Aspects of weight management in NCAA Division III collegiate wrestlers

Matthew Martone B.S., CSCS

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Aspects of weight management in NCAA Division III collegiate wrestlers

by

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Submitted in Partial Fulfillment of the
Requirements for the Master of Science in Exercise Science Degree

Kinesiology Department

STATE UNIVERSITY OF NEW YORK COLLEGE AT CORTLAND

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Objective: The purpose of this study was to observe the weight management habits of division III collegiate wrestlers during the week leading up to a competition. Methods: Subjects reported to the lab on Monday morning after an overnight fast. Subjects were measured for height and weight and were asked to provide urine and blood samples for analysis of hydration and creatine kinase activity, respectively. Throughout the week, subjects were weighed in and out of practice to monitor daily weight fluctuations. Subjects also self-recorded all food and drink consumption throughout the 5-day period. On Saturday morning, approximately 1 hour before weigh-ins for the competition, subjects reported to the lab after an overnight fast. They were measured for body weight and provided a second set of urine and blood samples. Results: Four subjects participated in the study while only two completed all aspects of the study. Subject 1 intended to lose over 7 kg in the 5-day period, however ended up dropping out of the study due to illness. Subject 3 lost roughly 2 kg throughout the week and induced severe dehydration and increases in creatine kinase activity, suggesting increased skeletal muscle damage. This subject also self-reported an average daily caloric intake of 1180 Kcals. Subject 4 lost roughly 1 kg throughout the study. This subject also induced severe dehydration but was able to reduce his creatine kinase activity, suggesting recovery of skeletal muscle. He self-reported eating an average caloric intake of 1635 Kcals. Subject 5 did not have to manage his weight. He showed low values of creatine kinase activity and did not induce severe dehydration during the 5-day period. Conclusion: Subjects who were consciously managing their weight experienced severe dehydration and appeared to be in a severe caloric deficit, even though they did not lose significant percentages of total body weight as compared to findings from other studies.
ACKNOWLEDGEMENTS

This research project was inspired by my experiences as a collegiate wrestler. Wrestling has been a defining part of my life and has shaped me into who I am today. The skills I gained as an athlete have given me the ability and the work ethic to begin and see through a project of this nature. This study has expanded the boundaries of research conducted at SUNY Cortland and I take pride in leaving this institution having made an impact.

I would like to thank my thesis committee: Dr. Phil Buckenmeyer, Dr. Larissa True, Dr. Ryan Fiddler, and Dr. Katherine Hicks. Without the support of these individuals, this project would not have been possible. Your support has given me the confidence to be ambitious and strive to exceed expectations.

To the Kinesiology faculty/staff: Your continued support over the course of my undergraduate and graduate studies have been instrumental to my success and ultimately my desire to pursue a doctorate within the field. I can only hope to inspire my future students the same way you have inspired me.
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CHAPTER 1
SUMMARY

Introduction

Rapid weight loss or “weight cutting” has been a topic of concern since the death of three collegiate wrestlers in 1997. Rapid weight loss (RWL) in wrestling is the act of aggressively reducing body weight over a short period of time before a competition with the intention of regaining the weight over the next 24-48 hours. The most common methods of RWL include dietary restriction through fasting and dehydration via exercise-induced sweating while abstaining from water consumption.

Since there is no professional league for wrestling, many athletes that compete for the national and Olympic teams come from collegiate programs and can be considered elite level athletes and train as such. Intense training requires sufficient nutritional intake for recovery and growth; however, it is unlikely that these athletes receive said nutritional intake. The collegiate season can last up to 5 months for those who make it to the national tournament and compete roughly once every 7 days. This process of losing and gaining weight repeatedly over the course of a season is known as weight cycling and has been shown to disrupt normal metabolic function (Steen & Brownell, 1990).

With the lack of recovery time and minimal nutrients, skeletal muscle damage has been a topic of many studies within this population. Creatine kinase (CK) is an enzyme that is used as an indirect biomarker of skeletal muscle damage. A study investigating skeletal muscle damage in wrestlers (Ozkan & Ibrahim, 2016) examined CK and correlated its concentration in the blood to percentage of total body weight lost. The next step for research on this topic is to determine if increased muscle damage caused from training and weight
management habits influences an athlete’s risk for injury. This would be evidence that diversion of proteins from their primary role of recovery and rebuilding to one of energy synthesis increases the risk for injury. According to the NCAA, wrestlers have the highest rate of injury at 13.1 per 1000 participants with nearly 50% of those injuries withholding the athlete from full participation for at least 7 days (Kerr et al., 2015).

Statement of the Problem

Wrestlers are sustaining significant injury at a much higher rate than the rest of the collegiate athlete population with the most common injuries being to muscle and connective tissue (Kerr et al., 2015). They are also the only NCAA sport to be subject to weight classes and subsequently have athletes engaging in RWL.

Purpose of the Study

The purpose of this study was to observe the dietary and training habits of collegiate wrestlers and examine the association between weight loss and muscle damage.

Hypotheses

Collegiate wrestlers who engage in RWL will experience greater amount of muscle damage as indicated by increased CK activity.

