETION HYPOTHESIS

The idea that during prolonged exercise the energy source (adenosine triphosphate, ATP) will eventually be depleted seems at first glance to be logical. Since part of the contraction process is an energy requiring system, when all the ATP is used up the result should be mechanical failure of the contractile apparatus. This is not the case because, it has been shown that free ATP levels

remain fairly constant and only show minimal decreases (Green *et al*., (1990), Byrd *et al*., (1989), and Vollestad *et al*., (1988)). Xu *et al.,* (1995) have demonstrated that SR vesicles have glycolytic enzymes (aldolase, GAPDH, PGK, phosphoglyceromutase, enolase, and pyruvate kinase) that are bound to the membrane that are just as, if not more effective in producing ATP to support Ca^{2+} uptake than are exogenous ATP sources (Fig. 12). This being the case, then maybe the ATPase activity associated with the SR membrane is more dependent on ATP that is generated from glycolysis than from free ATP within the cytosol of the SR. Hence, low levels of substrate (glycogen) could vastly affect this system which fuel glycolysis and provide ATP for Ca^{2+} uptake.

SR DYSFUNCTION HYPOTHESIS

Another and more feasible mechanism of causing fatigue is SR dysfunction. It has been shown that after prolonged intense exercise bouts there are some intrinsic alterations to the hardware that is involved in muscle contraction (SR and contractile proteins) that ultimately leads to a reduction in force output (Byrd *et al*., (1989), Westerblad *et al*., (2000), Favero (1999), Williams (1997), Williams *et al*., (1998), Williams *et al*., (1995) and Williams *et al*., (1993)). This damage manifests itself in the form of swelling, tearing and misalignment of proteins, and decreased Ca^{2+} release. Ultimately the production of force by the contractile apparatus is governed by the release of Ca^{2+} from the SR. There is a positive correlation between Ca^{2+} and force production. If the Ca^{2+} release channel is damaged then there could either be an

immediate influx of calcium into the cytosol causing an uncontrollable contraction or the channel could be inhibited in releasing Ca^{2+}. It is the later that has been shown. Muscles that have been exercised to exhaustion all show a decreased [Ca^{2+}] release as compared to a non-exercised muscle (Williams *et al.,* 1995). It has been shown that Ca^{2+} release can be restored after damage has occurred due to exercise by exposing the SR to caffeine (Williams *et al.,* 1993). Caffeine restores Ca^{2+} release and force to near normal levels which shows that the channel itself is not damaged but the SR has probably undergone some type of conformational change which prevents the release of calcium at the point of exhaustion. These results also lead to the notion that this reduction in calcium release is more of a protective mechanism. By reducing the amount of calcium released, ATP usage is reduced thus, sparing ATP and preventing any irreversible damage to the contractile proteins (Williams *et al.,* 1995). This decrease in calcium release appears to be a response mechanism to damage that occurs to the contractile hardware and not as a result of fatigue. The fact that Ca^{2+} release can be restored after a fatiguing bout, ATP levels are not depleted, NMJ function is unaltered, and glycogen stores are not completely depleted leads you to believe that the ECC system has a “protective: mechanism built in so that irreversible damage won’t occur. During fatigue, Ca^{2+} uptake is inhibited, thus Ca^{2+} stays in the myoplasm longer and causes damage to the contractile apparatus (misalignment of filaments, swelling, and tearing of proteins) by activating calpain and other Ca^{2+} activated proteases (phospholipase) (Gissel 2000). The longer Ca^{2+} stays around the more damage is done. The longer the

system is actively producing force without adequate clearing of Ca^{2+} the more likely ATP could be depleted which would cause a rigor bond and leave the muscle locked in the strong binding state. So in order to prevent this irreversible damage from occurring. Ca^{2+}-release is inhibited once [Ca^{2+}] reaches umolar levels.

GLYCOGEN AND THE SR

Of the two theories listed above, energy depletion as it relates to the production of ATP from glycogen appears to be more closely linked to fatigue. There are two primary stores of carbohydrates: glycogen stored in the muscle and liver glycogen, which has to be transported to the muscle in the form of glucose. Of these two sources muscle glycogen has been regarded as the limiting factor during prolonged exercise and endurance. Xu *et al.,* 1995 have demonstrated how specific the SR is designed to utilize glycogen and how the SR could be primarily dependent on muscle glycogen as opposed to free ATP in the cytosol. It has also been demonstrated that muscle glycogen concentration is positively correlated with endurance-exercise capacity (Ahlborg *et al*., (1977), Bergstrom *et al*., (1967), Hermansen *et al*., (1967) and Karlsson *et al*., (1971)). Meaning the higher the beginning levels of glycogen are, the longer an exercise bout could be endured and vice-versa. Thus, the depletion of muscle glycogen stores is regarded as a primary reason for fatigue during prolonged exercise Foster *et al.,* (1986)

Chin *et al.,* (1997) have also demonstrated the importance of glycogen in time to fatigue. Chin *et al.,* (1997) conducted an experiment where a muscle was exercised to exhaustion then bathed in a 5 mM solution of glucose for 60 minutes then exercised to exhaustion again. The two fatigue curves were identical. The experiment was then repeated except this time instead of allowing the muscle to replete itself with glucose it was bathed in a solution with no glucose then stimulated again. In this case, fatigue kinetics was accelerated by more than 50%. Barnes *et al*., (2001) did a similar experiment using skinned muscle fibers and reported similar results. This indicates the important role that glucose plays in endurance. When these data are combined with that of Xu *et al.,* (1995) it is easy to consider that there may be a direct link between glycogen and SR function. If the SR is specifically designed to utilize glycogen as a source of energy production then any part of the system that depends on ATP from glycolysis (e.g. Ca^{2+} uptake and release) should be adversely affected if the source of glycogen is depleted.

GLYCOGEN AND FORCE PRODUCTION

Not much has been done in regards to glycogen and force production. It has been suggested that lower glycogen levels will cause lower force generation, particularly during prolonged exercise. This assumption is based on several factors. If in fact the SR is designed to use endogenously produced ATP from glycolytic enzymes in the membrane that breakdown muscle glycogen then a decrease in glycogen will impair ATP production. Thus, Ca^{2+}-uptake will be

decreased. This will ultimately cause the SR to go into “protective mode,” which leads to decreased Ca^{2+}-release to spare ATP utilization and prevent irreparable damage to the muscle by Ca^{2+} activated proteases. Since force production is dependent on [Ca^{2+}], any depression in Ca^{2+}-release will cause less force. Hence, glycogen depletion should ultimately cause less force production. It was my intention to identify this link. Unfortunately, correlational data do not imply cause and effect. No definitive explanation to link glycogen depletion, fatigue, and force production has previously been identified.

SUMMARY

There is no direct evidence suggesting that glycogen depletion has detrimental effects on SR function. It was my intention to examine whether reduced muscle and SR glycogen content would limit SR function or muscle performance in rested muscle. Two groups of female Sprague-Dawley rats were fasted for 24 hr and exercised for 90 min (21 m/min at 10% grade) to reduce muscle glycogen; rats fasted after exercise formed the low glycogen (LG) group. Rats in the high glycogen (HG) group were allowed free access to food and a 5% sucrose solution. After glycogen depletion was determined, force production and SR function was accessed. Finally, endurance performance was tested. The first aim of this study was to determine if the proposed exercise protocol would cause an adequate decrease in total muscle and SR glycogen. The second and most important aim of this study was to determine the effect of this glycogen depletion on muscle and exercise performance.

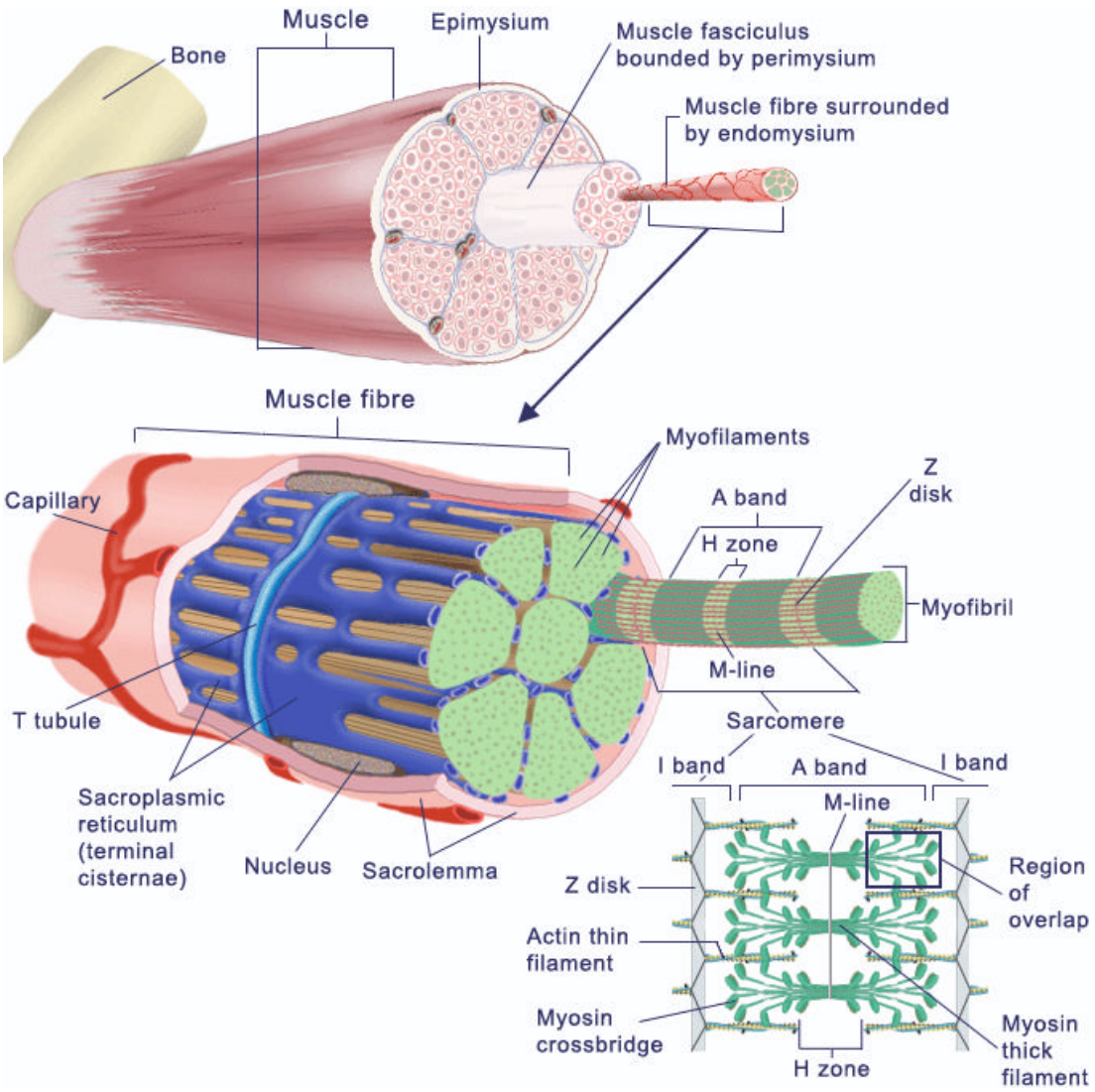

Figure 1. Skeletal Muscle Structure. Modified from Moffett et al., *Human Physiology*, 2nd edition, Mosbey, 1993, p 293. Copied from:

http://www.mmi.mcgill.ca/Unit2/Mandl/lect7musclestructureandfunctiona.htm

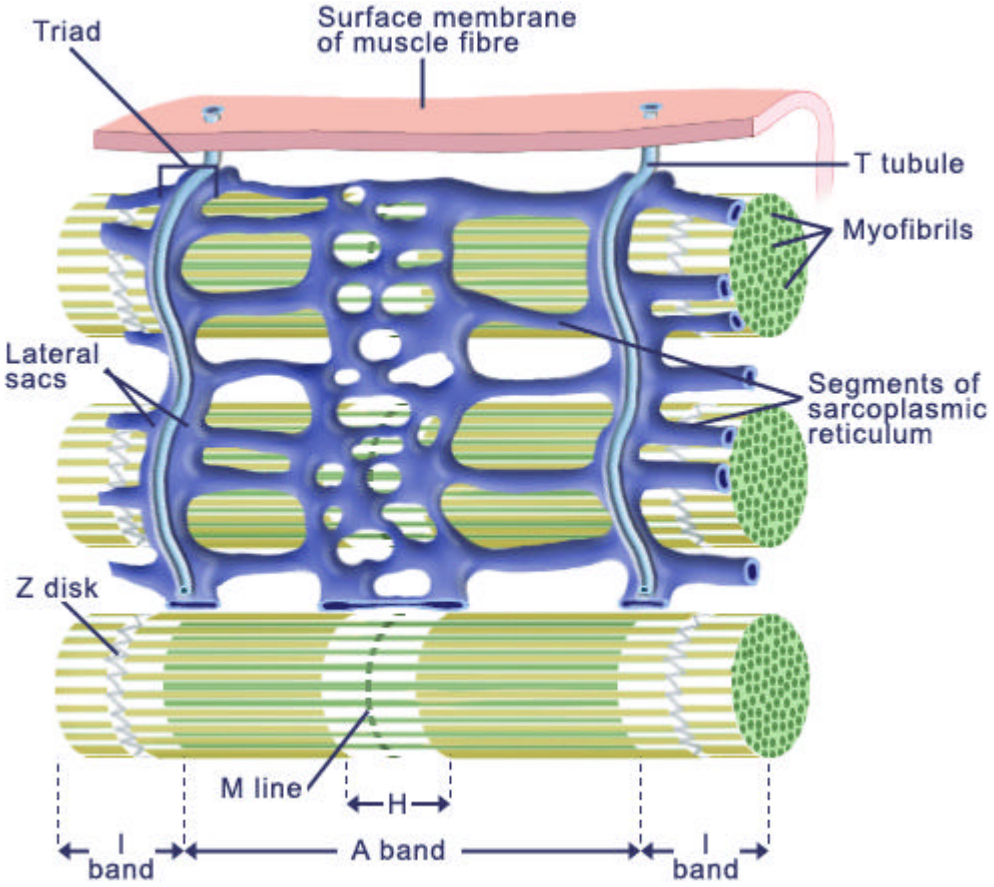

Figure 2. Striation pattern of skeletal muscle. Modified from Fig. 10-8 in Sherwood, p. 222. Copied from:

