The Effect of Glycogen Depletion on Sarcoplasmic Reticulum Function

by

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(ABSTRACT)

The role of glycogen in endurance performance has been accepted in theory. It has been shown that higher resting muscle glycogen levels prolong endurance performance. On the other hand, low glycogen levels have been associated with fatigue. Ultimately, a person's muscle glycogen level dictates the duration in which an activity can be maintained at a maximal effort, after which time, performance will decrease. As of yet, there has been no evidence as to what happens to the fatigued muscle. Force production in skeletal muscle is dictated by the release and uptake of Ca^{2+} from the sarcoplasmic reticulum (SR). Force production is proportional to $[Ca^{2+}]$, as $[Ca^{2+}]$ increases so does force. At the point of fatigue, there is a decrease in force production. Since fatigue has been associated with glycogen depletion, it is likely that SR function has been altered causing this decrease in force.

The purpose of this study was to determine the effect of glycogen depletion

on the SR. Twenty male Sprague-Dawley (Harlan Sprague-Dawley, Indianapolis, IN) rats weighing, 345 ± 70 gm were housed two per cage in the Virginia Tech Lab Animal Resources facility. They were fed ad libitum (Purina Rodent Laboratory Chow and water) until time of experiment. Ten of the rats were used as control animals and the other ten were assigned to the experimental group. Rats were allowed a minimum of 5 days to acclimate to their housing. On the morning of the day of testing, rats were selected in pairs according to the housing cage in an effort to decrease variations in food consumption. To reduce muscle glycogen levels, experimental rats were given an initial injection of either epinephrine (1μg/g: ip) while control rats were injected with saline (equal volume) at 0 hr. Thirty minutes later they received another injection of epinephrine or saline (0.5 μg/g: ip). At the end of the hour the rats were anesthetized with pentobarbital sodium (60 mg/kg:ip) for tissue harvesting. Upon reaching a surgical plane of anesthesia one gastrocnemious muscle was extracted for the muscle glycogen assay and the other removed for SR vesicle preparation. Rats were then euthanized with an overdose of pentobarbital sodium. The tissue was assayed for glycogen and glucose levels as well as for Ca^{2+} uptake and release and ATPase activity.

It was found that epinephrine animals had 23% less glycogen than did the control animals and almost twice the amount of glucose (control – 2.9 nmol/g and epinephrine – 5.9 nmol/g). Ca^{2+} uptake rates in epinephrine animals were

significantly decreased by 19.7% ($p < .05$). Control animals had a release rate of 77.15 ± 1.26 nmol/mg/min and epinephrine animals had a release rate of 75.01 ± 1.86 nmol/mg/min. Ca^{2+} release rates were decreased but not significantly. Ca^{2+} stimulated ATPase activity was significanlty decreased by 17.7% in epinephrine animals ($p < .05$).

This is one of the first studies that demonstrate that glycogen reduction in a rested muscle causes altered SR function similar to those caused by exercise. This study shows that low glycogen levels are associated with decreased SR function, which is the primary reason for causing the loss of force in muscle. Ultimately, this study suggests that glycogen loading will enhance endurance performance.

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INTRODUCTION

INTRODUCTION

In todays athletic community, one's ability to withstand long durations of high intensity exercise has become an ever important mission. An athletes capacity to sustain a maximal effort could determine how successful they are. This could mean the difference between being an elite athlete or weekend 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, diet can be more easily manipulated because a persons strength is for the most part determined by genetics. Taking this into consideration, much research has been done to determine the optimal diet regimen for certain events. As a result of this 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, the higher the starting muscle glycogen levels are the better. Endogenous muscle glycogen is the primary fuel source for contraction (Green *et al*., 1991). 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 (Hultman *et al*., 1983). 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 preexercise glycogen levels are in fact linked to endurance. These researchers varied beginning levels of glycogen in individuals and tested their fatiguability. They found that, higher beginning glycogen levels were correlated with increased endurance.

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 positive correlation between the myoplasmic [Ca^{2+}] and force generation. That is, the higher [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+}] are reduced and as a result, force generation is also reduced. Both Ca^{2+} release and sequestration are influenced by [ATP]. 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, this system could be vastly affected by low levels of substrate (glycogen) which fuel glycolysis and provide ATP for Ca^{2+} uptake.

JUSTIFICATION FOR RESEARCH

There is no direct evidence suggesting that glycogen depletion has detrimental effects on SR function. However, Xu *et al*., (1995) experiments imply that a depletion of substrate could inhibit normal functioning of the SR. The purpose of this investigation was to examine the effects of decreased glycogen in rested muscle on SR function. A procedure employed by Bonen *et al*., (1994) was used. This procedure involved injecting epinephrine into rats to induce glycogen depletion then harvesting tissue to analyze glycogen content to determine glycogen loss as compared to control animals. Bonen *et al*., (1994) have shown a 30% decrease in glycogen levels in animals that were injected with epinephrine. The first aim of the present study was to determine if epinephrine caused an adequate decrease in glycogen as reported by Bonen *et al*., (1994). The second aim was to determine the effect of this glycogen depletion on SR function. SR function was evaluated in glycogen deficient muscles by monitoring Ca^{2+} uptake and release rates in isolated SR vesicles.

RESEARCH HYPOTHESIS

The following null hypotheses were tested during this investigation:

H_0: Administration of epinephrine injections will not alter glycogen levels.

H_0: Administration of epinephrine injections will not alter Ca^{2+} uptake rates.

H_0: Administration of epinephrine injections will not alter Ca^{2+} release rates.

H_0: Administration of epinephrine injections will not alter Ca^{2+} ATPase activity.