Collegiate wrestlers who lose a greater percentage of body weight will experience greater muscle damage; indicated by increased CK activity.

Delimitations

The following were delimitations of the study:

1. Only men were observed in this study.

2. Subjects had 5 days to lose 6% of normal body weight to mimic a 1-week weight cut before competition.
Limitations

The following were limitations of the study:

1. Since this study was conducted towards the end of the collegiate season, wrestlers had already been weight cycling for 2 months. It is possible that the body underwent adaptations to prevent further muscle damage and reduce the effect of weight cutting on structural integrity.

2. The methods of weight cutting were at the discretion of the subjects; these methods were not directly monitored.

Assumptions

The following assumptions were made:

1. Participants accurately recorded food/drink consumption.

2. All individuals experienced a similar intensity of training during practices/lifts.

3. Participants used only legal methods to cut weight.

4. All equipment was calibrated and functioned properly.

Definition of Terms

Connective Tissue Damage  A loss in structural integrity of the structural proteins in and around muscle fibers, tendons, and ligaments

Creatine Kinase  An enzyme found in the muscle cell that facilitates a metabolic process. Has been used as an indirect marker of muscle damage.

Dehydration  The process of losing or removing water or moisture

Dietary Restriction  The practice of limiting caloric and nutritional consumption.

Energy Deficit  When the number of calories expended is greater than the amount consumed
**Erythropoiesis**  The production of red blood cells

**Gluconeogenesis**  The creation of glycogen from a non-carbohydrate source

**Hemolysis**  The breakdown of red blood cells

**Lean Body Mass**  A combination of all body mass with the exception of fat mass

**Muscle Damage**  A loss of structural integrity of muscle cells

**Proteolysis**  The process of breaking down proteins or amino acids

**Weight Cutting**  Rapid weight loss over a short period of time

**Weight Cycling**  The process of cyclically gaining and losing weight repeatedly over a period of time

**Significance of the Study**

This study was one of few to examine the association between percentage of body weight lost and indicators of muscle damage in a collegiate wrestling population. While statistical significance was not possible due to the limitations of the study, qualitative analysis of the few subjects who completed the study provided insight to the extreme dietary restrictions that these athletes impose on themselves. From this, it would be recommended that further research be dedicated to the dietary habits and restrictions that are found within this population.
CHAPTER 2
REVIEW OF LITERATURE

Introduction

This review of literature will provide a brief background on RWL, the physiological requirements of wrestling, muscle damage, and the effects of short-term nutrient deficiencies.

Wrestling is a physically demanding sport. At the collegiate level, a seven-minute match against a skilled opponent will be a fatiguing event. With the added stresses of potentially cutting 10-20 pounds of body weight (Steen & Brownell, 1990), the body is pushed to its limits. RWL consists of dietary restriction, dehydration, and increased physical activity to induce sweating. The process of RWL leaves the body in a negative energy balance and dehydrated state. It is also performed multiple times throughout a single season, so the body does not have the opportunity to rest and recover.

A collegiate wrestling season can last up to five months and consist of 15-20 competitions for which an athlete must weigh in. Most collegiate programs will have practice 5-6 days per week along with additional sessions for resistance training. It is safe to assume that collegiate programs practice and train at a high intensity. For roughly 16 weeks, the body is forced to perform at exceptionally high levels fueled by minimal nutrients, including water.

It is the belief of the researcher that compounding of degrading activities such as resistance training and RWL weaken the structural integrity of muscle fibers, thus increasing the risk for serious injury. This weakening due to insufficient recovery and repeated high intensity training sessions will produce increases in CK levels, which indirectly indicates
damage to the muscle. This claim is supported by Carbone et al., (2014), who found that negative energy balance and exercise produced increased total body proteolysis.

**Physiology of Wrestling**

To some, wrestling is considered the most physically demanding sport in the world. It requires strength, power, endurance, speed, technique and mental fortitude to win in wrestling. While wrestling is considered an anaerobic sport, athletes must have a moderate level of aerobic training. Horswill (1992) found average VO$_2$ max to be between 50-60 ml·kg$^{-1}$·min$^{-1}$ for collegiate wrestlers. This is higher than untrained individuals, but lower than aerobically-trained individuals in the same age range (Horswill, 1992). By partaking in some aerobic training, wrestlers become more efficient at aerobic metabolism.

Combat sports have bouts of frequent, quick, high-intensity action between longer periods of continuous motion and positioning. These high intensity moments label wrestling as an anaerobic sport. Wrestlers will train for speed, power, and strength in order to capitalize on these opportunities. This training also makes them more efficient at anaerobic metabolism, which is relied on during high intensity activity. Iide et al. (2008) showed similar physiological requirements when looking at simulated karate sparring.