http://www.mmi.mcgill.ca/Unit2/Mandl/lect7musclestructureandfunctiona.htm

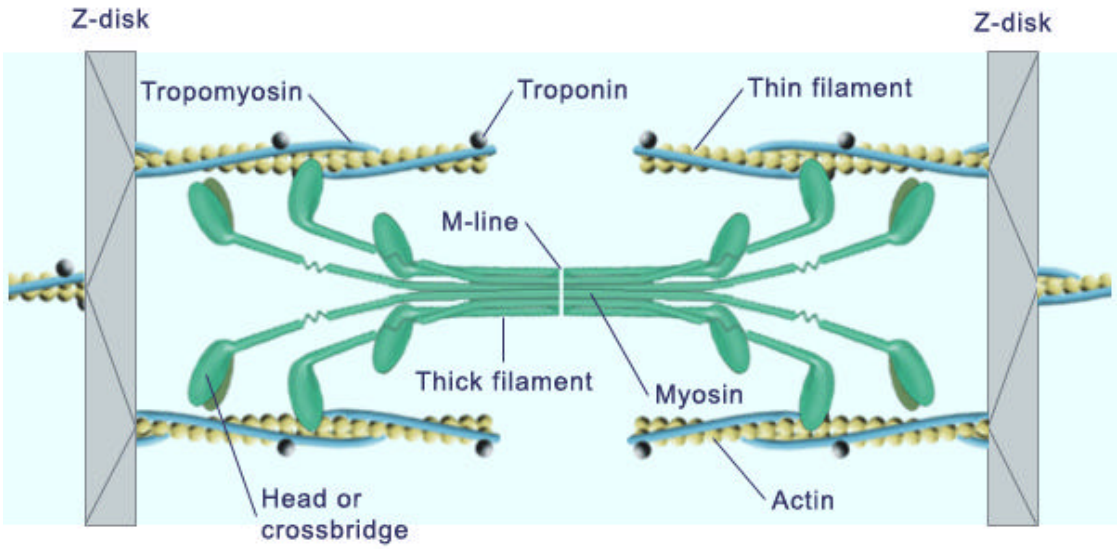

Figure 3. Diagram of sarcomere structure in skeletal muscle. Modified from Fig. 11-2 in Berne and Levy, *Principles of Physiology*, Mosbey, 1990, p. 155. Copied from:

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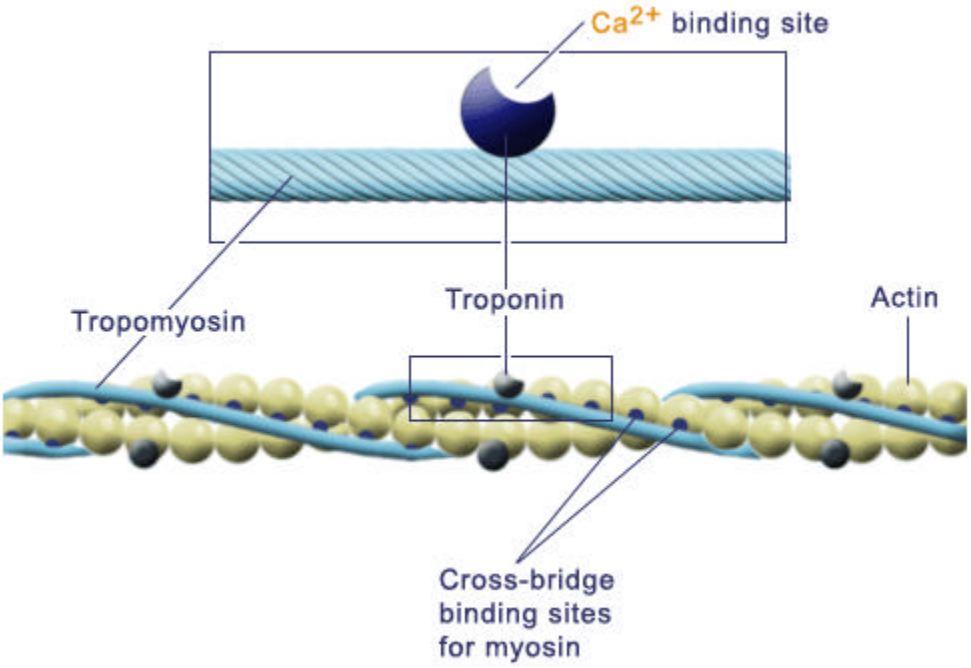

Figure 4. Diagram of actin filament. Modified from Fig. 11-13, in Vander, Sherman and Luciano, Human Physiology, 6th edition, Mcgraw-Hill, 1994, p. 313. Copied from:

http://www.mmi.mcgill.ca/Unit2/Mandl/lect7musclestructureandfunctiona.htm

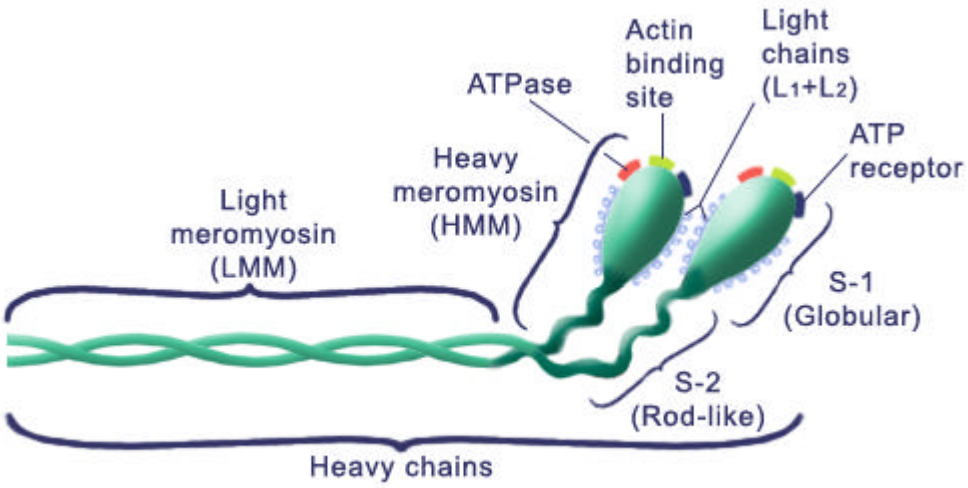

Figure 5. Diagram of the myosin molecule. Copied from:

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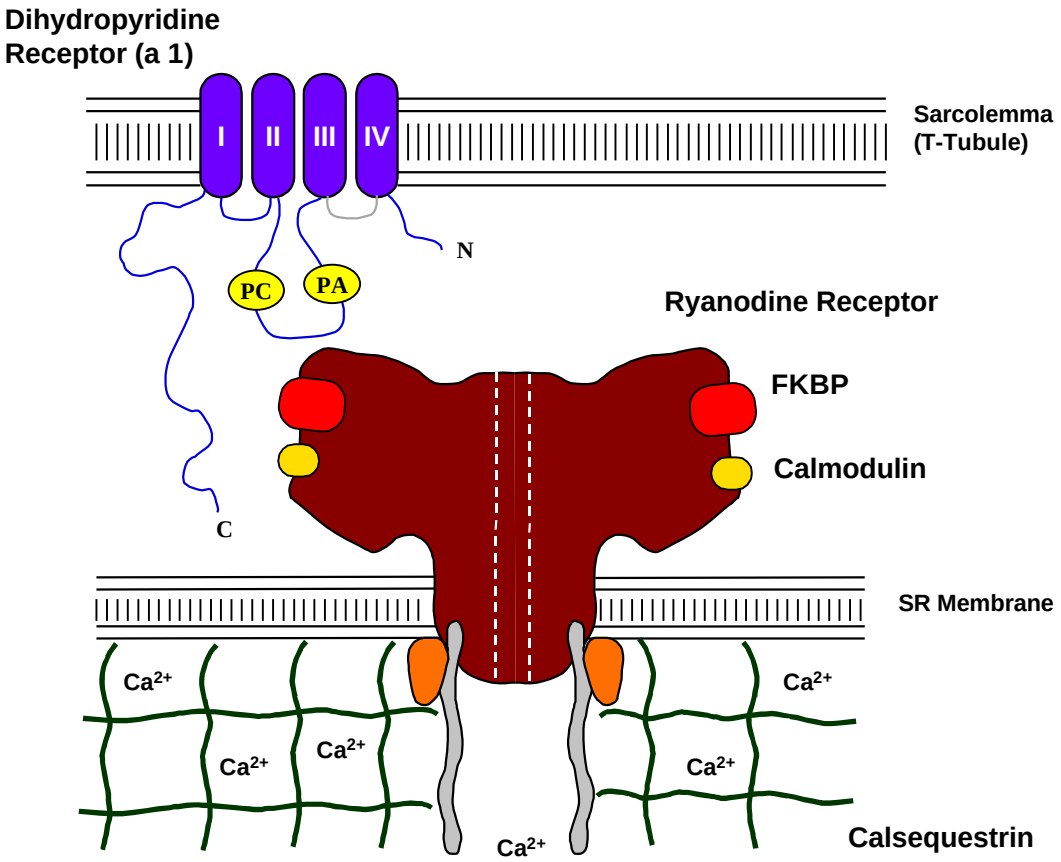

Figure 6. Diagram of the TT-SR junction.

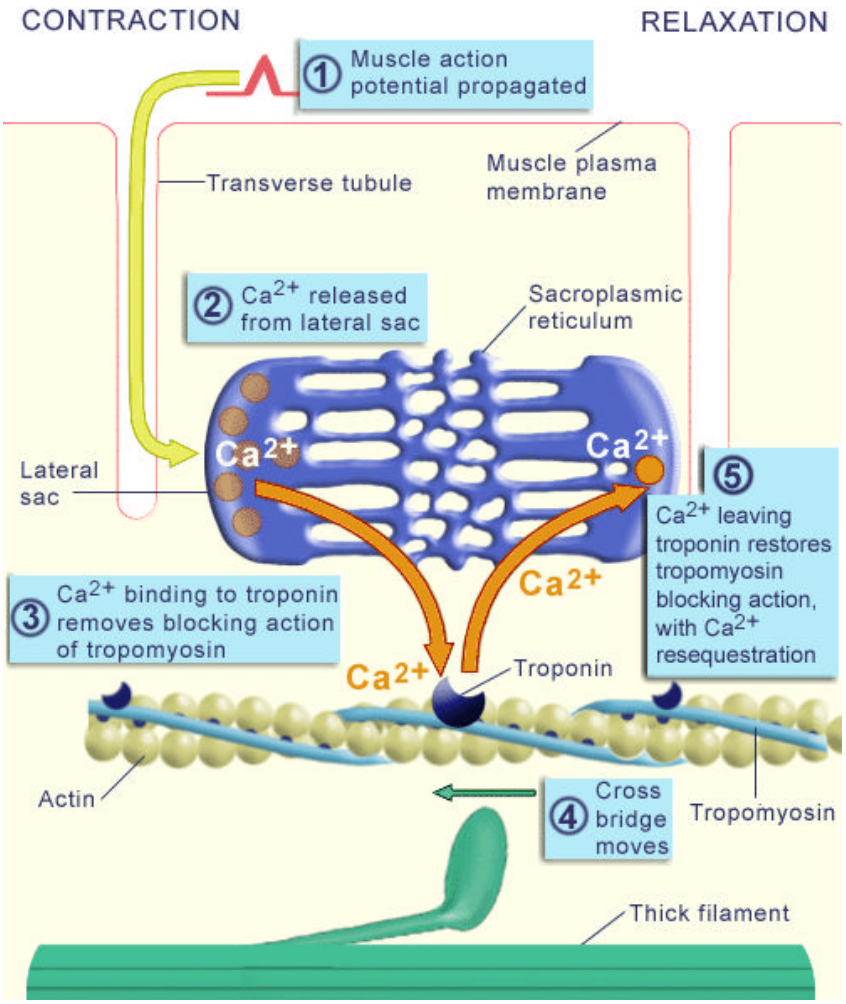

Figure 7. Diagram of ECC in skeletal muscle. Modified from Fig. 11-16 in Vander et al., p. 316. Copied from:

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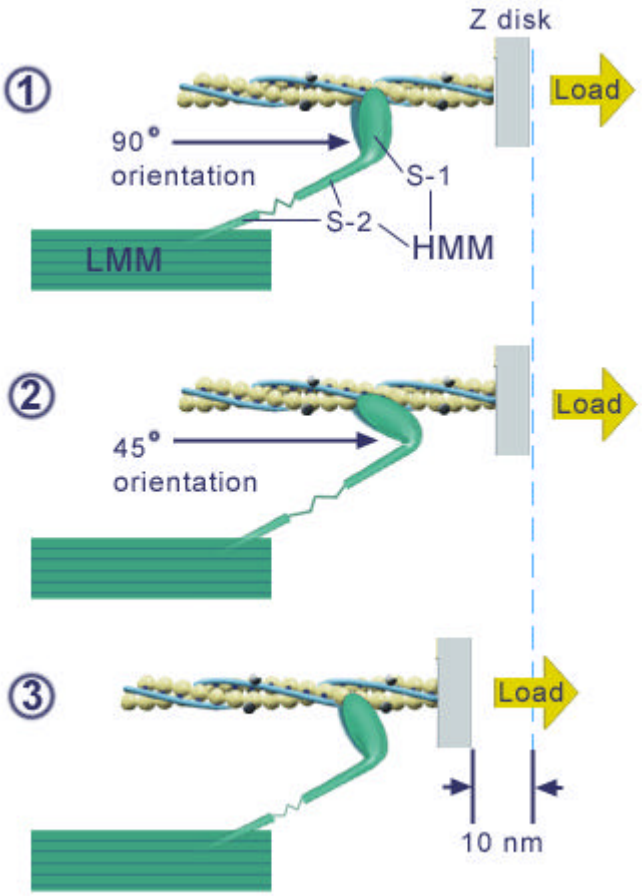