BASIC ASSUMPTIONS

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

1. All animals started with the same glycogen levels.
2. Epinephrine injections were administered accurately.
3. The SR prep yielded viable vesicles.
4. The glycogen assay indicated correct measurements.
5. Animals were free of disease.

Animals consumed the same amount of food.

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 the SR function. This study investigated the effects of decreased glycogen in rested muscle on SR function. It was hypothesized that a muscle depleted of glycogen would have decreased Ca^{2+} release and uptake thus, contributing to a loss in force (i.e fatigue). This finding would substantiate the link between glycogen and endurance.

CHAPTER TWO:

REVIEW OF LITERATURE

INTRODUCTION

The importance of nutrition in exercise has attracted considerable attention in ones attempt to be more competitive, especially in endurance events. These types of athletes (e.g. marathoners and triathaletes) depend almost solely on their ability to have more stamina, endurance, and be less fatiguable than their opponent. Thus, they tend to rely just as much on the nutritional aspects of training as they do physical training. The last obstacle, onset of fatigue, ultimately determines an endurance athlete's success. It has been shown by Bergstrom *et al*., (1969) that preexercise glycogen levels are linked to endurance. These researchers varied beginning levels of glycogen in individuals and tested their fatiguability and found that, higher beginning glycogen levels were correlated with increased endurance.

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. The cause of the loss in force remains to be somewhat of a mystery. It could be a result of training, genetics, environment, temperature, and a number of external factors. There are a number of things that can become impaired in the contraction process each of which can be categorized into one of two groups: central and peripheral. In the quest to determine the possible mechanisms of peripheral fatigue three theories have been postulated that attempt to describe why fatigue may occur they are: energy depletion, metabolite accumulation, and sarcoplasmic

reticulum (SR) dysfunction. This thesis proposes that the loss of muscle glycogen, a key substrate for energy production plays a very important role in endurance performance. As a consequence this literature review will examine the idea that the depletion of glycogen and SR dysfunction are closely related.

CENTRAL FATIGUE

Figure 1 provides a summary of the possible sites of muscular fatigue. As can be seen there are several sites where fatigue can occur before the signal reaches the periphery. This type of fatigue is often described by a lack of motivation on the subjects 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. 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 psychologically set. In other words, if a person thinks they

can only do 10 pull-ups then at 10 they will stop, not because they are tired but because that is the max that they have set.

PERIPHERAL FATIGUE

When considering the cascade of events in the contraction process of the fibers (Figure 1), 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. So if this is the case then the breakdown that is responsible for the lack of force during fatigue occurs in the 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-bridge due to Ca^{2+}

not being pumped back to the SR fast enough, and/or inadequate ATP, which is needed for dissociation of the cross-bridge. 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, 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 (Costil *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 muscle glycogen content the longer the subject will be able to exercise at that intensity (Costill *et al*., 1980).

Bergstrom *et al*., (1967) have shown that individuals with an initial glycogen content of about 100 mmol /kg could tolerate a 75% VO2max 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% VO2max 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 influences the fatigue process.

SARCOPLASMIC RETICULUM DYSFUNCTION

Another and more feasible mechanism of causing fatigue is SR dysfunction. After prolonged intense exercise bouts there are intrinsic alterations to the hardware that is involved in muscle contraction (SR and contractile proteins) that ultimately leads to a reduction in force output (Williams *et al*., 1995) (Williams *et al*., 1993). This damage manifests itself in the form of swelling, tearing and misalignment of proteins, and decreased Ca^{2+} release (Williams *et al*.,1995). 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 myoplasmic Ca^{2+} and force production. If the Ca^{2+} release channel is altered then there could either be an immediate influx of Ca^{2+} 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, typically show a decreased Ca^{2+} release as compared to a non-exercised muscle (Williams *et al*., 1993). Ca^{2+} release can be restored after exhaustive exercise has occurred 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 Ca^{2+} at the point of exhaustion. These results also lead to the notion that this reduction in Ca^{2+} release is more of a protective mechanism. By reducing the amount of Ca^{2+} released, ATP usage is reduced thus, sparing ATP and preventing any irreversible damage to the contractile proteins (Williams *et al*.,1995). This decrease in Ca^{2+} release appears to be a response mechanism to damage that occurs to the contractile hardware and not as a result of fatigue.

On the other hand, the Ca^{2+} pump appears to respond to exhaustive exercise with a decreased function. Booth *et al*., (1997) have shown that human skeletal muscle that has undergone prolonged exercise show a 17% and 21% decrease in Ca^{2+} uptake and Ca^{2+} ATPase activity respectively. Linked to this decrease in uptake and ATPase activity was a reduction in muscle glycogen at exhaustion. Byrd *et al*., (1989) have shown similar results with thoroughbred horses that were run to exhaustion on a treadmill. They reported significantly decreased Ca^{2+} uptake and Ca^{2+} ATPase activity after exhaustion also. To further prove their point they essentially repeated the experiment with rats. They found a decrease in Ca^{2+} uptake and Ca^{2+} ATPase activity also. Belcastro *et al*., (1993) have done similar experiments with rats and have found the same results. After prolonged intense exercise isolated SR vesicles showed decreased Ca^{2+} uptake and Ca^{2+}

ATPase activity (12% and 18% respectively). However, Dossett-Mercer et al., (1994) have shown conflicting results using rats. They found no change in Ca^{2+} uptake and Ca^{2+} ATPase activity after fatigue however, they showed significant decreases in muscle glycogen. This could be a result of using a muscle homogenate as opposed to isolating the SR to its purest form.