**Rapid Weight Loss**

Rapid weight loss, or weight-cutting, is a part of any sport that uses weight classes to separate athletes. Coaches and athletes alike believe that competing at a lower weight class will give them an advantage over the competition. Roughly 60% of all athletes who participate in combat sports go through some form of weight cutting (Gann, Tinsley, & La Bounty, 2015). Weight cutting is prevalent at most levels of competition, beginning as early as secondary school (Berkovich, Eliakim, Nemet, Stark, & Sinai, 2016). The National
Collegiate Athletic Association (NCAA) has banned several unsafe methods of weight cutting such as diuretics, sweat suits, and saunas (Schnirring, 1998). The most common legal methods of weight cutting include dietary restriction, dehydration, and increased training. The process of losing and regaining weight is known as weight cycling and can suppress resting metabolic levels (Horswill, 1992). With seasons lasting months, wrestlers may weight cut as many as 15 times a season (Steen & Brownell, 1990).

Dietary restriction is a method of weight loss where an individual drastically limits caloric and nutrient intake to reduce the possibility of weight gain. In combination with a continued high level of training, it imposes an energy deficit on the body. Training in an energy deficit has shown to lead to significant losses in both body fat mass and lean body mass (Carbone et al., 2014). When the body is limited on primary sources of energy, usually glucose or its storage form glycogen, it looks to break down other nutrients to resynthesize ATP. The secondary nutrient utilized for energy is fat, found in adipose tissue. Lipolysis is the breakdown of triglycerides to be used for energy. However, wrestlers tend to be very lean, having average body fat percentages ranging from 6% to 12.8% (Kordi et al., 2012). While triglycerides can provide energy for the body during periods of rest and low-intensity activity, fatty acid oxidation cannot keep up with the demands from a high intensity wrestling practice. If fat cannot be utilized, the body looks to break down amino acids as a fuel source. If amino acids are being utilized as a fuel source, then they cannot perform their primary function of repairing and building muscle and collagen. Proteolysis is increased during an energy deficit (Carbone et al. 2014; Horswill 1992). However, other studies have shown that increasing protein intake during caloric restriction and energy deficit may reduce the impact of utilizing amino acids as a fuel source (Helms, Zinn, Rowlands, & Brown, 2014). Overall,
dietary restriction in wrestlers results in training in negative energy balance, stimulating proteolysis, which is an inefficient method of metabolism and also diverts proteins from their primary task.

Dehydration under any circumstance is unhealthy for the body; however, athletes will intentionally induce excessive sweating to lose water weight 48-72 hours before competition. A significant consequence of intentional dehydration is hemolysis and reduction of hemoglobin mass (Reljic, Feist, Jost, Kieser, & Friedmann-Bette, 2016; Yankanich, Kenney, Fleck, & Kraemer, 1998). Hemoglobin is the carrier of oxygen in arterial blood and vital in the exchange of gases at the cellular level. With a reduced capacity to transport oxygen to working muscles, there can only be a decrease in athletic performance and delay recovery (Kraft et al., 2012; Pallares et al., 2016). Linger and Olson (2010) tracked fluid and dietary patterns over the course of a season and found that 92% of athletes participated in some form of intentional dehydration to lose weight for competition, whether it be induced sweats or restricting fluids. With a high percentage of athletes competing in a dehydrated state, it is assumed that they are performing at a sub-optimal level. Klinzing and Karpowicz (1986) support this assumption, showing that dehydration even after attempted rehydration can cause poorer performance. Throughout a season, the process of dehydration and rehydration cycles along with fluctuations in body weight. Hydration levels are shown to be low throughout the season and do not return to normal until roughly two weeks after the end of the season (Benoist, Schutter, Arabas, & Mayhew, 2014). Continuous dehydration and the resulting negative physiological responses ultimately leave wrestlers in a “deprived” state.

Collegiate training regimens are intense. During practice, athletes look to perfect their art, not necessarily cut weight. This means athletes look to add other training sessions
devoted to simply losing weight. Lingor and Olson (2010) showed that 69% of wrestlers partake in some form of strength training and 92% partake in some form of exercise to lose weight along with regular practice schedules. In all studies that simulated weight cutting for competition, all used some form of physical activity to induce the weight loss along with either fluid or dietary restriction.

**Muscle Damage**

Skeletal muscle damage has been well-documented as an effect of high intensity exercise (Brown et al. 1997; Tofas et al., 2008; Virtanen, Viitasalo, Vuori, Vaananen, & Takala, 1993). When the muscle sustains damage, enzymes leak into the blood stream. This has been attributed to either structural damage to the cell membrane or a metabolic effect increasing membrane permeability, allowing more enzymes to secrete into the blood stream (Armstrong, 1990).

Different modes of exercise have been shown to stimulate varying degrees of muscle damage. Virtanen et al. (1993) showed evidence that concentric exercise can stimulate increased muscle breakdown and Tofas et al. (2008) did the same with plyometric exercises. Brown et al. (1997) and Brown et al. (1999) found indirect evidence of muscle damage and collagen breakdown following 50 maximal eccentric voluntary contractions on an isokinetic dynamometer. Clarkson and Tremblay. (1988) found evidence of muscle damage following eccentric exercise focusing on the forearm flexors. Armstrong et al. (1983) looked at muscle damage from downhill running in rats and found that the eccentric-focused contractions involved in controlling speed were responsible for significant increases in biomarkers used to indirectly measure muscle breakdown. In untrained individuals, it appears that eccentric-
focused exercise stimulates higher amounts of muscle breakdown than concentric exercise (Brown et al., 1999).

