Effects of Pre-exercise Muscle Glycogen Status on Muscle Phosphagens, Sarcoplasmic Reticulum Function, and Performance During Intermittent High Intensity Exercise

Michelle R. Smith

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition, Foods & Exercise

Janet Walberg-Rankin Ph.D., Committee Chair
Jay Williams Ph.D.
Michael Houston Ph.D.

August 9, 1999
Blacksburg, Virginia
Effects of Pre-exercise Muscle Glycogen Status on Muscle Phosphagens, Sarcoplasmic Reticulum Function, and Performance During Intermittent High Intensity Exercise

Michelle Smith

(ABSTRACT)

Eight competitive cyclists performed two cycling trials, one following a high carbohydrate diet (H-CHO) and the other following a low carbohydrate diet (L-CHO). Trials consisted of repeated 60s maximal effort sprints to fatigue at a workload designed to elicit 125-135% VO2peak at 90rpm. Three min of recovery separated sprints. Muscle biopsies taken at rest (biopsy 1), 85% max interval rpm (biopsy 2), and 70% max interval rpm (biopsy 3) revealed a main effect of diet on muscle glycogen levels: 609 ± 38 mmol/kgdw H-CHO vs. 390 ± 42 mmol/kgdw L-CHO at biopsy 1, 383 ± 29 vs. 252 ± 28 mmol/kgdw at biopsy 2, and 346 ± 29 vs. 196 ± 18 mmol/kgdw at biopsy 3 (p<0.01). Similar decreases in muscle glycogen (45%), creatine phosphate (CP) (35%), and sarcoplasmic reticulum (SR) Ca2+ uptake (56%) were shown in both trials from biopsy 1 to 3. SR Ca2+ release decreased by 53% in H-CHO subjects and 36% in L-CHO subjects. Total exercise time tended to be longer in H-CHO than L-CHO subjects (57.5 ± 10 vs. 42.0 ± .89min) (p=0.09). H-CHO subjects exercised significantly longer than L-CHO subjects from biopsy 2 to 3 (33.6 ± 10 vs. 18 ± 3.6min) (p< 0.05). Results suggest that fatigue from 40-60min of intermittent 60s high intensity cycling intervals is associated with reductions in muscle glycogen, CP, and SR function, and that the latter part of performance is impaired by low muscle glycogen. These data do not support a relationship between muscle glycogen status and SR function in intermittent high intensity exercise.

Keywords: carbohydrate, creatine phosphate, calcium, cycle exercise, muscle biopsy
This research was funded by The American College of Sports Medicine Reebok Student Grant, Gatorade Sports Science Institute, and the James D. Moran Thesis and Dissertation Award.
ACKNOWLEDGEMENTS

Many people have been instrumental throughout my work on this thesis. Above all, I am truly blessed with the support of my wonderful family – Jim, Donna, and Jamie Smith. Although they have never become fans of the muscle biopsy idea, I will cherish how they have always been my greatest fans, no matter what the endeavor, and no matter what the outcome.

I have been so fortunate to have Helen Stevens as my research partner, roommate, and friend. She is a very enthusiastic and hard-working person; her patience and smiles are a big part of what I will remember about this project.

The expertise and generosity of my committee members – Janet Walberg-Rankin, Jay Williams, and Mike Houston – have enabled me to learn a tremendous amount during the past two years. Through her guidance and her example, Dr. Rankin has helped me become a better researcher, writer, and student. I appreciate her confidence in me to do a project while she was away, although she was so reliable and supportive that I barely even knew she was gone. I will take many things from Dr. Rankin into my professional career, because I know I’ve learned from an expert. I am very lucky to have been exposed to Dr. Williams’ knowledge, experience, and talents in the area of muscle fatigue through several classes and thesis work. He played an integral role in designing the study. Working with Dr. Houston this year allowed me to develop a better understanding of many important metabolic concepts and gain more confidence in the lab. He is a fantastic teacher and I admire how he is able to accomplish all of his responsibilities while still making time to teach courses and give students individual attention.

Janet Rinehart is the true unsung hero of this project and many others. I appreciate all of her help in the lab, the many early mornings and weekends, and her friendship. Her muscle biopsy skills have developed immensely and we have video footage and 48 successful biopsies to prove it! Her dedication and fun personality have added a lot to my experiences in graduate school.

I would like to thank our subjects from the Virginia Tech and East Coasters Cycling
teams. The effort they committed to the project was amazing. They made data collection fun and successful – they are outstanding athletes and admirable people.

There are several other individuals who committed an incredible amount of time to the project. I wish I could repay them for their kindness and I hope they are surrounded by the caliber of people in their work that I was blessed with in mine. Simon Lees and Randy Bird faithfully completed the most undesirable lab tasks, always with cheerful attitudes despite the long hours and early mornings. Christina Baum was a relentless pilot subject and photographer who could always be depended on for valuable advice. Dr. William Epstein was present for all of our lab days, despite having the flu and another job to take care of. Dr. Lawrence Cross and Greg Steeno patiently guided me through statistical analysis. Kathy Reynolds helped with pilot work and in teaching me the biopsy assistant technique. Ben Toderico dedicated two days to modifying our cycle. Jennifer Blevins spent time training us on lab equipment. There were several other students who served as assistants and pilot subjects and without them, the study would not have been possible.

I am privileged to know Katie Ollendick, Leslie Archilla, Lida Johnson, and Jeannemarie Beiseigel, my terrific roommates and pals. They showed me so many times during the past two years that a cup of coffee and the support of true friends can make hard times tolerable and good times unforgettable.

Finally, John Rockwell had what was probably the toughest job of all. He developed assays, taught lab techniques, served as a pilot subject, tested diet treatments, repaired computers, modified cycles, edited writing, and went out of his way countless times to make my life easier. I am glad his thesis came first because I would never have been able to do as much for him as he has done for me. May our roller coaster continue.
# TABLE OF CONTENTS

## ACKNOWLEDGEMENTS

### LIST OF TABLES

### LIST OF FIGURES

## CHAPTER 1:

### INTRODUCTION

STATEMENT OF THE PROBLEM

OBJECTIVES

HYPOTHESES

BASIC ASSUMPTIONS

DELIMITATIONS

LIMITATIONS

DEFINITIONS AND SYMBOLS

## CHAPTER 2:

### REVIEW OF LITERATURE

INTRODUCTION

GLYCOGEN AND HIGH INTENSITY EXERCISE

PHOSPHAGENS AND HIGH INTENSITY EXERCISE

SARCOPLASMIC RETICULUM FUNCTION AND FATIGUE

## CHAPTER 3:

### JOURNAL MANUSCRIPT
<table>
<thead>
<tr>
<th>APPENDIX C:</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STATISTICAL PROCEDURES AND RESULTS</strong></td>
<td>150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDIX D:</th>
<th>167</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INSTITUTIONAL REVIEW BOARD PROPOSAL</strong></td>
<td>167</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDIX E:</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INFORMED CONSENT &amp; HEALTH HISTORY QUESTIONNAIRE</strong></td>
<td>175</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDIX F:</th>
<th>189</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RECRUITMENT FLYER</strong></td>
<td>189</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDIX G:</th>
<th>191</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INSTRUCTIONS GIVEN TO SUBJECTS</strong></td>
<td>191</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDIX H:</th>
<th>197</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PILOT PERFORMANCE DATA</strong></td>
<td>197</td>
</tr>
</tbody>
</table>

| REFERENCES | 204 |

| VITA | 218 |
LIST OF TABLES

MANUSCRIPT TABLES

Table 1. Subject Characteristics 68
Table 2. Sample Subject Schedule 69
Table 3. Macronutrient Composition of Subjects’ Normal Diets 70
Table 4. Sample Diets for a Subject with a 3400 kcal Baseline Diet 71
Table 5. Overall Correlation Analysis (data from all biopsies included) 72

APPENDIX TABLES

Table 1. Individual Subject Characteristics 127
Table 2. Composition of Subjects’ Normal Diets 128
Table 3. Body Weight Data 129
Table 4. Individual Subject Overall Performance Data 130
Table 5. Individual Subject Performance Data – in Biopsy Segments 131
Table 6. Individual Subject Muscle Glycogen Data 140
Table 7. Individual Subject Muscle TC Data – NOT ADJUSTED 141
Table 8. Individual Subject Muscle CP Data – NOT ADJUSTED 142
Table 9. Individual Subject Muscle Cr Data – NOT ADJUSTED 143
Table 10. Individual Subject Muscle ATP Data – NOT ADJUSTED 144
Table 11. Individual Muscle CP Data – ADJUSTED 145
Table 12. Individual Muscle Cr Data – ADJUSTED 146
Table 13. Individual Muscle ATP Data – ADJUSTED 147
Table 14. Individual Subject Sarcoplasmic Reticulum Ca^{2+}-uptake Data 148
Table 15. Individual Subject Sarcoplasmic Reticulum Ca^{2+}-release Data 149
Table 16. Paired t-tests for Performance Data 151
Table 17. RM ANOVA Table for Muscle Glycogen Data 152
Table 18. Analysis of Contrasts for Muscle Glycogen Data 153
Table 19. RM ANOVA Table for Muscle CP Data 154
Table 20. Analysis of Contrasts for Muscle CP Data 155
Table 21. RM ANOVA Table for Muscle Free Creatine Data 156
Table 22. Analysis of Contrasts for Muscle Free Creatine Data 157
Table 23. RM ANOVA Table for Muscle ATP Data 158
Table 24. Analysis of Contrasts for Muscle ATP Data 159
Table 25. RM ANOVA Table for SR Ca^{2+}-uptake Data 160
Table 26. Univariate Contrasts for SR Ca^{2+}-uptake Data 161
Table 27. RM ANOVA Table for SR Ca^{2+}-release Data 162
Table 28. Analysis of Contrasts for SR Ca^{2+}-release Data 163
Table 29. Correlation Analysis for Biopsy 1 Data 164
Table 30. Correlation Analysis for Biopsy 2 Data 165
Table 31. Correlation Analysis for Biopsy 3 Data 166
LIST OF FIGURES

Figure 1. Intervals performed in both trials before declining to 70% max interval rpm

Figure 2. Work (kJ) performed in both trials before reaching 70% max interval rpm

Figure 3. Average amount of work (kJ) performed per interval in both trials

Figure 4. Intervals performed in both trials before declining to 85% max interval rpm

Figure 5. Work performed in both trials before declining to 85% max interval rpm

Figure 6. Intervals performed in both trials between 85% & 70% max interval rpm

Figure 7. Work performed in both trials between 85% & 70% max interval rpm

Figure 8. Muscle Glycogen at rest, 85% max interval rpm, & 70% max interval rpm

Figure 9. Muscle Creatine Phosphate at rest, 85%, & 70% max interval rpm

Figure 10. Muscle Free Creatine at rest, 85%, & 70% max interval rpm

Figure 11. Muscle ATP at rest, 85% max interval rpm, & 70% max interval rpm

Figure 12. Sarcoplasmic Reticulum Ca-uptake at rest, 85%, & 70% max interval rpm

Figure 13. Sarcoplasmic Reticulum Ca-release at rest, 85%, & 70% max interval rpm

Figure 14. Sample Performance Calculation

Figure 15. Sample Subject Diets for a Subject with a 3400 kcal Baseline Diet

Figure 16. Sample Subject Schedule

Figure 17. Volumes of Reagents used in Glycogen Extraction Procedure

Figure 18. Reactions involved in Glycogen Assay

Figure 19. Calculation of Glycogen Concentration

Figure 20. Reactions involved in CP/ATP Assay

Figure 21. Reactions involved in Cr Assay

Figure 22. Calculation of CP, Cr, and ATP Concentrations

Figure 23. Example of Ca^{2+}-uptake and release measurements

Figure 24. Intervals Performed by Subject 1

Figure 25. Intervals Performed by Subject 3

Figure 26. Intervals Performed by Subject 4

Figure 27. Intervals Performed by Subject 5

Figure 28. Intervals Performed by Subject 6
Figure 29. Intervals Performed by Subject 7 137
Figure 30. Intervals Performed by Subject 8 138
Figure 31. Intervals Performed by Subject 9 139
CHAPTER 1:

Introduction
Carbohydrate (CHO) status has repeatedly been shown to play a critical role in the performance of prolonged, moderate intensity exercise. Some research has suggested that CHO status also influences high intensity exercise performance. Specifically, subjects have been shown to fatigue more slowly when their muscle CHO stores (glycogen) are high or they are fed CHO during exercise. The underlying mechanisms of this benefit are not entirely clear.

One theory is that muscle glycogen may be associated with a cellular structure known as the sarcoplasmic reticulum (SR). The SR functions to mediate muscle contraction and relaxation by releasing and taking up calcium (Ca\(^{2+}\)). Exercise-induced changes in SR function have been implicated as a mechanism for muscle fatigue. Researchers have identified specific glycogen breakdown enzymes on the SR membrane and some studies have shown better maintenance of SR function when higher glycogen stores are available. Overall, few studies in this area have been performed.

A second theory is that CHO impairs fatigue by sparing muscle creatine phosphate (CP). Creatine phosphate significantly contributes to energy production at exercise onset since it can rapidly be activated to produce adenosine triphosphate (ATP), the form of energy needed for muscles to perform work. Glycogen breakdown is also a contributor to ATP production, but since it takes longer to reach its maximal rate, it does not significantly contribute until approximately 6 seconds after exercise onset. It is possible that when more glycogen is available, an earlier shift to CHO oxidation occurs, which may be favorable since the glycolytic system has a much greater capacity than the phosphagen system. Sparing CP may be especially beneficial to exercise involving intermittent bouts of activity due to greater CP availability with each bout.

**Statement of the Problem**

Since muscle fatigue is directly related to diminished athletic performance, decreased productivity, development of muscle soreness, and susceptibility to injury, determining causes of fatigue and ways to alleviate its effects are critical.

Sports nutrition researchers have studied different CHO treatments before and during exercise in attempt to delay fatigue through nutritional measures. Meanwhile, muscle physiologists continue to develop a case for SR dysfunction as a fatigue mechanism, typically in animal models. There is a need for research that links nutritional manipulation to cellular
physiology in human models. Furthermore, continued analysis of SR function, the role of CHO in high intensity exercise, and relationships among muscle fuels is needed. Elucidation of these mechanisms will yield more appropriately founded nutritional recommendations and exercise prescriptions.

**Objectives**
- To determine the influence of muscle CHO status on intermittent high intensity exercise
- To observe changes in SR function with fatigue resulting from intermittent high intensity exercise
- To examine associations among muscle glycogen, CP, ATP, and SR function with fatiguing intermittent high intensity exercise

**Hypotheses**
Ho: Initial muscle glycogen levels will not influence performance of intermittent high intensity cycling.
Ho: SR Ca\(^{2+}\)-uptake and release will not be affected by intermittent high intensity cycling.
Ho: Muscle CP and Cr levels will not be influenced by muscle glycogen status.
Ho: There will be no difference in SR function, muscle glycogen, CP, and ATP levels in a high vs. low muscle glycogen condition.

**Basic Assumptions**
The following assumptions were made in the study:

- Maximal effort was exerted in VO\(_{2peak}\), Wingate, and performance tests.
- Subjects refrained from creatine supplementation for at least 30 days prior to the study and did not use anabolic steroids.
- Subjects honestly reported their dietary intake for the 24 hours prior to their baseline ride.
- Subjects did not alter their training regimen on non-test days.
- Subjects did not alter fluid intake at any point during the study.
- Subjects refrained from physical activity and got the same amount of sleep during the 36 hours prior to their performance tests.
- Subjects fasted during the 12 hours prior to their performance tests.
- For the days when food was provided to subjects, all unconsumed food was returned.
- Muscle biopsies did not affect cycling performance.
- Similar muscle samples were obtained from repeated biopsies.
- Total muscle glycogen content reflects SR glycogen content.

**Delimitations**

The following delimitations were established for this study:

- A crossover design was implemented: subjects served as their own controls.
- Subjects were males, 18-30 years of age, who resided in Blacksburg, Virginia or were Virginia Tech students.
- Subjects were members of the Virginia Tech intercollegiate cycling team or Blacksburg East Coasters cycling team.
- Subjects had been performing cycling exercise for greater than two hours per day, four days per week for two years prior to the study and had a VO2peak greater than 52ml·kg⁻¹·min⁻¹.
- During the 22-30 days of study participation, food was provided by researchers only during the 36 hours prior to the two performance tests.
- Provided food was calorically matched to levels reported during subjects’ one-day dietary record.
- Two levels of dietary CHO were used: 5-10% and 85% of food energy needs (kcals).
- Ten different foods were provided for the high-CHO diet and eight different foods were
provided for the low-CHO diet; foods were substituted when food allergies or intolerances existed.
- Performance was measured from repeated 60-second bouts on a modified cycle ergometer.
- Subjects’ left legs were used for biopsies in their first performance trial and right legs for biopsies in their second performance trial; performance trials were conducted in random order.
- All muscle samples were obtained from the vastus lateralis muscle.

Limitations

The following limitations may be present in this study:

- Results may be generalized to individuals of similar gender, age, and training status.
- Although all subjects were Level I, II, or III cyclists, they varied to some extent in competitive caliber and experience.
- One familiarization trial and one baseline trial were conducted to make subjects comfortable with the exercise protocol and equipment and minimize any novel components of the exercise. Nevertheless, a learning effect may have occurred.
- Psychological approach to the second performance test may have been influenced by subjects’ experiences and perceptions in their first performance test.
- Liver glycogen levels were not determined.
- Muscle samples may have contained different proportions of Type I and Type II fibers.
- Subjects may have had different muscle fiber composition.
- Calcium uptake and release were the only measures of SR function.
- There was no direct measure of SR glycogen content.
**Definitions and Symbols**

**ATP**  
(Adenosine-triphosphate) phosphate compound that contains anhydride bonds that release energy when broken

**Ca\(^{2+}\)**  
(Calcium) ion stored in the sarcoplasmic reticulum of skeletal muscle cells; plays a significant role in muscle contraction and relaxation

**CP**  
(Creatine Phosphate) transfers a phosphate group to ADP to form ATP; catalyzed by the enzyme creatine kinase

**Glycogenesis**  
process of linking individual glucose molecules to synthesize glycogen in liver or skeletal muscle; requires the enzyme glycogen synthase

**Glycogenolysis**  
process of degrading glycogen into free glucose molecules; requires the enzyme glycogen phosphorylase

**Glycolysis**  
metabolic pathway beginning with glucose-6-phosphate and ending with lactate that yields 3 ATP per molecule of glucose; in aerobic conditions, pyruvate forms acetyl CoA which enters the TCA cycle.

**K\text{m}**  
(Michaelis constant) the substrate concentration when the initial velocity is \( \frac{1}{2} V_{\text{max}} \)

**Phosphagen**  
compound that generates ATP at a very high rate; phosphagen in mammalian skeletal muscle is creatine phosphate

**RER**  
(Respiratory Exchange Ratio) ratio of carbon dioxide expired to
oxygen consumed at the level of the lungs

(Sarcoplasmic Reticulum) longitudinal and transverse tubule system that stores calcium in skeletal muscle cells; calcium-release from the sarcoplasmic reticulum results in muscle contraction and calcium-uptake by the sarcoplasmic reticulum results in muscle relaxation
CHAPTER 2:

Review of Literature
Introduction

Carbohydrates (CHO) serve as an important fuel for exercise. Blood glucose provides fuel for brain cells, red blood cells, and exercising skeletal muscle cells. Breakdown of liver glycogen maintains blood glucose levels and breakdown of muscle glycogen yields substrate for glycolysis and oxidative phosphorylation.

Because the body has a limited storage capacity for CHO, it has been labeled as a limiting factor in endurance exercise. The association between muscle glycogen status and fatigue onset during endurance activity is well-established (37, 98). Muscle biopsies taken at exhaustion from prolonged submaximal running and cycling have revealed almost entire depletion of muscle glycogen (8). Additionally, subjects who better maintain blood glucose, liver glycogen, and muscle glycogen stores have been shown to exercise longer than subjects in poorer CHO status (37).

Since muscle glycogen stores are only moderately depleted (21-26%) with fatiguing high intensity exercise (50, 54, 94) and the duration of high intensity exercise is low, the contribution of muscle glycogen stores to fatigue development has been suggested to be minimal. Furthermore, blood glucose levels do not seem to be depressed with high intensity exercise. Researchers have even reported hyperglycemia during high intensity activity (5, 58). Some data, nevertheless, indicate a direct relationship between CHO status and high intensity exercise performance.

It is possible that high glycogen stores delay high intensity exercise fatigue through an association with muscle phosphagens. Creatine phosphate (CP) is a type of phosphagen found in muscle cells that functions to rapidly produce ATP. Since creatine kinase (CK), the enzyme that regenerates ATP from CP, has a very high activity in muscles, CP breakdown is instantaneously activated with exercise. This serves as a temporal buffer for ATP production during the brief lag before glycolysis and glycogenolysis more significantly contribute. Studies have shown that subjects who have higher glycogen stores better maintain CP levels throughout exercise than subjects with lower stores (65, 96).

Glycogen status may also contribute to delayed fatigue through an association with the muscle contraction/relaxation process, specifically functioning of the sarcoplasmic reticulum (SR). The SR stores calcium (Ca^{2+}) in muscle cells. Release of Ca^{2+} from the SR is a precursor
for muscle contraction, while Ca\textsuperscript{2+}-uptake by the SR induces muscle relaxation. Research has shown depressed SR function during fatigue (104). An association between glycogen and the SR has been theorized; thus, it is possible that SR dysfunction is related to exercise-induced glycogen depletion.

The following review of literature will focus on the role of muscle glycogen and CP in high intensity exercise, changes in SR function that occur with exercise, and relationships among diet, muscle fuels, and SR function.

**Glycogen and High Intensity Exercise**

**Manipulation of Muscle Carbohydrate Status Before Exercise**

*Benefits During High Intensity Performance Tests*

Researchers have reported an ergogenic effect of elevated muscle glycogen on time to fatigue during high intensity performance tests. Maughan and Poole (68), for example, had moderately trained male subjects consume either a high CHO (84% kcals), moderate CHO (43% kcals), or low CHO (2% kcals) diet for three days following an exhaustive glycogen-depleting cycling bout at 75% \( \text{VO}_2\text{max} \). No exercise was performed following the glycogen-depletion bout until day three when a supramaximal cycling test (104% \( \text{VO}_2\text{max} \) to exhaustion) was conducted. The high CHO group performed better than subjects who consumed moderate and low CHO diets (6.55 minutes, 4.87 minutes, and 3.32 minutes, respectively). Although muscle glycogen was not analyzed, performance differences were attributed to differences in muscle glycogen status among groups. Unfortunately, the order of dietary treatment was not randomized.

Langfort et al. (64) compared the effects of a three-day moderate CHO vs. low CHO diet on performance of short term high intensity cycling diet in sedentary college students. The moderate CHO diet was designed to mimic an average American diet (50% CHO) and the low CHO diet contained only 5% CHO. Following the diet period, subjects performed a 30-second Wingate test, a maximal effort cycling test designed to measure anaerobic power. Subjects on the moderate CHO diet produced significantly greater total power in the Wingate test (581 Watts)
as compared to the low CHO group (533 Watts). Researchers attributed these differences to diminished glycogen stores and a reduced rate of glycolysis, although no direct analyses were made. They further noted that performance benefits of the high carbohydrate diet may have been enhanced by the fact that subjects were untrained. It is noteworthy, however, that a performance decrement was found between moderate and low CHO groups. It would be interesting to compare the performance of a high CHO group as well. Additionally, maximal power produced during the first five-seconds of the Wingate was not significantly different between groups. Researchers explained that this demonstrates that muscle glycogen is not the primary fuel in the first five seconds of exercise.

In an attempt to more closely mimic athletes’ actual training regimens (many protocols have required subjects to abstain from exercise for a certain period of time), Pizza et al. (81) designed a cross-over study using eight competitive male runners taking part in two six-day treatment periods separated by two to three weeks each. During the six-day periods, subjects completed supervised outdoor runs at 75% VO$_{2\text{max}}$ ranging from 20 to 90 minutes in duration. They consumed either a self-selected mixed diet of 40% CHO (control) or three days on a 52% CHO diet followed by three days on a 73% CHO diet (HCHO). In order to minimize the effects of subjects’ preconceived expectations regarding CHO and exercise performance, the HCHO diet was administered in a double-blind fashion (CHO vs. placebo beverages) to four subjects during each of the two treatment periods. Performance tests consisting of 15 minutes of treadmill running at 75% VO$_{2\text{max}}$ followed by a run to exhaustion at 100% VO$_{2\text{max}}$ took place on day six of each treatment period. Subjects in the HCHO group ran for 5:04 minutes in the performance test which was significantly longer than the control group (4:40 minutes). Additionally, RER and carbohydrate oxidation were significantly higher during the 15 minute run in the HCHO group (0.96 and 3.49 g/min) compared to the control group (0.93 and 3.0 g/min).

Neufer et al. (75) analyzed performance in trained male cyclists five minutes following ingestion of either liquid CHO, solid CHO (confectionary bar), or a flavored placebo. The performance test consisted of 45 minutes of cycling at 77% VO$_{2\text{max}}$ on an electrically braked ergometer followed by a 15-minute performance trial on a isokinetic cycle ergometer designed to adjust resistance based on subjects’ effort. Total work performed by the liquid and solid CHO groups was significantly greater than that performed by the placebo group. There were no
performance differences between the liquid and solid CHO groups. Researchers had subjects complete an additional bout of the same performance test, but provided them with a high CHO meal (200 grams of CHO) four hours before and a confectionary bar five minutes before the performance test in attempt to simulate athletes’ pre-competition eating patterns. This group performed significantly greater total work than the other three groups. Muscle biopsies taken immediately before exercise onset and following the 45 minutes of submaximal cycling revealed no differences between groups in muscle glycogen status or usage. Thus, researchers attributed performance enhancement to increased glucose availability and oxidation rate. Serum glucose was significantly higher during the performance test in the groups that consumed liquid or solid CHO five minutes before the test. Glucose levels were not analyzed in the meal/solid CHO trial.

No benefit during high intensity performance tests

In a study by Hargreaves et al. (46), effects of glycogen status on short term high intensity exercise were examined. Well-trained male cyclists performed a 75-second maximal-effort performance test at 100% VO$_{2\text{max}}$ on a cycle ergometer modified to mimic cycles they were accustomed to riding. Twenty-four hours prior to the performance test, subjects performed 60 minutes of cycling at 70% VO$_{2\text{max}}$ followed by six 30-second sprints (to deplete muscle glycogen) after which they consumed either a high CHO diet (80% kcals) or a low CHO diet (25% kcals). All food was provided to subjects. Muscle biopsies taken before performance tests confirmed significantly different glycogen levels between groups (578 vs. 364 mmol/kgdw). There was no difference between the groups, however, in peak power or mean power during the 75 seconds. No difference in maximal accumulated oxygen deficit was found. The researchers concluded that muscle glycogen status did not influence short term high intensity exercise performance. These results contradict other findings possibly due to the high training status of the subjects used, a relatively high level of CHO consumed on the low CHO diet (other studies have used less than 10%), and the short duration of the performance tests. Although baseline glycogen levels were significantly different, they weren’t as drastically different as those reported by researchers who have found an influence of glycogen status. Additionally, while the 75-second bout closely parallels competition in several sprint sports and the low CHO diet is likely
somewhat similar to athletes’ actual training diets, the exhaustive glycogen depletion ride is very different from training that athletes customarily participate in 24 hours prior to performance.

It has been suggested that previous fatiguing exercise may contribute even greater to subsequent exercise performance than glycogen status. Grisdale et al. (45), for example, reported that subjects who performed exhaustive exercise (75 minutes of submaximal cycling, five one-minute sprints, and repeated leg lifts) 24 hours before an exhaustive static knee-extension test performed significantly fewer contractions than control subjects who had not done the exhaustive bout. Results were irrespective of glycogen status. One of the groups that performed the glycogen-depleting exercise consumed a 24-hour high CHO diet that resulted in a muscle glycogen level of 462 mmol/kgdw and the other group consumed a low CHO diet that resulted in a muscle glycogen level of 214 mmol/kgdw (glycogen levels were significantly different). The control group consumed a normal mixed diet. Unfortunately, the composition of the treatment and control diets were not included in the study manuscript, and control subjects’ muscle glycogen levels were not assessed.

A study by Ratz et al. (83) used previous exercise to deplete glycogen, but the exercise took place much longer than 24 hours prior to the performance test. The effects of a high CHO diet on the performance of high intensity exercise that consisted of cycling to exhaustion at 85 rpm with a resistance of 7 Watts/kg of body weight (approximately 75 seconds) was evaluated. Subjects used were a mixture of continuous and intermittent intercollegiate athletes (i.e.: speed skater, wrestler, distance runner, squash player, etc.). One week prior to the performance test, subjects completed a glycogen-depleting exercise bout of ten one-minute cycling intervals at 60 Wattls/kg of body weight. Muscle biopsies before and after the glycogen-depletion bout revealed significant depletion (1.63 vs. .95 g%). During the next six days, subjects consumed a low CHO diet (19% kcals) on days 1 through 3 and a high CHO diet (75% kcals) on days 4 through 6. A similarly matched control group completed the glycogen-depletion and low CHO portions of the study. It was confirmed through muscle biopsies in this group that the low CHO diet maintained low muscle glycogen levels. Results of the performance test indicate that subjects who were accustomed to continuous training cycled significantly longer than subjects who were intermittent-type athletes (time to exhaustion was 72.6 and 57.7 seconds, respectively). Furthermore, subjects who had a greater percentage of type II muscle fibers performed more
work than those with fewer type II fibers. Muscle glycogen status did not correlate with performance. Thus, the researchers concluded that muscle fiber composition and training status contribute more to high intensity exercise performance than glycogen status. Unfortunately, the low CHO control group did not perform the performance test, the diet pattern used does not mimic that of most athletes, and subjects maintained their normal training regimen during the treatment period which may have had a variable effect on muscle glycogen levels.

Symons and Jacobs (93) had moderately trained subjects perform high intensity quadriceps exercise tests consisting of isokinetic strength and endurance, isometric strength, and electrically evoked stimulation 48 hours after a glycogen-depleting cycling bout (90 minute submaximal cycling, five 5-minute sprints, and isokinetic leg extensions) in two dietary conditions: a mixed diet or low-CHO diet (approximately 10-15% kcals). For the mixed diet, subjects were instructed to consume their normal diets, but no diet records were kept for analysis of CHO content and compliance. No significant differences were found between groups in any of the tests despite a 35% difference in muscle glycogen following the diet manipulation (426 vs. 153 mmol glucose units/kgdw). Again, it is possible that performance was influenced by the previous exercise, particularly since the exercise was extremely intense and subjects were not well-trained.