Figure 8. Mechanism of force development by myosin in skeletal muscle. Modified from Fig 17-7b in Berne and Levy, Physiology, 3rd ed., Mosbey, 1993, p. 287. Copied from:

http://www.mmi.mcgill.ca/Unit2/Mandl/lect7musclestructureandfunctiona.htm

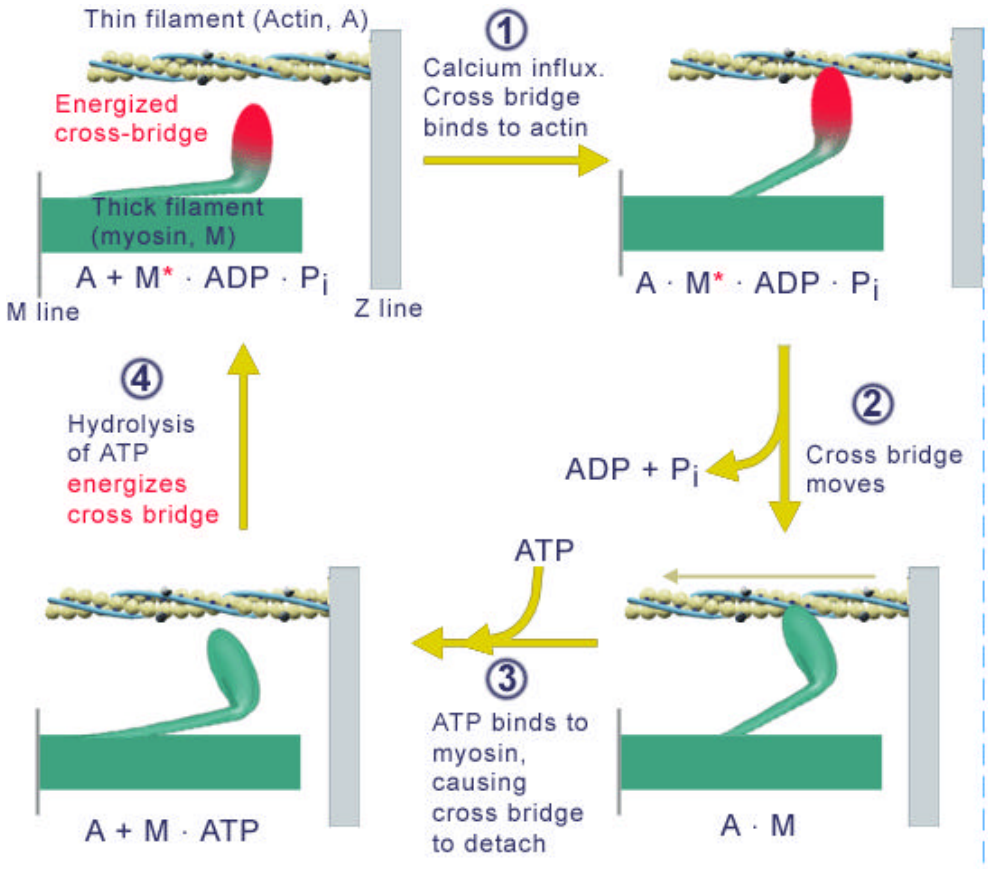

Figure 9. Cross-bridge cycle in skeletal muscle. Modified from Fig. 11-12 in Vander et al., p. 312. Copied from:

http://www.mmi.mcgill.ca/Unit2/Mandl/lect7musclestructureandfunctiona.htm

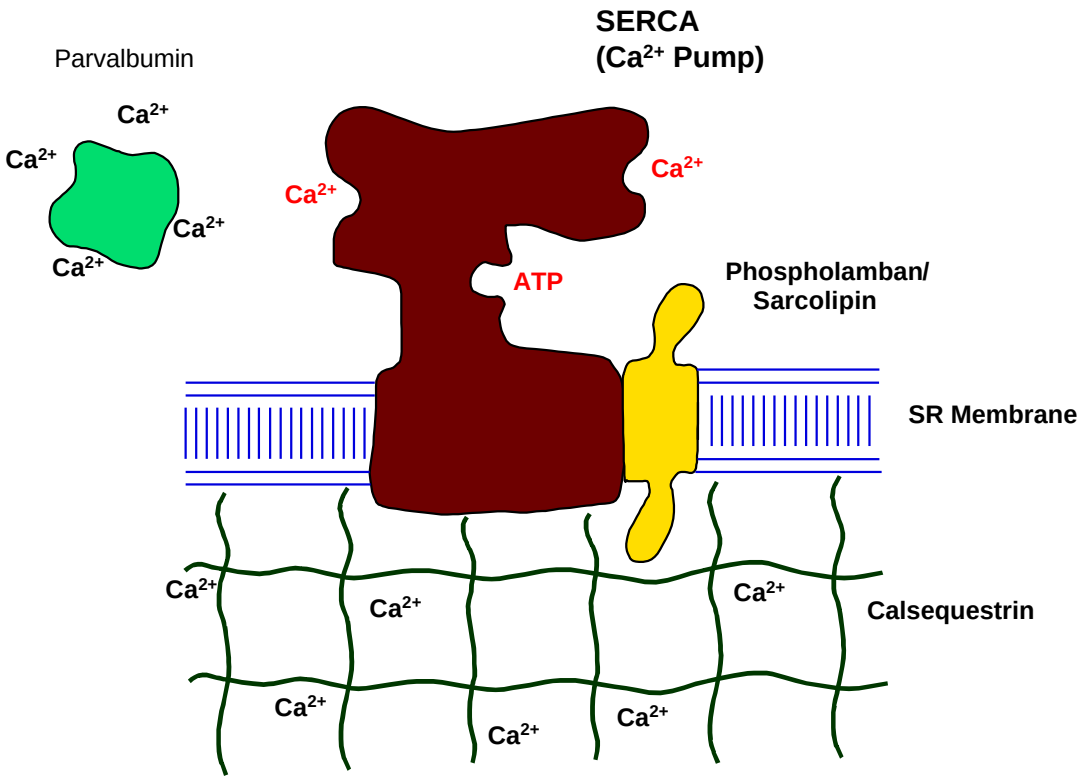

Figure 10. Proteins associated with the SR Ca^{2+} ATPase.

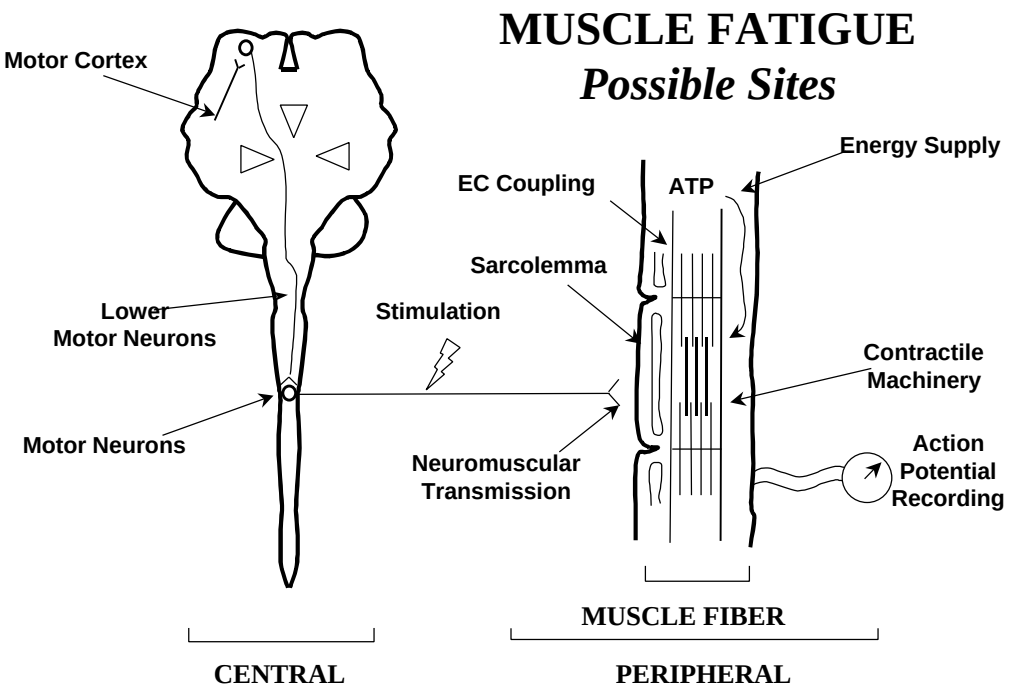

Figure 11. Possible sites of muscle fatigue. From Bigland-Ritchey, 1991.

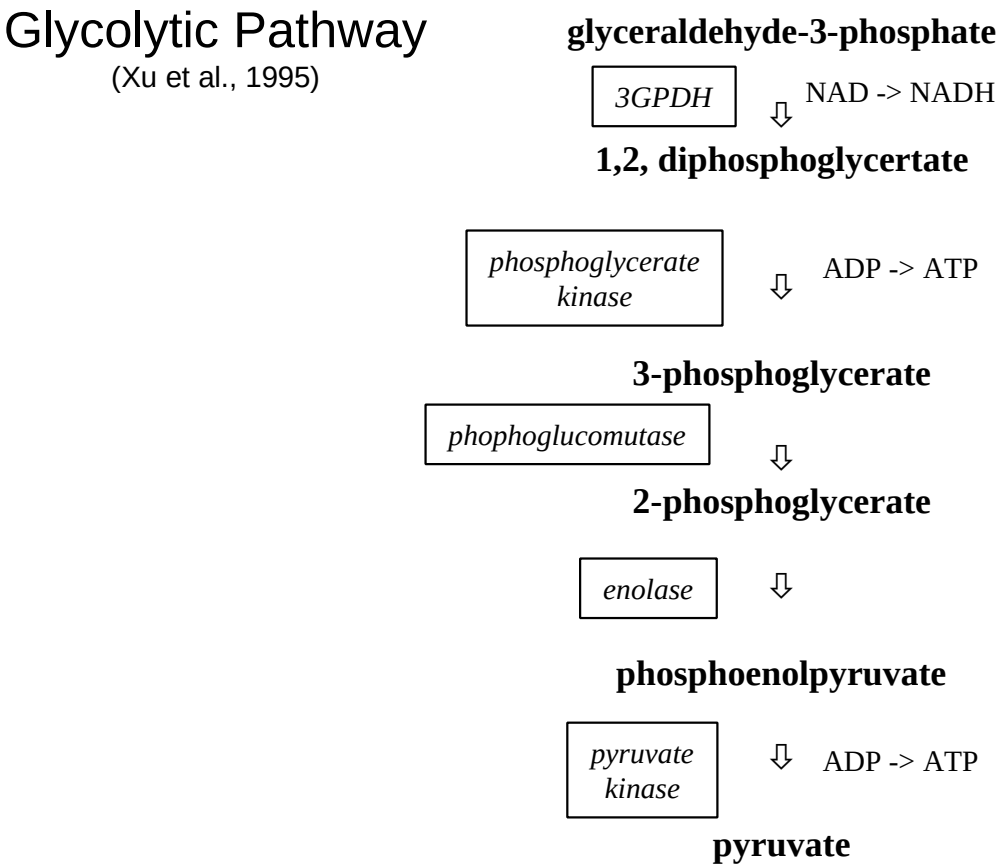

Figure 12. Glycolytic pathway of ATP synthesis.

CHAPTER THREE:

RESEARCH DESIGN AND METHODS

ANIMALS

Female Sprague-Dawley (Harlan Sprague-Dawley, Indianapolis, IN) rats weighing, 225 ± 70 gm were housed one per cage in the Virginia Tech Lab Animal Resources facility and exposed to a 12:12-h light-dark cycle. They were fed ad libitum (Purina Rodent Laboratory Chow) and water until the beginning of experiment. Exercise bouts and animal sacrificing were done between 8:00 – 9:00 a.m. The Virginia Tech animal use committee approved all animal treatment protocols.

GLYCOGEN DEPLETION PROTOCOL

Several protocols were used to create two groups of rats with high and low muscle glycogen content. Results of these efforts are shown in Figure 13 in chapter 4. As can be seen, these approaches failed to noticeably alter muscle glycogen in the treatment groups. Because liver glycogen is one of the primary sources for fuel, liver glycogen was measured to determine if this was a source for recovery from the glycogen depletion bout in the LG animals. Liver glycogen levels in the LG and HG groups were found to be 14.80 ± 2.23 μmol/g and 269.78 ± 67.22 μmol/g respectively. Based on the preliminary data, a protocol of fasting and exercise was chosen for this study. Rats were divided into two groups, either low glycogen (LG) or high glycogen (HG) based on the diet received after exercise. Twenty-four hours before the exercise bout food was removed from the cages then the animals were exercised for 90 minutes on a treadmill at 21 m/min at a 10% grade. After that the animals were either given

water only (LG) or standard rodent chow (ad lib) plus a 5% sucrose solution to enhance glycogen repletion (HG). Twenty- four hours later animals were either sacrificed for tissue harvesting or prepared for the stimulation protocol or participated in a timed treadmill run to exhaustion. Figure 14 describes both glycogen depletion protocols.