Researchers can measure muscle breakdown directly with a muscle biopsy or indirectly by measuring enzyme activity. Creatine kinase (CK) has been used as a biomarker to indirectly measure skeletal muscle damage (Armstrong et al., 1983; Brown et al., 1997; Brown et al., 1999; Clarkson & Tremblay, 1988; De Castro et al., 2011; Jones, Newham, Round, & Tolfree, 1986; Karamizrak et al., 1994; Ozkan & Ibrahim, 2016; Tofas et al., 2008; Virtanen et al., 1993).

While exercise can cause increases in serum enzyme levels, it has been shown that the response can be diminished as an adaptation of training. Clarkson and Tremblay. (1988) provided evidence that even low-intensity training will produce an adaptation; there will be a less significant increase in serum enzyme levels if the same exercise is performed at a higher intensity. This has come to be known as the “repeated bouts” effect. Karamizrak et al. (1994) looked at short-term supramaximal workloads and found that athletes did not have significant increases in serum enzyme levels compared to their sedentary control subjects who did see a significant increase in serum enzyme levels. The athletes, however, did have higher baseline serum CK levels that exceeded the controls’ pre- and post-exercise values. With rest, serum creatine kinase levels return to pre-exercise levels. De Castro et al. (2011) studied muscle recovery by monitoring serum CK levels. They found that following a full body workout, CK levels were still elevated 72 hours post-exercise. Brown et al. (1999) also noted significantly increased CK levels up to seven days post- eccentric-focused exercise. The time required for serum CK levels to return to normal levels appears to be dependent on exercise intensity and modality.
**Conclusion**

Research on collegiate wrestlers’ weight-loss habits have found significant results in regards to hydration, energy consumption, body composition, blood volume, and percentage of body weight lost; however, there is no research investigating muscle damage within this population. Research on this topic has been limited to athletes at the national and international level, which have different rules on methods of weight-loss. To fill this gap in the literature, this study used creatine kinase activity, a widely used biomarker, to indirectly measure skeletal muscle damage sustained by collegiate athletes in preparation for a competition.
CHAPTER 3

RESEARCH BRIEF

Subject Information

Subjects were recruited from an NCAA Division III collegiate varsity wrestling team. Inclusion criteria included:

- Not sustained any significant muscular injury that has withheld them from participation for one week for the past six months
- Must weigh more than 110 pounds
- Must be male

Subjects were also required to fill out a subject eligibility questionnaire (Appendix C) to determine suitability for the study. This questionnaire confirmed that subjects met the inclusion criteria and also asked their comfort level with having blood drawn for the study.

Methods

Subjects were observed over a 5-day period leading up to a post-season competition. After approval from the IRB (Appendix A), subjects completed necessary documents including informed consent (Appendix B), a subject eligibility questionnaire (Appendix C), and Par-Q (Appendix D). Those who met the inclusion criteria were measured for body composition using 3-site skinfold measurements at the triceps, abdomen, and subscapular.

Subjects reported to the lab on the morning of Day 1 after an overnight fast. Subjects’ height and weight were measured. They then provided a urine and blood sample for analysis of hydration and CK, respectively. Hydration was measured using urine samples after an overnight fast. Samples were analyzed using a pocket refractometer (Atago PAL-10S, Bellevue, WA, USA). CK was measured using an enzyme kit (Sigma-Aldrich MAK-116, St.
Louis, MO, USA). Subjects were instructed to keep a daily journal of all food and drink consumption and to not change any training or weight management habits. Throughout the week, subjects were weighed in and out of daily practices by the researcher to track weight fluctuations throughout the week. The scale used was a Befour PS6600 (Befour, Saukville, WI, USA).

On day 6, the morning of competition, subjects reported to the lab at a similar time roughly one hour before weigh-ins. Body weight was measured and subjects were asked to provide another urine and blood sample for analysis.

**Nutritional Analysis**

The nutritional analysis was done using Nutritics, an online software for nutritional analysis. Analysis consisted of total caloric and macronutrient consumption. These data were then compared to data sets from another study (Sagayama et al., 2017) to estimate caloric balance. Subjects were asked to provide information on food they consumed including type of food, number of servings, and brands of food if possible.

**Statistical Analysis**

Due to a low number of participants, it was not possible to run statistical analysis on these data. Data from this study were qualitatively analyzed by comparison to other studies examining the same outcome variables with similar subjects.
CHAPTER 4

RESULTS

Four subjects initially started the study \((n = 4)\), while only two subjects completed the study. One subject dropped out due to illness on the fourth day and another was unable to provide a post-one week blood sample. Anthropometric data is presented in Table 1. Pre- and post-one week weights, hydration status, and creatine kinase activity levels are reported in Table 2. Daily weight fluctuations, including weights pre/post practice are included and reported in Table 3. These daily weight fluctuations were further viewed in relation to subject target wrestling weights (Figure 1). CK activity levels were measured in triplicate and the average taken. Macronutrient deficiencies were determined based on daily recommended values by the American College of Sports Medicine (American College of Sports Medicine, 2014). Micronutrient deficiencies were determined by comparison to recommended nutritional intake for adults by the World Health Organization (World Health Organization, 2004).