In a study by Vandenberghe et al. (97), 32 university students (15 male and 17 female) were divided into two groups: a high CHO group that completed two days of glycogen depletion (prolonged cycling exercise and 50% kcals from CHO) followed by three days of glycogen supercompensation (no exercise and 70% kcals from CHO) and a normal CHO group that performed normal physical activity and consumed 50% kcals from CHO. Subjects performed a maximal effort performance ride at 125% VO2peak for either one minute and 45 seconds or until volitional exhaustion. The group that exercised for 1:45 had muscle biopsies taken from the vastus lateralis before and immediately following exercise; results indicated a similar extent of glycogen utilization by both groups, even though pre-exercise glycogen levels were significantly different (568 mmol/kgdw in supercompensated group vs. 364 mmol/kgdw in normal CHO group). Net glycogen breakdown was calculated to be 29 mmol/kgdw/minute in the supercompensated group vs. 28 mmol/kgdw/minute in the normal CHO group. There was no significant difference in performance (total work, time to exhaustion) between groups.
Unfortunately, the activity performed by the two groups during the five days before testing was extremely different and may have influenced results. Also, it is difficult to apply this protocol to actual athletic situations.

**Benefits During Intermittent High Intensity Performance Tests**

More consistent benefits of high CHO status have been reported when high intensity exercise is performed in a repeated, intermittent fashion. Although muscle glycogen levels have been infrequently assessed with this type of exercise, performance benefits have commonly been attributed to increased muscle glycogen stores. Jenkins et al. (57), for instance, found that moderately active subjects who were fed a 10-day high CHO (83% kcals) or moderate CHO (58% kcals) diet performed significantly more total work (compared to baseline) during a cycling test designed to mimic intermittent high intensity sports such as basketball, soccer, tennis, and hockey than subjects who consumed a low CHO diet (12%). Tests consisted of five 60-second maximal effort cycling bouts separated by five minutes of passive rest. Surprisingly, there was no difference between groups in plasma lactate and glucose. Muscle glycogen was not evaluated. Thus, researchers noted the importance of dietary CHO, and theoretically high muscle glycogen, for performance of intermittent supramaximal exercise, but recommended further research into involved mechanisms.

Bangsbo et al. (7) examined the effects of two-day consumption of a high CHO diet (65% kcals) as compared to a control diet (39% kcals) on long term intermittent intensity running performance in professional soccer players (subjects performed both diet periods separated by six to seven days). Subjects performed a 45-minute field protocol designed to mimic part of a soccer game (standing, jogging, sprinting, etc.) followed by a 35-minute variably paced treadmill run (0-25 km/hour) and a run to exhaustion that involved 15 second intervals at 18 km/hour separated by ten seconds of low speed rest. Subjects who had consumed the high CHO diet showed a significantly greater time to exhaustion than subjects who consumed the low CHO diet. The researchers did note, however, that three subjects (out of seven) did not show a significant performance difference between diets. They attributed this to a higher utilization of CHO, which
was based on higher RER values in these subjects throughout high CHO runs than control runs. It would have been interesting to see the benefits of an even higher CHO diet on this type of performance. The differences in muscle glycogen resulting from the two diets were not evaluated and again, no significant differences in blood lactate or glucose were detected.

In a second Bangsbo et al. (6) study, muscle biopsies were taken for analysis of muscle glycogen content and utilization. Six male subjects participated in a crossover study that involved one two-day high CHO period and one two-day normal CHO period. Diets successfully induced a significant difference in baseline muscle glycogen (176.8 vs. 87.0 mmol/kgww). Following the diet periods, subjects performed one-legged isometric exercise to fatigue (bout 1) and following 60 minutes of rest, repeated the same exercise protocol (bout 2). There was no difference in time to fatigue or glycogen utilization (as determined from muscle biopsies taken from the exercised leg) between groups in bout 1. However, in bout 2, high CHO subjects maintained their bout 1 performance and normal CHO subjects showed a 12% performance decrease (based on time to fatigue). Additionally, muscle glycogen decreased by 26.2 mmol/kgww in the high CHO group and 19.6 mmol/kgww in the normal CHO group, implying an increase in glycogen utilization based on glycogen status.

Walberg-Rankin et al. (99) had collegiate wrestlers consume either a high CHO (75% kcals) or moderate CHO (47% kcals) diet during a five-hour refeeding period following a three-day hypoenergy weight loss period. Performance tests (eight 15-second intervals of high intensity arm ergometry exercise separated by 20-second unloaded rest intervals) were performed before the weight loss period (I) and before (II) and after (III) the refeeding period. Subjects in the high CHO group increased their total work performance during the high intensity sprints over baseline performance, while performance of the moderate CHO group stayed depressed. There was no difference in peak power attained during performance tests. Researchers concluded that high CHO is superior to moderate CHO with respect to refeeding diets for performance maintenance in wrestlers.

No Benefit During Intermittent High Intensity Performance Tests

Some data do not support the ergogenic attributes of high CHO status during intermittent
high intensity performance tests. Snyder et al. (89) found no benefit of consuming a 19.7% CHO solution (5 ml/kg body weight) vs. a flavored, non-caloric placebo 15 minutes before a 25-minute variable intensity cycling ride (approximately 100 seconds of maximal effort sprinting separated by four-minute submaximal periods) in highly trained male cyclists. Researchers in this study suggested that there may have been insufficient time following supplement ingestion for glycogenesis to occur.

Dalton et al. (22) fed male resistance trainers either a CHO beverage (1 gram/kg body weight) or an artificially sweetened placebo 30 minutes prior to a resistance exercise test including repeated sets of parallel squats, bench press, leg press, and one-legged leg extensions. This test followed 11 days of resistance training (training every other day) and four days on an energy restricted formula diet (18 kcal/kg body weight, 54% kcals from CHO). There was no difference between groups in performance, leading researchers to conclude that CHO consumed 30 minutes prior to resistance exercise during a hypoenergetic period did not enhance performance. It was suggested that the lifting intensity used in this study may not have been great enough to promote a meaningful decrease in muscle glycogen. As an aside, exercise-induced changes in serum creatine kinase and cortisol were not different based on CHO consumption.

Wootton and Williams (108) evaluated effects of a three-day low CHO (79 grams), normal CHO (335 grams), and high CHO (592 grams) diet following a glycogen-depleting cycling bout (75% VO2max to exhaustion) on performance of repeated sprint cycling. Two 30-second Wingate tests with 15 minutes of passive rest were performed. No significant differences in peak, mean, or end power output and fatigue index were found. Blood lactate and glucose values were slightly higher throughout exercise in the normal and high CHO groups, but no significant differences existed. Total kcals consumed and percentage of kcals from CHO was not reported.

Performance effects of a 48-hour high CHO diet (7.66 grams CHO/kg body weight) on repeated sets of resistance exercise was compared to effects of a low CHO diet (0.37 grams CHO/kg body weight) in a study by Mitchell et al. (74). Levels were approximately 80% and 10% of kcals from CHO, respectively. In attempt to blind subjects to diet treatments, a non-caloric beverage was provided along with low CHO foods and subjects were told that different
Experimental beverages were being analyzed. Prior to beginning dietary treatment, subjects completed a 60-minute ride on a cycle ergometer at 70% VO$_{2\text{max}}$ followed by six one-minute sprints to deplete muscle glycogen. Performance tests consisted of five sets each of back squats, leg extensions, and leg presses. Resistance was set at subjects’ pre-determined 15-RM and repetitions were continued to failure. Despite an elevation in blood glucose following the performance test, there was no significant difference in lifting performance between groups. There was also no difference between groups in blood lactate, which researchers determined to indicate similar rates of glycolysis in both groups.

Carbohydrate Feedings During High Intensity Exercise

Carbohydrate feedings during exercise have been shown to improve exercise performance, theoretically by sparing muscle glycogen stores and enhancing the availability of glucose for oxidation (20). Nicholas et al. (76), for example, showed 21% lower glycogen utilization in subjects who consumed 75 grams of CHO during an 85-minute bout of intermittent high intensity running than subjects who consumed a placebo.

Similarly, Leatt and Jacobs (66) reported a greater decrease in muscle glycogen (decrease of 181 mmol/kg dw) in young soccer players who consumed 500 ml of 7% glucose polymer solution before and during halftime of a 90-minute game than players who consumed a placebo (decrease of 111 mmol/kg dw). However, some criticism of the study design is warranted. First of all, muscle biopsies were taken from the vastus lateralis of subjects. Gastrocnemius biopsies may be more appropriate for measuring changes in activities that primarily involve running (66). Vastus lateralis biopsies, on the other hand, are more suitable for cycling exercise since the quadriceps are heavily recruited in cycling. Additionally, post-game biopsies were taken as long as 45 minutes after exercise completion which may have allowed time for glycogen repletion.

Jeukendrup et al. (58) fed subjects (17 males and two females) either a 7.6% CHO-electrolyte solution or a flavor and color-matched placebo before and during a high intensity cycling bout designed to be completed in less than one hour. Subjects consumed 8 ml/kg body weight of the beverage during the warm-up period and 2 ml/kg body weight upon completion of 25%, 50%, and 75% of the performance ride. The amount of time required to complete the
performance was significantly greater in the group that consumed the placebo (60.15 minutes) as compared to the test beverage group (58.74 minutes).

In a study by Davis et al. (24), active college students (seven females and nine males) completed two high intensity interval tests (repeated 60-second cycling intervals at 120-130% VO$_{2\text{max}}$ with three minutes of rest to exhaustion) in random order. During one test, subjects consumed 4 ml/kg of an 18% CHO solution following the warm-up and a 6% CHO solution every 20 minutes during the performance test. During the other test, a flavored placebo was administered in the same amounts. Subjects who consumed the CHO beverage performed significantly longer than placebo subjects (87.0 vs. 59.8 minutes, respectively). Additionally, CHO beverage subjects showed higher plasma glucose and insulin throughout exercise and also reported a lower leg RPE than subjects in the placebo group.

Ergogenic Capabilities of Elevated Muscle Glycogen in High Intensity Exercise: Proposed Mechanisms

Although a variety of studies have explored the effects of muscle glycogen status on high intensity exercise performance, few have examined underlying mechanisms. In a discussion of muscle fatigue, Green (38) pointed out that although glycogen depletion is likely to be a factor in high intensity fatigue, specific mechanisms for this process are not yet known. As previously stated, glycogen depletion in high intensity-exercised muscle fibers is typically not nearly as great as depletion from endurance-exercised fibers. Clearly, muscle fatigue precedes complete glycogen depletion. Even at the levels of depletion reported by Greenhaff et al. (41) (50% reduction), glycogen deficiency does not appear limiting since substrate values exceed the K$_m$ for glycogen breakdown enzymes (37). The approximate K$_m$ for phosphorylase is only 2 to 8 mmoles glucosyl units/kgdw (84). According to Ren et al. (84), as long as 120-160 mmoles/kgdw are present, the rate of glycogenolysis likely will not be impaired.

An explanation may be that while the total amount of muscle glycogen depleted during high intensity exercise is typically minimal, the level of depletion directly from Type II muscle fibers may be selectively different (24, 69). Since high intensity anaerobic exercise depends heavily on recruitment of Type II fibers, insufficient CHO availability in these fibers may limit
performance. In a study by Greenhaff et al. (44), for example, muscle glycogen levels decreased by 61% more in Type II fibers than Type I fibers following 30 seconds of maximal sprinting.

Furthermore, there is little evidence that high glycogen levels cause a higher rate of glycogenolysis (97). Ren et al. (84), for example, showed no difference in the rate of glycogen utilization in groups with very low (VL), low (L), and normal (N) levels of muscle glycogen. Subjects’ muscle glycogen was manipulated to VL by 70 minutes of one-legged cycling exercise followed by three days on a low CHO diet. On day three, subjects performed two-legged cycling exercise to exhaustion; the leg that had not been previously exercised was considered L (237 mmol/kgdw vs. 155 mmol/kgdw in the VL leg). Subjects’ quadriceps were then electrically stimulated at a high intensity for 60 seconds; thigh muscle biopsies were taken before stimulation and following 10, 30, and 60 seconds of stimulation. This procedure was also performed in N glycogen status (350 mmol/kgdw) following a mixed diet and no previous exercise. Although muscle glycogen was significantly decreased during the 60 seconds in all three conditions, there was no difference in the rate of utilization among groups. Hespel et al. (51), however, showed higher total phosphorylase activity in glycogen-supercompensated as compared to glycogen-depleted (glycogen levels were manipulated by a combination of swimming and diet) rat muscles following 20 seconds of electrical stimulation (188 vs. 151 µmol/min/gdw). Also, the percent of total phosphorylase activity composed of phosphorylase a (active form) was higher in the supercompensated group (52 vs. 31%) (51).

As a mechanism, Ren et al. (84) suggested that glycogen phosphorylase is part of a glycogen-protein-SR complex in muscle cells. Depleted levels of muscle glycogen may cause dissociation of phosphorylase from this complex and consequently, decrease phosphorylase activity and glycogenolysis. Alternatively, it is possible that a low CHO diet inhibits glycogen utilization. Maughan et al. (69) proposed that a low CHO diet promotes fat utilization, which may lead to a build-up of Acetyl CoA units. Acetyl CoA is known to inhibit pyruvate dehydrogenase, resulting in decreased activity of the glycolytic pathway (69).

Researchers have theorized that changes in acid-base balance in response to different dietary treatments may lead to fatigue. Greenhaff (40) suggested that low CHO status causes a metabolic acidosis, making it more difficult to buffer lactate and H⁺. One specific consequence is that acid accumulation could lead to phosphofructokinase inhibition, thereby reducing
glycolytic anaerobic energy production and precipitating fatigue (53, 69). Accumulation of lactate and H⁺ may also have an inhibitory effect on the activity of glycogen phosphorylase (92), possibly leading to a reduction in the rate of glyogenolysis and consequently, glycolysis. Decreased activation of phosphorylase (phosphorylase b to phosphorylase a) has been reported in anaerobic exercise bouts and may be a result of acid build-up (84).

Animal muscle fibers electrically stimulated at high intensities have revealed a positive relationship between muscle glycogen levels and lactate accumulation (51). Greenhaff et al. (41) found that subjects who consumed a four-day 3% CHO diet had lower blood pH and base excess and performed worse on a maximal effort performance test than subjects who consumed moderate (45% kcals) and high CHO diets (70% kcals). Additionally, Horswill et al (52) reported that subjects (trained male wrestlers) who consumed approximately 40% of their kcals from CHO performed better on a six-minute intermittent arm ergometer performance test and had higher resting base excess than subjects who consumed approximately 65% of their kcals from CHO. In the aforementioned wrestler study by Walberg-Rankin (99), however, the altered high intensity exercise performance found when the CHO content of the refeeding diet was varied was not explained by acid-base changes (based on analysis of blood pH, bicarbonate, and base excess). Similarly, Vanderberghe et al. (97) found no difference in blood pH in 105 to 132-second exercise bouts between groups who had consumed a normal diet as opposed to a high CHO diet.

Mechanisms surrounding the role of muscle glycogen in intermittent high intensity exercise may be different than those involved in single high intensity bouts. Data on the extent of glycogen depletion in this type of exercise is variable and even somewhat inconsistent. For example, a 28% reduction in trained subjects following ten 60-second cycling intervals at 140 % VO₂max (68), a 35% decrease with three 60-second “all out” cycling intervals (47), a 46% decrease in recreational soccer players following a 90 minute game (56), and a 75% reduction in trained soccer players following a 105 minute soccer game (25). Depending on interval intensity and duration, length of rest between intervals, baseline muscle glycogen, and subjects’ training status, it is possible that glycogen resynthesis occurs during rest periods (25).
Phosphagens and High Intensity Exercise

Phosphagen Metabolism During Exercise

Although evidence of glycolytic activity immediately after exercise onset exists (muscle and blood lactate levels have been shown to be dramatically increased during 6-10 seconds of exercise) (33, 56), glycogen breakdown does not significantly contribute to energy production until about 8-12 seconds of exercise (87). It is clear, however, that the need for ATP during the first few seconds of activity is great. Resting muscle ATP levels are approximately 20 mmol/kgdw, but the demand for ATP upon onset of intense exercise may cause depletion of this amount in one to three seconds (63). Accordingly, the rate of ATP hydrolysis during initial intense exercise can be as much as one hundred times greater than the resting rate of 1 μmol·gram⁻¹·minute⁻¹ (53).

Given that research has continually shown that ATP levels are not severely depleted (below 40-50% of resting) during exercise of any type, duration, or intensity (37), it is apparent that muscle cells have alternative, more rapid means of ATP provision than glycogen breakdown. Following hydrolysis of ATP that is already present in the cell, it is the phosphagen system that makes the greatest contribution to ATP production during the first 6-8 seconds of exercise. Briefly, this system works through the transfer of a phosphate group from CP to ADP, which is present in the cell, to generate ATP (and free Cr). This reaction, catalyzed by the enzyme creatine kinase (CK), is reversible. Thus, as ATP is hydrolyzed for energy, the remaining phosphate group can bind with free Cr to regenerate CP. Since this system is rapidly activated to produce ATP, primarily due to the very high CK activity in muscle cells, the phosphagen system is thought of as a high power system. However, it is a low capacity system since muscle CP is rapidly depleted (53, 86).

The phosphagen system is thought to serve as an exercise buffer, specifically a proton buffer, spatial buffer, and temporal buffer (86). During intense exercise, decreased pH is accompanied by an increased concentration of protons, or hydrogen ions (H⁺), which may be directly related to diminished muscular performance. Since the phosphate component of CP
contains H⁺, CP regeneration absorbs some of the protons that may have precipitated fatigue (92). Next, CP is known to exist in higher concentrations in certain cellular compartments than in others. These compartments typically have a high need for ATP. For example, CP is thought to be associated with SR ATPase (38). Creatine Phosphate may provide a localized source of ATP in specific cellular regions that have a high requirement for it. Finally, since CP breakdown is so rapidly activated with exercise onset, ATP is available until other energy systems more significantly contribute.

Availability of CP can be a limiting factor in intense exercise, when the need for rapid ATP production is great. Ren et al. (84), for example, showed almost a complete depletion (96%) of CP levels following 60 seconds of exhaustive cycling. Although CP is regenerated during rest intervals, its availability may become especially limiting in repeated bouts of high intensity exercise, due to repetitive bursts of CP utilization exceeding rest period synthesis rate. Depending on the duration of rest periods, an explanation for dramatically diminished CP levels with exercise may be insufficient time between exercise intervals for CP regeneration to occur. Additionally, as the length of intermittent exercise increases, a decrease in cellular pH is common. This intracellular acidosis may inhibit CP regeneration (3). In specific studies: Spriet et al. (92) showed a 49% decrease in CP following two 30-second high intensity cycling intervals with a 54% decrease following a third interval; McCartney et al. (71) reported a 71% decrease in CP following one 30-second maximal cycling interval and a 93% decrease following a second interval; and Febbraio et al. (29) showed a 73-77% decrease in muscle CP following a set of four one-minute supramaximal cycling intervals.

In a study by Gaitanos et al. (34), a 73% decrease in power output was observed from the first to tenth interval in a set of repeated six-second maximal effort cycling intervals. Prior to exercise, CP was 76.5 mmol/kgdw. This decreased to 37.5 mmol/kgdw before the tenth sprint (a 51% decrease) and 12.2 mmol/kgdw after the tenth sprint (an 84% decrease from resting). Accordingly, ATP production from the phosphagen system was estimated as 44.3 mmol/kgdw during the first sprint and 25.3 mmol/kgdw during the tenth sprint. The amount of ATP produced from glycolysis (i.e.: glycogen breakdown) also decreased significantly from the first sprint (39.4 mmol/kgdw) to the tenth sprint (5.1 mmol/kgdw). Moreover, the observed decrease in power was likely due to the dramatic drop in anaerobic ATP production (CP and glycogen)
and increase in ATP production by oxidative phosphorylation. It should be noted, however, that CP was still making a considerable contribution to the total amount of ATP produced during the tenth sprint, but the total amount of ATP produced was only about 35% of that produced during the first sprint. Glycolysis, on the other hand, contributed very minimally to ATP production during the tenth sprint.

**Phosphagens in Overall Energy Metabolism**

Creatine phosphate stimulates glycogen breakdown via activation of the enzyme glycogen phosphorylase. Phosphorylase, the enzyme that catalyzes the first step in glycogenolysis, has two forms: phosphorylase b, the inactive form, and phosphorylase a, the phosphorylated active form. In resting muscle, about 25% of muscle phosphorylase is in the active form (59). A phosphate group derived from CP binds to the serine residue portion of phosphorylase b, converting it to phosphorylase a (63). When catalyzed by phosphorylase a, glycogen is converted to glucose-1-phosphate and ultimately, glucose-6-phosphate, a glycolysis substrate (59).

Degradation of CP also stimulates glycolysis at a second step: the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, a phosphorylation reaction catalyzed by the enzyme phosphofructokinase (PFK). Activation of PFK is in part related to the cellular increase in phosphate, derived from CP hydrolysis (63). Also, since $H^+$ is an inhibitor of PFK activity (37), CP indirectly stimulates PFK activity by buffering the cellular rise in $H^+$.

Following roughly 30 seconds of intense exercise, as oxygen reaches working muscles, there is a shift from anaerobic to aerobic metabolism. At this time, oxidative phosphorylation becomes the primary means of ATP generation. Substrates for oxidative phosphorylation include glycogen, amino acids, and predominantly, fat. Creatine phosphate has been shown to play a role in aerobic energy production via a pathway known as the phosphocreatine energy shuttle (10). In this process, a mitochondrial CK enzyme shuttles high energy phosphates from the mitochondria to the cytosol, where ADP becomes available to myofibrils, and other sites of ATP utilization (73). Free Cr is continuously shuttled back to the mitochondria so this process can repeat (49).

Moreover, it is important to note that energy systems (i.e.: phosphagen, glycolytic, and
oxidative phosphorylation) are interrelated and are active in some capacity throughout exercise. The proportion of energy production coming from each system is what changes with exercise. Serresse et al. (87), for example, compared energy utilization in 10, 30, and 90-second maximal cycling bouts. The 10-second bout involved 60% phosphagen, 28% glycolytic, and 2% oxidative phosphorylation activity. The 30-second bout required 23%, 49%, and 28% and the 90-second bout required 12%, 42%, and 46% for phosphagen, glycolytic, and oxidative phosphorylation, respectively. In general, the higher the intensity of exercise, the greater the contribution of the phosphagen system and glycolytic pathway. Oxidative phosphorylation prevails at lower intensities, in the vicinity of 60% of VO$_{2\text{max}}$ or lower (86). As the duration of submaximal exercise increases, the greater the oxidative phosphorylation contribution and lesser the phosphagen and glycolytic contribution, since these are more rapidly depleted of substrate.

Specific Relationship Between Muscle Glycogen and Phosphagen Status

An interrelationship between muscle glycogen and muscle phosphagens has been theorized. There is a limited amount of evidence suggesting that CHO status and metabolism may influence muscle phosphagen levels. Patients who suffer from glycogen metabolism disorders, such as individuals who cannot effectively break down glycogen due to phosphorylase deficiency (McArdle's disease), commonly show markedly low levels of muscle CP (67). Additionally, researchers have related decreased CP stores in fasted and previously exercised rats to depleted glycogen stores (62).

Studies have shown that following exhaustive exercise of varying intensity and duration, subjects who are glycogen depleted have lower CP levels than subjects who are less depleted (9, 96). In an endurance study by Tsintzas et al (96), runners who ingested a pre-exercise CHO solution showed improved performance at 70% VO$_{2\text{max}}$ compared to runners who consumed a placebo. The CHO group also showed higher muscle glycogen levels (173 CHO vs. 64 mmol/kgdw placebo) and CP levels (47 CHO vs. 32 mmol/kgdw placebo). There was no difference between groups in pre-exercise levels of glycogen and CP.

Larson et al. (65) fed subjects (males and females) either a high CHO diet (75% kcals) or
a high fat diet (70% fat, 15% CHO) for five days. Following the diet period, subjects performed paced quadriceps exercise with incremental resistance increases to exhaustion. The high CHO group exercised significantly longer than the high fat group (339 vs. 308 seconds, respectively). Muscle glycogen levels were not measured, but muscle CP, as measured by magnetic resonance spectroscopy, decreased significantly in both groups. However, the researchers noted a blunted decrease in the high CHO group and suggested that high CHO status is beneficial due to enhanced intracellular phosphorylation potential. It is possible that when more glycogen is available for oxidation, energy production from anaerobic and aerobic glycolytic pathways is greater and there is less dependence on muscle phosphagens for energy production, leading to a sparing of CP. Unfortunately, subjects used in the Larson et al. (65) study were of varying training levels, which may have influenced glycogen response to dietary manipulations.

This glycogen-phosphagen relationship exists in the reverse manner as well. In other words, enhanced CP stores may spare glycogen use. In a study by Febbraio et al. (29), untrained male subjects performed four one-minute cycling intervals at either 115 or 125% VO$_{2\text{max}}$ followed by a fifth interval to fatigue. This performance test was performed twice, in random order. One test was preceded by a 28-day wash out period and five days of creatine monohydrate supplementation at 20 grams/day and the other test followed a 28-day wash out period and five days on a placebo. Supplementation elevated muscle CP to 84 mmol/kgdw, which was significantly higher than placebo (69 mmol/kgdw). Creatine phosphate stores were approximately 25% of baseline following the first four intervals. Glycogen levels for the two groups were the same at baseline (482 and 480 mmol/kgdw, respectively), but following interval four, had decreased by 35% in the supplemented group and 46% in the placebo group (difference was a trend). This suggests that increasing CP stores may reduce glycogen utilization in intermittent high intensity bouts. There was no difference between groups in performance, however.

Conversely, there is data that does not support these findings. In the high intensity electrical stimulation study by Ren et al (84), CP levels and CP utilization rate were not influenced by varying muscle glycogen levels (baseline levels of 350, 237, and 155 mmol/kgdw). Additionally, Snow et al. (88) showed no difference in glycogen utilization (based on pre and post exercise muscle biopsies) or glycoglytic activity (based on blood lactate levels) in groups supplemented with 30 grams of creatine monohydrate as compared to a placebo.
Supplementation increased total muscle creatine levels by approximately 10%. No performance benefits were found and researchers concluded that increasing muscle creatine levels did not influence anaerobic muscle metabolism. Interestingly, supplementation increased total creatine by increasing free creatine. There was no significant enhancement of CP stores.
Sarcoplasmic Reticulum Function and Fatigue

Fatigue Definition and Theories

Skeletal muscle fatigue can be defined as the muscle’s inability to generate a desired power output (30) or an increase in the effort required to elicit the same force output (38). Allen et al. (1) characterized muscle changes occurring with fatigue into three categories: decreased maximal force, diminished shortening velocity, and decreased fiber relaxation. Many undesirable outcomes may be associated with these changes, namely diminished athletic performance, decreased exercise or work capacity, muscle soreness, and muscle injury. Therefore, determining ways to alleviate or delay muscle fatigue is relevant not only for athletes, but for recreational exercisers, rehabilitation patients, and laborers as well.

Providing accurate recommendations for minimizing fatigue requires an understanding of mechanisms underlying it. However, despite over a century of research, exact mechanisms of muscle fatigue are not entirely clear (106). Researchers have investigated both central and peripheral mechanisms as possible causes of fatigue. With regard to high intensity exercise, many have found little or no association between central failure and muscle fatigue. Williams and Klug (104) suggested that neuromuscular communication does not decline during exercise, given that the individual is well-motivated. Furthermore, data have shown that electrical activity that reaches the neuromuscular junction parallels electrical activation of muscle fibers, regardless of fatigue status (11). Kent-Braun (61), however, did attribute approximately 20% of the muscle fatigue experienced by subjects after four minutes of maximal ankle flexion exercise to central failure. This estimation was made based on electromyography (EMG) measurements. Likewise, the magnitude of the action potential that reaches the ryanodine receptors has been shown to be slightly reduced at fatigue (30). Nevertheless, the majority of fatigue research has focused on peripheral decline, since it is clearly a more significant contributor than central fatigue.

The site of peripheral mechanical failure may be a step within a fiber’s excitation-contraction coupling (e-c coupling) or in the interactions of filament cross bridges (30). Steps involved in e-c coupling include the transmission of an action potential down transverse-tubules (t-tubules), action potential detection by dihydorpyridine (DHP) receptors, opening of the Ca^{2+}-

28
release channel (ryanodine receptor), release of intralumenal Ca\(^{2+}\) from the terminal cisternae of the SR, and sequestering of myoplasmic Ca\(^{2+}\) back into the SR via Ca-ATPase (38). Cross bridge interactions include binding of Ca\(^{2+}\) to troponin-C on the actin filament, a subsequent shift in the binding of myosin to actin from a weak to strong-binding state via actomyosin-ATPase, and shortening of the crossbridges. Causes for failure at any of these steps may be classified into three theories: depletion theory; accumulation theory; or the theory of diminished cellular calcium handling. Overall, it is likely that the cause of muscle fatigue is multi-faceted and that the three theories may overlap.

**Depletion Theory**

The depletion theory is based on the principle that muscle fatigue occurs due to a deficiency of energy or fuels, including ATP, glycogen, and CP. It is commonly believed that muscles fatigue because they “run out of ATP”. However, it has been repeatedly shown that ATP levels are not entirely depleted with exercise of any intensity or duration (30, 104). In fact, in steady-state exercise, ATP production usually comes close to balancing ATP demand (104). In higher intensity or extreme duration exercise, at least 50-60% of ATP typically remains after exercise (34). In either case, sufficient ATP exists as a substrate to saturate ATPase enzymes involved in muscle contraction and relaxation (38).

Stores of muscle glycogen and CP have been shown to be exhausted with exercise and are clearly associated with muscle fatigue. However, depletion of glycogen and CP likely does not represent the complete fatigue mechanism. First of all, since ATP levels are generally maintained at relatively high levels during exercise, there must either be ample CP and glycogen to support ATP production, or sufficient localized substrate to support oxidative ATP production. It is possible that there is a compartmentalized depletion of ATP (17), glycogen, and CP (30, 37) in specific cellular regions that causes a decline in a muscle’s force and/or power output due to lack of localized energy production.

**Accumulation Theory**
Accumulation of certain metabolites, namely lactate, H\textsuperscript{+}, ADP, and inorganic phosphate (P\textsubscript{i}), may precipitate muscle fatigue. For example, breakdown products of ATP hydrolysis include ADP and P\textsubscript{i}. Build-up of cellular ADP has been associated with decreased fiber shortening velocity and excess P\textsubscript{i} has been shown to decrease maximal force (1). Changes in pH are believed to decrease maximal force and shortening velocity (30). Increased lactate concentration has been shown to decrease force, even when decreased pH does not accompany the lactate changes (26, 91).