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 for the most part, only showing minimal decreases (Green, 1990). Xu *et al*., (1995) have demonstrated that cardiac and skeletal SR vesicles have glycolytic enzymes (aldolase, 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 exogenous ATP sources. To determine the presence of these enzymes, the necessary substrates that fuel specific steps in glycolysis were added in the absence of exogenous ATP then Ca^{2+} uptake was monitored (Figure 2). For pyruvate kinase (the final ATP production step in glycolysis),

phosphoenolpyruvate (PEP) and ADP were added and Ca^{2+} ATPase activity was seen. Next, the substrate for enolase (2-phosphoglycerate) was added and Ca^{2+} ATPase activity was observed which indicates that PEP was formed from this reaction and was used for the synthesis of ATP by pyruvate kinase. Next, the substrate for phosphoglyceromutase (3-phosphoglycerate) was added and Ca^{2+} ATPase activity was observed, which indicates that 2-phosphoglycerate was formed and converted to PEP by enolase then PEP was converted to ATP by pyruvate kinase. Next, the presence of GAPDH and PGK (the second ATP production steps) was determined by adding the appropriate substrates for ATP production (glyceraldehyde 3-phosphate (GAP), NAD^+, P_i, and ADP). ATPase activity was then observed which indicates that GAP was converted to 1,2-diphosphoglycerate by GAPDH which in turn was converted to 3-phosphoglycerate by PGK (ATP producing step). This formation of ATP was also able to support Ca^{2+} ATPase activity. It was hypothesized that the 3-phosphoglycerate continued through the remainder of steps which ultimately produces another ATP by pyruvate kinase.

Because SR experiments require for the most part, exogenous ATP sources it was necessary for Xu *et al*., (1995) to determine whether endogenously produced ATP could provide more effective support for Ca^{2+} uptake than exogenously added ATP. They found that the presence of ATP and ADP as substrates, in regards to Ca^{2+} ATPase activity, was significantly less effective in

supporting Ca^{2+} uptake than with the presence of PEP and ADP. This indicates that ATP formed by SR associated pyruvate kinase from ADP and PEP is used more efficiently by the Ca^{2+} ATPase than exogenous ATP. This being the case, the ATPase activity associated with the SR membrane may be more dependent on ATP that is generated from glycolysis than from exogenous ATP. Hence, this system could be vastly affected by low levels of substrate (glycogen) which fuel glycolysis and provide ATP for Ca^{2+} uptake.

GLYCOGEN AND THE SARCOPLASMIC RETICULUM

Of these three theories, 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; 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 in time to exhaustion. 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 exercised to exhaustion. Time to exhaustion was decreased by more than 50% which indicates the important role that glucose plays in endurance.

SUMMARY

When the data from Chin *et al*., (1997) 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. It is my intention to determine if there is such a link. Correlational data do not imply cause and effect. No definitive explanation to link glycogen depletion and fatigue has been identified.

There is no direct evidence suggesting that glycogen depletion has

detrimental effects on SR function. It was my intention to investigate the effects of decreased glycogen in rested muscle on SR function. A procedure employed by Bonen *et al*., (1994) was used. This procedures involved injecting epinephrine into rats to induce glycogen depletion then harvesting tissue to analyze glycogen content to determine glycogen loss as compared to control animals. Bonen *et al*., (1994) have shown a 30% decrease in glycogen levels in animals that were injected with epinephrine. After glycogen depletion was determined then SR function was evaluated by monitoring Ca^{2+} uptake and release rates in isolated SR vesicles. The first aim of this study was to determine if epinephrine would cause an adequate decrease in glycogen as compared to Bonen *et al*., (1994). The second and most important aim of this study was to determine the effect of this glycogen depletion on SR function. Preliminary data has shown that both uptake rates and release rates are decreased by epinephrine injections. Associated with this is a 57% reduction in muscle glycogen levels. These results suggest that glycogen depletion affects the uptake rate substantially and supports the research stated earlier in this paper.