EXHAUSTION BOUT

The time to exhaustion was assessed using the same parameters as the first treadmill run (21 m/min at 10% grade), however the animals were allowed to run as long as they could. Exhaustion was determined by the inability of the animal to continue running on the treadmill despite external encouragement.

TISSUE HARVESTING

Animals were anesthetized using a Ketamine/Xylazine (60 mg/kg and 7.5 mg/kg) injection. Upon reaching a surgical plane of anesthesia the animals were either prepared for the in situ stimulation protocol or the gastrocnemius/plantaris muscles were extracted for total muscle or SR glycogen content or histochemistry. Rats were then euthanized with an overdose of the Ketamine/Xylazine solution.

SURGERY, STIMULATION PROTOCOL AND FORCE MEASUREMENTS

After the animal was anesthetized (Ketamine/Xylazine - 60 mg/kg and 7.5 mg/kg injection), preparation for the in situ stimulation protocol began. After the animals hindlimb was shaved and the epithelium was cut from the leg, the sciatic nerve was surgically exposed so that the stimulation electrode could be easily attached with minimal manipulation of the nerve. Next, a suture was tied and glued at the insertion of the calcaneus tendon, and the distal portion of the calcaneus bone was cut. The anterior portion of the leg was then separated from the posterior by cutting the tissue away from the tibialis anterior and groin area. The soleus muscle was then cut at the insertion point so as to not contribute to force production. The posterior compartment of the leg was separated up to the back of the knee, making sure to sever any nerves that ran to the anterior portion of the leg (this prevents the anterior muscles from contracting during the stimulation protocol and contributing to force production). The animal was then placed in a prone position in the stimulation chamber with the knee and distal end of the tibia securely clamped to prevent movement. The free end of the suture was then tied and glued at the knot to an isometric force transducer. The exposed sciatic nerve was then gently clamped with the stimulation electrode. The entire exposed surgical area was then covered with plastic wrap after a few drops of mineral oil was placed in and around the incision to the nerve. To maintain body temperature (37°C), a heating lamp was placed directly over the stimulation chamber. A temperature probe was placed directly on the surface of the exposed tissue, under the plastic wrap, to monitor temp.

After the appropriate length-tension relationship was established to produce a maximal twitch contraction, the stimulation protocol was initiated. Tetanic contraction were elicited using a Grass S48 stimulator, and force measurements were measured, displayed, and recorded using a Harvard apparatus isometric transducer, Tecktronix 2201 oscilloscope, and Labtech Notebook Pro. Twitch and tetanic contraction were measured pre and post stimulation protocol. The fatigue protocol for the in situ preparation was elicited at 20 Hz for 333 ms every 1 sec for 30 min. Tissue harvesting began immediately following post twitch and tetanic measurements. Tissues were then prepared for histochemical processing. Rats were then euthanized with an overdose of the Ketamine/Xylazine cocktail. A typical force response curve of the in situ stimulation protocol can be seen in Figure 15.

GLYCOGEN ANALYSIS

Tissue Preparation

Gastrocnemius and plantaris muscles from both groups were removed and homogenized with three, 20-s bouts (VirTis VirTishear) in 5 ml of 0.6 M perchloric acid solution per gram of tissue wet weight. Immediately after homogenization, 0.2 ml of this suspension was removed and stored at -80°C for glycogen hydrolysis and the remainder was centrifuged and stored at -80°C for determination of tissue glucose later. Whole muscle glycogen was then measured by digesting glycogen with glucoamylase then determining glucose

content spectrophotometrically as described by Keppler *et al*., (1984). A modification of this approach was used to measure SR glycogen.

Glycogen Hydrolysis

The 0.2 ml of sample that was set aside was mixed with $KHCO_3$ (43 mmol/l) and a amyloglucosidase solution containing 174 mmol/l acetic acid and glucoamylase (8.7 kU/l) (pH 4.8). Amyloglucosidase enzymatically degrades glycogen into glucose. Then the tube was stoppered and incubated with shaking at 40° for 2 hours, and then 1 ml of perchloric acid was added. The solution was vortexed then centrifuged for 10 minutes and the supernatant was used for glucose determination.

Glucose Determination

Fifty microliters of each sample was added to 1 ml of the ATP/NADP/G6P-DH/buffer solution (ATP - 1 mmol/l, NADP 0.9 mmol/l, TEA buffer 285 mmol/l, $MgSO_4$ 4 mmol/l, and G6P-DH 0.7 kU/l). The solution was mixed well and allowed to sit for 5-8 minutes then read with a spectrophotometer at 339nm (A_1). Five microliters of hexokinase solution (1.3 kU/l) was added and incubated for 5-10 min and absorbance read again (A_2). A_1 was subtracted from A_2 to get ΔA. Glycogen was calculated using this formula: $\Delta A_{gly} * 330.6 - \Delta A_{glu} * 20.1$.

SR ISOLATION

The following procedure was adapted from Williams *et al*., (1998). Gastrocnemius and plantaris muscles were removed and homogenized then

differential centrifugation was used to isolate SR vesicles. Specifically, the 8,000-12,000 g (heavy SR, HSR) and 12,000-49,000 g (light SR, LSR) fractions were collected. Tissues were then homogenized in 5 vol (wt/vol) of buffer containing 20 mM HEPES, 0.2% Sodium Azide (NaN_3), and 0.2 mM phenylmethylsulfonyl fluoride (PMSF) (pH 6.8). Using scissors, the tissue were minced as finely as possible, homogenized (3x 30-s), and centrifuged at 8,000 g (8,234 rpm) for 15 min. After the supernatant was filtered through 4 layers of pre-soaked (homogenization buffer) and wrung out gauze it was measured in a graduated cylinder and transferred to a clean centrifuge tube. Six hundred mM KCl (0.0447g KCl/ml) was added and the tube was inverted several times until KCl was in solution to solubilize any actomyosin complexes that were left. The supernatant was then centrifuged for 45 min at 12,000 g (10,360 rpm). The supernatant from this spin was carefully transferred to another clean tube (do not disturb pellet) and centrifuged at 49,000 g (21,000 rpm) for 60 min (LSR). The remaining pellet (HSR) was gently scraped from the tube after 500 µl of storage buffer (10 ml homogenization buffer plus 150 mM KCl and 300 mM sucrose) was added. Using a transfer pipet, the pellet was gently sucked up and down until the mixture was mostly homogeneous. This re-suspension was transferred to a 2 ml Potter-Elvehejem homogenizer then gently homogenized for approximately 15-20 up and down strokes, making sure not to cause bubbles, then 20 µl was transferred to an eppendorf tube for protein determination later (Bradford dye-binding assay adapted by Bio-Rad using BSA as a standard). The remainder

was aliquoted into 2-3 tubes and store at -80°C. This process was repeated for the final pellet (LSR), discarding the supernatant.

When preparing SR for glycogen analysis 1mM ethylenediaminetetraacetic acid (EDTA) was added to the homogenization buffer and the 300mM sucrose was omitted from the storage buffer as not artificially alter glycogen measurements.

SR GLYCOGEN ANALYSIS

A modification of the Keppler *et al*., (1984) muscle glycogen analysis was used to measure SR glycogen (Lees *et al*., 2001). Since these were purified samples it was not necessary to account for extracellular glucose as with the muscle glycogen samples. A volume containing 50 μg of re-suspended SR was incubated, coupled with shaking, in 0.5 ml of a 174 mM acetic acid, glucoamylase (8.7 kU/l) and $KHCO_3$ (43 mmol/l) solution (pH 4.8). Fifty microliters of the SR-glucoamylase solution was then added to 1 ml of TEA (tetraethylammonium) buffer (0.3 M tetraethylammonium, 4mM $MgSO_4$, 120 mM KOH, 1mM ATP, 0.9mM β-$NADP^+$, 700 U/l glucose-6-phosphate dehydrogenase (G-6-P-DH), and hexokinase (1.3 kU/l). After 10 min of incubation at room temperature, fluorescent emission of the reduced form of β-NADP was measured at 450 nm while the excitation wavelength was set at 365 nm.

SR Ca^{2+} UPTAKE

SR Ca^{2+} uptake rates were measured using the method described by Williams et al., 1998. Ca^{2+} uptake was measured in a HEPES buffer using Fura-2 as the extravesicular Ca^{2+} indicator. Ca^{2+} uptake was measured in 1 ml of incubation buffer containing 92.5 mM KCl, 18.5 mM Tris, 7.5 mM pyrophosphate, 1 mM $MgCl_2$, and 2 μM free Ca^{2+} (pH 7.0). Temperature was maintained at 37°C, and the buffer was continuously stirred. First 50 μg of SR protein was added and allowed to equilibrate for 3 min. Uptake was then initiated by the addition of 2 mM Na_2ATP and continued until no change in extravesicular free Ca^{2+} was observed. The steepest negative and positive slope of the free Ca^{2+} vs. Time curve was used to determine the rate of Ca^{2+} uptake. The total amount of Ca^{2+} sequestered was computed as the difference between the plateau portions of the curve measured before initiation of uptake and at the end of uptake. All rates and magnitudes of Ca^{2+} exchange were normalized by SR protein concentration. All samples were run in quadruplicate.

Extravesicular free Ca^{2+} was measured with the fluorescent Ca^{2+} indicator fura-2 (4 μM). Fluorescent changes were monitored with a Jasco CAF-100 fluorometer with excitation light filtered at 340 and 380 nm and emission light detected at 500 nm. The ration (R) of fluorescence due to excitation and at 340 and 380 nm was used to calculate free Ca^{2+} in the incubation medium according to the following formula: $[Ca^{2+}]_{free} = K_d \times \beta \times [(R - R_{min})/(R - R_{max})]$, where the fura 2- Ca^{2+} dissociation constant (K_d) was assumed as being 200 nm, R_{min} and R_{max} were the R values measured in the uptake buffer with 10 mM EGTA added and with 1mM Ca^{2+} added, respectively, β was the ratio of fluorescence measured at

380 nm excitation for the EGTA- and Ca^{2+} -supplemented buffers, and $[Ca^{2+}]_{free}$ was the free Ca^{2+} concentration.

Ca^{2+}ATPase ASSAY

This Ca^{2+}ATPase assay was adapted from Luckin *et al*., (1991). One ml of incubation buffer pH 7.0 (25 mM HEPES, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 0.2% NaA_2, 5 U/ml LDH, 7.5 U/ml PK, 0.6 mM NADH, 3.0 mM PEP, 2 μM Ionophore A23187) was added to a cuvette along with thirty micrograms of SR and allowed to equilibrate for 3 minutes in the spectrophotometer, which was set at 340 nm. Then 10 μl of ATP (1mM final concentration Na_2ATP) stock was added then the spectrophotometer was started for data collection. After 180 seconds 10 μl of $CaCl_2$ (~ 2 M) stock was added. After 8 minutes the graph appeared and the spectrophotometer was set up for the next sample.

HISTOCHEMISTRY

Muscles were mounted and frozen in liquid nitrogen in preparation for tissue slicing. Using a cryostat, sections were cut into 20 μm slices and mounted on microscope slides. To determine glycogen content qualitatively the Periodic Acid-Schiff Reaction for Glycogen (PAS) was used. After staining, photomicrographs were taken of the tissue sections.

SERCA AND GLYCOGEN PHOSPHORYLASE (GP) QUANTIFICATION

SERCA and GP were quantified using densitometric analysis of SDS-PAGE. SDS-PAGE gels were performed following a method outlined by Laemmli (1970). Twenty micrograms of protein was loaded into the gel and run on a mini-Protean II cell from Bio-Rad with a 5% acrylamide separating gel and a 4% acrylamide stacking gel. The running conditions were set at 45 mA (constant amps) until the tracking dye ran off the gel. Gels were then stained overnight in a solution containing the following: 0.1% Coomassie blue R-250, 40% methanol, and 10% acetic acid. After staining, the gels were destained until most of the background color was gone (≈ 1 hour) in a solution containing 50% H_20, 40% methanol, and 10% acetic acid. The bands corresponding to SERCA and GP were then scanned using the MultiImage Light Cabinet from Alpha Innotech and Analysis System. From these images, optical densities were determined from the appropriate bands.

STATISTICAL ANALYSIS

In situ stimulation and SR function data were collected by computer. Exhaustion bouts were measured using a timer. There were no effects of day of week on any parameter. Differences in each variable between conditions were determined by t-tests either by the Sigma Stat or Excel package, with a significance set at $p< 0.05$.

Muscle Glycogen Variation by Protocol

	Muscle Glycogen (umol/g wet mass)
(LG) Run + High Fat Diet **(HG) Run + High HCO Diet**	41.5 (97%) 42.7
(LG) Run + Fast **(HG) Run + HC Diet**	37.0 (87%) 42.6
(LG) Run + Fast **(HG) Run + Standard Diet**	28.9 (64%) 44.9
(LG) Fast + Run + Fast **(HG) Fast + Run + SD**	27.4 (47%) 58.5
(LG) Fast + Run + Fast **(HG) Fast + Run + SD + 5%**	27.2 (44%) 61.1

Figure 13. Summary of various protocols used to reduce muscle glycogen.