Theory of Diminished Cellular Calcium-Handling

Despite evidence that depletion and accumulation theories are valid explanations of muscle fatigue in some cases, it is apparent that they do not entirely account for the extent of changes in muscle function that occur. It is probable that depletion of certain cellular metabolites and accumulation of others contribute to a decrement in mechanical functioning of muscle’s contractile processes.

A likely contributor to muscle fatigue is changes in myoplasmic Ca\textsuperscript{2+}. There is a sigmoidal relationship between the concentration of myoplasmic Ca\textsuperscript{2+} and force production of muscle fibers (106). In general, the greater the myoplasmic Ca\textsuperscript{2+} concentration, the greater the force produced. Three theoretical fatigue mechanisms related to cellular Ca\textsuperscript{2+} metabolism have been established and include: a reduction in the amount of Ca\textsuperscript{2+} available to the contractile apparatus, a decline in the contractile apparatus’ sensitivity to Ca\textsuperscript{2+}, or a reduction in the maximum force attainable in response to Ca\textsuperscript{2+}-activation (101).

The isolation and manipulation of individual muscle fibers has provided insight into mechanical fatigue processes. Westerblad and Lannergren (101), for example, repeatedly stimulated single mouse fibers until tetanic force was decreased by 60%. At this time, Ca\textsuperscript{2+} sensitivity was decreased by nearly 100%, the amount of Ca\textsuperscript{2+} available to the SR was decreased by 50%, and the maximal Ca\textsuperscript{2+}-activated force was reduced by about 20%.
Evidence of Depressed Sarcoplasmic Reticulum Function with Fatigue

A magnitude of research has implicated the SR as the site of dysfunctional Ca\textsuperscript{2+} metabolism. Specifically, diminished SR Ca\textsuperscript{2+}-uptake, Ca\textsuperscript{2+}-release, and Ca-ATPase activity have been seen with fatigue. A variety of evidence has made the theory of SR dysfunction with fatigue convincing. First, muscles that are fatigued show markedly depressed force and Ca\textsuperscript{2+}-release. However, when the same fibers are placed in a bath with caffeine, which induces Ca\textsuperscript{2+}-release due to ryanodine receptor activation, force and Ca\textsuperscript{2+}-release return to near-normal levels (38, 104). This suggests that fatigued muscles experience a decline in the amount of Ca\textsuperscript{2+} made available to the contractile apparatus and based on the caffeine evidence, this decline seems related to changes in Ca\textsuperscript{2+}-release. Additionally, when muscle fibers are exposed to dantrolene sodium, an inhibitor of Ca\textsuperscript{2+}-release, changes in force, twitch, and relaxation time are much like changes seen in fatigued fibers (104). Finally, some researchers have suggested that intrinsic structural changes in the SR may occur (16, 17, 72). McCutcheon et al. (72) showed a dramatic dilation of the SR in thoroughbred horses after fatiguing exercise. Rises in myoplasmic Ca\textsuperscript{2+} may activate certain proteases that function to breakdown cell membranes (1). Structural deterioration of the SR would likely contribute to altered Ca\textsuperscript{2+} handling. Specifically, decreased membrane integrity may cause leakage of Ca\textsuperscript{2+} out of the SR.

Sarcoplasmic Reticulum Studies in Non-human Species

Diminished SR function has been shown in a variety of different species and with many different exercise protocols. Following, recent SR studies from various species are highlighted, with specific attention directed to differences in analytical procedures.
**Rat**

Numerous studies have reported exercise-induced SR dysfunction in mice and rats (16, 18, 30, 104), but different technical procedures have been used by different laboratories. Therefore, recent studies have compared different techniques and analytical procedures for measuring SR function. Chin and Green (19) compared the crude homogenate technique and the SR vesicle technique for analysis of rat SR function. Rats were divided into control and exercise groups and half from each group had crude homogenate Ca\(^{2+}\)-uptake and Ca-ATPase analysis, while the other half had SR vesicle Ca\(^{2+}\)-uptake and Ca-ATPase analysis. Control rats were analyzed for baseline resting values. Exercise rats ran on a treadmill at 8% grade at a speed of 21 to 28 meters/minute until exhaustion. Unfortunately, exhaustion time was different between analysis groups (100 minutes for homogenate group and 141 minutes for vesicle group). Neither technique revealed a significant difference in Ca-ATPase at fatigue, but a similar degree of decline in Ca\(^{2+}\)-uptake was seen with both techniques, with differences in absolute data values differing (homogenate: rest-9.5 to fatigue-6.9 nmol·mg\(^{-1}\)·min\(^{-1}\) vs. vesicle: rest-84.0 to fatigue-50.7 nmol·mg\(^{-1}\)·min\(^{-1}\)). These researchers suggested that there may not be a direct association between Ca-ATPase and Ca\(^{2+}\)-uptake in measurements made in vitro.

Byrd et al. (16) measured the differences in fibers taken from the superficial and deep regions of rat gastrocnemius and vastus muscles. Rats ran on a treadmill for 20 or 45 minutes, or to exhaustion, which was about 140 minutes. After 20 minutes, there was a decrease in Ca-ATPase activity in deep muscles, but no decrease in Ca\(^{2+}\)-uptake until the 45-minute mark in deep muscles. Both Ca\(^{2+}\)-uptake and Ca-ATPase activity were depressed at 140 minutes in deep muscles, but there was no reduction at any time point in superficial muscles. Researchers suggested that collection of deep muscle samples is required for analyzing SR function.

**Frog**

Baker et al. (4) mounted frog muscles in a bath anchored to a force transducer. Muscles were stimulated with .1-ms pulses at 100 Hz every eight seconds until force decreased by 50-60% of initial. A Ca\(^{2+}\) indicator was used to measure Ca\(^{2+}\) signals before and after stimulation.
There was a significant decrease in Ca\(^{2+}\) signals in fatigued muscles which researchers attributed to one of three things: slowed or diminished Ca\(^{2+}\)-uptake by the SR, leaving more Ca\(^{2+}\) in the myoplasm; increased leakage of Ca\(^{2+}\) into the myoplasm, indicating decreased SR integrity; or loss of Ca\(^{2+}\) in some other manner.

Ward et al. (100) removed sartorius muscles from a group of grass frogs. Muscles were mounted on a isometric force transducer and were exposed to a fatiguing twitch protocol which included 100-ms pulse trains at 100 Hz. Muscles were evoked at 2.0, 0.5, and 0.2mM trains per second. Some of the muscles were stimulated until force declined by about 80% of initial force, which required 1 minute (2.0mM), 4 minutes (0.5mM), and 17 minutes (0.2mM). Remaining muscles were stimulated for one minute which induced force reductions of 85% (2.0mM), 45% (0.5mM), and 10% (0.2mM). Following the fatigue protocol, muscles were analyzed for Ca\(^{2+}\)-uptake and release. Non-fatigued control muscles were used to measure resting Ca\(^{2+}\)-uptake and release. Muscles stimulated to an 80% force reduction showed similar decreases in Ca\(^{2+}\)-uptake and release at fatigue. In the muscles stimulated for one minute, Ca\(^{2+}\)-uptake and release paralleled the extent of force reduction. This led researchers to conclude that when muscles are fatigued to similar extents, like reductions in SR function occur, regardless of the intensity or duration of the contractions being performed. However, when not fatigued to the same extent, Ca\(^{2+}\)-uptake and release declines are determined by the degree of force reduction.

Results from a study by Williams (106) on electrically stimulated frog muscles also indicate a change in SR function with fatigue. A fatigue protocol that involved low intensity intermittent stimulation for varying a duration ranging from 20 to 180 minutes was administered to mounted grass frog muscle fibers. There was a decrease in the rate of Ca\(^{2+}\)-uptake by the SR and in the caffeine-sensitivity of Ca\(^{2+}\)-release in all samples. The fact that caffeine could not return Ca\(^{2+}\)-release to normal levels led Williams (106) to conclude that some sort of intrinsic structural change in the SR occurred with fatigue.
Horse

Byrd et al. (17) analyzed the effects of short term, high intensity running on SR function in thoroughbred horses. Eight horses were allowed a 5-minute warm-up at 40% VO\textsubscript{2max} followed by a run to exhaustion at 100% VO\textsubscript{2max} (131 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}). Mean running time was 4.65 minutes. Muscle biopsies in the amount of 1 gram were removed from the middle gluteal muscle before and immediately after exercise and following 30 and 60 minutes of rest. Analysis of Ca\textsuperscript{2+}-uptake and Ca-ATPase activity revealed a 43% decrease in Ca\textsuperscript{2+}-uptake and a 40% decrease in Ca-ATPase activity at fatigue. Both returned to baseline values following 60 minutes of recovery. These researchers suggested a possibility of muscle temperature and pH changes as a pre-cursor to SR dysfunction.

Horse SR function during high intensity exercise was also studied by Wilson et al. (107). Ten untrained quarter horses performed four repeated sprints on a treadmill (no details of the protocol were provided in the text). Following exercise, Ca\textsuperscript{2+}-uptake was decreased by 37% and Ca-ATPase activity was decreased by 27% from baseline. Interestingly, researchers had the same horses complete the same protocol following 12 weeks of conditioning. They noted a 14% increase in resting Ca\textsuperscript{2+}-uptake and a 38% increase in resting Ca-ATPase. They also found spared SR function with exercise, an increase in the amount of SR protein present, and a reduction in blood lactate, when compared to the untrained condition. This suggests that exercise training may have a positive effect on SR function.

Human Sarcoplasmic Reticulum Studies

Limited research on human SR function has been conducted, primarily due to the small sample size obtainable from human muscle biopsies. To date, no published data on SR Ca\textsuperscript{2+}-release is available, but Ca\textsuperscript{2+}-uptake and Ca-ATPase activity have been investigated. Some of the earliest data was reported in research abstracts by Parsons et al. (80) and Green et al. (36). In the Parsons et al. (80) study, moderately trained female cyclists performed exhaustive submaximal cycling at 75% VO\textsubscript{2max} to fatigue (2 to 3 hours). Muscle biopsies were
taken at baseline, twice during exercise, and at fatigue; 20±5 mg of tissue was used for Ca^{2+}-uptake analysis. Researchers reported a 70% decrease in Ca^{2+}-uptake following exercise, but unfortunately, values were not reported. Green et al. (36) had untrained male subjects perform similar exhaustive cycling exercise (30 minutes at 58% VO_{2max} and until fatigue at 72% VO_{2max}). Muscle samples obtained at baseline, following 30 minutes of cycling, and immediately after exercise revealed significant decreases in Ca-ATPase activity (5.99 \mu Mww\cdot min^{-1} at baseline, 5.04 at 30 minutes, and 4.17 at fatigue). Both groups of researchers concluded that SR function appears to be impaired with exercise in a manner similar to that shown in other species. Parsons et al. (80) further noted that there may be no differences based on gender, as well.

Booth et al. (12) further analyzed the effects of prolonged exercise on SR function. Ten untrained male college students cycled to exhaustion at 70% VO_{2max}, which was approximately 72 minutes. Before and after exercise, subjects' quadriceps muscles were electrically stimulated to contract isometrically (high and low intensity contractions); then, peak tension and half-relaxation time in a maximal isometric leg extension were measured. At exhaustion, peak contraction tension had decreased by 28-65%, depending on type and intensity of the contraction. There was no significant variation in half-relaxation time. Muscle biopsies taken prior to exercise and within 15 seconds of exercise completion showed significant decreases in Ca^{2+}-uptake (10.42 vs. 8.65 nmol·mg protein^{-1}·min^{-1}) and Ca-ATPase activity (88.83 vs. 70.18 nmol·mg protein^{-1}·min^{-1}). In this study, Ca^{2+}-uptake and Ca-ATPase activity were highly correlated. Earlier published data from the same subjects reported resting Ca^{2+}-uptake values of 1.81 \mu mol·min^{-1} when analyzed in terms of muscle wet weight. Overall, researchers noted that prolonged exercise reduced SR Ca^{2+}-uptake, but had no impact on the rate of muscle relaxation, which they had hypothesized would occur since Ca^{2+}-uptake and muscle relaxation are causally related.

Two human studies have shown depressed SR function with fatiguing high intensity exercise. Gollnick et al. (35) had five experienced subjects perform intense kicking exercise at a frequency of 1 Hz to fatigue (about 2.8 minutes). At exercise completion, muscle function was significantly diminished, which was indicated by a decrease in maximal voluntary contraction and half-relaxation time in an isometric quadriceps test performed before and after the kicking
exercise. There was a 58% reduction in Ca\(^{2+}\)-uptake at fatigue (9.78 vs. 5.70 nmol·mg protein\(^{-1}\)·min\(^{-1}\)) and this decrease inversely correlated with the change in half-relaxation time. Also, the researchers noted that 30 minutes after the completion of exercise, Ca\(^{2+}\)-uptake had returned to within 10% of baseline values. In contrast, the aforementioned Booth et al. (12) study showed a 21-25% depression in Ca\(^{2+}\)-uptake following 60 minutes of recovery from prolonged exercise.

Hargreaves et al. (47) analyzed SR function with repeated bouts of high intensity exercise. Six males performed four 30-second maximal effort cycling bouts. The first three bouts were separated by four minutes of rest. Thirty minutes of low intensity cycling (35% VO\(_{2\text{peak}}\)) and 60 minutes of passive recovery preceded the fourth bout. Muscle biopsies were obtained prior to the first, third, and fourth bouts. Ca\(^{2+}\)-uptake values prior to bouts 1 and 4 were similar, but a significant decline in Ca\(^{2+}\)-uptake from baseline to pre-bout 3 was found when values were expressed in terms of tissue wet weight (23.4 to 16.9 nmol·mgww\(^{-1}\)·min\(^{-1}\)). However, only a trend for a difference (p= 0.06) was found when values were expressed in terms of protein content (339 to 272 nmol·mgww\(^{-1}\)·min\(^{-1}\)). Values reported by Hargreaves et al. (47) in terms of protein content are quite different than those reported by other researchers who expressed Ca\(^{2+}\)-uptake in the same units (typical range is 1 to 18 nmol·mgww\(^{-1}\)·min\(^{-1}\)). This difference is likely due to differences in analytical procedure. Hargreaves et al. (47) used buffered tissue homogenates, which is similar to the method used by Parsons et al. (80), Green et al. (36), and Booth et al. (12), but other differences in homogenizing solution, buffer solution, homogenization, centrifugation, or calculations may have prevailed. Gollnick et al. (35) used an electrode technique with tissue homogenates, which is not common among other human studies, but found similar numerical results, nonetheless. Obviously, methods used by the studies published only in abstract form are not clear.

Only one set of data has shown no decrement in SR function with exercise. O'Toole et al. had seven untrained males complete two different intermittent high intensity cycling bouts. The first required 60 seconds of cycling at 120% VO\(_{2\text{max}}\) with 240 seconds of rest and 15 seconds of cycling at 120% VO\(_{2\text{max}}\) with 60 seconds of rest for a total of 60 minutes in both bouts. Researchers found no significant difference in Ca-ATPase following 1 minute or 60 minutes of cycling or following 30 minutes of recovery. Since there was no measure of muscle function and
no evidence of muscle fatigue at 60 minutes, it is possible that the extent of fatigue was not great enough to elicit changes in Ca-ATPase. Williams et al. (105) suggested that SR function may only be depressed when muscle force output is reduced by 50-60%. It is also possible that Ca-ATPase activity is less sensitive to fatigue-induced changes than Ca$^{2+}$-uptake. Again, since only an abstract is available, it is difficult to compare analytical procedure.

Relationship Between Muscle Glycogen and Sarcoplasmic Reticulum Function

There is evidence of direct association of glycogen with the SR. Maughan et al. (69) suggested a compartmentalized depletion of muscle glycogen during exercise, meaning that the amount of glycogen depleted from specific cellular regions differs from other regions and from the extent of depletion for the whole muscle. With regards to the SR, it is possible that glycogen depletion from the SR membrane causes a reduction in the amount of ATP available to fuel the Ca$^{2+}$ pump (38). This would explain why muscle performance and SR function decrease, while total muscle glycogen is not necessarily significantly depleted.

Researchers have located a SR-glycogenolytic complex, which contains glycogenolytic and glycolytic enzymes in addition to Ca-ATPase. This complex may have a structural and metabolic role in muscle cells. Rossi et al. (85) isolated glycogen phosphorylase and phosphorylase phosphatase (converts phosphorylase to its active form through phosphorylation) on the SR membrane. Low levels of glycogen may cause this complex to dissociate, impairing Ca-ATPase activity and consequently Ca$^{2+}$-uptake (84). Studies by Cuenda et al. (21) and Nogues et al. (77) showed that adding phosphorylase, phosphoglucomutase, and hexokinase to skinned rat fibers enables normal Ca$^{2+}$-uptake, even when ATP is not added. Additionally, Xu et al. (109) added glycolytic enzymes to a bath surrounding rabbit SR vesicles. Ca$^{2+}$-transport was increased following addition of all enzymes in glycolysis after aldolase including: aldolase, glyceraldehyde-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglyceromutase, enolase, and pyruvate kinase (PK). Furthermore, Ca$^{2+}$-transport was reduced by the addition of iodoacetic acid, which inhibits GAPDH. Of particular note are the enzymes PGK and PK, which catalyze ATP-producing substrate phosphorylation reactions. The ATP produced by these steps may fuel SR function. Researchers concluded that a functional
coupling of glycolysis and Ca\textsuperscript{2+}-uptake exists. In a second study, Xu et al. (110) used electron microscopic immunogold labeling on SR vesicles from rabbits. Analysis showed existence of PK, aldolase, and GAPDH on the cytoplasmic side of SR vesicles. Pyruvate kinase was located directly next to Ca-ATPase. These data support the theory of SR-associated glycolytic enzymes and an association with SR Ca\textsuperscript{2+}-balance.

Supporting data was also provided by Brautigan et al (14). Rested skinned rat muscle fibers were depleted of glycogen and glycogenolytic enzymes by treatment with alpha-amylase. This treatment caused a significant decrease in Ca\textsuperscript{2+}-uptake by the SR. However, when treated with glucose-6-phosphate (G-6-P), the first glycolytic substrate, Ca\textsuperscript{2+}-uptake was returned to normal. Researchers concluded that glycogen breakdown and glycolysis are related to the SR’s ability to sequester Ca\textsuperscript{2+}.

Opposing findings were reported by Chin and Allen (18). Single mouse fibers were used to analyze the effects of lowered muscle glycogen on SR Ca\textsuperscript{2+}-release. Muscles were stimulated to tetanus in a repeated manner until a 70\% reduction in force occurred. A significant decrease in the intracellular free Ca\textsuperscript{2+} occurred at this time, which was attributed to decreased Ca\textsuperscript{2-} release. Following one hour of recovery with 5.5mM glucose, force and glycogen levels had returned to normal (100\% and 157\% of baseline, respectively), but the concentration of intracellular free Ca\textsuperscript{2+} was still depressed by 20\%. Thus, the depression of Ca\textsuperscript{2-}-release was considered unrelated to glycogen.

Similar conclusions regarding the lack of an association between muscle glycogen and SR function were made by Hargreaves et al. (47) in the previously described study. When subjects were allowed a 90-minute rest period prior to performance of a fourth (of four) 30-second maximal effort cycling bout, Ca\textsuperscript{2-}-uptake and performance (indirect measure of muscle force) had returned to resting values, despite a significant depression (over 80\%) in muscle glycogen.

In the Byrd et al. (17) horse study, the significant decrease in Ca\textsuperscript{2-}-uptake and Ca-ATPase activity following a 4-5 minute high intensity treadmill run was not paralleled by a decrease in muscle glycogen. These researchers concluded that glycogen was likely not involved in the SR dysfunction they found. They noted that in an unpublished study performed in their lab, horses performed prolonged treadmill exercise which decreased muscle glycogen by over 75\%. Despite this large decrease in muscle glycogen, Ca\textsuperscript{2-}-uptake and Ca-ATPase changes were very similar to
the extent of decrease found with the high intensity activity. Of course, a limitation of the Hargreaves et al. (47) and Byrd et al. (17) studies is that they were only able to measure the total amount of glycogen in the muscle, rather than glycogen directly associated with the SR.
CHAPTER 3:

Journal Manuscript

Effects of Pre-Exercise Muscle Glycogen Status on Muscle Phosphagens, Sarcoplasmic Reticulum Function, and Performance During Intermittent High Intensity Exercise

Michelle R. Smith
Janet Walberg-Rankin, Ph.D.
Jay H. Williams, Ph.D.
Michael E. Houston, Ph.D.

Virginia Polytechnic Institute and State University
Blacksburg, Virginia
ABSTRACT

Eight competitive cyclists performed two cycling trials, one following a high carbohydrate diet (H-CHO) and the other following a low carbohydrate diet (L-CHO). Trials consisted of repeated 60s maximal effort sprints to fatigue at a workload designed to elicit 125-135% \( \text{VO}_2\text{peak} \) at 90rpm. Three min of recovery separated sprints. Muscle biopsies taken at rest (biopsy 1), 85% max interval rpm (biopsy 2), and 70% max interval rpm (biopsy 3) revealed a main effect of diet on muscle glycogen levels: 609 ± 38 H-CHO vs. 390 ± 42 mmol/kgdw L-CHO at biopsy 1, 383 ± 29 vs. 252 ± 28 mmol/kgdw at biopsy 2, and 346 ± 29 vs. 196 ± 18 mmol/kgdw at biopsy 3 (p<0.01). Similar decreases in muscle glycogen (45%), creatine phosphate (CP) (35%), and sarcoplasmic reticulum (SR) Ca\(^{2+}\)-uptake (56%) were shown in both trials from biopsy 1 to 3. SR Ca\(^{2+}\)-release decreased by 53% in H-CHO subjects and 36% in L-CHO subjects. Total exercise time tended to be longer in H-CHO than L-CHO subjects (57.5 ± 10 vs. 42.0 ± .89min) (p=0.09). H-CHO subjects exercised significantly longer than L-CHO subjects from biopsy 2 to 3 (33.6 ± 10 vs. 18 ± 3.6min) (p< 0.05). Results suggest that fatigue from 40-60min of intermittent 60s high intensity cycling intervals is associated with reductions in muscle glycogen, CP, and SR function, and that the latter part of performance is impaired by low muscle glycogen. These data do not support a relationship between muscle glycogen status and SR function in intermittent high intensity exercise.

Keywords: carbohydrate, creatine phosphate, calcium, cycle exercise, muscle biopsy
INTRODUCTION

A relationship between high muscle glycogen and endurance exercise performance has been clearly established (24). However, research on muscle glycogen’s involvement in high intensity exercise and intermittent high intensity exercise has revealed contradictory results. Improved performance was found in single-bout high intensity tests by Maughan and Poole (40), Langfort et al. (38), and Pizza et al (44) and in intermittent high intensity test by Jenkins et al. (34) and Casey et al. (10) when subjects had consumed high carbohydrate (CHO) diets that theoretically raised muscle glycogen stores. Hargreaves et al. (30) and Symons and Jacobs (52), on the other hand, did not find an ergogenic effect of elevated muscle glycogen on single-bout and intermittent high intensity exercise.

The mechanism by which elevated glycogen stores could enhance high intensity exercise performance is unclear. In prolonged submaximal exercise, high levels of initial muscle glycogen may prolong fatigue by maintaining glycogen stores for use in later stages of exercise, or by blunting the fall in blood glucose that commonly accompanies this type of exercise (24). However, since muscle glycogen is typically minimally depleted during high intensity exercise, glycogen availability is not thought to be limiting to performance (41). Given that the enzyme glycogen phosphorylase has a low $K_m$ for glycogen in muscle cells, it has been estimated that as long as muscle glycogen is higher than 120-160 mmol/kgdw, glycogenolytic rate is not impaired (45). Additionally, blood glucose levels typically do not decline in high intensity exercise to the same extent as that characteristic of prolonged exercise. Some researchers have even shown an increase in blood glucose regardless of initial muscle glycogen levels (4, 35). Thus, elevated muscle glycogen stores likely do not have the same effect on blood glucose during high intensity exercise as seen with prolonged exercise (54).

Whereas total muscle glycogen reduction is not typically dramatic with high intensity exercise, it is possible that selective or compartmentalized glycogen depletion occurs. For example, Type II muscle fibers, those critical for high intensity exercise, may be depleted of glycogen more rapidly than the overall muscle (19, 41). Additionally, glycogen may be depleted from specific cellular regions to a higher degree than other areas. One such region is the
sarcoplasmic reticulum (SR).

An extensive amount of animal research points to diminished SR function as a precipitator of muscle fatigue (8, 9, 11, 12, 46, 59, 60). Specifically, Ca$^{2+}$-uptake, Ca$^{2+}$-release, and Ca-ATPase activity have been depressed following fatiguing bouts of endurance, high intensity, and intermittent exercise of varying duration. Although few human studies are available, decreased Ca$^{2+}$-uptake and Ca-ATPase activity were shown with exhaustive submaximal cycling (5), and depressed Ca$^{2+}$-uptake was reported following two 30s cycling sprints (31) and 2.5-3min of high intensity kicking (23).

Evidence of a structural and metabolic relationship between muscle glycogen and SR function exists. Glycogen molecules, along with several glycogenolytic and glycolytic enzymes, have been located within the SR membrane, creating what is known as a SR-glycogenolytic complex (43, 46). Depletion of SR glycogen may influence membrane integrity and weaken the SR’s Ca$^{2+}$-handling ability (61). Also, glycogen may serve as a localized source of ATP for the Ca-ATPase pump, thereby enabling Ca$^{2+}$-uptake (13, 43). In animal studies, SR vesicles treated with alpha-amylase to break down glycogen showed impaired Ca$^{2+}$-uptake (7) and fatigued muscle fibers exhibited improved tetanic force and Ca$^{2+}$-handling when allowed to recover in a glucose bath as compared to a glucose-free bath (11). An effect of glycogen depletion on impaired Ca$^{2+}$-release has also been reported (11). Moreover, beginning exercise with higher levels of muscle glycogen may prevent a critical depletion of glycogen specifically associated with the SR.

Enhanced high intensity exercise performance has also been attributed to an increase in carbohydrate (CHO) oxidation when elevated glycogen stores are available (27, 33). High levels of glycogen may stimulate glycogen phosphorylase to oxidize more glycogen, while low levels of glycogen may inhibit glycogen phosphorylase (45). Since CHO is a primary fuel for ATP production at maximal and supramaximal intensities, increased CHO utilization may improve high intensity performance (45). Hargreaves et al. (30) noted, however, that although high muscle glycogen may enhance CHO utilization, increased lactate accumulation may offset performance-enhancing effects.

A limited amount of evidence has pointed to a relationship between muscle glycogen and creatine phosphate (CP) levels. Sparing of CP during exercise has been shown in subjects with
elevated resting muscle glycogen stores (39) and in subjects who consumed a CHO beverage before exercise (53). Tsintzas et al. (53) suggest that when more glycogen is available, CP may be depended on less for ATP production as CHO oxidation is increased.

The purpose of this investigation was to assess changes in muscle glycogen, phosphagens, and SR function occurring during fatiguing intermittent high intensity exercise and to determine effects of muscle glycogen manipulation on phosphagens, SR function, and the development of fatigue.

METHODS

Subjects

Eight competitive male cyclists between the ages of 18 and 30y volunteered to serve as subjects. Three subjects were members of the university’s Division I intercollegiate cycling team and five were members of a local pro-am cycling team and competed in either the Mountain Bike EXPERT category or USCF Category 3 or 4 Road Racing category. All subjects were currently cycling 4-6 times per week for at least two hours each ride (mostly on roads). The study took place approximately 1mo prior to university cyclists’ season and 1mo after pro-am cyclists’ season. Subject characteristics appear in Table 1.

Subjects gave their informed consent and were screened for contraindications to strenuous exercise, diet manipulation, and muscle biopsies. All methods and procedures were approved by the Institutional Review Board of Virginia Polytechnic Institute and State University.

Pre-testing

On the first day of the experiment, subjects performed a VO2peak test and a 30s Wingate test. The VO2peak test was performed as an incremental test to exhaustion on a Monark cycle ergometer. A MedGraphics cart was used for analysis of expired gases. Equipment was calibrated prior to each subject’s test. Following a low intensity warm-up, subjects cycled for
2min with a load of 2.0 kg. The load was increased by 0.5 kg every 2min, unless the subject’s heart rate did not increase by more than 8 beats/min in the first min of the stage. In that case, the 0.5 kg increase was made at 1min. Exhaustion was determined as the point when subjects were not maintaining the required 80 rpm, when VO₂ failed to increase over a 60s period, or when subjects volitionally terminated the test. A modified Monark cycle ergometer with freely hanging weights was used for the Wingate test. After the subject pedaled to top speed, a load of 8.75% of body weight was dropped. The number of pedal revolutions made during each 5s segment was manually recorded and converted into power (Watts). Only subjects whose VO₂peak was greater than 52 ml/kg-min and whose ratio of peak power at VO₂peak to average power during the Wingate test (average Watts for the six 5s segments) was greater than 0.40 were included in the study. Davis et al. (15) suggest that subjects with this VO₂peak:Wingate ratio typically better handle the protocol being used. A 30min recovery period separated the VO₂peak and Wingate tests.

Familiarization Trial

Approximately 20-30min after the VO₂peak and Wingate tests, subjects were allowed an opportunity to become familiarized with the experimental protocol. They performed a 3min warm-up at 70% VO₂peak followed by three or four 60s intervals at maximal effort with 3min of active recovery (pedaling at a self-monitored pace with no resistance) between intervals. Subjects were specifically instructed not to pace themselves, but to exert maximal effort throughout the 60s periods. They were allowed to stand up from the seat only during the first 3s of the intervals.

The workload used for the intervals was the load that would elicit 130% VO₂peak if 90 rpm was maintained. This workload was selected since it was the level at which pilot subjects were able to perform approximately 15 intervals. Ninety rpm was chosen since pilot subjects often reached a plateau at 85-95 rpm when giving maximal effort for repeated 60s intervals at this workload.

Baseline Trial

One week after the familiarization trial, subjects completed a baseline trial that mimicked
the experimental protocol. The protocol used was a modification of that previously used by Davis et al. (15) who had subjects perform repeated 60s intervals at 120-130% VO2max to fatigue. We selected this protocol since enhanced performance was attributed to CHO feeding: subjects performed 27min longer in the Davis et al. (15) study when ingesting a CHO beverage immediately before and every 20min of exercise. Davis et al. (15) had untrained subjects perform intervals at 80 rpm. Since our subjects were highly trained, they were instructed to provide maximal effort during intervals. Our pilot data showed that trained cyclists could continue the Davis et al. (15) protocol for well over 2h; our aim for total exercise time was 1h.