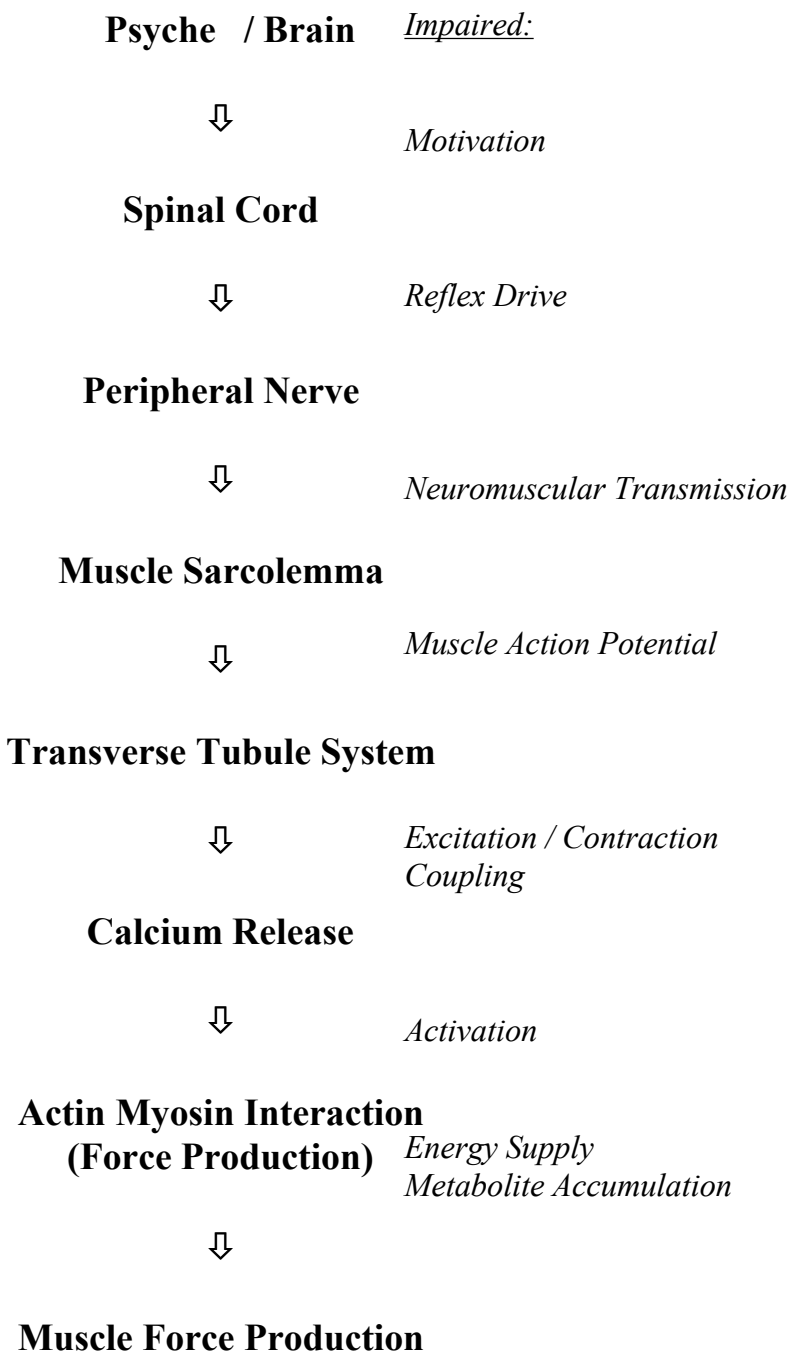

Figure 1. Possible sites of muscular fatigue.

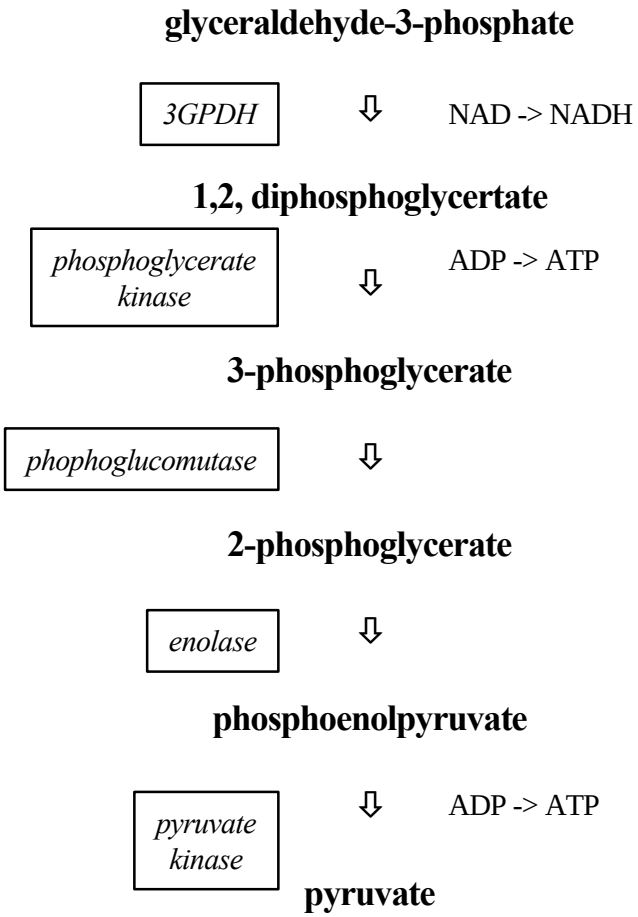

Figure 2. Sequence of the latter steps in glycolysis, including the metabolic intermediates, substrates and cofactors involved in each reaction.

CHAPTER THREE:

METHODOLOGY

ANIMALS

Twenty male Sprague-Dawley (Harlan Sprague-Dawley, Indianapolis, IN) rats weighing, 345± 70 gm were housed two per cage in the Virginia Tech Lab Animal Resources facility. They were fed ad libitum (Purina Rodent Laboratory Chow) and water until time of experiment. Ten of the rats were used as control animals and the other ten were assigned to the experimental group.

TISSUE HARVESTING

Rats were allowed a minimum of 5 days to acclimate to their housing. On the morning of the day of testing, rats were selected in pairs according to the housing cage in an effort to decrease variations in food consumption as much as possible. To reduce muscle glycogen levels, rats were given an initial injection of either epinephrine (1μg/g: ip), control animals received saline (equal volume) at 0 hr. Then 30 minutes later they received another injection of epinephrine or saline (0.5 μg/g: ip). At the end of the hour the rats were anesthetized with pentobarbital sodium (60 mg/kg:ip) for tissue harvesting. Upon reaching a surgical plane of anesthesia one gastrocnemious muscle was extracted for the muscle glycogen assay and the other removed for SR vesicle preparation. Rats were then euthanized with an overdose of pentobarbital sodium.

GLYCOGEN ASSAY

Tissue Homogenization

After the tissue was harvested it was blotted dry, weighed and thoroughly homogenized in a solution containing 5 volumes by weight of perchloric acid. Immediately after homogenization, 0.2 ml of this suspension was removed or frozen (80 C) and used for glycogen hydrolysis. The remainder was centrifuged for 10 minutes and the supernatant used for the determination of tissue glucose.

Glycogen Hydrolysis

The 0.2 ml of muscle homogenate was mixed with $KHCO_3$ (43 mmol/l) and amyloglucosidase solution (174 mmol/l) at acidic pH 4.8. Amyloglucosidase enzymatically degrades glycogen into glucose at low pH. Samples were stoppered and incubated with shaking at 40° for 2 hours, after which 1 ml of perchloric acid was added to terminate the reaction. The solution was then mixed by inversion or vortex then centrifuged for 10 minutes (1100 g force) and the supernatant was used for glucose determination.