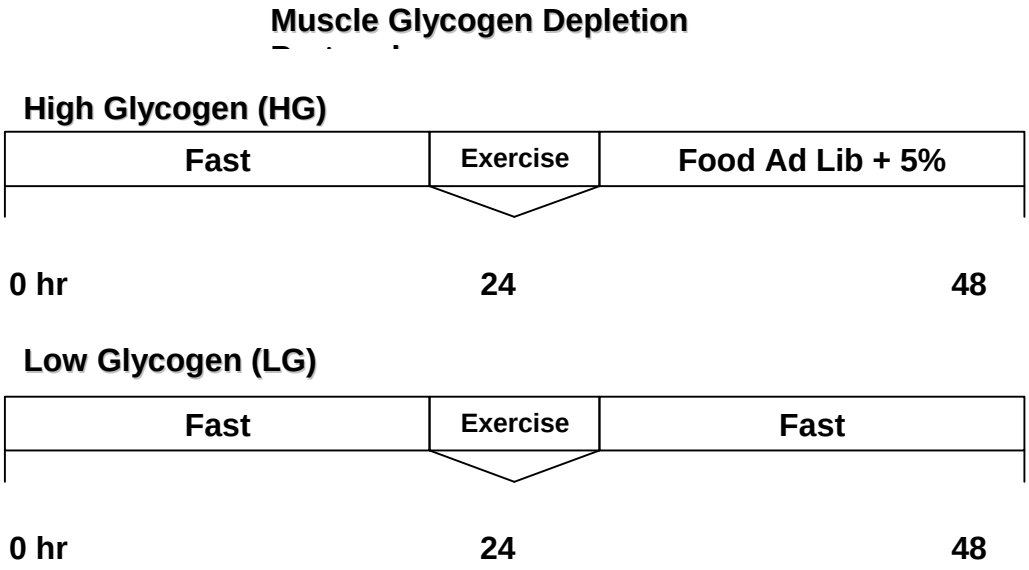

Figure 14. Overview of glycogen depletion protocol.

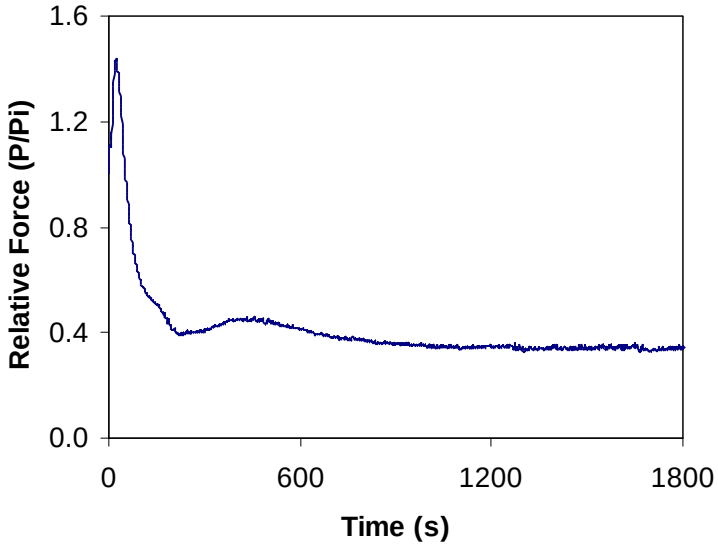

Figure 15. Typical force responses of a rat gastrocnemius during the in situ stimulation protocol. (P_i is the initial 20 Hz force)

CHAPTER FOUR:

RESULTS

APPEARANCE AND WEIGHT OF TISSUE

There was no difference in appearance of the extracted tissue. The HG group consumed 76.1% greater volume of the sucrose solution than the LG group did in consumption of water (Table 1) ($p < .05$). Despite this there were no apparent hydration problems. There were no significant differences in mean gastrocnemius masses (Table 2). The LG group consumed no food (by virtue of being fasted) as compared to the HG group which was allowed to consume standard diet (Table 1).

WHOLE MUSCLE GLYCOGEN

The development of a successful glycogen depletion protocol proved to be very challenging. As stated earlier several methods were tried before settling on the current protocol. The glycogen depletion protocol caused LG animals to have 42.4% less muscle glycogen than the HG animals (Table 2), $p< .05$.

To determine whether or not the glycogen assay was reliable, preliminary samples from different days were tested at various times. This showed stability of samples and precision of the assay. There was no difference in glycogen levels in control animals from one day to the next. In fact, the same sample was run 9 days after the original date with no discrepancy in results. This indicates that the assay is very reliable.

HISTOCHEMISTRY

Using PAS staining qualitative analysis of glycogen content revealed obvious differences in the HG and LG groups (Figure 16). However, while the LG group presented fibers that appeared to be stained lighter in color there were some fibers that were stained dark in color as in the HG animals. There appeared to be a dramatic difference between LG animals and animals that were subjected to the in situ protocol, indicating that while glycogen levels are lower than the HG group they are not completely depleted (Figure 16).

SR GLYCOGEN ANALYSIS

The SR isolation protocol separates the tissue into two fractions, HSR and LSR. The HSR fraction is typically used to determine Ca^{2+}-release rates as this fraction is concentrated with portion of the SR membrane associated with Ca^{2+}-release and LSR to determine Ca^{2+}-uptake rates as this fraction is rich in SERCA content. Both HSR and LSR glycogen contents were measured even though only the LSR fraction was used for uptake. LG animals had 89.6% and 90.1% less HSR and LSR glycogen levels than HG animals, $p < .05$ (Table 3).

SARCOPLASMIC RETICULUM YIELD

There was no significant difference in the amount of total protein recovered in the preparation of vesicles (LG, 3.21 ± 0.20 mg and HG, 3.21 ± 0.26 mg, n=6 for LG, n=6 for HG).

EXHAUSTION BOUT

The time to exhaustion bout revealed typical results in relation to total muscle glycogen levels and endurance activity. That is, higher initial muscle glycogen levels are associated with increased exercise performance. Notably, time to exhaustion during the subsequent treadmill run (21 m/min at 10% grade) was 79% lower in the LG group (35 ± 6.67 min vs. 166.75 ± 23.24 min, $p < .05$, n=4 for HG, n=4 for LG) as compared to HG animals.

STIMULATION PROTOCOL AND FORCE MEASUREMENTS

The results of the in situ stimulation protocol revealed no differences in resting or fatigued twitch and tetanic contractions or final force production (Table 4). Also, these values were not significantly different from those of control animals. However, there was a difference in the "staircase" between HG and LG groups. The "staircase" manifests itself as an initial rise in force immediately after the in situ stimulation protocol starts even though maximum length-tension was set. This effect diminishes almost as quickly as it presents itself, however it was slow enough to monitor the rate and peak. There was a 13.8% decrease in the peak relative tension (P/P_0), i.e. peak staircase, in the LG group, where P= the amount of tension when measured and P_0= maximum tension, (1.45 ± 0.06 P/P_0 (HG) and 1.25 ± 0.070 P/ P_0 (LG), $p< .05$). There was an increase in both resting and fatigued twitch and tetanic –dP/dt rates, 21.7% and 12.5% respectively, in the LG group (0.146 ± 0.009 mN/ms, 0.120 ± 0.0009 mN/ms, $p < .05$ and 0.619 ± 0.020 mN/ms, 0.550 ± 0.025 mN/ms, $p< .05$). The -dP/dt is the

rate of relaxation and this increased rate is consistent with the results for Ca^{2+} uptake and Ca^{2+} ATPase activity, both of which are increased in the LG group. Having an increased uptake rate is synonymous with saying you have a muscle that relaxes faster.

SERCA AND GLYCOGEN PHOSPHORYLASE (GP) QUANTIFICATION

SDS-PAGE revealed significant differences in the optical densities of the SERCA and GP bands associated with these proteins for HG and LG groups. Without utilizing other molecular techniques (i.e. western blot analysis or 2-D gel electrophoresis), it is impossible to determine the purity of these bands thus it should be noted that other proteins with similar molecular weights could have migrated to the same spots on the gel. The LG group had 33.3% more SERCA content than the HG group (1.88 ± 0.19 AU vs. 1.41 ± 0.15 AU, $p<.05$). Interestingly, the HG group had 47.1% more GP (0.85 ± 0.09 AU vs. 0.45 ± 0.060 AU, $p < .05$). This apparent increase in SERCA content supports the previous finding of an increased rate of relaxation.

Ca^{2+} UPTAKE RATES

LG animals displayed a 44.9% increase in uptake compared to HG animals, $p<.05$ (Table 5). Because it was shown previously that there was an increase in SERCA content and rate of relaxation (-dP/dt) in the LG group this increase in activity is not surprising. SERCA is clearing Ca^{2+} from the myoplasm at a faster rate, hence the muscle relaxes faster. Since the increase in activity is

greater than the increase in SERCA content (45% vs. 33%), part of the increase is attributed to more SERCA and the remainder is probably due to intrinsic changes in SERCA function.

Ca^{2+} ATPase ACTIVITY

In conjunction with the 44.9% increase in Ca^{2+} uptake there was a 51.2% increase in Ca^{2+} stimulated ATPase activity and a 48.0% increase in Basal ATPase activity with the LG animals, $p < .05$ (Table 5). This increase in ATPase activity may be a result of the increase in SERCA content. However, as mentioned before this increase in activity is greater than the increase in SERCA content (51% vs. 33%). Again, part of the increase is attributed to more SERCA protein and the remainder is probably due to intrinsic changes in SERCA function.

Table 1. Food and Water Consumption.

Parameter	HG	LG
Food (g)	16.39 ± 1.01*	0
Water (ml)	68.00 ± 9.85*	16.25 ± 2.28

Values are means ± SEM. *p<.05 between groups (n=9 for HG, n=8 for LG).

Table 2. Muscle Morphology.

Parameter	HG	LG	Control
Wet Mass (g)	1.89 ± 0.06	1.70 ± 0.10	1.86 ± 0.07
Dry Mass (g)	0.54 ± 0.03	0.46 ± 0.04	0.55 ± 0.04
Glycogen (µmol/g dry mass)	189.13± 9.35*	108.98 ± 6.21	160.45 ± 7.23

Values are means ± SEM. *p<.05 between groups (n=9 for HG, n=8 for LG).

Table 3. SR glycogen content.

Fraction	HG	LG	Control
HSR (µg/mg)	621.56 ± 147.10*	64.56 ± 14.29	597.32 ± 132.67
LSR (µg/mg)	809.93 ± 162.32*	80.17 ± 54.80	778.69 ± 137.92

Values are means ± SEM. Units are µg glycogen per mg total protein. *p<.05 between groups (n=6 for HG, n=6 for LG).

Table 4. Force Production Values

Parameter	HG	LG	Control
Twitch			
Rest (mN/gm DM)	8.04 ± 0.60	9.17 ± 0.88	8.21 ± 0.75
Fatigue (mN/gm DM)	3.56 ± 0.28	4.35 ± 0.52	4.03 ± 0.63
Fatigue (P/Po)	0.45 ± 0.10	0.47 ± 0.13	0.49 ± 0.16
Tetanus			
Rest (mN/gm DM)	32.51 ± 1.62	37.09 ± 3.42	34.18 ± 2.14
Fatigue (mN/gm DM)	17.53 ± 1.77	18.33 ± 2.11	18.02 ± 2.06
Fatigue (P/Po)	0.53 ± 0.16	0.49 ± 0.13	0.53 ± 0.20

Values are means ± SEM. Units are mN of force per gm of dry muscle mass (mN/gm DM) or fraction of rested force (P/Po) (n=9 for HG, n=8 for LG)..

Table 5. Measurements of SR function.

Parameter	HG	LG
Ca^{2+} Uptake (μmol/mg/min)	0.816 ± 0.092	1.182 ± 0.063*
Basal ATPase (μmol/mg/min)	0.175 ± 0.048	0.259 ± 0.053*
Ca^{2+}-stimulated ATPase (μmol/mg/min)	1.812 ± 0.238	2.740 ± 0.236*

Values are mean ± SEM. Units for uptake are in μmol Ca^{2+} per mg protein per min and for ATPase activity are in μmol ADP per mg protein per min. *$p<.05$ between conditions (n=6 for HG, n=6 for LG).

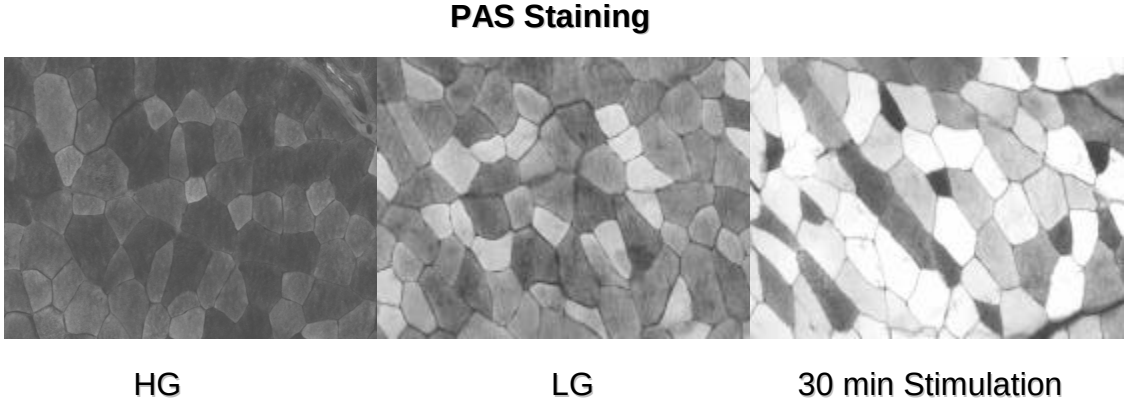

Figure 16. PAS staining in muscles from HG and LG animals as well as from and HG animal subjected to 30 min of stimulation. The intensity of staining is indicative of the fiber glycogen content.