Subjects fasted for 12h prior to reporting to the lab, but were encouraged to consume water and non-caloric/caffeine-free fluids up until the trial. They consumed their normal diet until the 12h prior to the trial. Subjects performed a 5min warm-up at 70% VO2peak. During the final 10s of min 3, 4, and 5 of the warm-up, subjects cycled at maximal effort. They were then allowed 5min for stretching.

Subjects performed repeated 60s intervals at maximal effort with 3min of recovery against the same workload used in the familiarization trial. The number of revolutions achieved during each interval was recorded from a mechanical counter mounted to the back of the ergometer and turned by the force of the left pedal. A researcher manually counted the number of revolutions achieved during each interval (int rpm) to insure accuracy. Subjects continued performing intervals until fatigue. For the purpose of this investigation, fatigue was considered the point when int rpm fell below 70% of first int rpm for two consecutive 1min intervals (i.e. if a subject performed 100 rpm on interval 1, they were considered fatigued when they fell below 70 rpm for two intervals).

Performance Trials

One to 2wk following the baseline trial, subjects completed two performance trials in a 12h fasted condition, separated by exactly 7d. Performance trials were completed in a random order: one trial was in a high CHO condition (H-CHO) and the other was in a low CHO condition (L-CHO).

Trials consisted of the same protocol described in the baseline trial section. Intervals performed during the baseline trial ranged from 3 to 20 (12min to 80min of exercise).
effort to induce homogeneous performance times and similar metabolic stress, the workload used for performance trials was adjusted depending on baseline trial performance. If subjects performed 10 or fewer baseline intervals (2 subjects), 125% VO$_{2peak}$ was used; if 15 or greater intervals were performed (2 subjects), 135% VO$_{2peak}$ was used; and if 11 to 14 intervals were performed (4 subjects), workload was not changed from 130% VO$_{2peak}$.

Subjects were verbally encouraged throughout trials. The researcher responsible for verbal encouragement was blind to subjects’ treatment condition.

**Glycogen Depletion Rides**

On the evening 36h prior to both performance trials, subjects completed a cycling bout designed to deplete leg muscles of glycogen. Subjects cycled at approximately 80 rpm at 70% VO$_{2peak}$ for 80min followed by four 60s maximal effort sprints with 3min of recovery between sprints.

Following the ride, subjects were given either a H-CHO or L-CHO snack that was approximately 10% of their normal daily caloric intake and a H-CHO or L-CHO diet for consumption the next day.

**Diet Manipulation**

The H-CHO diet consisted of 80-85% kcals from CHO, while the L-CHO diet contained 5-10% kcals from CHO. Total kcals provided was matched to a 24h diet record kept by subjects the day before the baseline trial (Table 3). Dietary records were analyzed with Nutritionist V computer software. Foods included in the H-CHO diet include pasta, tomato sauce, bananas, apple juice, bread, cereal, milk, and Gatorlode. The L-CHO diet consisted of eggs, cheese, mayonnaise, tuna, turkey, margarine, peanut butter, and rice cakes. A non-caloric beverage (32 oz.) was provided with both diets. In the case of vegetarian subjects or those with food allergies, problem foods were omitted and other foods from the list were increased. Subjects were instructed to consume all of the food given to them and to return any uneaten portions. Table 4 contains a sample H-CHO and L-CHO diet for a subject whose baseline diet was 3400 kcals.

**Water**
Water was provided ad libitum during all trials and consumption was encouraged during interval recovery periods. Subjects were urged to consume adequate fluids during and following glycogen depletion rides.

**Body Weight**

Subjects’ body weight was recorded upon arrival to the laboratory for pre-testing, baseline, and performance trials. Weight maintenance was encouraged and only fluctuations less than 1.5 kg were permitted.

**Muscle Biopsies**

Three muscle biopsies were taken during each performance trial. Subjects’ left leg was used for the first performance trial and right leg for the second performance trial. Prior to the warm-up, lidocaine was injected into subject’s vastus lateralis muscle and two 0.8cm incisions were made with a scalpel approximately 1.25cm apart vertically. A 60-100 mg muscle sample was removed from the lower incision using the percutaneous needle biopsy technique with suction. Incisions were covered with a piece of gauze and medical tape and subjects began warming up for the performance trial.

Biopsy 2 was taken after subjects’ interval performance (int rpm) had declined to 85% first int rpm and biopsy 3 was at exercise completion, when rpm declined to below 70% first int rpm for two consecutive intervals. Subjects were unaware of their performance throughout the trials and therefore did not know when biopsies were going to be taken. For these biopsies, subjects were asked to immediately stop cycling and remove their legs from pedal clips as researchers removed gauze and tape. A sample was removed and subjects were encouraged to continue cycling throughout the remainder of the recovery period. The first and second biopsies were taken from the lower incision (the needle was inserted at a different angle into the muscle for each biopsy) and the third biopsy was taken from the higher incision. Effort was made to perform this procedure as rapidly as possible.

Muscle samples were immediately cut into two sections with a scalpel. One portion was frozen in liquid nitrogen for later analysis of glycogen, CP, Cr, and ATP. The amount of time required for samples to be removed and placed in liquid nitrogen averaged <10s. Samples were
later freeze dried, powdered, and dissected free of connective tissue, blood, and other non-muscle constituents. Glycogen was analyzed using an extraction (amyloglucosidase) and enzymatic spectrophotometric technique (51). Analysis of CP, Cr, and ATP was performed using a perchloric acid/KHCO₃ extraction and enzymatic spectrophotometric technique (32). Each assay was performed on approximately 0.5 to 2.5 mg of freeze dried tissue. Glycogen was analyzed in triplicate (with 9% variance among triplicates); CP, Cr, and ATP were analyzed in duplicate (with 6% variance among duplicates). Creatine phosphate, Cr, and ATP were adjusted for peak total creatine values within the same trial.

Remaining portions of muscle samples (≈25 mg) were used for SR Ca²⁺-uptake and release measurements. Samples were immediately put in an ice-cold homogenizing buffer containing sucrose, HEPES, NaN₃, and PMSF, and minced with scissors while kept on ice. Samples were then homogenized using a Pro 200 homogenizer with a 5-mm probe. Following centrifugation at 1600g for 15min (2°C), the supernatant was removed and stored in a -80°C freezer. Total protein concentration was analyzed with the Bradford protocol as modified by Bio-Rad (6).

Analysis of Ca²⁺-uptake and release was performed using a technique described in detail by Ward et al. (55). Approximately 75-125 µg of homogenate protein was added to a buffer containing KCl, HEPES, Pyrophosphate, and MgCl₂. Free Ca²⁺ concentration was measured by a Jasco CAF-110 Intracellular Ion Analyzer in the presence of Fura (a fluorescent Ca²⁺ indicator). This measurement was performed before and after addition of Ca²⁺ and ATP as a means of quantifying the rate of Ca²⁺-uptake. Ca²⁺-release was stimulated by addition of AgNO₃.

**Statistical analysis**

A paired t-test and chi-squared test was performed to detect differences between trials in performance measurements. All other data were analyzed by repeated measures ANOVA to test for effect of group, time, and group by time interaction. Pearson’s correlation analysis was performed to determine associations between dependent measures. Significance was defined at the p<0.05 level.
RESULTS

Compliance

Subjects were verbally questioned regarding compliance to dietary and exercise instructions before performance trials. No deviance was reported at that time, with the exception of one subject who returned 1200 kcals of food provided for his first performance trial. This subject was provided with 1200 fewer kcals for his second performance trial. No other subject returned more than 200 kcals of unconsumed foods.

Body Weight

All subjects maintained their body weight within 1.5 kg during the study (baseline= 73.5 ± 1.6kg, H-CHO= 73.5 ± 1.7kg, and L-CHO= 72.9 ± 1.6kg). Seven subjects weighed slightly more before the H-CHO trial than the L-CHO trial, but this difference was not significant.

Performance

There was a tendency (p=0.09) for improved performance in the H-CHO trial (14.3 ± 2.5 intervals, 1312.2 ± 279.8 kJ total work) as compared to the L-CHO trial (10.4 ± 0.89 intervals, 957.8 ± 118 kJ total work) (Figures 1 and 2). Total exercise time tended to be 37% longer in the H-CHO trial (57.5 ± 10.0min) than the L-CHO trial (42.0 ± 3.6min) (p=0.09). There was no difference between trials in the average amount of work performed per interval (Figure 3). Six subjects performed better in the H-CHO trial, one performed better in the L-CHO trial, and one performed the same in both trials. Results of a chi-square test analyzing the number of subjects who did more total intervals in the H-CHO trial than the L-CHO trial revealed p<0.05. Performance differences were not influenced by the order in which trials were performed.

Peak power was achieved by all subjects during both trials’ first interval. There was no difference in peak power between trials (H-CHO, 641 ± 52.5 Watts vs. L-CHO, 652 ± 57.0 Watts). Biopsy 2, taken when int rpm declined to 85% of maximal interval rpm, occurred, on average, at the same time in both trials (5.9 ± 1.5 intervals, 23.6 ± 6.0min) (Figure 4). Total work completed up to this point was not different in the H-CHO and L-CHO trials (558.4 ± 84.2
vs. 571.5 ± 87.2kJ) (Figure 5). Performance differences between trials became apparent between biopsy 2 and 3 (when int rpm declined to 70% of first int rpm). Subjects cycled 86% longer following biopsy 2 in the H-CHO condition than the L-CHO condition (8.4 ± 2.5 intervals and 33.6 ± 10min vs. 4.5 ± .89 intervals and 18.0 ± 3.6min, respectively) (p<0.05) (Figure 6). Approximately 93% more work was completed between biopsy 2 and 3 in the H-CHO condition (753.5 ± 231.5kJ) than the L-CHO condition (389.1 ± 69.2kJ) (p<0.05) (Figure 7).

**Muscle Analyses**

A main effect of time and diet was found for glycogen (p< 0.001). Overall, glycogen decreased 35% from biopsy 1 to 2 (p<0.001), but the 6% decrease observed from biopsy 2 to 3 was not significant (Figure 8). There was no difference between trials in the rate of glycogen utilization (H-CHO, 5.7 ± 1.0 vs. L-CHO, 4.4 ± 1.4 mmol/kgdw·min). Glycogen was higher at all biopsies in the H-CHO trial than the L-CHO trial: 37% higher in biopsy 1 (609 ±38 vs. 390 ± 42 mmol/kgdw), 34% higher in biopsy 2 (383 ± 29 vs. 252 ± 28 mmol/kgdw), and 43% higher in biopsy 3 (346 ± 29 vs. 196 ± 18 mmol/kgdw).

A main effect of time was found for CP, Cr, ATP, Ca\(^{2+}\)-uptake, and Ca\(^{2+}\)-release (p<0.01). There was a tendency for a diet X time interaction for ATP (p= 0.08). A significant decrease in CP and increase in Cr occurred in both trials from biopsy 1 to 2 (p<0.01) (Figure 9). The overall 14% decrease in ATP was significant from biopsy 1 to 2 and 2 to 3 (p< 0.05) (Figure 11). CP decreased and Cr increased at a rate of 0.6 ± 0.1 mmol/kgdw·min. ATP decreased at a rate of 0.1± 0.06 mmol/kgdw·min. An overall decrease in Ca\(^{2+}\)-uptake was found from biopsy 1 to 2 (37%, p<0.001) and 2 to 3 (8%, p<0.001) (Figure 12). Ca\(^{2+}\)-release decreased by 38% from biopsy 1 to 2 (p<0.01), but remained unchanged from 2 to 3 (Figure 13). A significant correlation existed between CP and Ca\(^{2+}\)-uptake (r= 0.532, p< 0.01) (Table 5).

**DISCUSSION**

Results of this study indicate that performance during the latter stages of 40-60min of 60-s high intensity cycling intervals may be impaired by low muscle glycogen. Furthermore,
decreases in muscle glycogen, phosphagens, and SR function exist at fatigue with this type of exercise, but are not full explanations for the fatigue experienced, nor the performance differences observed between trials.

**Performance**

Subjects tended to perform more total work and more high intensity intervals when in a H-CHO condition than a L-CHO condition. Exercise time was 87% longer in the H-CHO trial than the L-CHO trial during the stage of exercise when performance decreased from 85% to 70% of initial, after approximately 6 intervals. Others have reported benefits of elevated CHO status in intermittent high intensity tests. Soccer players who consumed a H-CHO diet for 2d (elicited a 50% difference in resting muscle glycogen from players who consumed a 2d L-CHO diet) performed 6% more total work in an 85min intermittent intensity field and treadmill test (2). In a protocol similar to that used in our study, Davis et al. (15) found that subjects cycled significantly longer (87 vs. 60min) and performed about 50% more high intensity intervals when given CHO before and during exercise. Overall, our subjects tended to cycle over 15 minutes longer and perform 37% more high intensity intervals when beginning exercise with higher muscle glycogen. These differences, however, did not reach statistical significance. Power analysis indicated that ten subjects would have been required to obtain statistical significance for this analysis.

Jenkins et al. (34), in contrast, did not find enhanced performance of five 60s all-out cycling intervals (5min rest between intervals) following a 2d H-CHO diet compared to a moderate CHO diet. Total exercise time for these subjects was 30 minutes. Our results are similar in that we found no benefit of H-CHO during the first 25 minutes of high intensity intervals. Enhanced performance did not become evident until late in exercise (the last ≈15-30 minutes). Thus, differences in results are likely explained by variation in total exercise time. Additionally, muscle glycogen levels were not assessed by Jenkins et al. (34). It is possible that subjects’ resting levels were not as different as those in our study, particularly since a H-CHO diet was being compared to a moderate CHO rather than the L-CHO diet we used.

Our results are not similar to that of Casey et al. (10) who showed impaired performance in the first three of four 30s maximal intermittent cycling intervals (total exercise time = 14min)
following glycogen depleting exercise and a 3d L-CHO diet (8% kcals). It is likely that the glycogen depleting regimen used in this study induced lower muscle glycogen levels than that found in our study where the L-CHO diet was only followed for 1d. Unfortunately, muscle glycogen was not assessed, but these researchers (10) hypothesized that their performance decrement was due to levels of glycogen being so low that an inhibition of glycogen phosphorylase occurred, preventing sufficient CHO oxidation to support the high intensity intervals.

**Glycogen**

Our exercise and diet manipulation induced a 37% difference in resting muscle glycogen between the H-CHO and L-CHO treatments. Hargreaves et al. (30) also found a 37% difference in resting muscle glycogen levels in trained cyclists following depleting exercise and 24h on a H-CHO (80% kcals) or L-CHO diet (25% kcals).

The significant difference in muscle glycogen observed between treatments at rest remained throughout exercise. Muscle glycogen was 34% higher in biopsy 2 and 43% higher in biopsy 3 in the H-CHO trial than the L-CHO trial. Glycogen availability does not explain the performance differences we observed, since subjects reached the same level of fatigue (70% int rpm) despite significant differences in muscle glycogen levels.

Our subjects showed similar rates of glycogen utilization in both trials. Therefore, our data do not support the theory that having higher glycogen stores enhances the rate of glycogen utilization, which has been suggested to occur during intermittent and high intensity exercise (27, 29, 33). Ren et al. (45), however, found no difference in the rate of glycogen utilization in 10, 30, and 60s periods of high intensity quadriceps stimulation in subjects with normal and low muscle glycogen (350 vs. 155 mmol/kgdw). Bangsbo et al. (3) suggested that as long as the concentration of muscle glycogen exceeds 120-160 mmol/kgdw, glycogenolysis is not inhibited since the phosphorylase enzyme is saturated with substrate. Muscle glycogen levels in our study never fell beneath 190 mmol/kgdw, and therefore, ample glycogenolytic substrate apparently existed throughout exercise in both trials. Additionally, it has been suggested that glycogen breakdown in high intensity exercise is stimulated by increased catecholamine response, irrespective of initial muscle glycogen content (35).
We observed rapid glycogen utilization during the first six intervals. There was a 35% decrease in muscle glycogen from biopsy 1 to biopsy 2. Others have confirmed a rapid decrease in muscle glycogen with 30-60s sprints. McCartney et al. (42) found a 21% decrease in muscle glycogen following one 30s sprint and a 38% decrease following two 30s sprints. Febbraio et al. (17) showed a 40% decrease in muscle glycogen following four 60s sprints.

Glycogen utilization between biopsy 2 and biopsy 3, on the other hand, was minimal (6% in both trials). This may indicate a rise in CHO oxidation since the rate of glycogenolysis was reduced late in exercise. In a study by Gaitanos et al. (22), the contribution of glycolysis to ATP production in the tenth of a set of repeated 6s sprints decreased by 28% from the first 6s sprint. These researchers concluded that during interval ten, ATP was supplied primarily by the phosphagen system and oxidative metabolism and that the glycolytic contribution was minimal, which seems to be the case with our subjects as well. In both studies, glycogenolysis does not seem to have been limited by glycogen availability. Rather, an inhibition of glycogenolytic pathway likely occurred. Gaitanos et al. (22) suggest that this inhibition may have occurred at the phosphofructokinase (PFK) level due to an increased concentration of H+ and lactate, which likely accompanied the exercise bout. Increased H+ concentration may also inhibit glycogen breakdown by decreasing glycogen phosphorylase activity (51), or by decreasing the concentration of inorganic phosphate (Pi) in the monoprotonated form, which serves as a substrate for phosphorylase (36). Moreover, a decrease in the amount of CHO contributing to energy production may be responsible for the decreased intensity subjects were able to maintain (22).

**Phosphagens**

CP utilization appeared to be similar in both trials. Additionally, ATP levels decreased by only 14% during the exercise bout. Thus, it seems that availability of CP and glycogen was not limiting to performance since very low levels of these fuels never existed, and only minimal reductions in ATP occurred.

There was no effect of glycogen concentration on phosphagen status, since similar phosphagen levels were found in both trials. Some research supports a sparing of CP when more glycogen is available. Larson et al. (39), for example, showed a sparing of CP during
approximately 5min of high intensity quadriceps exercise in a group that had consumed high CHO for 5d compared to a group that consumed low CHO for the same amount of time. In a study by Tsintzas et al. (53), a CHO solution consumed prior to submaximal running induced higher CP levels at exhaustion than a placebo solution, despite a longer time to exhaustion in the CHO solution group (53). Comparisons between our study and the Tsitnzas et al. (53) study are complicated by the differing exercise protocols used. It is possible that elevated glycogen status may spare CP during moderate or low intensity exercise, but not during high intensity exercise. An obligatory glycogen breakdown has been shown to characterize high intensity exercise regardless of initial glycogen concentration (41). The acidic environment resulting from the high glycolytic rate may favor CP breakdown (48).

Given that a single 60s bout of high intensity exercise has been shown to place a dramatic drain on CP stores (45), our subjects likely experienced a high degree of CP resynthesis during interval recovery periods, since we observed only a 35% decrease in CP during the exercise bout. Gaitanos et al. (22) reported a CP resynthesis rate of 1.3 mmol/kg-s, so our 3min recovery periods may have enabled a significant resynthesis of CP between intervals. However, others have suggested a slowed rate of CP resynthesis with repeated high intensity sprints and consequently, more dramatic decreases in CP. For example, Spriet et al. (51) reported a 49% decrease in CP following two 30s cycling sprints, McCartney et al. (42) found a 93% decrease following two 30s maximal effort cycling sprints, and Febbraio et al. (17) showed a 73 - 77% decrease following four 60s high intensity cycling intervals. Since our subjects were given 3min rest periods between intervals, there may have been a greater degree of CP resynthesis between intervals than was found with Febbraio et al. (17) subjects, who were given only 1min rest periods between intervals. The training status of our subjects may have also enhanced their ability to maintain CP levels (17).

Although there is limited documented data to support the theory, some have suggested that post-exercise muscle samples must be taken and frozen very rapidly to avoid CP resynthesis (49). Thus, it is possible that the amount of time that was required for our researchers to remove muscle samples from subjects’ thighs and place them in liquid nitrogen (<10s) was excessive and CP values were misrepresented as too high. However, other researchers have reported that the process required as long as 20s in their investigations (5, 17, 42). Therefore, the duration of our
procedure seems similar to that used in other studies and theoretically, the CP values we obtained should be equally reliable. Future investigation should be conducted to determine exactly what time window is required for accurate CP analysis. For resting samples, it has been reported that CP levels in samples exposed to 60s of air best correspond to CP levels in fresh muscle (49). An alternative method for future studies may be making two biopsy passes for each set of exercise samples – one that is immediately frozen in the needle for metabolite analysis and a second that is used for less time-dependent analyses.

**SR Function**

Sarcoplasmic reticulum function was clearly depressed with the fatiguing exercise protocol used in this study. There was an overall 56% decrease in Ca\(^{2+}\)-uptake from biopsy 1 to biopsy 3. In a survey of several animal investigations, Williams and Klug (58) report a consistent 40-60% decrease in Ca\(^{2+}\)-uptake with fatiguing exercise. No human studies have examined Ca\(^{2+}\)-uptake with intermittent exercise of the duration we used, but Hargreaves et al. (31) reported a 25% decrease in Ca\(^{2+}\)-uptake after 1min of recovery from two 30s maximal effort intervals and Gollnick et al. (23) found a 42% decrease in Ca\(^{2+}\)-uptake immediately following a single high intensity kicking bout that lasted approximately 3min. We also found an overall 49% decrease in Ca\(^{2+}\)-release at biopsy 3. No human Ca\(^{2+}\)-release data is available to date, but this value is similar to that reported in animal studies (1, 58, 59).

Since there appeared to be no difference between trials in Ca\(^{2+}\)-uptake or the rate of decline in Ca\(^{2+}\)-uptake, our data do not support a relationship between muscle glycogen status and Ca\(^{2+}\)-uptake at any fatigue level induced by our protocol. Hargreaves et al. (31) also found no relationship between glycogen status and Ca\(^{2+}\)-uptake. Performance, muscle glycogen levels, and Ca\(^{2+}\)-uptake were depressed after three 30s cycling sprints. In a fourth 30s sprint following a 90min recovery period, Ca\(^{2+}\)-uptake remained depressed despite a rebound in performance and muscle glycogen levels. In contrast, Chin and Allen (11) reported an association among whole muscle glycogen, SR function, and force in animal fibers. Our study and the Hargreaves et al. (31) study, however, were limited by only measuring total muscle glycogen and not glycogen specifically associated with the SR. Friden et al. (19) and Maughan et al. (41) have reported human data that show selective and compartmentalized glycogen depletion in different cellular
regions, including the SR membrane, that is significantly greater than glycogen degradation in the whole muscle. Therefore, if glycogen is depleted from the SR more rapidly than from the whole muscle, techniques applied in the aforementioned studies would not detect this change.

Since trials were not significantly different in CP, Cr, and ATP levels at any biopsy, it is difficult to make inferences about an association among these metabolites and SR function. However, positive correlations existed between the percent decrease in CP from biopsy 2 to fatigue and the level of Ca\(^{2+}\)-uptake and release at fatigue. It is possible that SR function is impaired once CP falls below a certain level. In a study on skinned cardiac muscle fibers from rats, decreasing CP levels caused a decrease in Ca\(^{2+}\)-uptake. Similarly, Westerblad and Lannergren (56) showed decreased Ca\(^{2+}\)-release in rested animal fibers depleted of CP. This decrease was attributed to an inability to adequately buffer intracellular rises in ADP, due to CP depletion. Large rises in ADP have been suggested to occur during high intensity exercise (47), which may depress SR function and force production (1, 25).

The observed decrease in Ca\(^{2+}\)-uptake may be further attributed to a diminished rate of localized ATP hydrolysis (5) and thus, diminished SR Ca\(^{2+}\)ATPase activity. When adequate CP is present, it functions to maintain a favorable ATP/ADP ratio at cellular sites that require ATP consumption – for instance, the SR (56). Also, CP is closely associated with intracellular inorganic phosphate (P\(_i\)) levels. Excess P\(_i\) from CP or adenine nucleotide breakdown may lead to formation of a calcium-phosphate precipitate that reduces the amount of Ca\(^{2+}\) available for Ca\(^{2+}\)-release (1). Some research, however, is supportive of a P\(_i\)–transporter that actually causes an increase in the amount of Ca\(^{2+}\) released from the SR (20). Further research is necessary to investigate this relationship.

*Causes of Performance Differences Based on Glycogen Status*

Analyses included in our study do not easily explain performance differences observed between glycogen treatments, so it is possible that some other factor may be responsible. In general, blood glucose availability does not seem to be limiting during high intensity exercise (4). However, with the extended duration of our protocol, it is possible that blood glucose levels became a fatigue factor. We did not make direct blood glucose measurements, but it is possible that blood glucose may have been lower in the L-CHO group than the H-CHO group.
If a difference between trials in blood glucose levels existed, it is possible that neural drive may have been influenced by glycogen status. According to the Central Fatigue Theory, low levels of blood glucose may cause an increase in muscle uptake of branched chain amino acids (BCAA) and consequently, a decrease in blood BCAA (14). Lower BCAA may cause elevated brain uptake of free tryptophan, which could increase serotonin concentration and possibly cause earlier fatigue (14). Kent-Braun (37) suggests that central fatigue may contribute to as much as 20% of muscle fatigue. Thus, our L-CHO subjects may have experienced a greater degree of central fatigue than H-CHO subjects.

Finally, it is possible that differences in acid-base status explain the performance decrement observed in the L-CHO trial. Researchers have suggested that diets low in CHO and high in protein, such as the diet followed by our L-CHO subjects, may negatively influence acid/base balance, thereby decreasing performance (26-28, 41). Greenhaff et al. (26) had subjects follow a H-CHO (65% kcals) or L-CHO (10% kcals) diet for 3 days prior to a cycling test at 100% VO2max. L-CHO subjects showed lower resting pH, higher bicarbonate, PCO2, and base excess levels, and a faster time to fatigue than H-CHO subjects (26). An acidic environment may decrease performance by inhibiting the glycolytic pathway or by causing dysfunction of a contractile apparatus component (41).

**Causes of Fatigue**

It is unlikely that muscle fatigue can be explained by a single factor – many researchers agree that the cause is multi-faceted (18, 25, 58). The fatigue experienced by our subjects can partly be explained by the decreased anaerobic fuel utilization and increased reliance on oxidative ATP production late in exercise. It is also reasonable to conclude that the decline in SR function shown in our subjects at biopsy 3 contributed to fatigue development.

It is possible that dysfunction of a contractile component other than the SR also contributed to the decline in performance. Some researchers have reported a decrease in the affinity of troponin-C for Ca2+, which results in decreased Ca2+ sensitivity (1, 18, 59). Thus, even without a decline in Ca2+-release, cross bridges are less sensitive to the Ca2+ that is present, and contractile function is worsened (11).

Blood and muscle lactate and H+ were not assessed in this study, so specific changes
occurring in these metabolites during our protocol are unknown. However, it is reasonable to
expect that a dramatic decrease in lactate and $H^+$ occurred due to the intense nature of the exercise
(51). Increases in lactate (16, 50) and $H^+$ (1) have been suggested to impair SR function and
decrease force production. Thus, it is possible that changes in SR function observed in our study
may be explained by increased intracellular lactate and $H^+$.

It is important to note that in our study, muscle samples were taken as subjects reached a
certain fatigue level, regardless of total exercise time. Establishing a similar intermittent high
intensity protocol that enabled muscle samples to be taken after the same number of intervals in
both dietary conditions may give more insight into time-dependent changes in metabolites and
SR function.

**Summary**

Performance during the last 15 to 30 minutes of our intermittent high intensity cycling
protocol was enhanced by elevated muscle glycogen levels. Subjects experienced similar
decreases in muscle glycogen, CP, ATP, and SR function in both high and low muscle glycogen
conditions. Initial muscle glycogen levels did not appear to influence the rate of glycogen
utilization, nor did this affect SR function. Thus, other unmeasured factors must explain fatigue
during this type of exercise.