Glucose Determination

Glucose was measured in both the tissue glucose and glycogen hydrolysis samples. 50 µl of each sample was added to 1 ml of a ATP/NADP/G6P-DH/buffer solution (ATP, 1 mmol/l; NADP, 0.9 mmol/l; TEA buffer, 285 mmol/l;

$MgSO_4$, mmol/l; and G6P-DH, 0.7 kU/l). The solution was mixed well and allowed to sit for 5-8 minutes then absorbance was determined spectrophotometrically at 339 nm (A_1). This value represents the free G6P in each sample. 5 µl of hexokinase solution (1.3 kU/l) was then added and samples incubated for 5-10 minutes and absorbance read again (A_2). This value represents the free G6P plus free glycogen in the sample. To determine free glucose the difference between A_1 and A_2 was computed. Glycogen was then calculated by subtracting the free glucose in the tissue sample from the free glucose in the glycogen hydrolysis sample ($\Delta A_{gly} * 330.6 - \Delta A_{glu} * 20.1$). Absorbance values were converted to glucosyl units using Beer's law and an extinction coefficient of (6220/cm/M).

SARCOPLASMIC RETICULUM VESICLE PREPARATION

After muscles were removed they were weighed and homogenized using a Virtishear in an isolation buffer containing (in mM) 20 N-2-hydroxyethylpiperazine-N'-2ethanesulfonic acid (HEPES, pH 7.5), 250 sucrose, 0.2% sodium azide, and 0.2 phenylmethylsulfonyl flouride (PMSF) and centrifuged at 1600 g for 10min. The supernatant was then filtered using gauze and then centrifuged twice at 10,000 g for 20min after which the supernatant was collected. This fraction was again centrifuged at 48,000g for 90min. The pellet was then resuspended in isolation buffer, supplemented with 0.6 M KCl, and

centrifuged at 48,000 g (60min). The final pellet was resuspended in isolation buffer, frozen, and stored at -80°C. Protein concentrations were determined by Bradford protocol as described in the Bio-Rad kit.

SR Ca^{2+} uptake and release

Ca^{2+} uptake and release rates were 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. 50 μg of SR protein was added and allowed to equilibrate for 3 min. Uptake was initiated by the addition of 2 mM Na_2ATP and allowed to continue until no change in extravesicular free Ca^{2+} was observed. After uptake was completed, Ca^{2+} release was initiated by the addition of 5 μM $AgNO_3$. The rates of Ca^{2+} uptake and release were then determined by the steepest negative and positive slope of the free Ca^{2+} vs. time curve. All rates and magnitudes of Ca^{2+} exchange were normalized for SR protein concentration. All samples were run in quadruplicate.

Extravesicular free Ca^{2+} was measured with the fluorescent Ca^{2+} indicator fura 2 (2 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) is assumed as being 240 nm, R_{min} and R_{max} are the R values measured in the uptake buffer supplemented with 10 mM EGTA and 1mM Ca^{2+} added, respectively. β is the ratio of fluorescence measured at 380 nm excitation for the EGTA- and Ca^{2+} -supplemented buffers, respectively and $[Ca^{2+}]_{free}$ is the free Ca^{2+} concentration.

Ca^{2+}ATPase Assay

1 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. 30 μg of SR was added to the cuvette and was allowed to equilibrate for 3 minutes in the spectrophotometer which was set at 300 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.

STATISTICAL ANALYSIS

All data was collected by computer for subsequent analysis either by the Sigma Stat or Excel package using a t-test, with a significance set at $p< 0.05$.

CHAPTER FOUR:

RESULTS

APPEARANCE AND WEIGHT OF ANIMALS AND TISSUE

There were no significant differences in mean animal body mass and gastrocnemius mass (Table 1 and Appendix). Epinephrine injected rats appeared to be very lethargic and had significant increases in respiration as compared to the control animals. Breathing was also noted as being shallow. It should also be noted that epinephrine treated animals required approximately twice as much of the euthanizing drug (pentobarbitol sodium). There was no difference in appearance of the extracted tissue.

GLYCOGEN AND GLUCOSE LEVELS

Control animals had an average glycogen content of 32.9 ± 3.2 nmol/g as compared to epinephrine animals which were 23.8% lower (25.1 ± 2.4 nmol/g $p < .05$) (Table 2 and Figure 3). This evidence suggests that the epinephrine injections were successful in reducing glycogen. These results correspond with those of Bonen *et al*., (1994) who have shown a 30% decrease in glycogen levels after epinephrine injections. In support of these results, muscle serum glucose levels were also calculated. If the epinephrine injections did in fact breakdown glycogen into glucose then there should be a significant increase in glucose levels for the experimental animals as compared to the control animals. There was more than a 100% increase in mean muscle serum glucose levels for the epinephrine animals (epi- 6.0 ± 0.6 mmol/gm and control- 2.94 ± 0.5 mmol/gm) (Appendix).

RELIABILITY OF GLYCOGEN DATA

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 and I feel confident that the values given in this paper are accurate.

SARCOPLASMIC RETICULUM YIELD

There was no significant difference in the amount of total protein recovered in the preparation of vesicles (Appendix). If there had been a difference in the amount of protein yielded then any differences found in uptake or release would be difficult to attribute to the epinephrine injections. Because the pump is a protein, any detectable decrease in amounts will ultimately translate into decreased activity. For example, if the experimental animals had lower total protein recovery then a decrease in uptake rate could be attributed to less protein. To further ensure that there was no loss of SR protein samples were run on a SDS-PAGE. The results showed no difference in size of the bands associated with the Ca^{2+} ATPase or release channel between control and epinephrine animals and migration.