CHAPTER FIVE:

DISCUSSION

GLYCOGEN DEPLETION PROTOCOL

The task of creating an exercise protocol that produced noticeable differences in muscle glycogen levels for this study proved to be very difficult. At the onset of this project the idea was to manipulate the glycogen levels by subjecting the rats to a 90-minute treadmill run and then either a high fat (HF) or high carbohydrate (HC) diet. Two commercial diets were purchased, one was 76.2% carbohydrate and the other was 89.6% fat (primarily lard). The protocol was designed to deplete glycogen and then inflate glycogen levels in one group and maintain glycogen depression in the other. This first trial failed to yield sufficiently different glycogen levels (LG group was 97% of HG group). It was noticed that the rats were not sufficiently eating the HF diet yet glycogen levels were near normal. As a result, a second protocol was implemented. This procedure involved simply removing all food from the LG group for 24 hours. This proved to yield better but not desirable results. Glycogen levels in the LG group were 87% of the HG group. Stimulated by this quandry, the protocol was repeated and liver glycogen was measured in both groups. Liver glycogen levels in the LG group as compared to the HG group were almost completely depleted. During a 24-hour period the LG group was able to mobilize liver glycogen and synthesize muscle glycogen to return them to near initial ranges. It was also noted that the animals that were allowed access to the HC diet were not very receptive to the change in food so they were not eating as much as normal. The next protocol involved changing the HC diet back to the normal rodent chow. This produced a larger difference between the groups, however it was still

inadequate (LG group was 64% of the HG group). The next protocol utilized both fasting and exercise. Trying to address the liver utilization problem, food was removed 24-hours pre-exercise for both groups and the LG group was not allowed food after a treadmill run (90 min) and the HG group was allowed access to the normal rodent chow. This produced desirable glycogen depletion (LG group was 47% of HG group), however the HG group needed to somehow be elevated. The final protocol that was tried and decided upon involved exchanging the water with a 5% sucrose solution after the exercise bout for the HG group. This final protocol led to the significantly different muscle and SR glycogen levels used in this study.

HISTOCHEMISTRY

The appearance of HG and LG tissues after PAS staining was consistent with the glycogen depletion protocol. However, there were fibers within the LG tissue that appeared not to be depleted. This would imply that the entire muscle was not active to the same extent during the glycogen depleting exercise or that some fibers had markedly elevated glycogen levels prior to the start of activity. The sporadic dark staining in the LG animals was probably due to partial activation of the muscle because of the low intensity exercise protocol. This would cause localized glycogen depletion in the muscle due to partial activation. When muscle contracts there are several factors that determine utilization. One major determinant of force production is the number of motor units recruited. The more motor units recruited the more force. The frequency or intensity at

which the muscle is stimulated determines recruitment. Therefore, less intense exercise bouts recruit less motor units, which utilize less of the muscle as opposed to a high intensity exercise, which stimulates most if not all of the motor units of a muscle, producing maximum force. Motor unit type also determines force. Fast fatigue motor units (FF) produce the most force the fatigue resistant (FR) followed by slow (S) (FF> FR >S) and are recruited from slowest to fastest. The Henneman size principle is also a factor in determining force. This theory states that motor units with larger axons produce the most force and are connected to larger fibers. Coincidentally, these larger axons correlate with FF motor units and the small axons with S. So at low intensity, smaller less fatigable fibers are recruited. As intensity increases, the number of motor units increases, thus activating larger nerve bundles that correspond to larger fibers for increased force production.

EXERCISE PERFORMANCE

The 79% decrease in time to exhaustion in the LG group supports previous research showing that endurance capacity is determined by initial muscle glycogen content (Bergstrom *et al.,* (1967)). The lack of decreased muscle performance and increased SR function found in this study contradict this decreased exercise performance. Earlier in this paper, two types of fatigue were described, central and peripheral. This decrease in exercise performance found in this study is not likely due to peripheral fatigue because the cellular mechanisms involved with muscle contraction did not seem to be negatively

altered, in fact some of the functions were enhanced. Hence, the impaired exercise performance is likely attributed to central fatigue. Because the LG animals were glycogen depleted (muscle and liver) there is no compensatory mechanism that will increase blood glucose levels, which are likely to be very low. This decreased blood glucose manifests itself as lethargy and decreased motivation. Because the in situ protocol essentially eliminates motivational factors (central fatigue) when monitoring muscle performance any inhibited function can be attributed to cellular abnormalities, which in this study was not the case.

MUSCLE PERFORMANCE

The results of the stimulation protocol in regards to muscle performance conflict with those of exercise performance. There was a 79% decrease in time to exhaustion in the LG group as compared to no changes in force production or in fatigue kinetics. The in situ stimulation protocol was done at a much higher frequency of contraction than the depletion protocol, thus activating fibers that had not previously been glycogen depleted. This would mask any affects in depressed force production. However, the rate of relaxation in the LG group is congruent with those of the SR function results. There is an increased ATPase activity, which translates into a faster relaxation rate during contraction.

SR FUNCTION AND GLYCOGEN DEPLETION

This is the first study to show that SR function was enhanced in the LG group in regards to Ca^{2+}-ATPase activity and uptake rates. Despite reductions in whole muscle and SR glycogen, and GP content, LG animals displayed a 44.9% increase in uptake, 51.2% increase in Ca^{2+} stimulated ATPase activity, and a 48.0% increase in basal ATPase activity as compared to HG animals. In agreement with this increased Ca^{2+} pump function, the rate of relaxation during the in situ stimulation protocol was increased for both resting twitch and tetanic contractions in the LG group (21.7% and 12.5%, $p < .05$, respectively). The expectation was that when glycogen levels are reduced the uptake pump and ATPase activity would decrease. As stated before, Xu *et al.,* (1995) have shown that the SR has embedded in its membrane the necessary glycolytic enzymes to produce ATP and the SR are in fact more efficient at utilizing this glycolytically formed ATP than exogenous ATP. Xu *et al.,* (1995) have also shown that the uptake pump prefers to use ATP that is formed by the glycolytic enzymes in the SR membrane thus if there is a reduction in the substrate that fuels these enzymes then this glycolytic dependent pump suffers. The pump doesn't completely shut down because it is capable of utilizing cytosolic ATP but not as efficiently as the endogenously formed glycolytic ATP. Because glucose is the substrate that fuels these glycolytic enzymes, any decrease in the storage form of glucose (glycogen) could have a detrimental effect on the production of glycolytically produced ATP, causing the SR to function at a slower rate. However, the current results indicate another adaptation to decreased glycogen.

While Ca^{2+} release rates were not assessed in this study there has been speculation that glycogen depletion has a detrimental effect on SR Ca^{2+} release (Byrd *et al*., (1989), Westerblad *et al*., (2000), Favero (1999), Williams (1997), Williams *et al*., (1998), Williams *et al*., (1995) and Williams *et al*., (1993)). Chin *et al.,* (1997) have suggested that there is a functional coupling between ATP supplied by glycolysis and ATP utilized within the SR-t-tubule triadic gap (Han *et al.,* 1992). When glycogen is reduced below a critical level, ATP levels may transiently fall before [Ca^{2+}] and force output drops (Chin *et al.,* 1997). Under these conditions, the net reduction in ATP may inhibit excitation-contraction coupling (E-C coupling) processes or impair optimal Ca^{2+} release function (Smith *et al.,* 1985; Lannergren *et al.,* 1995). Although the energy depletion theory of muscle fatigue has been criticized due to the fact that ATP concentrations rarely fall by more than 30-50% (Vollestad *et al.,* 1988) and muscle glycogen is never entirely depleted. The concentration of glycogen and ATP within the narrow restricted space of the triadic gap may fall well below that in the bulk space. The compartmentalized supply and utilization of ATP in skeletal muscle triads is supported by observations that: (1) glycogen is bound in distinct regions of the I-band, a region corresponding to the terminal cisternae region of the SR (Friden *et al.,* 1989); (2) glycogen in the lateral I-band region is preferentially depleted following intense exercise in humans (Friden *et al.,* 1989); (3) ATP supplied by glycolytic enzymes is utilized to phosphorylate proteins in the triadic gap that may be required for E-C coupling (Han *et al.,* 1992). The role of muscle glycogen may therefore be to provide a rapid and local supply of ATP in the triadic gap for

important phosphorylation-dephosphorylation steps (Han *et al.,* 1992) or direct binding to the Ca^{2+} release channel (Smith *et al.,* 1985). Therefore, when localized glycogen stores are depleted, ATP supplies in the SR triadic region falls and a decrease in Ca^{2+} release and force results (Chin *et al.,* 1997).

There are several sites within the triad that could possibly cause EC coupling failure as a result of glycogen depletion and manifest as fatigue. As stated earlier, the DHPR is a voltage gated ion sensor for ECC. This receptor is comprised of several subunits, one of which is in direct contact with the RyR (α_{1s}) and has been shown to promote a conformational change that opens the RyR (Tanabe et al., 1990 and Nakai et al., 1998). Another subunit of the DHPR is the α_2-δ, which is highly glycosylated and has been shown to increase the amount of charge movement across the membrane (sarcolemma), which aids in depolarization. From the previous information it can be deduced that the α_2-δ subunit is closest to the t-tubule and the α_{1s} subunit is facing the RyR. If during glycogen depletion the α_2-δ subunit is deglycosylated then it is likely that the ability of the DHPR to respond to depolarization could be adversely affected. This could cause a slower depolarization or maybe even no depolarization. Either way the ability of the α_{1s} subunit to interact with the RyR to release Ca^{2+} will be decreased if not inhibited completely, thus a decrease in Ca^{2+} release, which translates into decreased force.

Another component in the ECC process that could be adversely affected by glycogen depletion is the RyR. There has been no evidence linking glycogen to the RyR, however there is glycogen that is associated with the SR membrane

(uptake pump) that could possibly, when depleted or lowered, alter function by either a biochemical or structural change in the RyR. This change would ultimately be manifested as a decrease in Ca^{2+} release, which translates into decreased force. Another possible adverse affect of glycogen depletion could be less evident directly at the RyR but the outcome would be the same. It has been shown that glycogen depletion inhibits Ca^{2+} uptake via decreased SR glycogen. This being the case [Ca^{2+}] would increase to μmolar levels, which causes a negative feedback on Ca^{2+} release via a Ca^{2+}-calmodulin (CaM) interaction at the RyR (to be discussed next). Net result, decrease in Ca^{2+} release, which translates into decreased force.

If glycogen depletion does not directly affect the function of the RyR a negative feedback mechanism may ultimately decrease Ca^{2+} release via increased [Ca^{2+}]. CaM is a protein that has binding sites on the RyR and has been shown to increase Ca^{2+} release at nmolar concentrations and inhibit at μmolar concentrations. There are two states that CaM can been found on the RyR, one is the Ca^{2+} free state (nmolar [Ca^{2+}]) and the other is the Ca^{2+} bound state (μmolar [Ca^{2+}]). In the free state CaM enhances Ca^{2+} release from the RyR but in the bound state it inhibits release. Glycogen depletion has been shown to compromise Ca^{2+} uptake, thus [Ca^{2+}] will increase, shifting CaM to its bound form, which decreases Ca^{2+} release.

FKBP is an integral protein of the RyR that has been shown to stabilize the RyR in the closed or open state and to be coupled with the DHPR. However, when this protein is removed (rapamycin) Ca^{2+} release is decreased due to loss

of coordinated channel opening. Glycogen depletion may cause the FKBP to dissociate from the RyR, thus causing Ca^{2+} release to be decreased.

GP PROTEIN CONTENT

As expected GP content was decreased in the LG group and supports the idea that GP is an enzyme that is involved in glycogen break down and is dissociated from the SR during exercise (Cuenda *et al*., 1993; Lees *et al*., 2001). GP is activated during exercise via adenyl cyclase activation. The activation of adenylyl cyclase stimulates the production of cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate activates protein kinase-A, which activates phosphorylase kinase by phosphorylating it. Phosphorylase kinase then activates GP, which in turn stimulates glycogen breakdown for energy production in the exercising muscle. At the same time protein kinase-A inactivates glycogen synthase by phosphorylation, which inactivates it, thus shutting off glycogen synthesis. The same signal that activates glycogen breakdown also inactivates protein phosphatase–I, which would normally unphosphorylate everything that was activated. It does this by activating phosphatase inhibitor protein, which binds to active phoshatase-I, rendering it inactive. It has been implied that GP may be bound to the SR via glycogen. Cuenda *et al*., (1993) have shown that SR glycogenolysis may cause GP to be released into the myoplasm. This loss in GP content should also manifest itself as a decreased GP activity, as was proven by Cuenda *et al*., (1993). A decrease in GP activity would ultimately decrease energy production for ATPase activity.

However, in the present study, possible changes in GP associated with the SR did not adversely affect force, fatigue, or SR function in fact, it was likely the cause for the increased SERCA content to be discussed later.

SR FRACTIONATION

Utilizing an isolated SR preparation to determine responses to contractile activity is a widely accepted practice. However, there are several factors that have to be assumed when interpreting results. One major assumption is that all necessary components remain intact and are functional. If this is not the case, any disruption in function of the SR could be attributed to missing proteins or enzymes. It has been shown that the SR has glycolytic enzymes associated with the membrane that are used to produce ATP for various functions (e.g. Ca^{2+} uptake). This ATP that is formed from endogenous sources is more efficient at supporting Ca^{2+}-uptake than exogenous ATP. If during the isolation process these enzymes are lost or reduced, the ATPase activity may be lower, possibly disrupting Ca^{2+} release. This, turn will cause a decrease in force production.

If CaM were reduced away the activation of the RyR would be affected causing a decrease Ca^{2+}release rate thus, producing less force. Also, CaM activated kinases (phoshorylase kinase – activates glycogen breakdown and myosin regulatory light chain kinase-likely to be responsible for the ratcheting of the myosin head) will display decreased or no activity. If FKBP is lost during isolation Ca^{2+} release will be decreased.

Parvalbumin is a protein that is involved with shuttling Ca^{2+} to the Ca^{2+} uptake pump by binding to it in the myoplasm. If this protein is lost during isolation, Ca^{2+} could stay around too long and inhibit release from the RyR, as stated earlier. Causing a decrease in force production.