This research was funded by the American College of Sports Medicine Reebok Student Grant, Gatorade Sports Science Institute, NIH AR41727, and the James D. Moran Thesis and Dissertation Award. Gatorlode used in the study was donated by Gatorade Company - Barrington, IL. Researchers would like to thank Janet Rinehart for laboratory assistance and for performing muscle biopsies. Helen Stevens, Simon Lees, and Randy Bird assisted with data collection and analysis.
REFERENCES


27. **Greenhaff, P.L., M. Gleeson, and R.J. Maughan.** The effects of diet on muscle pH and


52. Symons, J.D. and I. Jacobs. High-intensity exercise performance is not impaired by low


61. **Xu, K.Y. and L.C. Becker.** Ultrastructural localization of glycolytic enzymes on
### Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (kg)</th>
<th>Body fat %</th>
<th>Relative VO$_{2peak}$ (ml/kg·min)</th>
<th>Absolute VO$_{2peak}$ (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>23.9</td>
<td>73.5</td>
<td>8.5</td>
<td>58.0</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>1.6</td>
<td>1.6</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>DAY</td>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pre-test (VO$_{2peak}$, Wingate);</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Familiarization trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Baseline Trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Glycogen depletion ride - 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Diet manipulation - A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Performance trial - 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Glycogen depletion ride - 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Diet manipulation - B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Performance trial - 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total kcals</td>
<td>% CHO</td>
<td>% Fat</td>
<td>% Protein</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>Mean</td>
<td>3474</td>
<td>62</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>± S.E.M</td>
<td>169</td>
<td>2.4</td>
<td>2.7</td>
<td>.94</td>
</tr>
</tbody>
</table>
Table 4. Sample Diets for a Subject with a 3400 kcal Baseline Diet

<table>
<thead>
<tr>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H-CHO (80-85% Carbohydrate)</strong></td>
<td><strong>(5-10% Carbohydrate)</strong></td>
</tr>
<tr>
<td>4 cups Honey Nut Cheerios</td>
<td>4 eggs</td>
</tr>
<tr>
<td>1 ½ cups skim milk</td>
<td>24 ounces tuna</td>
</tr>
<tr>
<td>5 slices whole wheat bread</td>
<td>5 ounces turkey</td>
</tr>
<tr>
<td>¼ cup jelly</td>
<td>3 ounces cheese</td>
</tr>
<tr>
<td>1 pound pasta</td>
<td>½ cup margarine</td>
</tr>
<tr>
<td>2 ½ cups tomato sauce</td>
<td>½ cup peanut butter</td>
</tr>
<tr>
<td>24 ounces Gatorlode</td>
<td>3 Tablespoons mayonnaise</td>
</tr>
<tr>
<td>3 bananas</td>
<td>1 rice cake</td>
</tr>
<tr>
<td>36 ounces apple juice</td>
<td>1 slice whole wheat bread</td>
</tr>
<tr>
<td>32 ounces crystal lite</td>
<td>4 Hershey’s kisses</td>
</tr>
<tr>
<td></td>
<td>32 ounces crystal lite</td>
</tr>
</tbody>
</table>
### Table 5. Overall Correlation Analysis (data from all biopsies included)

<table>
<thead>
<tr>
<th>Overall</th>
<th>Glycogen</th>
<th>Creatine Phosphate</th>
<th>ATP</th>
<th>Calcium-uptake</th>
<th>Calcium-release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>X</td>
<td>r = 0.454</td>
<td>r = 0.488</td>
<td>r = 0.385</td>
<td>r = 0.435</td>
</tr>
<tr>
<td>Creatine Phosphate</td>
<td>r = 0.454</td>
<td>X</td>
<td>r = 0.470</td>
<td>r = 0.532 *</td>
<td>r = 0.443</td>
</tr>
<tr>
<td>ATP</td>
<td>r = 0.488</td>
<td>r = 0.470</td>
<td>X</td>
<td>r = 0.473</td>
<td>r = 0.490</td>
</tr>
<tr>
<td>Calcium-uptake</td>
<td>r = 0.385</td>
<td>r = 0.532 *</td>
<td>r = 0.473</td>
<td>X</td>
<td>r = 0.644 *</td>
</tr>
<tr>
<td>Calcium-release</td>
<td>r = 0.435</td>
<td>r = 0.443</td>
<td>r = 0.490</td>
<td>r = 0.644 *</td>
<td>X</td>
</tr>
</tbody>
</table>

r = Pearson’s Correlation Coefficient

* = p< 0.05
Figure 1. Number of intervals performed in both trials before declining to 70% max interval rpm

$p = 0.09$

for difference between trials
Figure 2. Amount of work (kJ) performed in both trials before reaching 70% max interval rpm

$\text{Total Work Performed}$

![Bar chart showing total work performed in kJ for H-CHO and L-CHO trials. The chart indicates that there is no significant difference ($p = 0.09$) between the trials.]
Figure 3. Average amount of work (kJ) performed per interval in both trials.
Figure 4. Number of intervals performed in both trials before declining to 85% max interval rpm.
Work Performed before Biopsy 2

Figure 5. Amount of work performed in both trials before declining to 85% max interval rpm
Figure 6. Number of intervals performed in both trials between 85% max interval rpm & 70% max interval rpm

* = p < 0.05
Figure 7. Amount of work performed in both trials between 85% max interval rpm & 70% max interval rpm

* = p < 0.05
Figure 8. Muscle Glycogen at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001); main effect of diet (p< 0.001)

* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.001)

n = 7 for L-CHO biopsy #3
n = 8 for all others
Figure 9. Muscle Creatine Phosphate at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001)
* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.001)

n = 7 for H-CHO biopsy #2 and L-CHO biopsy #3
n = 8 for all others
Figure 10. Muscle Free Creatine at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001)
* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.001)

n = 7 for H-CHO biopsy #2 and L-CHO biopsy #3
n = 8 for all others
**Figure 11.** Muscle ATP at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001); Diet X Time interaction: p= 0.078

* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.05)

$ = significantly different from biopsy 2 for both H-CHO and L-CHO (p< 0.05)

n = 7 for H-CHO biopsy #2 and L-CHO biopsy #3

n = 8 for all others
Figure 12. Sarcoplasmic Reticulum Calcium-uptake at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001)
* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.001)
$ = significantly different from biopsy 2 for both H-CHO and L-CHO (p< 0.01)

n = 6 for H-CHO biopsy #2
n = 7 for L-CHO biopsy #2
n = 8 for all others
Sarcoplasmic Reticulum Calcium-Release

Figure 13. Sarcoplasmic Reticulum Calcium-release at rest, 85% max interval rpm, & 70% max interval rpm

Main effect of time (p< 0.001)

* = significantly different from biopsy 1 for both H-CHO and L-CHO (p< 0.001)

n = 6 for H-CHO biopsy #2
n = 7 for L-CHO biopsy #2
n = 8 for all others
CHAPTER 4:

Summary and Recommendations
Consuming a high carbohydrate (CHO) diet is commonly recommended for endurance athletes’ training diets and particularly, for their pre-game meals. Having elevated glycogen stores has been repeatedly shown to enhance endurance performance, while having low glycogen stores may impair performance (37). However, CHO are often considered less important for athletes who perform high intensity activities (74). In fact, the popularity of several high-protein, moderate CHO diets is widely increasing among athletes of high intensity sports (5).

Some studies have shown clear benefits of elevated CHO status during high intensity exercise performance. Subjects in a study by Pizza et al. (81), for example, ran longer in a treadmill performance test at 100% VO$_{2\text{max}}$ following six days on a high CHO diet compared to six days on a moderate CHO diet (≈300 vs. 280 seconds, respectively). Langfort et al. (64) showed enhanced performance (increased power output) in a 30-second Wingate test following three days on a moderate CHO vs. low CHO diet. An even more convincing case has been established for performance of intermittent high intensity exercise. Subjects performed approximately 50% longer in a protocol involving 60-second supramaximal cycling bouts when consuming a CHO vs. placebo beverage at the start of exercise and every 20 minutes during exercise (24). Jenkins et al. (57) reported improved performance (total work) in five 60-second maximal effort cycling sprints when a three-day high or moderate CHO vs. low CHO diet was consumed. In these studies, enhanced performance was at least partially attributed to elevated muscle glycogen stores, although direct measurements were not made.

Some data do not support ergogenic effects of elevated CHO status for high intensity exercise. Hargreaves et al. (46) showed no benefit of a high CHO vs. low CHO diet in performance of a 75-second all-out cycling sprint despite a 37% difference in baseline muscle glycogen levels between groups. Additionally, there was no difference in performance of four
maximal-effort 1.6 kilometer cycling intervals following consumption of a CHO beverage or placebo (89).

There are several mechanisms that may explain the value of high muscle glycogen during intense exercise. First, when exercising at high intensity, muscles are primarily fueled by CHO and creatine phosphate (CP). Fat and amino acids minimally contribute to fuel production during intense activity, which makes adequate CHO stores seem critical to performance. The duration of high intensity exercise, however, is relatively low and muscle glycogen is likely only minimally depleted (≈20-35% has been reported). Thus, for years, researchers have not considered muscle glycogen levels to be performance-limiting (84). It is possible, however, that glycogen is depleted from different muscle fibers at different rates (69). Thus, whole muscle glycogen may be only slightly depleted while some fibers are severely depleted. If type II muscle fibers are depleted more rapidly than type I fibers, which has been reported (96), performance of high intensity exercise (involves heavy recruitment of type II fibers) may be impaired.

It has also been suggested that glycogen may be depleted from different cellular regions at different rates. One specific region in question is the sarcoplasmic reticulum (SR). The SR functions to store and metabolize calcium (Ca^{2+}) in muscle cells. Release of Ca^{2+} from the SR precedes muscle contraction, while Ca^{2+}-uptake by the SR initiates muscle relaxation. Some research supports an association between SR function and glycogen breakdown (21, 77, 109, 110). Most SR studies have been conducted on animals. Human studies have been limited by the small sample size obtainable from humans.

Other data suggest that having high muscle glycogen may spare the use of muscle CP during exercise (9, 65). A mechanism for this effect is not entirely clear, but Tsintzas et al. (96) suggest that when more glycogen is available, a higher rate of glycogen utilization occurs. If
glycogen breakdown is supplying the ATP needed for high intensity exercise, there may be decreased dependence on CP for fuel production (96). Maintaining higher CP levels throughout exercise may extend high intensity exercise duration or enable better recovery during rest periods. It may also improve performance of high intensity bursts occurring during an exercise bout, with examples being top-speed sprints in a soccer or hockey match and the “kick” at the end of a race (65).

Given the aforementioned findings and relationships, the purpose of our study was to compare effects of high muscle glycogen status to low muscle glycogen status on performance of approximately 40-70 minutes of intermittent high intensity cycling. We also investigated some specific metabolic mechanisms in an attempt to explain muscle fatigue experienced during intermittent high intensity exercise.

Eight competitive male cyclists were recruited from the Virginia Tech cycling team and the local East Coasters Pro-Am cycling team. Although subjects were not in the competitive portion of their season, they were cycling 4-6 times per week for at least two hours each ride. Subjects performed two intermittent high intensity cycling performance trials; one trial followed exhaustive exercise and a 36-hour low CHO diet (L-CHO) and the other followed exhaustive exercise and a 36-hour high CHO diet (H-CHO).

Performance trials consisted of repeated one-minute cycling sprints to fatigue. Fatigue was considered the point in time when interval pedal revolutions per minute (int rpm) decreased to 70% of their initial level. There was a tendency (p= 0.09) for better performance in the H-CHO condition than the L-CHO condition (=14 vs.10 intervals and 1200 vs. 900 kJ of total work, respectively). In the later portion of the exercise bout (after int rpm decreased to 85% of initial), H-CHO subjects performed 47% longer than L-CHO subjects (p<.05).
From muscle biopsies taken at rest, during exercise (when int rpm decreased to 85% of initial), and at fatigue (when int rpm decreased to 70% of initial), it was determined that muscle glycogen was approximately 40% higher throughout exercise in the H-CHO trial than the L-CHO trial. However, there was no difference between trials in the rate of glycogen utilization. Glycogen was utilized at a rate of approximately 0.5mmol·kg⁻¹·dw⁻¹·min⁻¹ during both trials (includes intervals and rest periods).

Overall, performance trials induced significant decreases in muscle CP (35%) (p<0.01) and ATP (14%) (p<0.05). Diminished SR function was also shown, based on decreases in Ca²⁺-uptake (56%) (p<0.01) and Ca²⁺-release (49%) (p<0.01) at fatigue. There were no differences between trials in CP, ATP, or Ca²⁺-uptake levels at any biopsy or in their rate of change during exercise. At fatigue, H-CHO subjects showed a 41% higher rate of Ca²⁺-release than L-CHO subjects (p<0.05), but there were no differences at any other time point.

It should be noted that the third biopsy samples were taken during both trials when subjects were at the same level of fatigue (based on int rpm) rather than after the same duration of exercise. Although CP, ATP, and Ca²⁺-uptake were similar when subjects were at the same level of fatigue (70% initial int rpm), it required a longer amount of time for H-CHO subjects to reach that level than L-CHO subjects. Had biopsy samples also been taken following the same duration of exercise in both trials, higher levels of CP, ATP, and Ca²⁺-uptake were likely to exist in the HG condition. Biopsies taken during exercise, on the other hand, were taken after an average of six intervals in both conditions. Subjects reached 85% initial int rpm (biopsy 2) at the same or within one interval in both the H-CHO and L-CHO trials. Thus, these biopsies represented the same fatigue level and approximately the same duration of exercise for both trials.
In the original study design, the second biopsy was going to be removed after the same number of intervals in both trials. This interval was to match the baseline trial interval when int rpm declined to 85% of initial int rpm. In other words, if a subject reached 85% initial int rpm during the sixth baseline trial interval, biopsies would be removed following the sixth interval in the H-CHO trial and L-CHO trial. However, researchers were limited by variation between subjects in baseline trial performance. Since some subjects performed as few as three total intervals and others performed 20 total intervals (total exercise time ranged from 12 to 80 minutes), a decision to adjust the workload used for subsequent performance trials was made. Workload was decreased for subjects who performed fewer than 10 intervals, increased for subjects who performed greater than 15 intervals, and unchanged for subjects who performed between 11 and 14 intervals. The goal was to match exercise time as closely as possible between subjects, although the crossover design did help with subject variability since subjects served as their own comparative counterpart for the two different trials. Ideally, subjects would have performed a second baseline trial before performance tests, but this was not possible since subjects were near the start of their competitive season and did not wish to participate in the study during their competitive training. The third biopsy was taken when originally planned, when int rpm decreased to 70% of initial in each trial.

Regarding trial performance, it appeared that elevated muscle glycogen was favorable to depleted muscle glycogen during our intermittent high intensity cycling protocol. Muscle glycogen status seemed to be especially important during later stages of the exercise bout, based on enhanced performance during this time in the HG condition. The decrease in muscle glycogen that occurred from biopsies taken during exercise to those taken at fatigue, however, was not significant. It is possible that glycogen status influenced a factor associated with fatigue
late in exercise. However, of those measured in our study, muscle glycogen status had no effect on muscle CP and ATP levels or Ca\(^{2+}\)-uptake at any biopsy. Although there was no difference between conditions in the rate of SR functional decline, the absolute rate of Ca\(^{2+}\)-release was higher in the H-CHO than the L-CHO condition at fatigue (this could not be analyzed statistically). If maintaining a normal rate of Ca\(^{2+}\)-release is dependent on the presence of a certain amount of muscle glycogen, L-CHO subjects may have fatigued more rapidly due to a lesser amount of Ca\(^{2+}\) being released from the SR. A lower rate of Ca\(^{2+}\)-release could depress muscle force due to insufficient contractile apparatus activation.

It is possible that the trend toward performance enhancement observed among H-CHO subjects may be attributed to factors not investigated in our study. For example, some component of the contractile apparatus other than the SR may have been sensitive to glycogen concentration. Those that have been specifically suggested include the activity of acto-myosin ATPase and Ca-ATPase, the affinity of troponin-C for Ca\(^{2+}\), and myofibrillar Ca\(^{2+}\)-sensitivity (1). However, since there was no difference between trials in the rate of muscle glycogen and ATP utilization regardless of initial concentrations, it seems unlikely that mechanical failure due to inadequate glycogenolysis occurred. Similarly, there are several muscle metabolites known to interfere with contractile function, namely \(\text{H}^+\), lactate, \(\text{P}_i\), and ADP (1). Levels of these metabolites may be affected by manipulation of muscle glycogen status, but we have no evidence, based on our analyses, that this was the case. For example, H-CHO subjects may be expected to oxidize more CHO and therefore produce more intramuscular lactate than L-CHO subjects. However, since there was no difference in glycogenolytic rate between trials, there was likely no difference in the rate of CHO oxidation and lactate accumulation. Likewise, the similarity between trials in phosphagen levels may indicate similar \(\text{P}_i\) and ADP levels.
The performance differences we observed may be explained by effects of the dietary manipulation other than muscle glycogen status. For example, L-CHO subjects may have experienced a greater degree of central fatigue during the exercise bout. According to the Central Fatigue Theory, low levels of blood glucose (which may have occurred in our L-CHO subjects) could cause an increase in muscle uptake of branched chain amino acids (BCAA) and consequently, a decrease in blood BCAA levels (23). Lower BCAA may cause elevated brain uptake of free tryptophan, which could increase brain serotonin release (23). Increases in serotonin levels have been associated with exercise fatigue. Kent-Braun (61) suggested that central fatigue may contribute to as much as 20% of muscle fatigue.

It is also possible that L-CHO subjects experienced mood alterations attributable to the dietary manipulation used in our study. Keith et al. (60) reported that following one week on a L-CHO diet, highly trained female cyclists’ responses on a Profile of Mood States (POMS) questionnaire revealed greater ratings of tension, depression, and anger than ratings made after a one week H-CHO diet. Low POMS scores have been related to depressed performance of exercise lasting longer than 40 minutes in both male and female subjects (82). Although our L-CHO diet lasted only 36 hours, subjects’ mood state may have been negatively influenced.

Researchers have suggested that diets low in CHO and high in protein, such as the diet followed by our L-CHO subjects, may negatively influence acid/base balance, thereby decreasing performance (40-43, 69). Greenhaff et al. (41) had subjects follow a H-CHO (65% kcals) or L-CHO (10% kcals) diet for 3 days prior to a cycling test at 100% VO_{2\text{max}}. L-CHO subjects showed lower resting pH, higher bicarbonate, PCO₂, and base excess levels, and a faster time to fatigue than H-CHO subjects (41). An acidic environment may decrease performance due to
inhibition of the glycolytic pathway at the enzyme phosphofructokinase (PFK), or by causing
dysfunction of a contractile apparatus component (69).

Dehydration is widely believed to contribute to decreased performance of prolonged exercise (2). Some researchers have found similar decrements with intermittent high intensity exercise. Maxwell et al. (70) found that hypohydrated subjects performed significantly worse than euhydrated subjects on an intermittent supramaximal running test. A possible mechanism for this effect may be that dehydrated subjects may exhibit poorer temperature control. Elevated body temperature has been associated with muscle fatigue, including a possible decrease in SR function (1). Current research suggests that high body temperature may increase the rate of glycogen utilization, which causes a more rapid depletion of muscle glycogen at high temperature than low temperature (28, 79). Although our subjects were encouraged to consume copious amounts of fluids (water, non-caloric beverages, caffeine free beverages, and beverages provided by researchers as part of experimental diets) during the 36 hours of dietary manipulation, no records of fluid consumption were kept. Since more fluids were provided for the H-CHO trial (apple juice, Gatorlode, milk, Crystal Light) than the L-CHO trial (Crystal Light only), subjects may not have matched their fluid consumption for both trials. There was, however, no significant difference between trials in body weight, which would be expected if subjects had actually experienced dehydration. The slight elevation in body weight observed in seven of the subjects before the H-CHO trial (roughly 0.5 kg) is likely due to the increased amount of water associated with storing muscle glycogen.

Moreover, fatigue during our intermittent high intensity protocol appeared to be related to a significant decline in muscle glycogen, CP, and SR function. Elevated baseline muscle glycogen seemed favorable to depleted muscle glycogen with respect to performance, but a
A mechanistic explanation for this effect was not elucidated by our investigation. Nevertheless, athletes who participate in intermittent high intensity activities such as soccer, basketball, lacrosse, and hockey may benefit from high muscle glycogen and therefore, from consuming high CHO training and pre-game diets.

Recommendations for Future Research

1. The exercise and diet manipulation used in our study elicited a 37% difference in resting muscle glycogen. Using a diet manipulation period longer than 36 hours may cause a greater difference in muscle glycogen. Symons and Jacobs (93) reported a 64% difference in resting muscle glycogen following depleting exercise and 2 days on a H-CHO vs. L-CHO diet and Bangsbo et al. (6) found a 50% difference following exercise and 2 days on a H-CHO vs. moderate CHO diet. Inducing a greater degree of baseline difference may be valuable for a mechanistic investigation involving muscle glycogen: alterations in performance, SR function, and phosphagen status may be more pronounced.

2. For a more practical investigation involving muscle glycogen and high intensity exercise, it would be beneficial to examine performance effects of diets containing a level of CHO closer to what athletes typically consume (approximately 85% CHO and 10% CHO were used for our study). Comparing a moderate CHO diet (40-45% kcals) to a high CHO diet (65-70% kcals) may be especially valuable. In order to develop recommendations for high intensity athletes’ training diets and pre-exercise meals, performance effects should be evaluated following variable periods of diet manipulation.
3. There is controversy regarding the effects of glycogen status on the rate of glycogenolysis in high intensity exercise. Some researchers have reported that increasing muscle glycogen levels causes an increased rate of glycogen utilization in high intensity exercise (51, 89). Additionally, the same is true with prolonged, moderate intensity exercise (37). We and others (84, 97), however, did not find this. It is possible that in our study, glycogen was not depleted to a point where utilization rate was affected. If lower levels of initial muscle glycogen are used in future research, it may be possible to determine if there is some critical glycogen level below which utilization rate is influenced.

4. Levels of CP and Cr in our samples may have been altered by the length of time required to perform muscle biopsies and place samples into liquid nitrogen. Determining how rapidly phosphagen levels are affected would be useful in designing experiments and developing muscle biopsy techniques. There is a small amount of published data that assess the effects of time and air exposure on resting muscle phosphagens. Soderlund and Hultman (90) reported that CP levels did not change during one to six minutes of air exposure. Furthermore, they suggested that a one-minute delay in freezing may more accurately reflect the true CP content in resting muscle (90). Although there is no documented data on the effects of freezing delay on exercised samples, it is possible that low levels of CP may be more susceptible to rapid CP resynthesis. In this case, an excessive freezing delay may cause assayed values to be falsely high. Research on the rate of CP resynthesis in exercised muscles is needed. Additionally, in future muscle biopsy experiments, it may be advisable to
take repeated samples at the same time point and use the first sample for volatile measurements and the second for more stable analyses.

5. Although there is extensive research involving Cr supplementation, there is a limited amount involving muscle biopsy analysis of phosphagen metabolism during exercise. We found a 35% decrease in CP from baseline to fatigue. Others have reported a 54% decrease in CP following three 30-second cycling intervals (92), a 75% decrease following three 60-second cycling intervals (29), and a 96% decrease after one supramaximal 60-second bout (84). Valuable information on CP’s contribution to single-bout high intensity may be obtained from research that utilizes the same subjects, exercise intensity, and exercise mode in a range of different exercise durations, from as little as 10-15 seconds to 3-4 minutes. Additionally, having subjects perform repeated bouts of high intensity exercise would give insight into the rate of CP decline during variable intensity exercise and the extent of CP recovery during rest periods, especially if muscle biopsies were repeated throughout exercise before and after high intensity intervals and at the end of rest periods.

6. There is a limited amount of research available on glycogen and phosphagen metabolism in actual field settings. Most research is performed in a laboratory setting on a cycle ergometer, such as the one used in our study, on a treadmill, or with some other type of laboratory equipment. Although results from these studies may be applicable to other settings, it would be beneficial to study muscle changes in athletes in their competitive environments during actual performance bouts. This research could assess actual changes in metabolites and SR function so laboratory studies can be designed to accurately reflect these changes.
7. Much of the research on SR function in fatigue has been conducted on skinned animal fibers, which has enabled the discovery of many important mechanisms. However, as discussed by Allen et al. (1), studies on skinned fibers may be limited in their ability to mimic ionic and systemic changes that occur in intact muscles during fatigue. Thus, researchers have performed studies on intact animal muscles to explore many fatigue mechanisms. In order to confirm that human SR function and fatigue development parallel that of lower species, modifying analysis techniques for human samples is necessary. In our study, we analyzed Ca²⁺-uptake and Ca²⁺-release on approximately 25mg of muscle tissue. Significant decreases in Ca²⁺-uptake (56%) and Ca²⁺-release (49%) were found, similar to data reported for animal models. We lacked enough tissue to analyze Ca-ATPase. Developing a reliable method for Ca²⁺-uptake, release, and ATPase activity analysis on a small amount of tissue would facilitate further research in this area. There is currently no published data on Ca²⁺-release in humans.

8. Future investigations on the association between muscle glycogen metabolism and SR function should be conducted, despite the negative results found in our study and the Hargreaves et al. (47) study. Both of these studies were limited by analyzing only whole muscle glycogen, but not glycogen specifically associated with the SR. Changes in whole muscle glycogen content may not reflect SR glycogen levels if the rate of glycogen depletion in this region is selectively more rapid. This assessment would be possible by assaying glycogen directly from SR homogenates. Again, development of a method that allows this analysis on small muscle samples is needed.
9. Our results indicate a clear decline in SR function due to an intermittent high intensity exercise bout, but we did not determine a specific mechanism for this finding. Researchers have associated lactate (26, 91) and \( \text{H}^+ \) (1) accumulation with SR dysfunction in animal models. Decreased pH and increased lactate concentrations are common with high intensity exercise and may have been related to changes we found in \( \text{Ca}^{2+} \)-uptake and release. Additionally, intense exercise bouts have been shown to induce increased free radical activity; free radicals have been associated with SR damage and dysfunction in animals.

10. Studies have shown that a high CHO diet may be more protective against free radical damage than a low CHO diet in resting conditions, although these results have not been confirmed during exercise. It is possible that our H-CHO subjects experienced a smaller degree of free radical activity, and therefore were more protected against mechanical dysfunction than L-CHO subjects. We did not assess changes in muscle pH, lactate, or free radical activity that occurred during our exercise protocol, but it may be a beneficial addition to future studies.

11. Some researchers have suggested that \( \text{P}_i \) is directly associated with muscle fatigue and SR function (1). It has been theorized that a Ca-\( \text{P}_i \) precipitate may occur during fatiguing exercise and cause a decrease in the amount of \( \text{Ca}^{2+} \) available for release from the SR and consequently, diminished force production (32, 33). Others have described \( \text{P}_i \) as having a stimulatory effect on \( \text{Ca}^{2+} \)-release by stimulating ryanodine receptors (31). Research into the role of \( \text{P}_i \) with fatiguing exercise of varying intensity and duration is needed. Much of the available research has involved prolonged, submaximal exercise. Researchers have also not used a human model for investigation of \( \text{P}_i \) and the SR.
12. In our study, biopsies were taken at the same level of fatigue, regardless of total exercise time. Establishing a similar intermittent high intensity protocol that enabled muscle samples to be taken after the same number of intervals in both dietary conditions may give more insight into time-dependent changes in metabolites and SR function. One suggestion would be to have subjects perform the L-CHO trial first with biopsies taken at 85% and 70% of initial int rpm. In their second trial, the H-CHO trial, biopsies could be taken at the intervals that matched 85% and 70% of initial int rpm in the L-CHO trial, along with a subsequent sample being taken when int rpm declined to 70% initial int rpm during that specific (H-CHO) trial. Measurements could be compared after the same amount of exercise time and when muscles were at the same level of fatigue. Clearly, this design is limited by a lack of trial randomness, but it would allow more complete insight into metabolic and cellular changes that occur with fatigue. Due to the extremely different CHO content of diets used in our study, it was impossible to keep subjects blind to what CHO manipulation they were assigned. Thus, removing random trial assignment may have little effect on study reliability. If time was permitted, however, making use of two baseline trials (one with original workload and the second with an adjusted workload) to develop a better idea of performance in the diet-manipulated trials may be optimal.

13. We used subjects’ performance in VO_{2peak} and Wingate tests to determine their ability to handle the intensity of our performance test, based on previous work by Davis et al. (24). This method proved to be effective for subject selection, but not for designing performance trial workload. All subjects performed their baseline trial at 130% VO_{2peak}. Their
performance at this workload did not correlate with their performance on the VO_{2peak} and Wingate tests. In other words, subjects with higher VO_{2peak} or Wingate scores did not necessarily perform better in the baseline trial than subjects with lower VO_{2peak} and Wingate scores. We determined that subjects’ ventilatory threshold was most predictive of baseline trial performance. For future studies involving repeated one-minute supramaximal intervals, incorporating ventilatory threshold measurements into the workload design may be advisable.
APPENDIX A:

Detailed Description of Research Methods and Procedures
**Subject Selection and Screening**

Eight males between the ages of 18 and 30 were selected. Subjects were trained cyclists who had been cycling three or more times per week (two hours each time) for at least three years. Three subjects were members of the intercollegiate Virginia Tech cycling team and five subjects were members of the competitive East Coasters cycling team.

Subjects completed a health history questionnaire that screened for contraindications to strenuous exercise, muscle biopsy, and diet manipulation. Some conditions screened for include: diabetes, cardiovascular problems, orthopedic limitations and injuries, eating disorders, and organ malfunctions. Subjects were not attempting to alter their body weight in any way and had not used any supplements (including creatine monohydrate) other than multi-vitamins for at least 30 days. All subjects signed an informed consent form.

**Subject Pre-testing**

Subjects’ were weighed in their cycling clothing without shoes. Body fat percentage was evaluated based on 3-site skinfold measurements made in duplicate and estimated by the Siri equation.

Subjects completed an exercise pre-test which included a VO$_2$peak test on a Monark cycle ergometer and a 30-second Wingate test on a modified Monark cycle ergometer. For the VO$_2$peak test, subjects were allowed a three to five minute warm-up at a low resistance of their choice. They were then asked to maintain cycling at 90 rpm (in beat with a metronome) with a 2.0 kg load. The load was increased 0.5 kg every two minutes. The 0.5 kg increase was made after one minute if the resistance produced a heart rate increase of less than 8 bpm over the previous stage. Subjects continued cycling until volitional exhaustion or until researchers determined they were
not maintaining 90 rpm. All tests lasted between 8 and 11 minutes. Only subjects whose relative VO₂peak was greater than 52 ml·kg⁻¹·min⁻¹ were included in the study.

Thirty minutes after completion of the VO₂peak test, the Wingate test was administered. Subjects began cycling maximally against no resistance. Once researchers determined that subjects had reached maximal speed, a load of .75 grams/kg body weight was manually dropped. Subjects continued cycling maximally for 30 seconds. Researchers recorded the number of revolutions achieved during the six 5-second periods and determined watts (W) based on this data. Only subjects whose ratio of W at VO₂peak to average W during the Wingate was greater than 0.40 were included in the study. The ratio method, which has been previously used by Davis et al. (1997), was designed to evaluate subjects’ anaerobic and aerobic capacities and predict their ability to handle the interval protocol being used in the study. A sample calculation for pre-testing and other trial values is included in Figure 14.

**Familiarization Trial**

On the same day that the VO₂peak and Wingate pre-testing was performed, subjects completed a trial designed to introduce subjects to the experimental protocol. After they felt recovered from the pre-testing (usually about 20 minutes), subjects performed a 3-minute warm-up at 70% VO₂peak. Next, they performed three 60-second intervals at maximal effort with 3 minutes of active recovery (pedaling at a self-monitored pace with no resistance) between intervals. Subjects were specifically instructed not to pace themselves, but to exert maximal effort throughout the 60 seconds. They were allowed to stand up from the seat only during the first 3 seconds of the intervals.
Resistance used for the intervals was the load that would elicit 130% VO$_{2peak}$ if 90 rpm was maintained. This resistance was used since pilot subjects were able to perform approximately 15 intervals at 130% VO$_{2peak}$. Ninety rpm was selected since pilot subjects often reached a plateau of 85-95 rpm when giving maximal effort for repeated 60-second intervals with this load.

**Baseline Trial**

One week after the familiarization trial, subjects completed a baseline trial that mimicked the experimental protocol. Subjects fasted for 12 hours prior to reporting to the lab, but were encouraged to consume water and non-caloric/caffeine-free fluids. Subjects performed a 5-minute warm-up at 70% VO$_{2peak}$. During the final 10 seconds of minutes 3, 4, and 5 of the warm-up, subjects cycled at maximal effort. They were then allowed five minutes for stretching.

Subjects performed 60-second intervals at maximal effort against the same load that was used in the familiarization trial. Again, 3 minutes of active recovery was allowed between intervals. This protocol was based on one previously used by Davis et al. (24) since CHO status was shown to influence performance of it.