Table 1.

Anthropometric Data for All Subjects

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.74</td>
<td>1.7</td>
<td>1.74</td>
<td>1.93</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.30</td>
<td>65.70</td>
<td>61.20</td>
<td>128.8</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>15.7</td>
<td>11.4</td>
<td>11.1</td>
<td>28.8</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>69.7</td>
<td>58.2</td>
<td>54.4</td>
<td>91.7</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>12.6</td>
<td>7.50</td>
<td>6.80</td>
<td>37.1</td>
</tr>
</tbody>
</table>
Table 2.

Pre- and Post-One Week Data for Weight, Hydration, and Creatine Kinase Activity

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Pre-Weight</th>
<th>Post-Weight</th>
<th>Pre-Hydration</th>
<th>Post-Hydration</th>
<th>Pre- CK Activity</th>
<th>Post- CK Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>82.3</td>
<td>X</td>
<td>1.012</td>
<td>X</td>
<td>120</td>
<td>X</td>
</tr>
<tr>
<td>Subject 3</td>
<td>65.7</td>
<td>64</td>
<td>1.021</td>
<td>1.027</td>
<td>64.1</td>
<td>120</td>
</tr>
<tr>
<td>Subject 4</td>
<td>61.2</td>
<td>60.2</td>
<td>1.021</td>
<td>1.03</td>
<td>97.5</td>
<td>75.5</td>
</tr>
<tr>
<td>Subject 5</td>
<td>128.8</td>
<td>127.1</td>
<td>1.021</td>
<td>1.021</td>
<td>32.3</td>
<td>X</td>
</tr>
</tbody>
</table>

Note. Weights are in kg. Hydration is measured by specific gravity. CK activity is measured in U/L.

Table 3.

Weight Fluctuations 5-Days Pre-Competition Before and After Practice

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Mon In</th>
<th>Mon Out</th>
<th>Tues In</th>
<th>Tues Out</th>
<th>Wed In</th>
<th>Wed Out</th>
<th>Thurs In</th>
<th>Thurs Out</th>
<th>Fri In</th>
<th>Fri Out</th>
<th>Sat In</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81.0</td>
<td>79.4</td>
<td>79.6</td>
<td>77.7</td>
<td>77.6</td>
<td>75.3</td>
<td>77.1</td>
<td>76</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>66.2</td>
<td>64.9</td>
<td>65.7</td>
<td>64.2</td>
<td>65.4</td>
<td>63.9</td>
<td>66.1</td>
<td>64.5</td>
<td>65.2</td>
<td>63.6</td>
<td>64.0</td>
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<td>61.2</td>
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<td>128.5</td>
<td>127.9</td>
<td>128.3</td>
<td>127.6</td>
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<td>127.7</td>
<td>127.5</td>
<td>127.4</td>
<td>127.8</td>
<td>127.1</td>
<td></td>
</tr>
</tbody>
</table>

Note: Weights are in kg.

Subject 1

Subject 1 began the study 7.3 kg over his target weight class. Pre-week hydration analysis showed he was in a hydrated state. His pre-week CK activity levels were elevated relative to his teammates, indicating greater muscle damage. Over four days, this subject lost 5 kg, roughly 6% of his body weight. Subject 1 dropped out of the study due to illness. No post-week data or diet journal was collected.
Subject 3 reported on the first day 1.6 kg over his intended weight class. Subject was dehydrated but did not show evidence of elevated creatine kinase activity levels. This subject showed progress in reducing his body weight gradually over the first three days of the study, however was almost back to his pre-one week weight on Thursday weighing in to practice. At the end of the week, the subject submitted a 5-day diet journal which was analyzed for macro and micronutrient intake (Table 3). The subject averaged less than 1200 kcals·day$^{-1}$ and only 60 grams·day$^{-1}$ of protein. Micronutrient analysis showed he met his mineral requirements, but was deficient in vitamins A, D, K, C, B$_1$, B$_2$, B$_3$, B$_5$, B$_6$, and B$_7$. The morning of competition, the subject provided urine and blood samples for analysis of hydration and creatine kinase activity levels, which indicated severe dehydration and an 87% increase in creatine kinase activity levels from pre-week measurements. This indicates that the subject likely used both caloric restriction and severe dehydration in order to reduce his weight down to his target weight class. It also shows that he experienced an increase in muscle damage as indicated by his increased creatine kinase activity levels.
Table 4.