Phospholamban is another integral protein found within the SR membrane that plays an important role in Ca^{2+} uptake. When it is phosphorylated it enhances uptake and when it is dephosphorylated it inhibits uptake. This is another protein that will be affected by the loss of kinases (PKC, PKA-also important in mobilizing G-1-P for energy production, PKG, CaM-kinase). If this protein is simply lost during isolation or preparation of the sample, uptake will be decreased causing a decrease in force.

POSSIBLE ADAPTATIONS TO GLYCOGEN DEPLETION

Consistent with the increased uptake, and ATPase activity there was an increase in SERCA content. This increase in pump content could possibly explain the enhanced Ca^{2+} sequestration. It appears that the tissue has attempted to adapt to the depleted glycogen levels by upregulating SERCA content that translates into increased total pump activity. One possible mechanism for this apparent adaptation is presented by Malyshev *et al*., (2000). They showed an upregulation of gene expression of Ca^{2+}ATPase in skeletal and cardiac muscle, six (6) and two (2) hours after exposure to nitric oxide (NO). Nitric oxide synthase (NOS) has been shown to increase in response to acute exercise and decreased glycogen (Roberts *et al.,* 1999). NOS is responsible for

the production of NO. There are different forms of NOS, however skeletal muscle expresses two types, endothelial NOS (eNOS) and neuronal NOS (nNOS) (Kobzik *et al*., 1994 and Kobzik *et al*., 1995). The distribution of the two types range from being localized at the subsarcolemmal and at the neuromuscular junction region of skeletal muscle to being uniformly distributed within the muscle fibers as well as in the vasculature (Kobzik *et al*., 1994, Kobzik *et al*., 1995, and Kusner *et al*., 1996). Even though it has been demonstrated that both fast and slow muscles express eNOS and nNOS (Kobzik *et al*., 1994, Kobzik *et al*., 1995, and Wolf *et al*., 1998), there is evidence that nNOS levels are higher in fast-glycolytic muscles (Kobzik *et al*., 1994) and eNOS is associated with slow-oxidative muscles (Kobzik *et al*., 1995). This study uses a muscle that is primarily fast twitch but does have a mixture of slow fibers as well. In this same study they hypothesize that there is a negative correlation between muscle glycogen content and NOS meaning as glycogen levels decrease, NOS activity increases. They make this assumption because they have also demonstrated that exercise-stimulated glucose transport is NO dependent (Roberts *et al*.,1999). So as glycogen levels are depleted NOS activity increases and NO is produced which causes glucose transport to the muscle. As a result, they suggest that glycogen status in skeletal muscle may be a signal for induction of glucose transport during exercise. If this were in fact the case, this would explain why the SR vesicles from the LG group have increased SERCA content. However, it should be noted that the present study provides no direct evidence to support such a mechanism.

It is also possible that the differences in SERCA content between conditions does not reflect an upregulation of SERCA in the LG animals but a down regulation in the HG animals. One possible response to elevated glycogen levels may be to down regulate SERCA content, hence activity. Pharmacology tells us that whenever there is an abundance of substrate for a particular receptor the number of receptors is often times decreased. This could be happening with the HG animals. Substrate levels are above normal so the concentration of "receptor", in this case, SERCA doesn't need to be as high as normal therefore, the protein could be degraded until glycogen levels are lower. One way to check this is to compare SERCA content from HG animals to control animals. If SERCA content were lower in the HG animals then there would definitely be evidence of down regulation.

While there is little or no phospholamban (PLB) in skeletal muscle, another possible mechanism for the increased uptake and ATPase activity in the LG group might be due to PLB activation (Stokes 1997). Using electron microscopy Stokes has proposed a model for SERCA and PLB orientation to one another within the SR membrane. His model implies that there is a dynamic equilibrium between two orientations of PLB. PLB can either be found in a pentamer form (storage form) in the SR membrane or as monomers. Both SERCA and PLB are transmembrane proteins that have their interaction sites facing the myoplasm. In the monomeric unphosphorylated form, PLB is able to migrate freely in the membrane. By doing so it is able to interact with the SERCA via transmembrane helices and at a specific site in the myoplasmic head. It is

this interaction that is thought to inhibit SERCA activity and Ca^{2+} uptake. Once PLB is phosphorylated (Ser-16 site at base of myoplasmic helix) via protein kinase A (PKA), it is destabilized and partially unwound at the myoplasmic helical phosphorylation site (Stokes 1997). It is this unwinding that is thought to cause the PLB to dissociate from the SERCA and promote activity (Stokes 1997). Phosphorylation of PLB is also thought to push the monomeric form back to the storage form (pentameric form) (Stokes 1997). It is plausible to hypothesize that maybe during the exercise protocol PKA is left activated in the LG group in an effort to increase SERCA activity. If PKA is left activated PLB is likely to be in the phosphorylated form which would promote uptake and push PLB back to its storage form (Stokes 1997). Leaving PKA in the activated form would also promote more glycogen breakdown, which in the LG group would not be a desirable effect but this is just theory for now. On the other hand, maybe the HG group has more of the PLB in the unphosphorylated form, contributing to a partially active SERCA. So maybe SERCA activity is not enhanced in the LG group but the SERCA in the HG group is impaired. It should also be noted that PLB levels have been shown to be minimal in the rat gastrocnemius.

IMPLICATIONS FOR FATIGUE

The current methods of increasing endurance performance are based on evidence showing that higher initial muscle glycogen levels lead to increased exercise performance (Bergstrom *et al*., 1967). This correlation has been supported by numerous studies (Bergstrom *et al*., 1967 and Hermansen *et al*.,

1967) and as a result, “glycogen loading” is commonly practiced by endurance athletes (Hermansen *et al.,* 1967). A proposed mechanism for this correlation suggests that there is a reduction in muscle performance as a result of decreased muscle glycogen. Chin *et al.,* (1997) demonstrated the importance of muscle glycogen in determining muscle performance *in vitro*. Isolated muscle fibers were stimulated until force production was 30% of initial levels and corresponding glycogen levels were decreased by 27%. Fibers were allowed to recover in a 5 mM solution of glucose for 60 minutes and then stimulated again. Glycogen levels recovered to 100% of initial and the subsequent fatigue curve was identical to the pre-fatigued curve. The experiment was then repeated except this time instead of allowing the muscle to recover in glucose it was bathed in a solution with no glucose then the stimulation protocol was repeated. Final tetanic force production was decreased by 45% and muscle glycogen levels maintained the 27% reduction as seen before. These results indicate that in isolated fibers, glycogen levels determine muscle performance, i.e. force production, and the ability to sustain a contraction, hence determining the on-set of fatigue. In contrast, the present study found that lowered glycogen levels did not affect contraction parameters of *in situ* stimulated muscles despite lowering exercise tolerance in intact animals. The major difference between the two isolated tissue studies is that the blood supply to the tissue is maintained in the present study, which allows alternative substrates, i.e. blood glucose and free fatty acids (FFA), to be utilized by the muscle for ATP production. Whereas, in

Chin *et al*., (1997), the fatigability is solely dependent on muscle glycogen and when it is depleted, compromised function is manifest.

The conflicting results between muscle and exercise performance propose that fatigue may be related to muscle glycogen but not by altering muscle contraction. The present study suggests that there is an alternative pathway that causes decreased exercise performance as it relates to muscle glycogen and muscle performance. The reduction in exercise performance could be due to by products of an altered substrate dependence of contraction that cause "central fatigue" (Newsholme *et al*., 1987). One mechanism for initiating central fatigue as a result of sustained moderate to high intensity exercise is the activation of the serotonergic system (Fernstrom *et al.,* 1971 and 1972). Serotonin or 5-hydroxytryptamine (5-HT), which is found in the brain in conjunction with tryptophan, has been linked to mental fatigue and causes feelings of exhaustion and lethargy (Young 1986). A precursor for serotonin formation is the presence of free tryptophan in the plasma (Fernstrom 1990). Amino acids do not readily enter the brain due to the blood-brain barrier (BBB), however they can cross the BBB via a transporter, i.e. large neutral amino acids (LNAA). According to Jakeman (1998), the LNAA-transporter is a saturable carrier that shares its transporter function between six amino acids: tyrosine, tryptophan, phenylalanine, and the branched-chain amino acids (BCAA) leucine, isoleucine and valine. The binding affinity to the LNAA transporter is dependent on the concentration of each amino acid relative to each other; hence the most abundant amino acid will cross the BBB. Of the six competitor amino acids,

tryptophan has the lowest plasma concentration, 50μM, of which 90% is bound to albumin and the resulting 10% (5μM) in the free form. The remaining amino acids are present in the plasma at a 10-fold greater concentration, i.e. 500μM, which correlates to a 1:100 ratio of free tryptophan to LNAA. Studies have shown that this ratio can be manipulated in rats by altering the plasma concentration of amino acids such that tryptophan is transported into the brain for synthesis of serotonin (Chauloff 1989). As stated earlier, the majority of tryptophan found in the plasma is bound to albumin, however there is a tendency for the free form of tryptophan to be liberated during exercise. According to Struder *et al*., (2001) and Snyder (1998), this increase in the free form of tryptophan is due to andrenergically induced lipolysis of free fatty acids (FFA) that compete for binding sites on albumin. Because FFA's have a higher affinity for albumin than tryptophan, the ratio of tryptophan to BCAA can increase rapidly during and after sustained exercise thus catalyzing the serotonergic pathway, i.e. central fatigue (Blomstrand 2001). Carbohydrates and fat provide the necessary energy that is needed to maintain a given intensity during sustained exercise, 50-60% and 40-50% respectively (Hagerman 1992). During the initial stages of exercise, the preferred energy source is muscle glycogen/carbohydrate (Costill 1986) however there is a transition towards mobilizing liver glycogen and FFA's as a means to replenish or prevent complete depletion of muscle glycogen in the latter stages of the exercise bout. This phenomenon has been termed "glycogen sparing" (Costill *et al*., 1977). It is this transition to utilizing alternative substrates, i.e. FFA, which causes the liberation of tryptophan from albumin for transport to

the brain and subsequent synthesis of serotonin. While “glycogen sparing” appears to be advantageous for preventing glycogen depletion, hence inhibiting the on set of peripheral fatigue it could potentially induce “central fatigue”. It should be noted that the liberation of FFA and competition between FFA and tryptophan for binding sites on albumin can be suppressed by carbohydrate feeding during and after exercise, thus enhancing endurance performance (Jakeman 1998).

If this mechanism for the on set of central fatigue during sustained exercise is correct, i.e. increased levels of brain serotonin, then the decreased exercise tolerance by the LG animals in this study is expected. Although measurements of plasma free FFA, free tryptophan, brain serotonin, and BCAA’s were not measured in the present study; it is likely that the LG animals had high levels of plasma FFA and an increased plasma free tryptophan to BCAA ratio before and after the subsequent treadmill run. Alternatively, the HG animals had a higher exercise tolerance because of the carbohydrate feeding which likely resulted in no upward shift in the free tryptophan to BCAA ratio.

Another aim of this study was to examine the hypothesis that lowered muscle glycogen was causally related to decreased SR function. Previously, other studies have shown that muscle fatigue is associated with decreased SR function (Williams *et al*., 1997). However, these studies measured SR function in isolated samples that were stimulated *in vitro*, i.e. no blood supply, and in the immediate post-exercise state. In addition to glycogen being low, a variety of metabolic breakdown products, i.e. lactic acid and P_I, could have accumulated in

the absence of a blood supply in this state. These metabolic by products could have indirectly caused changes in SR function, i.e. decreased SERCA activity. From this, it would be difficult to attribute any alteration in SR function to glycogen depletion alone. Therefore this study utilized an *in situ* stimulation protocol and *rested* muscles that had been glycogen depleted to remove any effects of possible accumulated metabolic by products found in the *in vitro* post-exercise state. No reduction of SR function was associated with the glycogen-depleted state suggesting that glycogen depletion does not diminish SR function. However, SR function was enhanced in the glycogen depleted state. This may be due to a direct effect of glycogen depletion or possibly an indirect effect of the exercise protocol used to induce glycogen depletion. If samples were taken directly after the glycogen depletion protocol was complete, decreases in SR function would possibly be manifest. However, the muscles were allowed to recover, and as a result, it appears that this study has uncovered an unusual response.

The enhanced SR function, i.e. increased rate of relaxation and Ca^{2+}-ATPase activity, found in the LG animals, may be a response to glycogen depletion in order to prevent the activation of calcium-activated proteases due to prolonged increased [Ca^{2+}]. There are two possible mechanisms for this response, one is directly related to glycogen and the other is an induced alteration. One mechanism for the enhanced SR function found in this study was speculated to be in response to glycogen depletion that led to the apparent dissociation of GP from the glycogenolytic complex. It was stated earlier that SR

has glycogenolytic complexes on the membrane that is adjacent to the SERCA pump that provide a preferred ATP source for SERCA activity (Xu *et al.,* 1995). This complex has a ready supply of glycogen, means of mobilizing it, i.e. GP and glycogen debranching enzyme, and localized glycolytic enzymes that synthesize ATP for SERCA activity (Fig. 17) (Entman *et al*., 1980 and Wanson *et al*., 1972). GP is thought to be bound to glycogen particles, in its b-form (inactive, unphosphorylated), that are themselves attached to the membrane via a hydrophobic tail associated with the glycogen associated form of protein phosphatase I (Cuenda *et al*., 1991, Cuenda *et al*., 1995, Entman *et al*., 1980, Wanson *et al*., 1972, Meyer *et al*., 1970, Hubbard *et al*., 1989, Hubbard *et al*., 1990). Activation of GP (phosphorylation) with muscle contraction is likely to cause glycogenolysis of SR glycogen, thus reducing its affinity to the SR membrane (Cuenda *et al*., 1991 and Cuenda *et al*., 1994). This enzyme along with SR glycogen was found to be decreased in LG animals, hence the ability to supply ATP for SERCA activity, i.e. Ca^{2+}-uptake was likely to be diminished. This finding supports the study done by Lees *et al*., (2001), which reported that glycogen and GP associated with skeletal muscle SR is reduced after fatiguing activity. This decrease in the preferential ATP supply possibly caused SERCA content to be up regulated as an adaptive response to increase the ability of the SR to extract Ca^{2+} from the myoplasm to prevent Ca^{2+} proteases from being activated.