The number of revolutions achieved during each interval was recorded from a mechanical counter mounted to the back of the ergometer. As subjects cycled, the counter lever was turned by force of the left pedal. A study helper manually counted the number of revolutions for each interval to insure accuracy. The counter was reset to 0 during recovery periods. Subjects continued performing intervals until fatigue, the point when their interval rpm twice fell below 70% of their first interval rpm. In other words, if a subject performed 100 rpm on interval 1, they
were considered fatigued when they fell below 70 rpm twice. Subjects were verbally encouraged throughout intervals and rest periods. Water was allowed ad libitum during the trial.

Body weight was recorded before the trial. Weight maintenance was encouraged and only fluctuations of less than 1 kg were allowed.

**Performance Trials**

One to two weeks following the baseline trial, subjects completed two performance trials, separated by one week. One trial was in a low carbohydrate condition (L-CHO) and the other was in a high carbohydrate condition (H-CHO); trials were performed in random order. Subjects fasted during the 12 hours prior to performance trials.

Trials consisted of the same warm-up and cycling protocol described in the baseline trial section. However, resistance used for performance trials was determined by subjects’ performance in their baseline trial. If subjects performed less than or equal to 10 intervals, 125% VO$_{2peak}$ was used; if 15 or more intervals were performed, 135% VO$_{2peak}$ was used; and if subjects performed 11 to 14 intervals, resistance was not changed from 130% VO$_{2peak}$. Water was allowed ad libitum. Subjects were verbally encouraged throughout the intervals and rest periods. The researcher primarily responsible for verbal encouragement was blind to subjects’ treatment condition.

Two subjects were given additional recovery time during their first performance trial due to nausea and vomiting. These subjects were given an identical amount of recovery time at the same point during their second performance trial.

Body weight was recorded before the trials. Weight maintenance was encouraged and only fluctuations of less than 1 kg were allowed.
Glycogen Depletion Rides

Thirty-six hours prior to both performance trials, subjects completed a cycling bout designed to deplete leg muscles of glycogen. Subjects cycled at 80 to 90 rpm at 70% VO$_{2\text{peak}}$ for 80 minutes followed by four 60-second maximal effort sprints separated by 3 minutes of active recovery. Water was allowed ad libitum.

Following the ride, subjects consumed either a high carbohydrate (H-CHO) or low carbohydrate (L-CHO) snack that was approximately 10% of their normal daily caloric intake. The next day, subjects continued consuming either a H-CHO or L-CHO diet. All food was supplied by the researchers and is described in detail in the Diet Manipulation section below.

Sample Performance Calculation for Subject X

Subject X:

Body Weight: 70 kg

VO$_{2\text{peak}}$: 65 ml/kg·min (relative); 4.6 L/min (absolute)
*only subjects over 52 ml/kg·min were included in the study

Power at VO$_{2\text{peak}}$: 405 Watts
\[
\text{Watts} = \frac{\text{kg·m/min}}{6}
\]
\[
\text{kg·m/min} = \text{kg} \cdot \text{m/rev} \cdot \text{rev/min}
\]
\[
\text{kg} = \text{load on ergometer, m/rev} = 6 \text{ for Monarch ergometer, and rev/min} = \text{prescribed pace}
\]

\[
\text{kg·m/min} = 4.5\text{kg} \cdot 6\text{m/rev} \cdot 90\text{rev/min}
\]
\[
\text{kg·m/min} = 2430
\]
\[
\text{Watts} = 2430 \div 6
\]
\[
\text{Watts} = 405
\]

Average power during Wingate: 765.5 Watts
For each 5 second period:

\[ \text{Watts}_{\text{First 5 seconds}} = Load \ (kg) \cdot \text{revolutions} \cdot 11.765 \]

\[(\text{revolutions} = \text{number of pedal rotations in 5 second period)}\]

Load (kg) = 0.0875 \cdot \text{body weight (kg)}

Load (kg) = 0.0875 \cdot 70 \text{ kg}

Load (kg) = 6.1 \text{ kg (13.5 lbs.)}

\[ \text{Watts}_{\text{First 5 seconds}} = 6.1 \text{ kg} \cdot 14 \text{ revolutions} \cdot 11.765 \]

\[ \text{Watts}_{\text{First 5 seconds}} = 1004.7 \]

Watts for other 5-second segments = 861.2, 717.7, 717.7, 645.9, 645.9

Average of six 5-second segments = 765.5 Watts

Ratio of power at VO2peak to average power during Wingate:

\[ \text{Ratio} = \frac{405 \text{ Watts}}{765.5 \text{ Watts}} \]

\[ \text{Ratio} = .53 \]

*only subjects with a ratio >.40 were included in the study*

Determination of Familiarization and Baseline Interval Load:

130% VO2peak (based on protocol used by Davis et al., 1997)

\[ \text{VO2peak} = 4.6 \text{ L/min, 405 Watts} \]

Based on linear regression (using Watts and VO2 at peak and three other time points during the VO2peak test, 130% VO2peak = 5.9 kg (13.0 lbs.))

*90 rpm was selected since pilot subjects reached a plateau ~90 rpm*

Determination of Performance Interval Load:

<table>
<thead>
<tr>
<th>Baseline performance</th>
<th>Performance interval load</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 10 )</td>
<td>125% VO2peak</td>
</tr>
<tr>
<td>11-14</td>
<td>130% VO2peak</td>
</tr>
<tr>
<td>( \geq 15 )</td>
<td>135% VO2peak</td>
</tr>
</tbody>
</table>

*90 rpm was selected since pilot subjects reached a plateau ~90 rpm*

**Figure 14:** Sample Performance Calculation
Diet Manipulation

Subjects kept detailed dietary records for the 24 hours before their baseline trial. Diets were analyzed with Nutritionist V computer software for total kcals, % CHO, % fat, and % protein. The number of kcals consumed during the analyzed day was replicated for the 36-hour period before each performance trial (mean kcals = approximately 3500). During this period, subjects consumed either a H-CHO diet (80-85% kcals from CHO) or a L-CHO diet (5-10% kcals from CHO). Diets were assigned in random order. Food was distributed to subjects following glycogen depletion rides.

Foods consumed on the H-CHO diet included pasta, tomato sauce, bananas, apple juice, bread, cereal, milk, and Gatorlode. The L-CHO diet consisted of eggs, cheese, mayonnaise, tuna, turkey, margarine, peanut butter, and rice cakes. Both groups received non-caloric drink mix. A sample H-CHO and L-CHO is shown in Figure 15. Only vegetarian subjects or those with food allergies had specific foods omitted. Foods that required preparation (i.e.: eggs and pasta) were prepared ahead of time for subjects who lived in dormitories and had no access to cooking facilities. Subjects were instructed to consume all of the food given to them and to return any uneaten portions. They were permitted to consume spices and non-caloric and caffeine-free beverages, but no other foods aside from what was given to them. Subjects were encouraged to consume ample fluids.
<table>
<thead>
<tr>
<th></th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>H-CHO (80-85% Carbohydrate)</strong></td>
<td><strong>L-CHO (5-10% Carbohydrate)</strong></td>
</tr>
<tr>
<td>4 cups Honey Nut Cheerios</td>
<td>4 eggs</td>
<td></td>
</tr>
<tr>
<td>1 ½ cups skim milk</td>
<td>24 ounces tuna</td>
<td></td>
</tr>
<tr>
<td>5 slices whole wheat bread</td>
<td>5 ounces turkey</td>
<td></td>
</tr>
<tr>
<td>¼ cup jelly</td>
<td>3 ounces cheese</td>
<td></td>
</tr>
<tr>
<td>1 pound pasta</td>
<td>½ cup margarine</td>
<td></td>
</tr>
<tr>
<td>2 ½ cups tomato sauce</td>
<td>½ cup peanut butter</td>
<td></td>
</tr>
<tr>
<td>24 ounces Gatorlode</td>
<td>3 Tablespoons mayonnaise</td>
<td></td>
</tr>
<tr>
<td>3 bananas</td>
<td>1 rice cake</td>
<td></td>
</tr>
<tr>
<td>36 ounces apple juice</td>
<td>1 slice whole wheat bread</td>
<td></td>
</tr>
<tr>
<td>32 ounces crystal lite</td>
<td>4 Hershey’s kisses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 ounces crystal lite</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 15:** Sample Subject Diets for a Subject with a 3400 kcal Baseline Diet
<table>
<thead>
<tr>
<th>DAY</th>
<th>DAY 1</th>
<th>Pre-test (VO₂peak, Wingate); Familiarization trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Baseline Trial</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Glycogen depletion ride - 1</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Diet manipulation - A</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Performance trial - 1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Glycogen depletion ride - 2</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Diet manipulation - B</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Performance trial - 2</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 16: Sample Subject Schedule**

**Muscle Biopsies**

Three muscle biopsies were taken during each performance trial. Subjects’ left leg was used for the first performance trial and right leg for the second performance trial. Prior to beginning the cycling warm-up, subjects were placed in a supine position on a table. Following cleansing, lidocaine was injected into subject’s vastus lateralis muscle. After waiting for 3 to 5 minutes, two approximately ¼ inch incisions were made with a scalpel approximately ½ inch apart vertically. A 60-100 mg muscle sample was removed from the lower incision (closer to the knee) using the percutaneous needle biopsy technique with suction. A 6-mm biopsy needle was used. Incisions were covered with a piece of gauze and medical tape, and subjects began performing the warm-up and performance trial as soon as they felt ready (usually about 5 minutes).
The second muscle biopsy was taken after subjects’ interval performance (rpm) had declined to 85% of their first interval rpm and the third biopsy was taken at fatigue, exercise completion, when performance had declined to below 70% of first interval rpm twice. Subjects were unaware of their performance throughout the trials and therefore did not know when biopsies were going to be taken. Due to having to predict subjects’ performance on upcoming intervals (since the biopsy technician needed to have ample time to prepare for biopsy, but not so much time that sterile needles were exposed to air for too long), there were a few cases when biopsies were not taken at exactly 85% and 70% first interval rpm.

For the second and third biopsies, subjects were asked to immediately stop cycling, lean back into the arms of a catcher, and remove their legs from pedal clips. Gauze and tape were rapidly removed and a sample was taken as quickly as possible. If little or no muscle was obtained from a biopsy pass, one repeat pass was made. The first and second biopsies were taken from the lower incision and the third biopsy was taken from the higher incision (closer to the hip). Effort was made to perform this procedure as quickly as possible.

Muscle samples were immediately removed from the biopsy needle, placed into a cool petri dish, and cut into two sections with a scalpel. One portion was placed in a cryovile and frozen in liquid nitrogen for later analysis of glycogen, CP, Cr, and ATP. This required about 5 seconds or less. Total time from the time subjects stopped cycling to the point when samples were placed in liquid nitrogen averaged less than 10 seconds. Others have required up to 20 seconds for this process (12, 29, 47).

The remaining portion was used for SR Ca^{2+}-uptake and release measurements. Samples were weighed and put in an ice-cold homogenization buffer containing 250mM sucrose, 20mM HEPES, 2% NaN, and .2mM PMSF (pH = 7.0). The volume of buffer used was equal to the
sample weight in mg to a factor of 10 µl. Samples were repeatedly minced with scissors while kept on ice and homogenized with a Pro 200 homogenizer and 5-mm probe (three 12-second bursts at 3,000 rpm). Following centrifugation at 1600g for 15 minutes (2°C), the supernatant (homogenate fraction) was removed. The homogenate was divided into two microcentrifuge tubes and stored in a -80°C freezer; one tube contained 10 µl of homogenate (to be used for analysis of protein content) and the other contained all remaining homogenate (to be used for Ca^{2+}-uptake and release analyses).

**Preparation for Muscle Metabolite Analyses**

*Freeze Drying*

Samples were removed from the -80°C freezer and immediately placed in individual 12 X 75 glass test tubes covered with cheese cloth and held closed with rubber bands. Test tubes were placed in a Styrofoam cup containing liquid nitrogen (5 test tubes per cup). Styrofoam cups were placed in the freeze dryer, which had been cooled to below -30°C. A vacuum of 10-100 microns was applied to samples for at least 12 hours.

When removed from the freeze dryer, samples were stored in a sealed container with desiccant. The container was left at room temperature while tissue was powdered. Following powdering, it was stored in the -80°C freezer. Sample dry weight was equal to approximately 4 to 5 times less than wet weight.
**Powdering Tissue**

Samples were dissected free of fat, blood, and connective tissue with forceps and a scalpel blade. Some samples contained as much as 25% connective tissue, which is lighter in color and tougher than muscle tissue. Blood is a much darker color than muscle tissue and fat is white and flaky. Few samples contained significant amounts of blood and fat.

Samples were manipulated to a fine powder suitable for metabolite analysis by grinding with a scalpel in a petri dish. On occasion, powdering was difficult due to static causing scattering of tissue out of the petri dish. When this occurred, static spray and sheets were applied to lab benches and gloves. Powder was weighed, placed in a microcentrifuge tube, and either used for analyses immediately or stored in a desiccated container in the -80°C freezer.

**Muscle Glycogen Analysis**

*(Method of Spriet et al., 1989 – reference 92)*

**Extraction**

Freeze dried and powdered samples (8 samples at a time) were removed from the -80°C freezer. Approximately 1.5 mg of tissue was placed in a plastic microcentrifuge tube. Remaining tissue was returned to the freezer and saved for CP, Cr, and ATP analyses. Samples were allowed to warm to room temperature in a desiccated container, which required approximately 30 minutes.

Microcentrifuge tubes were removed from the desiccated container and 100 µl of 0.1M NaOH, labeled G5, was added to samples and vortexed. Samples were incubated in an 80°C water bath for 10 minutes. Background glucose and hexose monophosphates were destroyed at this time. After cooling, 0.1M HCl (G6) and 0.2M citric acid mixed with 0.2M Na₂HPO₄ (G7,
pH = 5.0) was added to samples. The amount of G5, G6, and G7 added to samples is based on values shown in Figure 4. The exact amount of G6 and G7 used was determined each day the assay was performed. If equal volumes of G5 and G6 did not neutralize each other (pH = 7), the exact volume required for neutralization was determined. The volume of G5 shown in Figure 17 was always used, but the amount of G6 used was adjusted based on the neutralization ratio. Then, the volume of G7 needed was adjusted. G6 and G7 were always added in a 1:3 ratio.

After adding amyloglucosidase (AGS) in the volume shown in Figure 4, samples were inverted and incubated at room temperature for 60 minutes. During this time, glycogen breakdown occurred.

<table>
<thead>
<tr>
<th>Dry Muscle (mg)</th>
<th>G5 (µl)</th>
<th>G6,G7 (µl)</th>
<th>AGS (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2.0</td>
<td>100</td>
<td>1:3 ratio (~400)</td>
<td>15</td>
</tr>
<tr>
<td>2.01-3.0</td>
<td>120</td>
<td>1:3 ratio (~480)</td>
<td>15</td>
</tr>
<tr>
<td>3.01-4.0</td>
<td>160</td>
<td>1:3 ratio (~640)</td>
<td>20</td>
</tr>
<tr>
<td>4.01-5.0</td>
<td>200</td>
<td>1:3 ratio (~800)</td>
<td>25</td>
</tr>
</tbody>
</table>

**Figure 17:** Volumes of Reagents used in Glycogen Extraction Procedure

*Spectrophotometric Analysis*

While the sample was incubating for 60 minutes, the glycogen reagent was prepared and stored on ice. Some solutions required for the reagent could remain stable in the refrigerator or freezer and were therefore prepared in larger volumes ahead of time and stored. These included: 0.375M Triethanolamine mixed with 0.15M KOH, 0.113M Magnesium Acetate, and 0.004M EDTA (G1, pH = 8.2, using KOH to make more basic), 0.05M ATP(H1), and 0.06M DTT (H4). On the day of the procedure, 0.03M NAD (F8) was made fresh. For the reagent, 1,600 µl of G1,
100 µl of H1, 100 µl of H4, 200 µl of F8, and 3,000 µl of distilled water were mixed in a 10 ml test tube.

Enzymes used were Glucose-6-Phosphate Dehydrogenase (G6PDH) and hexokinase (HK). One thousand units of G6PDH and 1000 units of HK were diluted with 1000 µl of distilled water each. Equal volumes of enzyme solution were mixed. Enzyme aliquots were stored in microcentrifuge tubes in the -80° C freezer. Enzymes were stored on ice.

Sample (50 µl) was added to 330 µl cuvettes along with 250 µl of glycogen reagent. The mixture was stirred with a plastic stirring rod and after 1 minute, baseline absorbance was read at 340 nm. Following addition of 2 µl of the G6PDH/HK enzyme mixture, absorbance was recorded every minute for 8 minutes. The reaction was considered complete when absorbance changes were less than .003 per minute. Further changes were attributed to drifting.

The assay was based on the following reactions:

\[
\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-Phosphate} + \text{ADP}
\]

\[
\text{Glucose-6-Phosphate} + \text{NAD} \xrightarrow{\text{G6PDH}} \text{Phosphogluconolactone} + \text{NADH}
\]

Figure 18: Reactions involved in Glycogen Assay
Calculation

Using the change in absorbance readings for NADH, a calculation was applied to quantify glycogen concentrations (Figure 19). A dilution factor (DF) was required for the calculation: DF

\[
DF = \frac{\text{mg dry tissue} + \mu l \text{ G5} + \mu l \text{ G6} + \mu l \text{ G7} + \mu l \text{ AGS}}{\text{mg dry tissue}}.
\]

\[
\text{mmol/kgdw} = \left[ \frac{\Delta A \times TV}{3.4 \times SV} \right] \times DF
\]

\( \Delta A \) = Change in absorbance recorded from spectrophotometer
\( TV \) = Total volume in cuvette (sample + reagent) in \( \mu l \)
\( SV \) = Sample volume in cuvette in \( \mu l \)
\( DF \) = Dilution factor, defined above

Figure 19: Calculation of Glycogen Concentration

Muscle Creatine Phosphate, Free Creatine, and ATP Analysis
(method of Harris et al., 1974 – reference 48)

Extraction

Freeze dried and powdered samples (6 samples at a time) were removed from the -80°C freezer. Approximately 1.5 mg of tissue (or the amount that remained following glycogen analysis) was placed in a 12 X 75 glass tube and was allowed to warm to room temperature in a desiccated container, which required 30 minutes. To degrade CP, perchloric acid (PCA) was added to each sample in the amount of 160 \( \mu l \). If greater than 3.0 to 4.0 mg of tissue is used, the recommended volume of PCA is mg of tissue powder X 40. However, since less than 2.0 mg of tissue was used for all samples in this analysis, multiplying by 40 did not provide enough supernatant for duplicate analysis. Samples were allowed to react with PCA for 5 minutes while
kept on ice. Test tubes were gently mixed throughout the 5 minutes, with careful attention paid
to prevent tissue from adhering to test tube sides. Samples were then centrifuged at 5,000 rpm
for 5 minutes at 4° C (centrifuged was pre-cooled).

The supernatant was removed from each sample using a Pasteur pipette, transferred to a
tared 12 X 75 test tube, and weighed. The weight of the sample in mg was divided by 1.025
(density) to obtain extract volume in µl. The extract volume was divided by 4 to determine the
volume of KHCO₃ in µl required to neutralize the solution. Prior to beginning the extraction
procedure, 4 ml of PCA were added to 1 ml of KHCO₃ to determine if the two neutralize each
other. If the pH of the solution was not between 6.5 and 7.0, fresh KHCO₃ was prepared.
Refrigerated KHCO₃ solutions can become acidic with time.

The appropriate volume of KHCO₃ was added to the supernatant. The mixture was gently
vortexed until bubbling terminated and then centrifuged at 5,000 rpm for 15 minutes at 4° C.
The supernatant was removed with a Pasteur pipette, transferred to a 12 X 75 test tube, and
stored in ice (for up to 4 hours) for spectrophotometric analysis. The extraction and
spectrophotometric procedure were always completed on the same day, but practice data showed
that samples could be stored in a -80° C freezer for up to 2 weeks and still produce reliable data.

It was necessary to use glass test tubes for all steps of the extraction since some
components react with plastic.

Spectrophotometric Analysis

Two different assays were performed on the same extract. One assay measured CP and
ATP and the second measured Cr. Each assay required a different reagent; these reagents were
made fresh on the day of the procedure. However, some solutions required for the reagents could
remain stable in the refrigerator or freezer and were therefore prepared in larger volumes ahead of time and stored. These included: 0.05M DTT (F1), 0.01M ADP (F3), 0.125M Glucose (F4), and 1M Triethanolamine mixed with 0.1M Magnesium Acetate and 0.01M EDTA (D1, pH = 7.5) for the CP/ATP assay and 2M KCl (D3) and 0.025M PEP-tri-cyclohexyl-ammonium salt (F6) for the Cr assay. Other solutions were made fresh on the day of the procedure: 0.025M NADP (F2) for the CP/ATP assay and 0.012M NADH (F7) and 0.32M Glycine mixed with 0.016M Magnesium Acetate (D4, pH = 9.0 to 9.1) for the Cr assay.

For the CP/ATP reagent, 500 µl of D1, 100 µl of F1, 200 µl of F2, 20 µl of F3, 200 µl of F4, 3,980 µl of distilled water, and 17 µl of Glucose-6-Phosphate Dehydrogenase were mixed in a 10 ml test tube. For the Cr reagent, 2,000 µl of D4, 100 µl of D3, 400 µl of F5, 300 µl of F6, 90 µl of F7, 3,100 µl of distilled water, 6 µl of Lactate Dehydrogenase, and 4.9 µl of Pyruvate Kinase were mixed in a 10 ml test tube. Reagents were kept on ice.

After mixing the reagents, enzymes were prepared. For the Creatine Phosphokinase (CPK) enzyme solution, lyophilized CPK was mixed with D5 in the amount of 17 mg/ml. D5 consisted of 0.5% NaHCO3 and 0.05% Bovine Serum Albumin which was prepared ahead of time and stored at 4°C. Hexokinase (HK) was prepared by diluting liquid HK with distilled water in a 1:1 ratio. Enzymes were kept on ice.

The CP/ATP assay was performed in duplicate on all samples and then the Cr assay was performed in duplicate on all samples. For the CP/ATP assay, 25 µl of sample was added to a 330 µl cuvette along with 225 µl of CP/ATP reagent. Following stirring with a plastic stirring rod and waiting ~1 minute, baseline absorbance was read at 340 nm. In order to quantify the concentration of ATP in the sample, HK (3.6 µl) was added to the cuvette and stirred. Absorbance was recorded every minute until no further absorbance changes occurred (~5
minutes). To quantify the concentration of CP in the sample, 2.25 µl CPK/D5 was then added to the cuvette and stirred. Changes in absorbance were recorded each minute for the next 8 to 15 minutes (varied by sample size and CP concentration). The reaction was considered complete when changes in absorbance were less than .006 per minute. Further changes were attributed to drifting.

For the Cr assay, 20 µl of sample was added to a 330 µl cuvette along with 300 µl Cr reagent. Following stirring with a plastic stirring rod and waiting 1 minute, baseline absorbance was read at 340 nm. In order to quantify the concentration of Cr in the sample, 11.3 µl CPK/D5 was added to the cuvette and stirred. Changes in absorbance were monitored each minute for the next 6 to 10 minutes (varied by sample size and Cr concentration). The reaction was considered complete when absorbance changes were less than .006 per minute. Further changes were attributed to drifting.

Assays were based on the following reactions:

\[
\text{CP + ADP} \xrightarrow{\text{CPK}} \text{Cr + ATP}
\]

\[
\text{ATP + Glucose} \xrightarrow{\text{HK}} \text{ADP + Glucose-6-Phosphate}
\]

\[
\text{Glucose-6-Phosphate + NADP} \xrightarrow{\text{G6PDH}} \text{Gluconolactone + NADPH}
\]

**Figure 20:** Reactions involved in CP/ATP Assay
ADP + PEP $\xrightarrow{PK}$ ATP + Pyruvate

Pyruvate + NADH $\xrightarrow{LDH}$ Lactate + NAD

**Figure 21:** Reactions involved in Cr Assay

**Calculation**

Using the change in absorbance readings for NADPH, NADH, and ADP, a calculation was applied to quantify CP, Cr, and ATP concentrations (Figure 21). The same calculation was used for CP, Cr, and ATP. A dilution factor (DF) was required for the calculation: DF = [(mg dry tissue + µl PCA added)/mg dry tissue] * 1.25. The constant of 1.25 was calculated by (µl PCA + µl KHCO₃)/µl PCA.

\[
\text{mmol/kgdw} = \left(\frac{\Delta A \times TV}{E \times SV}\right) \times DF
\]

\(\Delta A = \) Change in absorbance recorded from spectrophotometer

TV = Total volume in cuvette (sample + reagent) in µl

E = Extinction coefficient or molar absorption coefficient (always 6.22 cm² for this reaction at 340 nm)

SV = Sample volume in cuvette in µl

DF = Dilution factor, defined above

**Figure 22:** Calculation of CP, Cr, and ATP Concentrations

*Adjusting Phosphagen Values*
Since TC values should not be different in biopsies taken during the same bout of exercise, all phosphagen data were adjusted to coincide with the peak TC value obtained within each exercise bout. This procedure has been used by others (28).

As an example, if biopsy 1 TC was 120 μmol/g-dw, biopsy 2 TC was 115 μmol/gdw, and biopsy 3 TC was 125 μmol/gdw, CP, Cr, and ATP values for biopsy 1 and 2 were adjusted proportionally so that TC was equal to biopsy 3. The mean correction factor was 6.5%.

It may have also been acceptable to adjust phosphagen values from both exercise bouts (all six biopsies) since TC levels are unlikely to change during one week’s time without supplementation. However, this made a negligible difference in obtained values, so adjustments were limited to individual bouts.

Sarcoplasmic Reticulum Calcium Uptake and Release Analysis
(Method of Ward et al., 1998 – reference 100)

Protein Analysis
The 25 μl aliquot of homogenate reserved for protein analysis was removed from the freezer and allowed to thaw. Five ml of Bio-Rad reagent was added to 1.5 ml cuvettes. To one group of cuvettes, 0, 10, 20, 30, 40, and 50 μl of BSA was added and mixed. The selection of BSA was made since it has a known protein concentration. These mixtures were read at a wavelength of 595 nm to establish a standard curve. To remaining cuvettes, 5 μl of homogenate was added. Protein concentration of homogenates was based on the BSA standard curve.
Determination of the Rate of Calcium Uptake and Release

Approximately 75-125 µg of homogenate protein was added to a buffer containing warmed 100mM KCl, 20mM HEPES, 7.5mM Pyrophosphate, and 1mM MgCl₂ (pH = 7.0). This buffer was stored at 4°C for up to 7 days. After inverting to mix, 10 µl of 200µM fura, a fluorescent Ca²⁺ indicator, and 2 µl of 20µM CaCl₂ were added to the cuvette, which was inverted again.

The cuvette was placed in a Jasco CAF-110 Intracellular Ion Analyzer with excitation light filtered at 340 and 380 nm and emission light detected at 500 nm. In this analyzer, changes in free Ca²⁺ were quantified via detection of Fura. The Jasco was connected to a computer that recorded Ca²⁺ concentration measurements in 6-minute increments. Approximately 20 seconds after placing the cuvette in the Jasco and initializing the 6-minute interval in the computer, 10µl of 1mM ATP was added to the cuvette to initiate Ca²⁺-uptake. Three minutes later, 10 µl of 2,500µM AgNO₃ was added to the cuvette to initiate Ca²⁺-release. The cuvettes contained a miniature stir-bar and solutions were continually mixed. Changes in Ca²⁺ recorded by the computer looked similar to what is shown in Figure 23.

![Graph](image)

Figure 23: Example of Ca²⁺-uptake and release measurements (Reference: Ward et al., 1998)
The downward slope that begins before minute 1 represents Ca\(^{2+}\)-uptake initiated by adding ATP. The upward slope that begins after minute 3 represents Ca\(^{2+}\)-release initiated by adding silver.