Nutritional Analysis for Subject 3

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
<th>Kcals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>142 g</td>
<td>568</td>
</tr>
<tr>
<td>Fats</td>
<td>38 g</td>
<td>342</td>
</tr>
<tr>
<td>Proteins</td>
<td>60 g</td>
<td>240</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.35 g</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>927 mg</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>636 mg</td>
<td></td>
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<tr>
<td>Phosphorus</td>
<td>542 mg</td>
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</tr>
<tr>
<td>Magnesium</td>
<td>103 mg</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>7.3 mg</td>
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</tr>
<tr>
<td>Zinc</td>
<td>4.1 mg</td>
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</tr>
<tr>
<td>Vitamin D</td>
<td>2.6 µg</td>
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</tr>
<tr>
<td>Thiamin B1</td>
<td>.45 mg</td>
<td></td>
</tr>
<tr>
<td>Riboflavin B2</td>
<td>.71 mg</td>
<td></td>
</tr>
<tr>
<td>Niacin B3</td>
<td>0 mg</td>
<td></td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>.45 mg</td>
<td></td>
</tr>
<tr>
<td>Biotin B7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>1.5 µg</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.8 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Total Kcals</strong></td>
<td></td>
<td><strong>1180</strong></td>
</tr>
</tbody>
</table>

Subject 4

Subject 4 reported to the lab less than 1 kg over his intended weight class. He informed researchers that he typically wrestles at the weight class below but moved up in order to fill the lineup for the team. His pre-week hydration analysis showed moderate dehydration and creatine kinase activity levels showed evidence of elevated values, suggesting sustained muscle damage. Weight fluctuations were recorded daily throughout the week and showed that he gradually maintained his weight throughout the week and naturally made weight on the morning of the competition. At the end of the week, the subject submitted a self-recorded diet journal. This subject averaged roughly 1600 calories·day⁻¹ and
72 grams·day$^{-1}$ of protein. He did meet his daily recommended values for most micronutrients, however was deficient in vitamins D, K, B$_3$, and B$_7$. On the morning of competition, the subject reported for post-one week data collection and provided a urine and blood sample for analysis. Subject was severely dehydrated but showed a 22% reduction in levels of CK activity levels from pre-week assessments. These data suggest he underwent caloric restriction and dehydration to manage his weight but was able to reduce his CK activity levels from pre-week values.

**Table 5.**

Nutritional Analysis for Subject 4

<table>
<thead>
<tr>
<th>Nutrition</th>
<th>Amount</th>
<th>Kcals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>202 g</td>
<td>808</td>
</tr>
<tr>
<td>Fats</td>
<td>57 g</td>
<td>513</td>
</tr>
<tr>
<td>Proteins</td>
<td>72 g</td>
<td>288</td>
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<tr>
<td>Sodium</td>
<td>1.62 g</td>
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<tr>
<td>Potassium</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Phosphorus</td>
<td>1141 mg</td>
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<tr>
<td>Magnesium</td>
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<tr>
<td>Iron</td>
<td>12.5 mg</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>11.7 mg</td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1.3 µg</td>
<td></td>
</tr>
<tr>
<td>Thiamin B1</td>
<td>1.2 mg</td>
<td></td>
</tr>
<tr>
<td>Riboflavin B2</td>
<td>1.4 mg</td>
<td></td>
</tr>
<tr>
<td>Niacin B3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>1.9 mg</td>
<td></td>
</tr>
<tr>
<td>Biotin B7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>4.8 µg</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>146 mg</td>
<td></td>
</tr>
<tr>
<td><strong>Total Kcals</strong></td>
<td><strong>1635</strong></td>
<td></td>
</tr>
</tbody>
</table>
Subject 5

Subject 5 reported to the first day of the study roughly 0.7 kg underweight. Heavy weights are unique in wrestling since they do not have to manage their weight due to the high weight limit. Pre-week hydration analysis showed he was moderately dehydrated and CK activity levels were not elevated. This subject did not appear to have issues with managing his weight throughout the week and even weighed in for the competition 2.4 kg underweight. His weight fluctuations were monitored daily throughout the week and these data are reported in Table 2. He submitted a 5-day diet journal; however, his dietary habits were not analyzed. The morning of competition, the subject provided a urine sample for hydration analysis, but was unable to provide a blood sample. Subject maintained his level of hydration from pre- to post- one week. He did not provide a post-one week blood sample so CK activity was not analyzed for this time point.

Figure 1. Daily weight fluctuations in relation to target weight.
CHAPTER 5
DISCUSSION

Rapid weight loss and weight management is a critical part of competing in the sport of wrestling. Its prevalence at different levels of the sport show that the practice is introduced at a young age and seemingly encouraged as the level of competition increases up to the elite level. From a theoretical perspective, the hypocaloric state and dehydration observed in this study can lead to severe problems with major physiological processes including erythropoiesis, protein synthesis, and metabolism. Inhibition of these processes can lead to decreased performance and increased risk of serious injury in these athletes.

The weight loss patterns in this study have been described by other studies examining weight loss patterns in elite wrestlers. Yankanich et al. (1998) showed subjects losing 6% of body weight implemented a variety of weight loss habits. The three weight loss methods that they devised from their study were gradual, moderate, and rapid. Their results showed that individuals who lost weight gradually over a longer period of time were able to lose more weight when compared to the “rapid” group, who aimed to lose the majority of their weight in the last 24 hours. Ozkan and Ibrahim. (2016) showed the prevalence in weight cutting at the elite level. Of the 72 subjects observed, 29 had lost at least 3% of their natural body weight and 31 were dehydrated. Lingor and Olson. (2010) observed caloric and fluid restriction in collegiate wrestlers. They found an average weekly weight loss of 4.7% of natural body weight. Caloric intake ranged during the week from 4000kcal·day\(^{-1}\) to 874 kcal·day\(^{-1}\) while fluid intake dropped by nearly 50% from the beginning to end of the week.