Ca^{2+} UPTAKE AND RELEASE RATES

The average uptake rate for the control animals was 92.2 nmol/mg/min as compared to the epinephrine animals which displayed a 19.7% difference in uptake (74.0 nmol/mg/min) $p < .05$ (Table 2 and Appendix). Because it was shown previously that there was no loss in protein between groups this reduction in uptake rate is likely due to differences in pump activity. There was no statistical difference with release rates (Control - 77.2 nmol/mg/min and Epinephrine - 75. 0 nmol/mg/min).

ATPase ACTIVITY

In conjunction with the 19% difference in Ca^{2+} uptake there was an 18% difference in Ca stimulated ATPase activity with the epinephrine animals (Control - 4.4 μmol/mg/min and epinephrine - 3.7 μmol/mg/min) $p < .05$ (Table 2 and Appendix). This gives more evidence that the uptake pump is dependent on ATP formed by glycolytic enzymes. The Ca^{2+}ATPase is an enzyme that breaks down ATP into ADP to fuel energy requiring reactions. Because the uptake pump requires ATP and a difference in rates has been established due to glycogen depletion which leads to decreased ATP, then when the pump slows down the ATPase activity should do the same.

CORRELATION BETWEEN GLYCOGEN AND UPTAKE AND RELEASE

To better illustrate the relationship between glycogen levels and SR function a correlation coefficients were computed (Figures 4 & 5). This shows that there is a positive correlation between glycogen levels and SR function. As glycogen levels decrease so does uptake and release rates. The correlation between uptake and glycogen is slightly better than the release nonetheless there is a trend seen with both. This is evidence that directly links glycogen levels to SR function.

Table 1. Mean body and tissue weights

Condition	Body Mass (g)	Gastrocnemius Mass (g)
Control	341.3	1.976 ± 0.129
Epinephrine	351.5	1.906 ± 0.106

Table 2. Mean uptake and release rates and corresponding ATPase activity with standard deviations.

Condition	Ca^{2+} Release nmol/mg/min	Ca^{2+} Uptake nmol/mg/min	Mg ATPase μmol/mg/min	Ca^{2+} ATPase μmol/mg/min
Control	77.15 ± 1.26	92.18 ± 7.19	1.087 ± 0.089	4.438 ± 0.396
Epinephrine	75.01 ± 1.87	73.97 ± 5.98	1.122 ± 0.055	3.653 ± 0.185

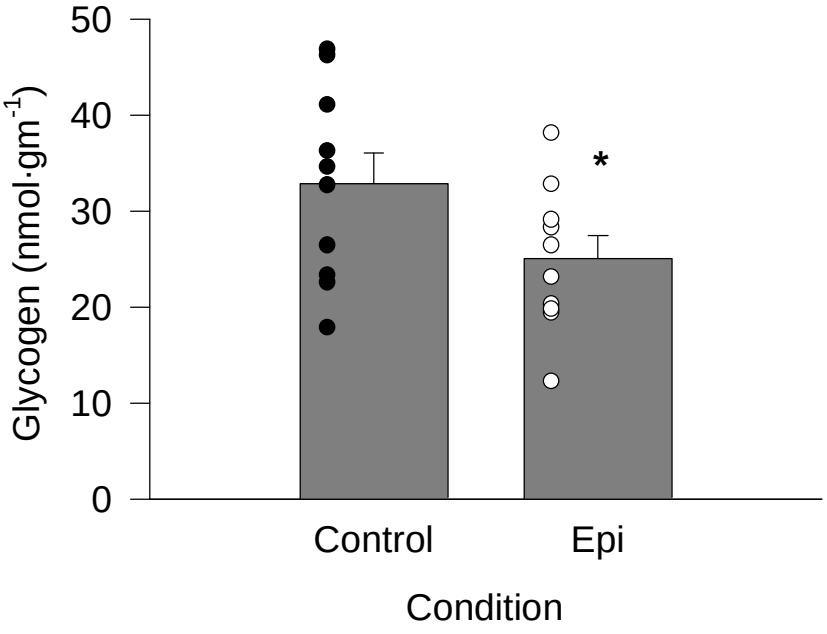

Figure 3. Gastrocnemius glycogen content in control and epinephrine treated animals. Shown are mean±SE as well as individual values. *p<.05.

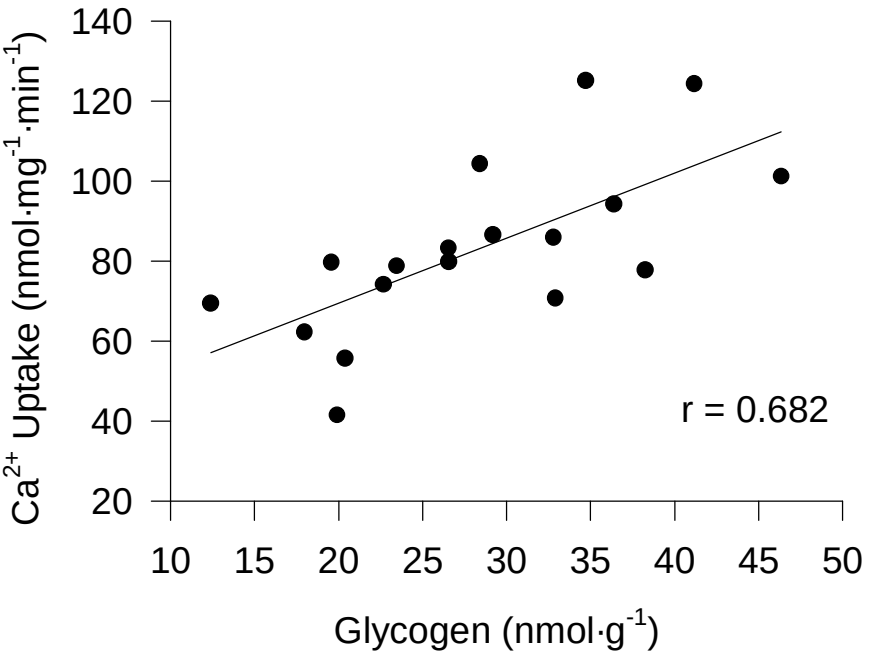

Figure 4. The relationship between muscle glycogen content and the rate of SR Ca^{2+} uptake. ($p<.05$)