A computer program written by Dr. Jay Williams was used to convert changes in free Ca\(^{2+}\) to rates in nmol/min·mgprotein.
APPENDIX B:

Raw Data Tables
**Table 1.** Individual Subject Characteristics

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Body Fat (%)</th>
<th>Relative VO$_{2\text{peak}}$ (ml/kg·min)</th>
<th>Absolute VO$_{2\text{peak}}$ (L/min)</th>
<th>Max Wingate Power (Watts)</th>
<th>Average Wingate Power (Watts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>71.0</td>
<td>7.8</td>
<td>60.1</td>
<td>4.3</td>
<td>1094</td>
<td>851</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>75.0</td>
<td>7.5</td>
<td>64.3</td>
<td>4.8</td>
<td>932</td>
<td>777</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>78.5</td>
<td>7.8</td>
<td>53.3</td>
<td>4.2</td>
<td>974</td>
<td>866</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>70.5</td>
<td>12.1</td>
<td>51.7</td>
<td>3.6</td>
<td>1037</td>
<td>864</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>68.5</td>
<td>5.5</td>
<td>61.5</td>
<td>4.2</td>
<td>988</td>
<td>753</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>70.5</td>
<td>5.3</td>
<td>53.9</td>
<td>3.8</td>
<td>1021</td>
<td>826</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>72.5</td>
<td>7.2</td>
<td>61.3</td>
<td>4.4</td>
<td>1112</td>
<td>828</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>81.5</td>
<td>14.9</td>
<td>57.8</td>
<td>4.7</td>
<td>1253</td>
<td>913</td>
</tr>
<tr>
<td>Mean</td>
<td>23.9</td>
<td>73.5</td>
<td>8.5</td>
<td>58.0</td>
<td>4.3</td>
<td>1051.4</td>
<td>834.8</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.60</td>
<td>1.59</td>
<td>1.17</td>
<td>1.61</td>
<td>.14</td>
<td>35.7</td>
<td>18.1</td>
</tr>
</tbody>
</table>
Table 2. Composition of Subjects’ Normal Diets Analyzed from a 24-hour Dietary Record

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Total kcals</th>
<th>% CHO</th>
<th>% fat</th>
<th>% protein</th>
<th>protein (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4129</td>
<td>49</td>
<td>42</td>
<td>13</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>3171</td>
<td>59</td>
<td>25</td>
<td>16</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>3488</td>
<td>58</td>
<td>25</td>
<td>20</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>3277</td>
<td>67</td>
<td>22</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>6</td>
<td>3472</td>
<td>68</td>
<td>16</td>
<td>16</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>4100</td>
<td>69</td>
<td>21</td>
<td>12</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>3016</td>
<td>67</td>
<td>24</td>
<td>14</td>
<td>1.4</td>
</tr>
<tr>
<td>9</td>
<td>2805</td>
<td>62</td>
<td>27</td>
<td>15</td>
<td>1.3</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>3432.3</strong></td>
<td><strong>62.4</strong></td>
<td><strong>25.3</strong></td>
<td><strong>14.8</strong></td>
<td><strong>1.7</strong></td>
</tr>
<tr>
<td>± SEM</td>
<td><strong>168.8</strong></td>
<td><strong>2.4</strong></td>
<td><strong>2.7</strong></td>
<td><strong>.94</strong></td>
<td><strong>.12</strong></td>
</tr>
</tbody>
</table>
Table 3. Body Weight Data

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Baseline Body Weight (kg)</th>
<th>H-CHO Trial Body Weight (kg)</th>
<th>L-CHO Trial Body Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71.0</td>
<td>71.0</td>
<td>70.5</td>
</tr>
<tr>
<td>3</td>
<td>75.0</td>
<td>75.5</td>
<td>74.0</td>
</tr>
<tr>
<td>4</td>
<td>78.5</td>
<td>78.5</td>
<td>77.0</td>
</tr>
<tr>
<td>5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
</tr>
<tr>
<td>6</td>
<td>68.5</td>
<td>70.0</td>
<td>67.5</td>
</tr>
<tr>
<td>7</td>
<td>70.5</td>
<td>69.0</td>
<td>71.5</td>
</tr>
<tr>
<td>8</td>
<td>72.5</td>
<td>70.5</td>
<td>70.5</td>
</tr>
<tr>
<td>9</td>
<td>81.5</td>
<td>82.5</td>
<td>81.5</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>73.5</strong></td>
<td><strong>73.5</strong></td>
<td><strong>72.9</strong></td>
</tr>
<tr>
<td><strong>±SEM</strong></td>
<td><strong>1.59</strong></td>
<td><strong>1.72</strong></td>
<td><strong>1.58</strong></td>
</tr>
</tbody>
</table>
Table 4. Individual Subject Overall Performance Data

<table>
<thead>
<tr>
<th>Subject #</th>
<th>HG # of intervals</th>
<th>HG total work (kJ)</th>
<th>HG average work per interval (kJ)</th>
<th>LG # of intervals</th>
<th>LG total work (kJ)</th>
<th>LG average work per interval (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>673.5</td>
<td>84.2</td>
<td>10*</td>
<td>904.3</td>
<td>90.4</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>1698.8</td>
<td>106.2</td>
<td>14*</td>
<td>1361.8</td>
<td>97.3</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>2032.8</td>
<td>101.6</td>
<td>12*</td>
<td>1250.9</td>
<td>104.2</td>
</tr>
<tr>
<td>5</td>
<td>9*</td>
<td>707.2</td>
<td>78.6</td>
<td>8</td>
<td>752.2</td>
<td>94.0</td>
</tr>
<tr>
<td>6</td>
<td>18*</td>
<td>1406.0</td>
<td>78.1</td>
<td>12</td>
<td>956.6</td>
<td>79.7</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>809.9</td>
<td>81.0</td>
<td>8*</td>
<td>710.1</td>
<td>78.9</td>
</tr>
<tr>
<td>8</td>
<td>7*</td>
<td>609.5</td>
<td>87.1</td>
<td>7</td>
<td>535.7</td>
<td>76.5</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>2559.6</td>
<td>94.8</td>
<td>12*</td>
<td>1190.9</td>
<td>99.2</td>
</tr>
<tr>
<td>Mean</td>
<td>14.25</td>
<td>1312.2</td>
<td>88.9</td>
<td>10.38</td>
<td>957.8</td>
<td>90.0</td>
</tr>
<tr>
<td>± SEM</td>
<td>2.53</td>
<td>279.8</td>
<td>3.8</td>
<td>.885</td>
<td>118.0</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*= performance trial completed first
Table 5. Individual Subject Performance Data – in Biopsy Segments

<table>
<thead>
<tr>
<th>Subject #</th>
<th>HG # of intervals to 85% first int rpm</th>
<th>LG # of intervals to 85% first int rpm</th>
<th>HG work to 85% first int rpm (kJ)</th>
<th>LG work to 85% first int rpm (kJ)</th>
<th>HG # of intervals between 85 &amp; 70% first int rpm</th>
<th>LG # of intervals between 85 &amp; 70% first int rpm</th>
<th>HG work between 85 &amp; 70% first int rpm (kJ)</th>
<th>LG work between 85 &amp; 70% first int rpm (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6</td>
<td>522.3</td>
<td>577.3</td>
<td>2</td>
<td>4</td>
<td>151.2</td>
<td>326.9</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7</td>
<td>895.8</td>
<td>721.6</td>
<td>8</td>
<td>7</td>
<td>803.0</td>
<td>640.2</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>10</td>
<td>947.5</td>
<td>1075.7</td>
<td>11</td>
<td>2</td>
<td>1085.3</td>
<td>175.2</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
<td>257.8</td>
<td>322.9</td>
<td>6</td>
<td>5</td>
<td>449.4</td>
<td>429.6</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>6</td>
<td>421.4</td>
<td>506.3</td>
<td>12</td>
<td>6</td>
<td>984.6</td>
<td>450.3</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4</td>
<td>433.8</td>
<td>349.7</td>
<td>5</td>
<td>5</td>
<td>296.9</td>
<td>287.0</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>5</td>
<td>453.3</td>
<td>397.7</td>
<td>2</td>
<td>2</td>
<td>156.2</td>
<td>138.0</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>6</td>
<td>458.5</td>
<td>620.5</td>
<td>22</td>
<td>6</td>
<td>2101.1</td>
<td>665.7</td>
</tr>
<tr>
<td>Mean</td>
<td>5.87</td>
<td>5.87</td>
<td>548.8</td>
<td>571.5</td>
<td>8.50</td>
<td>4.62</td>
<td>753.5</td>
<td>389.1</td>
</tr>
<tr>
<td>± SEM</td>
<td>.667</td>
<td>.743</td>
<td>85.7</td>
<td>87.1</td>
<td>2.32</td>
<td>.653</td>
<td>231.6</td>
<td>69.2</td>
</tr>
</tbody>
</table>

*= performance trial completed first
Figure 24: Intervals Performed by Subject 1
Figure 25: Intervals Performed by Subject 3
Figure 26: Intervals Performed by Subject 4
Subject 5

Figure 27. Intervals Performed by Subject 5
Figure 28. Intervals Performed by Subject 6
Figure 29. Intervals Performed by Subject 7
Figure 30. Intervals Performed by Subject 8
Figure 31. Intervals Performed by Subject 9
Table 6. Individual Subject Muscle Glycogen Data

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>683</td>
<td>371</td>
<td>349</td>
<td>323</td>
<td>275</td>
<td>286</td>
</tr>
<tr>
<td>3</td>
<td>788</td>
<td>220</td>
<td>451</td>
<td>356</td>
<td>111</td>
<td>181</td>
</tr>
<tr>
<td>4</td>
<td>496</td>
<td>337</td>
<td>269</td>
<td>233</td>
<td>198</td>
<td>151</td>
</tr>
<tr>
<td>5</td>
<td>654</td>
<td>409</td>
<td>378</td>
<td>545</td>
<td>353</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>640</td>
<td>384</td>
<td>193</td>
<td>278</td>
<td>242</td>
<td>145</td>
</tr>
<tr>
<td>7</td>
<td>624</td>
<td>493</td>
<td>396</td>
<td>557</td>
<td>317</td>
<td>188</td>
</tr>
<tr>
<td>8</td>
<td>490</td>
<td>412</td>
<td>334</td>
<td>382</td>
<td>208</td>
<td>224</td>
</tr>
<tr>
<td>9</td>
<td>495</td>
<td>441</td>
<td>396</td>
<td>449</td>
<td>307</td>
<td>197</td>
</tr>
<tr>
<td>Mean</td>
<td>609</td>
<td>383</td>
<td>346</td>
<td>290</td>
<td>252</td>
<td>196</td>
</tr>
<tr>
<td>± SEM</td>
<td>38</td>
<td>29</td>
<td>29</td>
<td>42</td>
<td>28</td>
<td>18</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cell resulted from insufficient sample size.
Table 7. Individual Subject Muscle TC Data – NOT ADJUSTED

Total Creatine

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126</td>
<td>120</td>
<td>122</td>
<td>131</td>
<td>138</td>
<td>112</td>
</tr>
<tr>
<td>3</td>
<td>129</td>
<td>131</td>
<td>126</td>
<td>128</td>
<td>132</td>
<td>141</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>128</td>
<td>131</td>
<td>130</td>
<td>139</td>
<td>126</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>142</td>
<td>123</td>
<td>144</td>
<td>144</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>124</td>
<td>118</td>
<td>99</td>
<td>111</td>
<td>136</td>
<td>117</td>
</tr>
<tr>
<td>7</td>
<td>123</td>
<td>132</td>
<td>117</td>
<td>141</td>
<td>138</td>
<td>126</td>
</tr>
<tr>
<td>8</td>
<td>102</td>
<td>no data</td>
<td>139</td>
<td>137</td>
<td>136</td>
<td>146</td>
</tr>
<tr>
<td>9</td>
<td>129</td>
<td>116</td>
<td>122</td>
<td>127</td>
<td>140</td>
<td>131</td>
</tr>
</tbody>
</table>

Mean: 126.0 126.7 122.4 131.1 137.9 128.4
± SEM: 4.6 3.5 4.1 3.6 1.23 4.6

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 8. Individual Subject Muscle CP Data – NOT ADJUSTED

Creatine Phosphate

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>59</td>
<td>49</td>
<td>65</td>
<td>59</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>74</td>
<td>42</td>
<td>82</td>
<td>71</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>50</td>
<td>45</td>
<td>72</td>
<td>62</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>55</td>
<td>63</td>
<td>98</td>
<td>63</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>45</td>
<td>63</td>
<td>98</td>
<td>63</td>
<td>no data</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>62</td>
<td>47</td>
<td>85</td>
<td>67</td>
<td>47</td>
</tr>
<tr>
<td>8</td>
<td>84</td>
<td>no data</td>
<td>102</td>
<td>90</td>
<td>51</td>
<td>84</td>
</tr>
<tr>
<td>9</td>
<td>82</td>
<td>57</td>
<td>43</td>
<td>80</td>
<td>71</td>
<td>52</td>
</tr>
<tr>
<td>Mean</td>
<td>80.4</td>
<td>57.4</td>
<td>53.8</td>
<td>80.4</td>
<td>59.4</td>
<td>49.1</td>
</tr>
<tr>
<td>± SEM</td>
<td>3.3</td>
<td>3.5</td>
<td>7.4</td>
<td>3.8</td>
<td>4.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 9. Individual Subject Muscle Cr Data – NOT ADJUSTED

Free Creatine

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>61</td>
<td>73</td>
<td>66</td>
<td>78</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>57</td>
<td>84</td>
<td>47</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>78</td>
<td>85</td>
<td>58</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>87</td>
<td>60</td>
<td>46</td>
<td>81</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>73</td>
<td>60</td>
<td>39</td>
<td>105</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>70</td>
<td>68</td>
<td>56</td>
<td>71</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>no data</td>
<td>37</td>
<td>47</td>
<td>85</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>59</td>
<td>79</td>
<td>47</td>
<td>68</td>
<td>78</td>
</tr>
<tr>
<td>Mean</td>
<td>45.1</td>
<td>69.3</td>
<td>38.3</td>
<td>50.8</td>
<td>78.1</td>
<td>79.0</td>
</tr>
<tr>
<td>± SEM</td>
<td>4.2</td>
<td>4.2</td>
<td>5.6</td>
<td>3.0</td>
<td>4.7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 10. Individual Subject Muscle ATP Data – NOT ADJUSTED

ATP

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO Biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>26</td>
<td>22</td>
<td>25</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>28</td>
<td>24</td>
<td>31</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>29</td>
<td>21</td>
<td>25</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>25</td>
<td>29</td>
<td>25</td>
<td>24</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>32</td>
<td>26</td>
<td>20</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>29</td>
<td>25</td>
<td>33</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>no data</td>
<td>31</td>
<td>37</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>31</td>
<td>22</td>
<td>29</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Mean</td>
<td>30.8</td>
<td>28.6</td>
<td>25.0</td>
<td>28.1</td>
<td>24.8</td>
<td>24.7</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.7</td>
<td>3.2</td>
<td>1.9</td>
<td>2.3</td>
<td>1.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cell resulted from insufficient sample size.
Table 11. Individual Subject Muscle CP Data – ADJUSTED FOR PEAK TOTAL CREATINE

Creatine Phosphate (adjusted)

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO Biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>62</td>
<td>50</td>
<td>69</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>74</td>
<td>44</td>
<td>90</td>
<td>77</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>51</td>
<td>45</td>
<td>77</td>
<td>62</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>57</td>
<td>76</td>
<td>98</td>
<td>63</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>47</td>
<td>49</td>
<td>88</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>62</td>
<td>55</td>
<td>85</td>
<td>69</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>115</td>
<td>no data</td>
<td>102</td>
<td>96</td>
<td>55</td>
<td>84</td>
</tr>
<tr>
<td>9</td>
<td>82</td>
<td>63</td>
<td>45</td>
<td>88</td>
<td>71</td>
<td>56</td>
</tr>
<tr>
<td>Mean</td>
<td>85.6</td>
<td>59.2</td>
<td>58.3</td>
<td>86.3</td>
<td>60.8</td>
<td>53.2</td>
</tr>
<tr>
<td>± SEM</td>
<td>5.3</td>
<td>4.2</td>
<td>5.6</td>
<td>2.8</td>
<td>4.6</td>
<td>6.4</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 12. Individual Subject Muscle Cr Data – ADJUSTED FOR PEAK TOTAL CREATINE

Free Creatine (adjusted)

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO Biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>64</td>
<td>75</td>
<td>69</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>58</td>
<td>87</td>
<td>52</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>80</td>
<td>85</td>
<td>62</td>
<td>77</td>
<td>110</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>91</td>
<td>72</td>
<td>46</td>
<td>81</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>78</td>
<td>76</td>
<td>48</td>
<td>105</td>
<td>106</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>70</td>
<td>77</td>
<td>56</td>
<td>72</td>
<td>88</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>no data</td>
<td>37</td>
<td>50</td>
<td>91</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>66</td>
<td>83</td>
<td>52</td>
<td>68</td>
<td>84</td>
</tr>
<tr>
<td>Mean</td>
<td>46.8</td>
<td>72.2</td>
<td>74.1</td>
<td>54.2</td>
<td>79.7</td>
<td>86.8</td>
</tr>
<tr>
<td>± SEM</td>
<td>3.5</td>
<td>4.2</td>
<td>5.6</td>
<td>2.8</td>
<td>4.6</td>
<td>6.4</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 13. Individual Subject Muscle ATP Data – ADJUSTED FOR PEAK TOTAL CREATINE

ATP (adjusted)

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO Biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>24</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>28</td>
<td>23</td>
<td>28</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>28</td>
<td>21</td>
<td>23</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>24</td>
<td>25</td>
<td>24</td>
<td>24</td>
<td>no data</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>24</td>
<td>21</td>
<td>24</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>29</td>
<td>22</td>
<td>33</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>no data</td>
<td>31</td>
<td>35</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>28</td>
<td>21</td>
<td>26</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>29.3</td>
<td>27.4</td>
<td>23.0</td>
<td>26.3</td>
<td>24.1</td>
<td>22.6</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.1</td>
<td>0.8</td>
<td>1.2</td>
<td>2.1</td>
<td>0.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

All values are expressed in mmol/kgdw.

The “no data” cells resulted from insufficient sample size.
Table 14. Individual Subject Sarcoplasmic Reticulum Ca\textsuperscript{2+}-uptake Data

Ca\textsuperscript{2+}-uptake

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.07</td>
<td>1.46</td>
<td>1.31</td>
<td>3.17</td>
<td>2.45</td>
<td>2.07</td>
</tr>
<tr>
<td>3</td>
<td>3.44</td>
<td>2.32</td>
<td>1.20</td>
<td>1.85</td>
<td>1.20</td>
<td>.820</td>
</tr>
<tr>
<td>4</td>
<td>3.44</td>
<td>no data</td>
<td>1.22</td>
<td>2.07</td>
<td>1.11</td>
<td>.890</td>
</tr>
<tr>
<td>5</td>
<td>2.08</td>
<td>.460</td>
<td>.250</td>
<td>.290</td>
<td>.100</td>
<td>.080</td>
</tr>
<tr>
<td>6</td>
<td>2.26</td>
<td>1.80</td>
<td>1.07</td>
<td>1.12</td>
<td>.670</td>
<td>.500</td>
</tr>
<tr>
<td>7</td>
<td>1.51</td>
<td>1.12</td>
<td>.990</td>
<td>2.76</td>
<td>1.84</td>
<td>1.28</td>
</tr>
<tr>
<td>8</td>
<td>3.58</td>
<td>no data</td>
<td>2.85</td>
<td>2.80</td>
<td>no data</td>
<td>.610</td>
</tr>
<tr>
<td>9</td>
<td>1.99</td>
<td>1.07</td>
<td>2.56</td>
<td>2.56</td>
<td>2.61</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Mean 2.55 1.37 1.20 2.08 1.42 .920

± SEM .29 .26 .26 .34 .35 .21

All values are expressed in nmol/min·mg·protein.

The “no data” cells resulted from insufficient sample size.
Table 15. Individual Subject Sarcoplasmic Reticulum Ca\(^{2+}\)-release Data

<table>
<thead>
<tr>
<th>Subject #</th>
<th>H-CHO biopsy 1</th>
<th>H-CHO biopsy 2</th>
<th>H-CHO biopsy 3</th>
<th>L-CHO biopsy 1</th>
<th>L-CHO biopsy 2</th>
<th>L-CHO biopsy 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.56</td>
<td>3.74</td>
<td>2.87</td>
<td>2.58</td>
<td>2.59</td>
<td>2.20</td>
</tr>
<tr>
<td>3</td>
<td>3.41</td>
<td>2.08</td>
<td>2.00</td>
<td>3.31</td>
<td>3.31</td>
<td>1.15</td>
</tr>
<tr>
<td>4</td>
<td>2.58</td>
<td>no data</td>
<td>1.15</td>
<td>.600</td>
<td>.600</td>
<td>.510</td>
</tr>
<tr>
<td>5</td>
<td>.900</td>
<td>.410</td>
<td>.170</td>
<td>.360</td>
<td>.360</td>
<td>.150</td>
</tr>
<tr>
<td>6</td>
<td>3.99</td>
<td>2.37</td>
<td>1.78</td>
<td>.400</td>
<td>.400</td>
<td>.360</td>
</tr>
<tr>
<td>7</td>
<td>3.23</td>
<td>2.73</td>
<td>2.61</td>
<td>3.13</td>
<td>3.13</td>
<td>1.67</td>
</tr>
<tr>
<td>8</td>
<td>4.48</td>
<td>no data</td>
<td>4.26</td>
<td>3.66</td>
<td>no data</td>
<td>1.30</td>
</tr>
<tr>
<td>9</td>
<td>4.74</td>
<td>2.62</td>
<td>3.16</td>
<td>2.05</td>
<td>2.05</td>
<td>3.26</td>
</tr>
<tr>
<td>Mean</td>
<td>3.49</td>
<td>2.32</td>
<td>2.25</td>
<td>2.81</td>
<td>1.78</td>
<td>1.32</td>
</tr>
<tr>
<td>± SEM</td>
<td>.45</td>
<td>.45</td>
<td>.40</td>
<td>.54</td>
<td>.49</td>
<td>.37</td>
</tr>
</tbody>
</table>

All values are expressed in nmol/min·mg·protein.

The “no data” cells resulted from insufficient sample size.
APPENDIX C:

Statistical Procedures and Results
A paired t-test was performed to detect differences between groups in performance data. All other measures were analyzed by repeated measures analysis of variance (ANOVA) to test for effect of group, time, and group by time interaction. Pearson’s correlation analysis was performed to determine associations between dependent measures. Significance was defined at the p< 0.05 level.

### Table 16. Paired t-tests for Performance Data

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Intervals</td>
<td>7</td>
<td>1.978</td>
<td>.088</td>
</tr>
<tr>
<td>Total Exercise Time</td>
<td>7</td>
<td>1.978</td>
<td>.088</td>
</tr>
<tr>
<td>Total Work</td>
<td>7</td>
<td>1.914</td>
<td>.093</td>
</tr>
<tr>
<td>Average Interval Work</td>
<td>7</td>
<td>.371</td>
<td>.721</td>
</tr>
<tr>
<td>Trial Completed First vs. Second</td>
<td>7</td>
<td>.920</td>
<td>.388</td>
</tr>
</tbody>
</table>
### Table 17. RM ANOVA Table for Muscle Glycogen Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>359285.3</td>
<td>359285.3</td>
<td>42.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>400371.8</td>
<td>200185.9</td>
<td>29.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>21619.1</td>
<td>10809.6</td>
<td>1.57</td>
<td>0.229</td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>178682.8</td>
<td>6872.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 18. Analysis of Contrasts for Muscle Glycogen Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
<td>Mean</td>
<td>1</td>
<td>472926.6</td>
<td>472926.6</td>
<td>25.4</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>33599.9</td>
<td>33599.9</td>
<td>1.81</td>
<td>0.202</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td>Mean</td>
<td>1</td>
<td>23103.2</td>
<td>23103.2</td>
<td>2.21</td>
<td>1.161</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>135861.4</td>
<td>10450.9</td>
<td>0.00</td>
<td>0.949</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2;
Time 2 = from time 2 to time 3)
Table 19. RM ANOVA Table for Muscle CP Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>36.4</td>
<td>36.4</td>
<td>0.16</td>
<td>0.700</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>7052.6</td>
<td>3526.3</td>
<td>28.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>8.9</td>
<td>4.5</td>
<td>0.04</td>
<td>0.958</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>3008.2</td>
<td>125.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 20. Analysis of Contrasts for Muscle CP Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Mean</td>
<td>1</td>
<td>7491.3</td>
<td>7491.3</td>
<td>29.2</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>12.9</td>
<td>12.9</td>
<td>0.05</td>
<td>0.826</td>
</tr>
<tr>
<td>Time 2</td>
<td>Mean</td>
<td>1</td>
<td>737.3</td>
<td>737.3</td>
<td>2.40</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.018</td>
<td>0.018</td>
<td>0.00</td>
<td>0.994</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2;  
Time 2 = from time 2 to time 3)
Table 21. RM ANOVA Table for Muscle Free Creatine Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>470.6</td>
<td>470.6</td>
<td>4.38</td>
<td>0.058</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>7052.6</td>
<td>3526.3</td>
<td>28.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>8.95</td>
<td>4.47</td>
<td>0.04</td>
<td>0.958</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>3008.2</td>
<td>125.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 22.** Analysis of Contrasts for Muscle Free Creatine Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
<td>Mean</td>
<td>1</td>
<td>7491.3</td>
<td>7491.3</td>
<td>29.2</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>12.9</td>
<td>12.9</td>
<td>0.05</td>
<td>0.826</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td>Mean</td>
<td>1</td>
<td>737.3</td>
<td>737.3</td>
<td>2.40</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.994</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2; 
Time 2 = from time 2 to time 3)
Table 23. RM ANOVA Table for Muscle ATP Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>28.6</td>
<td>28.6</td>
<td>1.67</td>
<td>0.221</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>177.9</td>
<td>8.9</td>
<td>11.0</td>
<td>0.0004</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>33.6</td>
<td>16.8</td>
<td>2.07</td>
<td>0.078</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>194.4</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 24. Analysis of Contrasts for Muscle ATP Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Mean</td>
<td>1</td>
<td>119.5</td>
<td>119.5</td>
<td>8.19</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>11.3</td>
<td>11.3</td>
<td>0.78</td>
<td>0.395</td>
</tr>
<tr>
<td>Time 2</td>
<td>Mean</td>
<td>1</td>
<td>61.6</td>
<td>61.6</td>
<td>6.02</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>66.5</td>
<td>66.5</td>
<td>6.50</td>
<td>0.026</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2; Time 2 = from time 2 to time 3)
Table 25. RM ANOVA Table for SR Ca$^{2+}$-uptake Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.897</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>8.7</td>
<td>4.4</td>
<td>38.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.86</td>
<td>0.427</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>2.5</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 26. Univariate Contrasts for SR Ca\textsuperscript{2+}-uptake Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Mean</td>
<td>1</td>
<td>6.4</td>
<td>6.4</td>
<td>36.1</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.3</td>
<td>0.3</td>
<td>1.71</td>
<td>0.218</td>
</tr>
<tr>
<td>Time 2</td>
<td>Mean</td>
<td>1</td>
<td>2.7</td>
<td>2.7</td>
<td>13.4</td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.968</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2;  
Time 2 = from time 2 to time 3)
Table 27. RM ANOVA Table for SR Ca\textsuperscript{2+}-release Data

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>4.7</td>
<td>4.7</td>
<td>1.11</td>
<td>0.315</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>13.1</td>
<td>6.6</td>
<td>18.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diet * Time</td>
<td>2</td>
<td>0.1</td>
<td>0.05</td>
<td>0.16</td>
<td>0.856</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>7.8</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 28. Analysis of Contrasts for SR Ca\(^{2+}\)-release Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Mean</td>
<td>1</td>
<td>13.7</td>
<td>13.7</td>
<td>32.8</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.42</td>
<td>0.530</td>
</tr>
<tr>
<td>Time 2</td>
<td>Mean</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.97</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.21</td>
<td>0.650</td>
</tr>
</tbody>
</table>

(Time 1 = from time 1 to time 2;  
Time 2 = from time 2 to time 3)
Table 29. Correlation Analysis for Biopsy 1 Data

<table>
<thead>
<tr>
<th>Biopsy 1</th>
<th>Glycogen</th>
<th>Creatine Phosphate</th>
<th>ATP</th>
<th>Calcium-uptake</th>
<th>Calcium-release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>X</td>
<td>r = -0.050 p = 0.853</td>
<td>r = 0.331 p = 0.210</td>
<td>r = 0.109 p = 0.689</td>
<td>r = 0.230 p = 0.391</td>
</tr>
<tr>
<td>Creatine Phosphate</td>
<td>r = -0.050 p = 0.853</td>
<td>X</td>
<td>r = 0.196 p = 0.466</td>
<td>r = 0.001 p = 0.997</td>
<td>r = -0.080 p = 0.768</td>
</tr>
<tr>
<td>ATP</td>
<td>r = 0.331 p = 0.210</td>
<td>r = 0.196 p = 0.466</td>
<td>X</td>
<td>r = 0.496 p = 0.051</td>
<td>r = 0.567 p = 0.022</td>
</tr>
<tr>
<td>Calcium-uptake</td>
<td>r = 0.109 p = 0.689</td>
<td>r = 0.001 p = 0.997</td>
<td>r = 0.495 p = 0.051</td>
<td>X</td>
<td>r = 0.404 p = 0.121</td>
</tr>
<tr>
<td>Calcium-release</td>
<td>r = 0.230 p = 0.391</td>
<td>r = -0.080 p = 0.768</td>
<td>r = 0.567 p = 0.022</td>
<td>r = 0.404 p = 0.121</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 30. Correlation Analysis for Biopsy 2 Data

<table>
<thead>
<tr>
<th>Biopsy 2</th>
<th>Glycogen</th>
<th>Creatine Phosphate</th>
<th>ATP</th>
<th>Calcium-uptake</th>
<th>Calcium-release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>X</td>
<td>( r = -0.172 )</td>
<td>( r = 0.459 )</td>
<td>( r = -0.209 )</td>
<td>( r = 0.068 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.539 )</td>
<td>( p = 0.085 )</td>
<td>( p = 0.493 )</td>
<td>( p = 0.826 )</td>
</tr>
<tr>
<td>Creatine Phosphate</td>
<td>( r = -0.172 )</td>
<td>X</td>
<td>( r = -0.286 )</td>
<td>( r = 0.358 )</td>
<td>( r = 0.460 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.539 )</td>
<td>( p = 0.302 )</td>
<td>( p = 0.229 )</td>
<td>( p = 0.114 )</td>
</tr>
<tr>
<td>ATP</td>
<td>( r = 0.459 )</td>
<td>( r = -0.286 )</td>
<td>X</td>
<td>( r = -0.003 )</td>
<td>( r = 0.325 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.085 )</td>
<td>( p = 0.302 )</td>
<td>( p = 0.991 )</td>
<td>( p = 0.278 )</td>
<td></td>
</tr>
<tr>
<td>Calcium-uptake</td>
<td>( r = -0.209 )</td>
<td>( r = 0.358 )</td>
<td>( r = -0.003 )</td>
<td>X</td>
<td>( r = 0.543 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.493 )</td>
<td>( p = 0.229 )</td>
<td>( p = 0.991 )</td>
<td>( p = 0.055 )</td>
<td></td>
</tr>
<tr>
<td>Calcium-release</td>
<td>( r = 0.068 )</td>
<td>( r = 0.460 )</td>
<td>( r = 0.325 )</td>
<td>( r = 0.543 )</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>( p = 0.826 )</td>
<td>( p = 0.114 )</td>
<td>( p = 0.278 )</td>
<td>( p = 0.055 )</td>
<td></td>
</tr>
</tbody>
</table>
Table 31. Correlation Analysis for Biopsy 3 Data

<table>
<thead>
<tr>
<th>Biopsy 3</th>
<th>Glycogen</th>
<th>Creatine Phosphate</th>
<th>ATP</th>
<th>Calcium-uptake</th>
<th>Calcium-release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>X</td>
<td>r = 0.244 p = 0.435</td>
<td>r = -0.044 p = 0.875</td>
<td>r = 0.154 p = 0.583</td>
<td>r = 0.416 p = 0.123</td>
</tr>
<tr>
<td>Creatine Phosphate</td>
<td>r = 0.244 p = 0.381</td>
<td>X</td>
<td>r = 0.601 p = 0.018</td>
<td>r = 0.435 p = 0.105</td>
<td>r = 0.385 p = 0.157</td>
</tr>
<tr>
<td>ATP</td>
<td>r = -0.044 p = 0.875</td>
<td>r = 0.601 p = 0.018</td>
<td>X</td>
<td>r = 0.090 p = 0.751</td>
<td>r = 0.020 p = 0.944</td>
</tr>
<tr>
<td>Calcium-uptake</td>
<td>r = 0.154 p = 0.583</td>
<td>r = 0.435 p = 0.105</td>
<td>r = 0.090 p = 0.751</td>
<td>X</td>
<td>r = 0.727 p = 0.001</td>
</tr>
<tr>
<td>Calcium-release</td>
<td>r = 0.416 p = 0.123</td>
<td>r = 0.385 p = 0.157</td>
<td>r = 0.020 p = 0.994</td>
<td>r = 0.727 p = 0.001</td>
<td>X</td>
</tr>
</tbody>
</table>
APPENDIX D:

Institutional Review Board Proposal
Title: The effect of muscle glycogen depletion on fatigue and sarcoplasmic reticulum function during intermittent high intensity exercise

Investigators:
Michelle Smith, M.S. candidate  
Janet Walberg-Rankin, Ph.D., advisor

Justification:  
Recent sports nutrition research has focused on the role of carbohydrates in intermittent high intensity exercise such as basketball, soccer, hockey, football, and lacrosse. Several studies have associated inadequate muscle carbohydrate stores (known as muscle glycogen) with fatigue in this type of exercise. Few studies have examined the specific mechanisms relating to muscle glycogen depletion and fatigue.  