The current study presents findings on weight loss habits that align with other research on the
topic. From this, it can be concluded that collegiate wrestlers, regardless of division, appear to use similar weight cutting practices in order to make weight for competition.

The creatine kinase activity levels found in this study correlate well to results found in other literature. Ozkan and Ibrahim. (2016) correlated dehydration and weight loss to CK levels in elite wrestlers. Their study found a direct relationship between percent body weight lost and CK levels. Individuals in that study who lost less than 1% of body weight showed an average CK value of 191.6 while individuals who lost 1-3% averaged 214.4 U/L. Subjects 3 and 4, who provided post-one week blood samples for CK analysis, were found to be well below each of the averages. A possible explanation for this is the “repeated bouts” effect. Since collegiate wrestlers undergo weight cycling on a weekly basis, compared to elite level wrestlers who do not have weekly competitions, their bodies may have produced an adaptation in order to control muscle damage and reduce the effects of their training and weight loss habits on physiological markers. This phenomenon has been shown by Clarkson and Tremblay. (1988). Their study showed that exposing muscles to a stress will reduce the damaging effects of later bouts of that same exercise or stress.

Hydration levels found in this study also translate well into other literature. Yankanich et al. (1998) examined pre-competition weight loss and vascular fluid volume changes in Division I wrestlers. Hydration was monitored through changes in blood fluid volumes and expressed in percent change in osmolality. While there were no significant differences in osmolality from pre- to post- one week weight reduction, there were significant changes in total blood volume, indicating dehydration. It is worth noting that their post-one week weight reduction data collection took place 15 hours after weigh-ins, where individuals had already begun to regain some of their lost body weight as compared to the current study
which collected samples one hour before weigh-ins. Another study that aligns with the findings of the current study was conducted by Lingor and Olson. (2010). Their study looked at fluid and diet patterns over the course of a season. Their findings show that wrestlers within the 24 hours of weigh-ins will drastically reduce fluid intake. Significant dehydration, measured by urine osmolality (Uosm), showed that subjects met the standard for significant dehydration (>860 mOsm·kg⁻¹) within the 48-hour window pre-competition. The correlation between osmolality and urine specific gravity was calculated by Lingor and Olson. (2010) and had an R² value of .9381. A measurement of 800 mOsm·kg⁻¹ translates roughly to a urine specific gravity of 1.020. From this, it is apparent that the subjects in the current study who had urine specific gravity values above 1.020 were significantly dehydrated.

The analysis of the self-reported diet journals showed evidence of a hypocaloric state with deficiencies in both macronutrient and micronutrient intake. Sagayama et al. (2017) examined energy requirements in Japanese collegiate wrestlers. Anthropometric data on the subjects was similar to subjects 3 and 4 from the current study. Reported training frequency for these individuals was similar to the training regimen for the current study. The researchers calculated total energy expenditures (3901 Kcal·day⁻¹) using the doubly- labeled water method. Energy intake was calculated using self-recorded food surveys and visual records using a camera. Energy intake for the lightweight subjects was found to be an average of 3260 kcal/day. Comparing the findings of this study to the current one, there is a large discrepancy in caloric intake between the two. While the Japanese wrestlers were in a caloric deficit, they were still able to cover basal metabolic costs and appeared to be well-nourished. Their daily caloric intake averaged 3260 Kcals·day⁻¹. In the current study, subjects 3 and 4, whose self-recorded food journals were analyzed, found they consumed roughly half
the calories of their Japanese counterparts. Assuming that their training was similar, it should have a similar caloric cost and both groups should have relatively similar total energy expenditures and costs. A plausible explanation for the findings of the current study is the inaccuracy of reporting food intake by the subjects. Sagayama et al. (2017) also address this topic, explaining their belief that self-recorded figures could be inaccurate by 25% of caloric intake.

If the food journals were accurate for the current study, the subjects in question would have several nutrient deficiencies. The hypocaloric state and protein deficiency along with daily high intensity exercise will leave the body in a negative energy balance. Carbone et al. (2014) found increases in total body proteolysis as a result of short-term energy deficit. In that study, while caloric intake was reduced, subjects had higher than average protein intake (1.5 g·kg⁻¹·day⁻¹). In the current study, subjects 3 and 4 averaged roughly 1 g·kg⁻¹·day⁻¹ of protein. Carbone et al. (2014) come to the conclusion that while short-term energy deficit will increase whole-body proteolysis, extended periods of energy deficit will produce a protein-conserving adaptation, that will allow the body to protect muscle mass during extended periods of energy deficit.