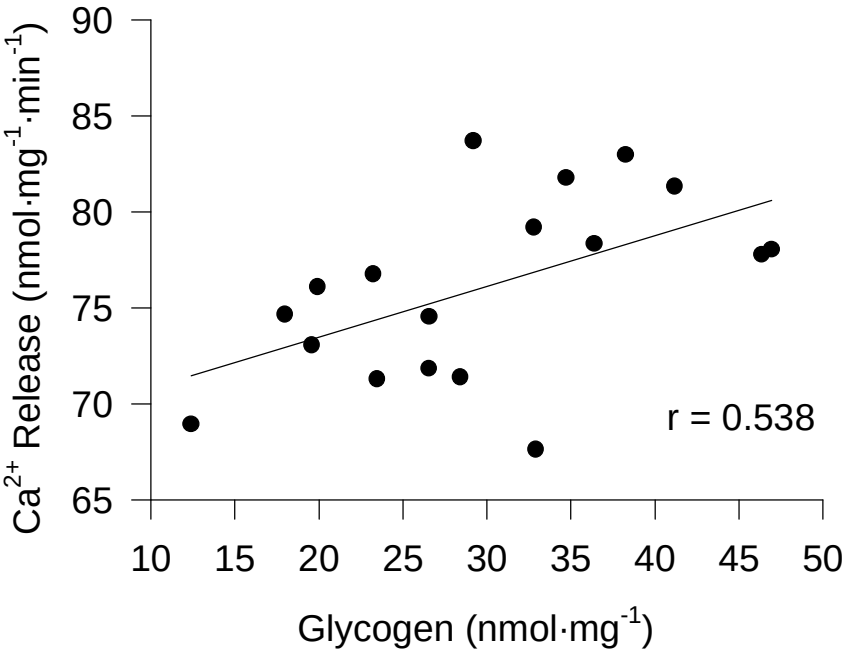

Figure 5. The relationship between muscle glycogen content and the rate of SR Ca^{2+} release. ($p<.05$)

CHAPTER FIVE:

DISCUSSION

EFFECTS OF EPINEPHRINE ON GLYCOGEN AND GLUCOSE

Epinephrine is a catecholamine that is naturally released in the body from the adrenal medulla, and is considered to be the primary factor in mobilizing glucose from the liver. Epinephrine in comparison to norepinephrine, which also stimulates the glycolytic rate, seems to be more responsive to changes in the plasma glucose concentration. A low plasma glucose concentration stimulates a receptor in the hypothalamus to increase epinephrine secretion (Powers *et al*., 1994). Epinephrine then binds to beta receptors in the liver and muscle and stimulates the breakdown of glycogen into glucose for release into the plasma or myoplasma. Powers et al., (1994) have shown that epinephrine levels increase linearly with exercise, however, this study involved rested muscle.

Epinephrine injections proved to be effective in reducing glycogen levels in resting muscle. This decrease in glycogen in unexercised muscle is not as great as in a muscle that has undergone high intensity exercise (60% - 80% reduction). Glycogen reduction associated with epinephrine would most likely correspond to low intensity exercise. If this epinephrine associated glycogen depletion is enough to cause diminished SR function without the damage that is associated with high intensity exercised muscle (swelling and tearing) then this demonstrates that there is a direct link between glycogen depletion and the SR. This link can be made because all of the variables that normally elicit SR dysfunction in exercised muscle

have been eliminated, except the reduction of glycogen. Even so, the reduction in glycogen associated with exercise is significantly higher than in this experiment yet the SR still displays some malfunction at this lower reduction of glycogen.

EFFECTS ON SARCOPLASMIC RETICULUM

The data show a very strong correlation between glycogen levels and SR function. As glycogen levels decrease SR function decreases. There appears to be a better relationship with the uptake of Ca^{2+} than with release. This information is interesting in lugh of that found by Xu *et al*., (1995). They showed that the SR has embedded in its membrane the necessary glycolytic enzymes to produce ATP and the SR is 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. This decreased SR function manifests itself at the Ca^{2+} uptake pump (ATP dependent) most noticeably.

Ca^{2+} release is also ATP dependent and shows some depression in activity. It is possible that the presence or absence of glycolytically formed ATP has an effect on Ca^{2+} release. In this experiment, Ca^{2+} release was depressed slightly but it had a strong correlation with glycogen levels. As glycogen decreased so did Ca^{2+} release. Chin *et al*., (1997) have attempted to explain this mechanism. They 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 drop (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 criticised 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 supply in the SR triadic region falls and a decrease in Ca^{2+} release and force results (Chin *et al*., 1997).