Muscle physiologists, meanwhile, have related muscle fatigue with altered functioning of a cellular component known as the sarcoplasmic reticulum. The sarcoplasmic reticulum is involved in calcium release and uptake, and therefore, contraction and relaxation by muscle cells. Studies on animal muscle cells have revealed that impaired sarcoplasmic reticulum function correlates with muscle fatigue. Unfortunately, this process has not been verified through many human studies due partly to the difficulty associated with assessing sarcoplasmic reticulum function in the small muscle samples obtainable through muscle biopsy. Our laboratory has successfully performed sarcoplasmic reticulum analysis on samples as small as 10-15 mg. An average of 50-75 mg of tissue is typically acquired through the human muscle biopsy procedure.  

Some researchers have associated glycogen with the sarcoplasmic reticulum. It has been reported to exist on the sarcoplasmic reticulum membrane. Also, the energy produced from the breakdown of glycogen has been theorized to fuel the pump that uptakes calcium into the muscle cell, a crucial part of the muscle contraction-relaxation process. Therefore, depletion of glycogen, such as that which occurs during exercise, may be a mechanism for the impaired sarcoplasmic reticulum function that is involved in fatigue. To our knowledge, no research has examined the relationship between muscle glycogen status and human sarcoplasmic reticulum function.
Our research proposes to combine the fields of sports nutrition and muscle physiology by evaluating the effects of glycogen depletion on time to fatigue in an intermittent high intensity exercise bout and analyzing if glycogen depletion correlates with fatigue-induced changes in the sarcoplasmic reticulum.

Results from our study would be valuable to both fields. With a better understanding of the effects of glycogen status, sports nutritionists could make more accurate dietary recommendations concerning carbohydrates consumed before and during exercise. Muscle physiologists would have a human model of what they have already analyzed in animals. Further, they could perform more studies with different glycogen manipulations and analyze the effects on the sarcoplasmic reticulum to define the extent of their association. Ultimately, discovering the causes of fatigue and determining ways to alleviate it is critical. Fatigue is not only associated with diminished performance by athletes in intermittent high intensity exercise; it is also a precursor for muscle soreness and susceptibility to orthopedic and musculotendinous injury.

**Procedures:**
**Subject selection and pre-testing**

Twelve 18-25 year-old male experienced cyclists (training at least 3 times per week for 2 years) will be recruited. Fliers briefly explaining the experimental procedures (including the muscle biopsy procedure) will be posted in fitness settings on campus and around town to notify the public of the study. Interested individuals will contact the investigators via telephone or email to obtain more details about the study. Those still interested will attend a group information session where the study will be described in full and an opportunity to sign informed consent forms will be given. Subjects who sign the informed consent form will undergo a health screening and will be excluded if conditions such as diabetes, cardiovascular disease, orthopedic limitations, mental illness, or other contraindications to the experiment exist. Additionally, subjects who report allergy to Novocain or iodine will also be excluded.

**Exercise protocol**

All 12 subjects will participate in four exercise trials: a familiarization trial, a practice trial, and two performance tests. The familiarization trial will allow subjects to become comfortable with the experimental procedures and equipment. At this time, subjects will have the opportunity to practice the protocol, but will not exercise to exhaustion.
The practice trial will be an imitation of the actual performance tests. Subjects will perform intermittent high intensity cycling intervals of 1 minute at 120-130% VO2 max separated by 3 minutes of rest. Intervals will be continued to exhaustion.

Performance tests (the third and fourth exercise trials) will be performed in a glycogen-depleted state and a non-depleted state in random order. In other words, six subjects will perform the glycogen-depleted test first and the other six will perform the non-depleted test first. The glycogen-depletion method used will be a modification of one previously used in our lab involving cycling at 70% VO2 max for 90 minutes followed by six one minute maximal effort sprints in the evening, 16 hours prior to the performance test. The non-depleted group will rest the night before the performance test. In order to maintain glycogen levels, subjects in both groups will consume a low carbohydrate snack (<15g CHO) 12 hours before the performance test and fast overnight until after the test.

Water

Water will be available to subjects ad libitum during the practice trial. The amount consumed will be recorded and this amount will be provided during the two performance tests.

Diet

Subjects will be instructed to keep a diet record for 24 hours prior to the practice trial and to consume identical diets during the 24 hour periods before both performance tests.

Body weight

Body weight will be recorded before the familiarization trial and before each performance test. Weight maintenance will be encouraged and any subject whose weight varies more than 1 kg will be excluded from the study.

Muscle samples

Muscle biopsies will be taken from the vastus lateralis muscle (in the thigh) before each performance test, at 75% of average time to exhaustion (determined from the practice trial), and at exhaustion. Subjects' right leg will be used for biopsy during the first performance test and left leg will be used for the second performance test.
The procedure will involve injecting a local anesthetic into the muscle following cleansing. Subjects will feel a minor sting, similar to a bee sting. A half inch incision in the numbed area will be created with a scalpel blade. A hollow biopsy needle will be inserted and samples of 50-75 mg, on average, will be suctioned from this incision. All three biopsies for each performance test will be obtained from different angles in the same incision.

The incision will be closed using steri-strips and pressure will be applied with sterile gauze. A cold pack will be placed over the incision. Before subjects leave the laboratory, a pressure wrap will be placed around the leg. Instructions on how to properly care for the incision and warning signs of infection will be given. Telephone numbers of the investigators and attending physician will be given to subjects in case they have any concerns.

Muscle samples will be divided in half. One half will be immediately frozen in liquid nitrogen for later analysis of glycogen. The other half will be used for sarcoplasmic reticulum analysis.

Biopsies will be performed by Janet Rinehart, a certified medical laboratory technician. Ms. Rinehart was trained in the muscle biopsy procedure in 1995 and has performed around 100 since then. No adverse consequences of the procedure have been reported. She is currently involved in performing biopsies for a study in our lab involving creatine supplementation. A physician will be present for all biopsies.

**Risks and benefits:**

Subjects will be financially compensated $150 for completion of the study. They will also receive information on their fitness level based on preliminary testing.

There is very little risk involved with the VO2 max testing especially since trained athletes who are accustomed to cycling equipment and high intensity exercise are being used. Fatigue, muscle soreness, and muscle strains could result from the preliminary testing or the actual protocol, but, again, this is unlikely since subjects regularly train on the equipment and at the intensity being used.

One risk involved with the muscle biopsy technique is an allergic reaction to the local anesthetic. Subjects will be questioned on allergies before the procedure. To prevent infection, another possible risk of the muscle biopsy technique, subjects will be educated on the proper care of their incision. They will be required to return to the lab for two days following the biopsy to have their incisions examined. A small scar will result from the procedure and subjects will be informed of this before the study. Further, all biopsies and examinations will be performed by a certified medical laboratory technician.
Any medical expenses will not be covered by Virginia Tech unless the university is negligent.

Confidentiality:
Data from subjects in this study will be seen only by the principal investigator and faculty advisor. Otherwise, subjects will remain anonymous, identified only by number.

Biographical sketch:
Michelle R. Smith, M.S. candidate in Nutrition for Sports and Chronic Disease option in the department of Human Nutrition Foods, and Exercise. She graduated from Virginia Tech last Spring with a bachelor's degree in Dietetics and a minor in Exercise Science. She has been involved in the fitness setting for several years as a member of the Virginia Tech Track & Field Team and through work as a fitness trainer at a public gym. She also worked as a Nutrition Technician at a Baltimore hospital during school breaks for the last 3 years. During the Spring semester, she will be assisting with two studies in the department involving diet manipulation, exercise testing, and muscle biopsy.

Janet Walberg Rankin, Ph.D., Faculty Advisor and Associate Professor in the Department of Human Nutrition, Foods, and Exercise since 1982. She earned a bachelor's degree in Zoology from Duke University and a doctorate in Nutrition with a minor in Exercise Physiology from the University of California at Davis. Her research is primarily in sports nutrition and weight control and has been published in International Journal of Sports Nutrition, Medicine and Science in Sports and Exercise, and International Journal of Sports Medicine. She teaches Exercise Physiology and Nutrition & Physical Performance to undergraduates and Metabolic Aspects of Exercise to graduate students.

Proposed timetable:
January, 1998
* submit study for approval by the Institutional Review Board
* submit study for Reebok Research Grant
July-August, 1998
* develop performance test and prepare equipment
* learn glycogen and SR analysis techniques
* perform pilot work
September-October, 1998
* recruitment of subjects
* orientation and informed consent meeting
* preliminary testing
November-
December, 1998
* data collection
(see table 1)
January-March, 1999
* analysis of frozen muscle samples
February-April, 1999
* statistical analysis of data
May-August, 1999
* writing of manuscript and thesis

**Data Collection Period**
(November-December, 1998)

Sample Schedule for 1 Subject
*each subject's involvement will be about 30 days

day 1
Preliminary testing - measurement of VO2 max
day 7
FAMILIARIZATION TRIAL
day 13
24 hour diet record
day 14
PRACTICE TRIAL
  -record body weight
  -establish time to exhaustion
day 20
follow diet from day 7
evening - consume low CHO snack
day 21
PERFORMANCE TEST 1: NON-DEPLETED TRIAL
days 22 & 23
report to lab for examination of biopsy incision
day 27
follow diet from day 7
evening - perform glycogen-depleting exercise bout  
    consume low CHO snack
day 28
PERFORMANCE TEST 2: GLYCOGEN-DEPLETED TRIAL
days 29 & 30
report to lab for examination of biopsy incision
APPENDIX E:

Informed Consent & Health History Questionnaire
Virginia Polytechnic Institute and State University
Informed Consent for Participants of Investigative Projects
Department of Human Nutrition, Foods and Exercise

Title: The Effect of Muscle Glycogen Status on Muscle Phosphagens, Sarcoplasmic Reticulum Function, and Performance During Intermittent High Intensity Exercise

Investigators: Michelle Smith, M.S. candidate
               Janet Walberg Rankin, Ph.D. (faculty advisor)

I. Purpose

Muscle Fatigue

Some research shows that the level of muscle carbohydrate stores (glycogen) may play a role in fatigue during exercise involving intermittent bursts of high intensity effort. Many muscle physiologists have linked muscle fatigue to a change in a cellular component known as the sarcoplasmic reticulum. The sarcoplasmic reticulum is directly involved in the contraction-relaxation process, so any disturbance in this may lead to fatigue, the inability to maintain maximal force output. Some researchers have theorized a relationship between glycogen status and proper sarcoplasmic reticulum function, but few studies have been performed to analyze this. Particularly limited is the amount of sarcoplasmic reticulum analysis performed on human muscle; most studies have been performed on animal models. One purpose of our study is to analyze the effects of glycogen status on human sarcoplasmic reticulum function during fatigue from intermittent high intensity exercise.

**Eight well-trained male cyclists, ages 18-30, will serve as subjects in the study.**
II. Procedures

Testing

The study will require a 4-week commitment (one or two approx. 2 hr. periods each week). During this time, you will participate in preliminary testing and three exercise trials, each separated by one week. The PRELIMINARY TESTING will involve two maximal tests (VO_{2peak} and Wingate) on a cycle ergometer so we can evaluate your fitness level which we need to know for setting exercise levels later in the study. The second exercise trial is a PRACTICE TRIAL, an imitation of the actual performance test. You will exercise to fatigue on a stationary bicycle ergometer. The exercise will consist of intervals of 1 minute of maximal effort separated by 3 minute rest periods. The second and third exercise trials will be PERFORMANCE TESTS in which you will again perform the interval exercise bouts.

Water and Diet Records

During the practice trial, you can drink as much water as you want. We will keep record of how much you drink and provide you with this amount during the performance tests to make each bout as similar as possible. Also, you will be asked to record everything you eat during the 24 hours before the practice trial so we can evaluate your normal caloric consumption with a computerized nutritional analysis program.

Glycogen-Depletion Rides / Diet

Thirty-six hours before the performance tests, you will complete an exercise bout designed to deplete muscle glycogen. You will cycle at a submaximal pace for 80 minutes followed by four one-minute intervals of cycling at maximal effort. After completing this ride and during the remaining 36 hours before the performance test, you will consume only foods supplied by the investigators and perform no exercise.
**Body Weight**

We will record your body weight before the familiarization trial and before each performance test. Weight maintenance will be encouraged and any subject whose weight varies more than 1 kg (a little more than 2 pounds) will be excluded from the study.

**Muscle Biopsies**

In order to analyze your muscle glycogen levels and sarcoplasmic reticulum function, we will take muscle biopsies before each performance test, during the test at 85% of your first interval performance, and at fatigue (70% of your first interval performance). The muscle biopsy procedure is very safe and has been performed numerous times in our laboratory without incident. Your thigh will be shaved, cleaned, and injected with a local anesthetic (lidocaine) which will feel like a bee sting. Two 1/4 inch incisions will be made in the numbed area with a small scalpel. A hollow needle will be inserted through the incision to suction out a small piece of your muscle. Some cramping and discomfort may be involved in the procedure. Two of the biopsies in each performance test will be taken from the first incision and the final biopsy will be taken from the second incision. We will use your left thigh for the first performance test and your right thigh for the second. After the performance tests, pressure and ice will be applied to the area to prevent swelling. The incisions will be closed with steri-strips (similar to Band-Aids) and covered with a pressure wrap. The pressure wrap can be removed after 8 hours, but the steri-strips should remain in place for 3 days. The incisions will heal within a few days, but a small scar will remain and fade over time. Performing activity may reduce stiffness in the leg involved in the procedure. More instructions will be given at the time and site of the biopsies.

**III. Risks**

*Fatigue, muscle soreness, and muscle strains or pulls may result from the performance test. This, however, is unlikely since you are accustomed to the type of exercise and equipment being used.*
*Bruising and muscle soreness from the muscle biopsy procedure is possible. Because a certified, experienced technician will be performing the biopsies, adverse effects such as these should be minimized. A physician will be present in case of emergency. We will examine the incision two days after the procedure to prevent infection.

*An allergic response may result from the local anesthetic. Thus, it is crucial that you inform us of any known allergies prior to the study.

Note that a telephone will be available in all testing sites to alert emergency services (Virginia Tech rescue squad) of any medical emergencies. Investigators involved in the study have been trained in first aid and CPR.

The University will not be responsible for any medical expenses you may have unless the University has been negligent. In the past, no muscle biopsy subjects have required medical attention.

IV. Benefits and Compensation

For participation, you will receive:

* $150 for full completion of the study, including all exercise trials. If you drop out or are asked to leave the study prior to completion, you will receive a portion of the $150.

* data on your aerobic and anaerobic fitness level

*data on your body fat percentage

*computerized dietary analysis
* a summary of the procedures, data, and conclusions from the study that you may find valuable to your training

**No guarantee of benefits of the study have been made with intent to encourage you to participate in the study.

V. **Extent of Anonymity and Confidentiality**

Only the investigators will be allowed to view data linked with your identity. No data will be released to anyone but the investigators without your written consent. You will be identified by a random number, not by name, in all reports.

VI. **Freedom of Withdrawal**

You are free to withdraw at any time from this study for any reason without penalty. Investigators may ask you to leave the study for reasons including illness, failure to cooperate with procedures, failure to attend required sessions, and poor compliance to diet instructions.

VII. **Approval of Research**

This research project has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods and Exercise.

VIII. **Subject's Responsibilities**

1. Attendance at an information session detailing the study.

2. Subjection to VO$_{2peak}$ and Wingate tests, body weight and body fat measurements, and muscle biopsies.

3. Completion of two glycogen-depletion rides and three interval performance tests.
4. Exertion of maximal effort during all performance tests.

5. Informing the researchers of any medical conditions or injuries that are pre-existing or may arise during the study that may affect the results, or of any known transmittable diseases such as HIV or hepatitis.

6. Recording food consumed before the baseline trial.

7. Complying to prescribed diets

8. Reporting to the laboratory two days following the muscle biopsies for examination of incisions.

9. Informing investigators of any problems associated with the site of muscle biopsies.

10. Informing investigators at any time throughout the study of lack of compliance or failure to fully participate in the study.

Should I have any questions about this research or its conduct, I may contact:

Principal investigators:
Michelle Smith, M.S. candidate - HNFE department (540)951-4581 / mismith2@vt.edu

Faculty members:
Dr. Jay Williams, Associate Professor - HNFE department
(540)231-8298 / jhwms@vt.edu

Institutional Review Board:
Tom Hurd, Chairman - IRB (540)231-9359
IX. **Subject's Permission**

I have read and understand the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

Signature: _______________________ Date:___________

Witness:________________________
Name: _____________________ Age: _______ Birth Date: __________

Address: _______________________________ e-mail: ________________

Phone Numbers: Home: _________________ Work :______________

Person to Contact in Case of an Emergency: ______________________________

Relationship: _________________________ Phone: ________________

Primary Care Physician: ______________ Phone: ______________

Medical Insurance Carrier: ______________ SSN: ________________

MEDICAL HISTORY

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease or any heart problems:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatic Fever:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory disease or breathing problems:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circulation problems:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease or problems:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Urinary problems: ______  ______
Musculoskeletal problems: ______  ______
(Any muscle or skeletal limitations: i.e. Orthopedic injuries, osteoporosis)
Fainting and Dizziness: ______  ______
High Cholesterol: ______  ______
Diabetes: ______  ______
Thyroid problems: ______  ______
Allergies (e.g. Novocain) ______  ______
Mental illness: ______  ______
Hypoglycemia: (i.e. low blood sugar) ______  ______
Epilepsy or seizures: ______  ______

If you answered “yes” to any of the previous questions, please indicate the date and describe:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Please list any hospitalizations/operations/recent illnesses (type/date):
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Yes          No
Have you ever been diagnosed as having high blood pressure?    _____    _____
Date: __________________
Are you currently being treated for high blood pressure?    _____    _____

If “yes”, please explain:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

For what reason(s) are you taking this medication?
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Health Habits

Yes          No
Are you on any special type of diet?    _____    _____

If “yes”, please describe:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Do you drink alcoholic beverages? ______ Yes ______ No

How many drinks per week? _____________

Do you smoke cigarettes? ______ Yes ______ No

Packs per day: ______________

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (times per week)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you engage in regular exercise? ______ Yes ______ No

If “yes”, please list:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (times per week)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you ever faint, short of breath, or chest discomfort with exertion? ______

If “yes”, please explain: _______________________________________________________

________________________________________________________________________

Are there any orthopedic limitations you have that may restrict your ability to perform exercise on stationary cycle and if “yes”, please explain:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
**Family History**

Has anyone in your family been diagnosed or treated for any of the following?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Relationship</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please sign to indicate that the above information is correct:

________________________  __________________  __________
Print name     Signature     Date
Supplementary Questionnaire

Have you ingested creatine supplements during the past 2 months? Yes  No

Are you on any special type of diet? Yes  No
If yes, describe __________________________________________________________

Are you a vegetarian? Yes  No

Do you eat red meat? Yes  No  How often? _______________________

Are you trying to lose weight? Yes  No

Has your weight fluctuated more than:
   10 pounds in the past 12 months? Yes  No
   2 months? Yes  No

What is the most you have weighed since you were 18 years old? ___________

What is the least you have weighed since you were 18 years old? ___________

Have you used a dietary supplement in the past? Yes  No
Which one(s)? _______________________
When? ___________________________

Do you have a fear of blood withdrawal with a needle? Yes  No

Have you received Novocain at the dentist’s office? Yes  No

Did you have an allergic reaction to Novocain? Yes  No
APPENDIX F:

Recruitment Flyer
We are looking for

CYCLISTS

to participate in a Sports Nutrition study
sponsored by the Virginia Tech
Human Nutrition, Foods & Exercise Department

Who is eligible?

- Males, ages 18-30

What are your responsibilities?

- Blood and muscle tissue sampling
- 2 days without exercising outside of the study
- 2 days of consuming foods supplied by the study

What will you receive?

- $150 for completion of the study
- Computerized nutritional analysis
- VO_{2\text{max}} (aerobic fitness) testing
- Wingate (anaerobic fitness) testing
- 2 days worth of free meals
- Gatorlode and Gatorade supplements

INTERESTED? Need more information? Contact:

Helen Stevens: 552-9017 --- hstevens@vt.edu
Michelle Smith: 951-4581 --- mismith2@vt.edu
APPENDIX G:

Instructions Given to Subjects
* Given to subjects at Preliminary Meeting

Guidelines for Pre-testing Lab Day

Do not consume food 2 hours prior to reporting to the lab.

Do not exercise the morning of the test.

Do not consume caffeine for the 24-hour period prior to the test.

Wear appropriate clothing: biking shorts, t-shirt (whatever you’re used to cycling in). Please bring regular running shoes since your cycling shoes may or may not be compatible with our cycle.

Test date and time: ______________________________

230 War Memorial Gym

Any questions?

*Michelle Smith, 951-4581

*Helen Stevens, 552-9017
*Given to subjects at pre-testing

For the meals we provide, would you prefer NOT to receive any of the following due to FOOD ALLERGY OR SEVERE DISLIKE?

Apple Juice
Banana
Canned Tuna
Crystal Light
Eggs
Gatorade
Gatorlode
Honey Nut Cheerios Cereal
Jelly
Margarine
Mayonnaise
Pasta
Peanut Butter
Rice Cakes
Skim Milk
Sliced Turkey
Spaghetti Sauce
Yellow Cheese
Whole wheat Bread
*Given to subjects at Pre-testing*

**Dietary Record Sheet**

Please record EVERYTHING YOU EAT OR DRINK from the time you wake up until midnight on ________________.

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Preparation method or brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>22.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*Given to subjects at Baseline Trial*

During the **36 hours before**
& the **24 hours after**
your two *performance rides*:

**DO:**

Consume ALL supplied foods.
Return any uneaten foods, no matter how much.
Consume the amount of fluids you normally consume.
Consume non-caloric foods in addition to supplied foods, if desired
(ex: crystal light/ decaffeinated tea, coffee, soda).
Get your normal amount of sleep.

**DO NOT:**

Exercise.
Consume any caffeine (tea, coffee, soda).
Consume any nutritional supplements (includes vitamins and minerals).
Consume any food in excess of what is supplied.
Consume any alcoholic beverages.
Take any over-the-counter or prescription pain or anti-inflammatory medications.
Take any antihistamines.

**If you have any questions, do not hesitate to contact:**

**Michelle Smith**

951-4581
mismith2@vt.edu

**Helen Stevens**

552-9017
hstevens@vt.edu
APPENDIX H:

Pilot Performance Data
The following is data collected from several pilot subjects who were used to develop
details of the protocol used for performance trials (including interval duration, recovery
duration, workload, paced intervals vs. maximal effort, etc.).

**Subject 1**

**Characteristics:**
- Female – 69 kg
- Moderately trained cyclist
- $VO_{2\text{peak}} = 47 \text{ ml\cdot kg}^{-1}\cdot\text{min}^{-1}$ (recently measured)

**Protocol:**
- 120% $VO_{2\text{peak}}$: 3 X 60 seconds with 3 minutes rest at 80 rpm
- Workload = 3.6 kg/5.2% body weight
- Same workload was also attempted with 1 60-second all out interval

**Results/Comments:**
- Goal was to see how intervals felt for cycling-trained subject
- 80 rpm was too slow – she recommended 90 rpm
- Paced intervals felt much easier than all out interval

**Subject 2**

**Characteristics:**
- Female – 59 kg
- Moderately trained swim/run/bike
- $VO_{2\text{peak}} = 44 \text{ ml\cdot kg}^{-1}\cdot\text{min}^{-1}$ (recently measured)

**Protocol:**
- **Trial 1:**
  - 120% $VO_{2\text{peak}}$: 10 X 60 seconds with 3 minutes rest at 80 rpm
  - Workload = 3 kg/5.1% body weight
- **Trial 2:**
  - 5 days after Trial 1
  - Same workload 60-second intervals to fatigue
Results/Comments:

Trial 1:
Subjects stopped by experimenter at 40 minutes (too easy)

Trial 2:
Subject rode until rpm decreased by 40%
Completed 37 intervals (2 hours 28 minutes)
Rpm range = 129-73
  First 5 were hard, then got easier
  Felt as if she was pacing, not giving max effort
  Very sore the next day

Subject 3
Characteristics:
  Female – 64 kg
  Weight lifting/jogging
  VO_{2peak} = 40 \text{ ml·kg}^{-1} \cdot \text{min}^{-1} \text{ (recently measured)}

Protocol:
  Trial 1
  120\% \text{ VO}_{2peak}: 10 \times 60 \text{ seconds with 3 minutes rest at 80 rpm}
  Workload = 3.2 \text{ kg/5.0\% body weight}
  Trial 2
  6 days after trial 1
  Same workload – 60 second intervals with 3 minutes rest to fatigue

Results/Comments:

Trial 1
Subject stopped b experimenter at 40 minutes (too easy)

Trial 2
Subject rode until rpm decreased by 40%
Did 19 intervals (76 minutes)
Rpm range = 129-76
First 3 were hard, then full recovery between intervals

Subject 4
Characteristics:
  Male – 91 kg
  Weight lifting/running
  VO\text{\textsubscript{2peak}} = 48 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (recently measured, but after Trial 1)

Protocol:
  Trial 1
  130\% VO\text{\textsubscript{2peak}}: 60 second intervals with 3 minutes rest at 90 rpm
  Workload = 5.8 kg/6.4\% body weight

  Trial 2
  115\% VO\text{\textsubscript{2peak}} for 3 60 second intervals
  130\% VO\text{\textsubscript{2peak}} to fatigue

Results/Comments:
  Trial 1
  Goal was to go to fatigue of 40\% rpm decrease
  Subject voluntarily stopped after 7 intervals (28 minutes)
    Felt lightheaded, had dry heaves, very pale
  Overestimated his VO\text{\textsubscript{2peak}}; should’ve used 5.3 kg

  Trial 2
  Did 15 total intervals
    Felt much better than trial 1

Subject 5
Characteristics:
  Female – 68 kg
  VO\text{\textsubscript{2peak}} = 50 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} (measured 1 year ago)

Protocol:
  Trial 1
120% VO$_{2peak}$: 60-second intervals with 3 minutes rest at max effort
Workload = 3.6 kg/5.3% body weight

**Trial 2**
5 days after Trial 1
130% VO$_{2peak}$: 90 second intervals with 3 minutes rest at 90 rpm
Workload = 4.0 kg/6.0% body weight

**Results/Comments:**

**Trial 1**
Subject stopped by experimenter at 84 minutes (too easy)
Did 21 intervals
rpm range = 131-102 (84-94 rpm for 18 intervals)

**Trial 2**
Goal was to go to fatigue of 40% rpm decrease
Subject stopped by experimenter after 18 minutes (too hard)
Did 4 intervals (rpm range = 130-90)
Could barely pedal by the end of all intervals
Did not feel like she was pacing – gave maximal effort for 90 seconds

**Subject 6**

**Characteristics:**
Male – 82 kg
VO$_{2peak}$ = 60 ml·kg$^{-1}$·min$^{-1}$ (recently measured)

**Protocol:**
130% VO$_{2peak}$: 60 second intervals with 3 minutes rest at max effort
Workload = 6.7 kg/8.0% body weight
Switched to 5.4 kg/6.5% body weight after interval 2

**Results/Comments:**
Goal was to go to fatigue of 40% rpm decrease
Subject voluntarily stopped after 12 minutes (3 intervals)
Felt nauseous and vomited
Said 1 kg less would be better
Felt max intervals are better for muscle damage protocol, but as a cyclist, he’d never have a minute where he pedals all out from the gun

**Subject 7**

**Characteristics:**
- Male – 69 kg
- Well-trained mile runner
- \( \text{VO}_2\text{peak} = \text{unknown} \)

**Protocol:**
- 60 second intervals with 3 minutes rest at max effort
- Pedaled backward during rest period
- Workload = 4.1 kg/6.0% body weight

**Results/Comments:**
- Subject voluntarily stopped after 24 minutes (6 intervals)
- rpm range = 130-95
  - Felt nauseous and vomited
  - Legs very tired – shaking after interval 2
  - Breathing recovered fully during rest

**Subject 8**

**Characteristics:**
- Female – 66 kg
- Well-trained cyclist/triathlete
- \( \text{VO}_2\text{peak} = 54 \text{ ml·kg}^{-1}·\text{min}^{-1} \) (approximate based on what she said her training was and her VO2peak used to be)

**Protocol:**
- **Trial 1**
  - 130\% \( \text{VO}_2\text{peak} \) for 60 second intervals with 3 minutes rest
  - Workload = 4.0 kg/6.0% body weight
  - Rest was active pedaling at subject’s choice with no resistance
Trial 2
Same protocol after glycogen depletion ride of 80 minutes at 70% VO2peak with
4 max effort 60 second sprints and a H-CHO diet

Trial 3
Same protocol after glycogen depletion ride of 80 minutes at 70% VO2peak with
4 max effort 60 second sprints and a L-CHO diet

Results/Comments:
Subject completed 17 intervals in trial 1, 23 in trial 2, and 16 in trial 3
Felt lethargic and thirsty on L-CHO diet

Subject 9
Characteristics:
Male – 70 kg
Well-trained cyclist
VO2peak = 66 ml·kg⁻¹·min⁻¹ (recently measured in our lab on his bike)

Protocol:
125% VO2peak for 60 second intervals with 3 minutes rest – max effort
Rest was active pedaling at subject’s choice with no resistance

Results/Comments:
Completed 24 intervals (fatigue = 30% decrease in rpm)
Felt great throughout – appeared to be pacing
REFERENCES


78. Oba, T., M. Koshita, and M. Yamaguchi. H$_2$O$_2$ modulates twitch tension and increases P$_o$ of Ca$^{2+}$ release channel in frog skeletal muscle. Am. J. Physiol. 271(Cell Physiology): C810-C818, 1996.


Vita

Michelle Rene Smith was born on March 29, 1975 in Baltimore, Maryland. She graduated Summa Cum Laude from Virginia Tech in 1997, earning a Bachelor of Science degree in Dietetics. While pursuing a Master of Science degree in Human Nutrition, Foods & Exercise, her concentration area was Nutrition for Sports and Chronic Disease. Her future plans include a dietetic internship at the National Institutes of Health.