Another mechanism for the increased SERCA content found in this study involves the production of NO in response to acute exercise and low glycogen

levels (Roberts *et al*., 1999 and Malyshev *et al*., 2000). These studies implicate NO as the stimulus for the upregulation of SERCA content in response to exercise and low glycogen. If the liberation of NO is negatively correlated with glycogen levels, i.e. as glycogen decreases, NO increases, then the increased SERCA content in this study could be explained. Since the LG animals in this study are in a glycogen depleted state it is possible that the proposed NO mechanism could have induced up-regulation of SERCA content. Doing a time dependent exercise study measuring NO, glycogen, and SERCA content, could test whether there is a correlation. For a complete profile of NO, glycogen, and SERCA content, samples should be taken at 0 time then at intervals throughout the exercise duration to exhaustion and then up to 24 hrs post exercise.

SUGGESTIONS FOR FUTURE RESEARCH

This study provided conflicting information concerning the link between glycogen, muscle performance and SR function. However, this study would be worth extending but some additional measurements. The first change would involve increasing the intensity of the exercise depleting protocol. Alternatively, a different protocol could be developed that matched glycogen depletion and in situ stimulation intensities. It is possible that the disparity of the intensity of exercise between the glycogen depletion protocol and the in situ protocol masked the expected response in the LG animals to low glycogen, i.e. decreased muscle performance.

In an effort to try to track the possible adaptation effects seen in this study samples of SR would need to be collected immediately after the exercise and in situ protocols for measurements of: GP and SERCA activity, Ca^{2+}-release, NOS activity, NO production, and gene expression of SERCA. Measuring SERCA content in control animals in comparison to HG animals, in an effort to determine whether up or down regulation of SERCA is occurring, would also be necessary. To determine whether or not PLB is a factor it would be nice to check first if it is present and if it is, what form is it in (phosphorylated or unphosphorylated).

A proposed protocol for determining if the serotonergic pathway, i.e. central fatigue, is activated in the LG animals is to repeat the subsequent exhaustion treadmill bout; harvesting samples from three groups, one LG and two HG groups. At the time the LG group stops running animals from both groups should be taken then allow the remaining HG group to continue until exhaustion, noting run times in all groups. Plasma concentration of FFA, albumin bound FA, free tryptophan, and brain serotonin will either implicate or dispute central fatigue.

Overall this study has provided new insight into glycogen and skeletal muscle function and further studies in the previously described direction is justified.

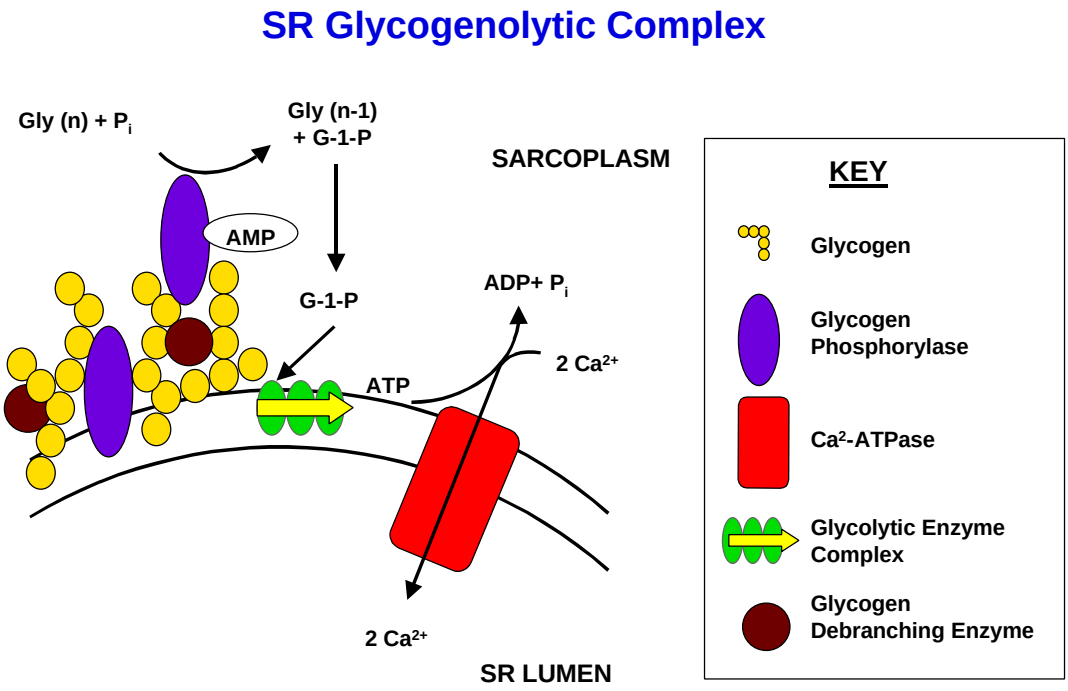

Figure 17. Diagram of the SR glycogenolytic complex.

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APPENDIX:

RAW DATA

Animal	Cond	Food (g)	Water (ml)	Wet Mass (g)	Dry Mass (g)	Dry/Wet Mass
37	HC	11.4	94	2.05	0.63	0.31
38	HC	17.3	86	1.93	0.53	0.28
39	HC	19.4	58	2.07	0.60	0.29
40	HC	18.7	30	2.07	0.66	0.32
47	HC	16.6	74	1.72	0.48	0.28
48	HC	15.0	76	1.64	0.46	0.28
49	HC			2.03	0.54	0.27
50	HC			1.67	0.44	0.26
51	HC			1.81	0.48	0.27
Mean		**16.39**	**69.67**	**1.887**	**0.535**	**0.283**
SEM		**1.19**	**9.36**	**0.060**	**0.026**	**0.006**
35	LC	0.0	10	2.33	0.72	0.31
36	LC	0.0	10	2.04	0.58	0.28
41	LC	0.0	20	1.59	0.41	0.26
42	LC	0.0	14	1.56	0.39	0.25
43	LC	0.0	12	1.64	0.41	0.25
44	LC	0.0	14	1.60	0.41	0.26
45	LC	0.0	22	1.59	0.43	0.27
46	LC	0.0	28	1.49	0.37	0.25
Mean		**0.00**	**16.25**	**1.730**	**0.465**	**0.266**
SEM		**0.00**	**2.28**	**0.104**	**0.043**	**0.008**

Table 6. Measurements of food and water intake and muscle morphology.

						Glycogen						
Animal	**Cond**	**SERCA**	**GP**	**Animal**	**Cond**	**LSR**	**HSR**	**Animal**	**Cond**	**Uptake[1]**	**ATPase**	**Basal**
4	HG	1.00	1.00		HG	335.7	442.5	24	HG	0.834	1.320	0.016
2	HG	1.66	0.50		HG	811.7	579.3	26	HG	0.842	1.950	0.124
6	HG	1.65	1.01		HG	1886.4	1625.8	30	HG	1.048	2.110	0.297
8	HG	1.20	1.11		HG	205.9	134.9	1	HG	0.713	1.360	0.175
4	HG	1.87	0.70		HG		325.3	2	HG	0.945	1.350	0.116
6	HG	1.08	0.76					3	HG	0.518	2.780	0.325
Mean		**1.410**	**0.847**	**Mean**		**809.93**	**621.56**	**Mean**		**0.82**	**1.81**	**0.18**
SEM		**0.148**	**0.095**	**SEM**		**381.73**	**261.43**	**SEM**		**0.08**	**0.24**	**0.05**
3	LG	2.03	0.47		LG	26.9	83.2	22	LG	1.311	2.380	0.027
5	LG	1.15	0.37		LG	12.6	31.4	28	LG	1.152	3.500	0.408
7	LG	1.63	0.59		LG	244.0	95.3	32	LG	1.062	3.470	0.349
9	LG	2.01	0.39		LG	37.2	84.4	4	LG	1.087	2.290	0.286
5	LG	1.90	0.24		LG		28.5	5	LG	1.048	2.410	0.251
7	LG	2.56	0.62					6	LG	1.445	2.390	0.234
Mean		**1.880**	**0.447**	**Mean**		**80.18**	**64.56**	**Mean**		**1.18**	**2.74**	**0.26**
SEM		**0.191**	**0.059**	**SEM**		**54.84**	**14.29**	**SEM**		**0.07**	**0.24**	**0.05**

[1] units: µmol/mg/min

Table 7. Measurements of SR structure and function.

											Twitch		Tetanic	
Animal	**Cond**	**Rest (mN/g DM)**	**Tetanic Fatigue (mN/g DM)**	**Fatigue P/Po**	**Rest (mN/g DM)**	**Twitch Fatigue (mN/g DM)**	**Fatigue (P/Pt)**	**Stair-case (P/Po)**	**Fatigue Final Force (P/Po)**	**Rate Const (/s)**	**+dP/dt (mN/ms)**	**-dP/dt (mN/ms)**	**+dP/dt (mN/ms)**	**-dP/dt (mN/ms)**
37	HC	26.28	17.85	0.679	6.23	3.88	0.623	1.30	0.31	1.00	0.234	0.132	0.409	0.597
38	HC	32.35	16.48	0.509	7.74	3.55	0.459	1.57	0.31	1.19	0.176	0.148	0.382	0.476
39	HC	27.59	17.82	0.646	6.18	3.64	0.589	1.18	0.24	1.66	0.265	0.119	0.460	0.477
40	HC	26.27	18.75	0.714	7.06	2.77	0.392	1.44	0.34	1.32	0.184	0.157	0.328	0.612
47	HC	35.75	21.99	0.615	6.66	2.77	0.416	1.36	0.33	1.02	0.196	0.090	0.379	0.587
48	HC	37.79	12.04	0.319	7.79	2.41	0.309	1.75	0.47	1.31	0.247	0.079	0.375	0.591
49	HC	31.96	19.75	0.618	9.49	5.17	0.545	1.63	0.41	1.72	0.197	0.097	0.321	0.563
50	HC	38.28	18.17	0.475	9.92	4.25	0.428	1.38	0.28	1.46	0.161	0.135	0.453	0.448
51	HC	36.31	16.40	0.452	11.29	3.57	0.316	1.42	0.34	1.17	0.263	0.120	0.416	0.591
Mean		**32.509**	**17.694**	**0.558**	**8.040**	**3.557**	**0.453**	**1.45**	**0.34**	**1.32**	**0.214**	**0.120**	**0.391**	**0.549**
SEM		**1.618**	**0.906**	**0.043**	**0.600**	**0.283**	**0.037**	**0.06**	**0.02**	**0.09**	**0.013**	**0.009**	**0.016**	**0.021**
35	LC	17.56	9.89	0.563	4.93	2.65	0.538	1.04	0.33	1.40	0.217	0.133	0.231	0.657
36	LC	27.25	12.34	0.453	5.93	2.39	0.403	1.16	0.41	1.48	0.236	0.136	0.480	0.615
41	LC	42.03	28.12	0.669	11.33	6.46	0.570	1.17	0.27	1.32	0.197	0.172	0.324	0.626
42	LC	43.26	22.88	0.529	9.83	3.91	0.398	1.53	0.26	1.54	0.187	0.145	0.404	0.619
43	LC	40.44	27.66	0.684	11.31	5.74	0.508	1.14	0.27	1.81	0.228	0.168	0.280	0.639
44	LC	40.80	27.95	0.685	11.37	5.43	0.478	1.17	0.27	1.32	0.192	0.110	0.319	0.582
45	LC	38.94	21.03	0.540	9.39	3.72	0.396	1.53	0.26	1.54	0.255	0.163	0.424	0.580
46	LC	46.45	21.23	0.457	9.29	4.47	0.481	1.26	0.26	1.93	0.206	0.140	0.373	0.551
Mean		**37.091**	**21.387**	**0.573**	**9.173**	**4.346**	**0.471**	**1.25**	**0.29**	**1.54**	**0.215**	**0.146**	**0.354**	**0.609**
SEM		**3.424**	**2.479**	**0.034**	**0.877**	**0.516**	**0.024**	**0.07**	**0.02**	**0.08**	**0.008**	**0.007**	**0.029**	**0.012**

Table 8. Measurements of muscle performance.

VITA

Timothy Wayne Batts was born August 9, 1970 to Mr. and Mrs. Jerome C. Batts. After growing up in Petersburg, Virginia and graduating from Petersburg High School, he pursued an undergraduate degree in Biology at Old Dominion University and transferred to Virginia State University where he graduated with distinction and received his bachelors' degree in biology in 1994.

After graduation he decided to take a break from school and work. He accepted a job as an Assistant Chemist at Petersburg's Wastewater Treatment Plant. In the Spring of 1995, after talking with Dr. Laurence Moore he decided to pursue a graduate degree in Biochemistry at Virginia Tech. Shortly after he got into the program he realized that he hated biochemistry. He was advised to talk with Jay Williams in Human Nutrition, Foods, and Exercise about transferring to that department. After a lot of red tape and proving himself, he was accepted into the master's program. After completing his masters in the fall of 1997 he decided to pursue his doctorate. In anticipation of his completion, he has matriculated to The University of Virginia where he is currently a Post-Doc in the Cardiovascular Research Center.