IMPLICATIONS FOR FATIGUE

This is one of the first studies that demonstrate that glycogen reduction in a rested muscle causes altered SR function similar to those caused by exercise. Chin *et al*., (1997) have 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 in time to exhaustion. 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 exercised to exhaustion. Time to exhaustion was decreased by more than 50% which indicates the important role that glycogen plays in endurance. This study provides a direct link between glycogen depletion and fatigue. In comparison to the study done by Chin *et al*., (1997) this study

implicates glycogen levels as the key factor in fatigue. This study used rested muscle whereas Chin *et al*., (1997) used exercised muscle but the same results were achieved, altered function. It has been well documented that lower glycogen levels are associated with fatigue however there has never been any evidence to provide insight into how the muscle is affected internally to cause this decrease in force. This study shows that low glycogen levels are associated with decreased SR function, which is the primary reason for causing the loss of force in muscle. Ultimately, this study suggests that glycogen loading will enhance endurance performance.

SUGGESTIONS FOR FUTURE RESEARCH

This study provided very useful information concerning the link between glycogen and endurance performance. However, there are some things that I would change if I were to repeat this experiment. The first change I would make involves the housing and feeding of the animals. The problem with this study was that there was no way to monitor the dietary intakes of the paired rats. As a result, glycogen levels showed some variability. To solve this problem, next time I would house each animal separately and monitor food consumption the day before the experiment.

Another suggestion would be to remove the livers from the animals to measure glycogen and glucose levels. Since this study involved rested muscle

maybe next time exercised muscle could be used. Better yet, the diaphragms could be removed from the rested animals to see what differences there would be. This might provide different results because while the animals are not exercised the epinephrine causes increased respiration which causes the diaphragm to contract. Another suggestion would be to use exercised animals. This way glycogen depletion would be more representative of what happens in humans. This would allow better comparison to Chin et al., (1997) results.

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APPENDIX:

RAW DATA

Table 1. Gastrocnemius muscle mass and SR protein yield values in control and epinephrine treated animals.

No.	Cond	[Protein] (µg/µl)	Mass (gm)	Vol (ul)	Yield (mg/g)
1	Control	2.68	1.52	400	0.70
3	Control	1.92	1.59	400	0.48
5	Control	3.70	2.70	750	1.03
7	Control	2.88	2.55	750	0.85
9	Control	2.65	1.95	400	0.54
11	Control	3.33	1.61	400	0.83
13	Control	3.90	1.79	400	0.87
15	Control	4.84	1.81	400	1.07
17	Control	2.86	2.28	400	0.50
19	Control	1.92	1.96	400	0.39
Mean		**3.069**	**1.976**		**0.727**
SE		**0.285**	**0.129**		**0.075**
2	Epi	3.06	1.57	400	0.78
4	Epi	2.38	1.59	400	0.60
6	Epi	3.09	2.54	750	0.91
8	Epi	2.88	2.43	750	0.89
10	Epi	4.84	1.70	400	1.14
12	Epi	5.08	1.84	400	1.11
14	Epi	5.00	1.77	400	1.13
16	Epi	4.92	1.72	400	1.14
18	Epi	2.97	1.87	400	0.64
20	Epi	3.06	2.03	400	0.60
Mean		**3.729**	**1.906**		**0.894**
SE		**0.342**	**0.106**		**0.073**
% Diff		**21.5**	**-3.5**		**22.9**
t-test		**0.078**	**0.340**		**0.064**

Table 2. Measurements of gastrocnemius SR function as well as glycogen and glucose content in control and epinephrine treated animals.

No.	Cond	Uptake (nmol/mg/min)	Release (nmol/mg/min)	Glycogen (nmol/g)	Glucose (nmol/g)	Basal ATPase (µmol/mg/min)	CaStim ATPase (µmol/mg/min)
1	Control	101.21	77.79	46.35	1.59	0.51	3.60
3	Control		78.05	46.94	1.33	1.10	3.78
5	Control	124.36	81.34	41.17	6.43	1.06	7.54
7	Control	125.16	81.78	34.70	1.67	0.76	4.92
9	Control	94.22	78.35	36.38	2.63	1.02	4.31
11	Control	85.98	79.20	32.79	2.25	1.31	3.62
13	Control	83.34	71.86	26.53	2.89	1.35	3.48
15	Control	78.83	71.31	23.45	2.99	1.30	5.31
17	Control	74.23		22.68	2.77	1.06	4.21
19	Control	62.26	74.67	17.97	4.84	1.40	3.61
Mean		**92.177**	**77.150**	**32.896**	**2.939**	**1.087**	**4.438**
SE		**7.189**	**1.259**	**3.196**	**0.499**	**0.089**	**0.396**
2	Epi	104.34	71.40	28.41	7.30	1.06	3.12
4	Epi		76.76	23.23	3.22	0.98	3.24
6	Epi	77.82	82.98	38.24	3.42	0.99	3.19
8	Epi	86.53	83.69	29.19	7.18	1.34	3.54
10	Epi	79.84	74.55	26.56	6.83	0.95	3.96
12	Epi	70.76	67.63	32.91	4.44	1.10	3.84
14	Epi	79.71	73.07	19.56	4.90	0.95	4.00
16	Epi	69.47	68.94	12.38	5.15	1.37	4.84
18	Epi	55.72		20.39	8.04	1.11	3.96
20	Epi	41.57	76.10	19.91	9.19	1.37	2.84
Mean		**73.973**	**75.013**	**25.078**	**5.967**	**1.122**	**3.653**
SE		**5.981**	**1.869**	**2.377**	**0.640**	**0.055**	**0.185**
% Diff		**-19.7**	**-2.8**	**-23.8**	**103.0**	**3.2**	**-17.7**
t-test		**0.035**	**0.179**	**0.033**	**0.001**	**0.372**	**0.045**

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 and a minor in chemistry.

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. He soon grew bored with this and after talking with Dr. Laurence Moore he decided to pursue a graduate degree in Biochemistry. 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 his self he was accepted into the master's program. During his academic career in this department he decided to upgrade his status to doctoral student. After he defends his thesis he will continue on for his doctorate and eventually get a job in the field of muscle physiology.