Subjects 3 and 4 both exhibited micronutrient deficiencies. Some of the nutrients, including vitamins A, B, C, D, K, have important roles in maintaining the homeostasis of major physiological processes such as carbohydrate and fat metabolism, immune function, bone mineralization, muscle contractions, and nerve conduction (World Health Organization., 2004). While short-term deficiencies of these important vitamins may not lead to chronic illness, they can contribute to increase risk of acute sickness, decreased performance, and increased risk of injury.
These findings along with other studies show the potential detrimental effects and adaptations of rapid weight loss, dehydration, and negative energy balances. Even in situations of minimal weight loss shown in this study, the methods by which wrestlers manage their weight are putting them at risk for serious injury and health concerns.

**Conclusion**

NCAA Division III collegiate wrestlers are still utilizing popular methods of weight management and experience the same negative side-effects seen in other populations. Severe dehydration and malnutrition were seen in this study and the findings corroborate well with other research on this topic.

There are unfortunately many limitations to this study which prevent any statements on significant findings. Due to an elongated approval process for this study, its start date and timeframe were delayed by 9 weeks. This change in timeframe put a large limitation on the study. Since these athletes had been weight cycling for over two months and the physical adaptations of training having already occurred, any possible significant changes in outcome variables, specifically CK may have been affected. Another limitation is the number of subjects. It isn’t possible to find statistical significance with a cohort of four subjects. With the subject requirements of the study including blood draws the morning of a competition, it isn’t surprising to not have many subjects volunteer. Many athletes would rather focus on the competition rather than have obligations for a scientific study.

While this study was severely limited, it did have strong aspects to it. For starters, it was the first research project on the SUNY Cortland campus to include the analysis of human blood samples. Not only did it expand the research conducted on campus, but it directly involved multiple departments including Kinesiology, Biology, and Chemistry. It was
necessary to create a protocol for conducting blood draws on campus, so that future researchers would have a clear-cut procedure for organizing such a study. This protocol was approved by the Kinesiology Department and IRB and can be used by future students and staff as a guideline for conducting human blood-based research on campus. It also directly involved student-athletes at SUNY Cortland. They were able to be involved with research and receive results on the effects that their training and preparation had on their bodies.

**Future Research**

Future directions for research on weight management in wrestlers needs to consider better methods of measuring energy intake and caloric balance. The self-recorded diet journals received from subjects in this study were shocking, and if assumed to be accurate, would be indicative of extreme negative energy balances and possibly fall under the category of anorexia. Another direction for future research should look to create large data sets, in order to look for predictive measures of increased risk for injury. Thresholds for dehydration, severity of caloric deficit, and percentage of total body weight loss should be determined and findings could impact NCAA protocols and rules for weight management and weight class stratification.
List of References


Lingor, R. J., & Olson, A. (2010). Fluid and diet patterns associated with weight cycling and changes in body composition assessed by continuous monitoring throughout a college wrestling season. *Journal of Strength and Conditioning Research, 24*(7), 1763-1772.


MEMORANDUM

To: Matt Martone
Phil Buckenmeyer

From: Peter Ducey, Reviewer on behalf of
Institutional Review Board

Date: 12/20/2017

RE: Institutional Review Board Approval

In accordance with SUNY Cortland’s procedures for human research participant protections, the protocol referenced below has been approved for a period of one year:

Title of the study: The Association between Rapid Weight Loss and Muscle and Connective Tissue Damage in Collegiate Wrestlers

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<th>Level of review:</th>
<th>Expedited</th>
<th>Protocol number:</th>
<th>17824</th>
</tr>
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<tr>
<td>Project start date:</td>
<td>Upon IRB approval</td>
<td>Approval expiration date*:</td>
<td>12/19/2018</td>
</tr>
</tbody>
</table>

* Note: Please include the protocol expiration date to the bottom of your consent form and recruitment materials. For more information about continuation policies and procedures, visit www.cortland.edu/irb/Applications/continuations.html

The federal Office for Research Protections (OHRP) emphasizes that investigators play a crucial role in protecting the rights and welfare of human subjects and are responsible for carrying out sound ethical research consistent with research plans approved by an IRB. Along with meeting the specific requirements of a particular research study, investigators are responsible for ongoing requirements in the conduct of approved research that include, in summary:

- obtaining and documenting informed consent from the participants and/or from a legally authorized representative prior to the individuals’ participation in the research, unless these requirements have been waived by the IRB;
- obtaining prior approval from the IRB for any modifications of (or additions to) the previously approved research; this includes modifications to advertisements and other recruitment materials, changes to the informed consent or child assent, the study design and procedures, addition of research staff or student assistants, etc. (except those alterations necessary to eliminate apparent immediate hazards to subjects, which are then to be reported by email to irb@cortland.edu within three days);
- providing to the IRB prompt reports of any unanticipated problems involving risks to subjects or others;
- notifying the IRB of continued research under the approved protocol to keep the records active; and,
- maintaining records as required by the HHS regulations and NYS State law, for at least three years after completion of the study.
Appendix B

INFORMED CONSENT

You are invited to participate in a research project about the association between rapid weight loss and muscle/connective tissue damage in collegiate wrestlers. This research is being conducted by Matt Martone for his Master’s thesis project. The project is being supervised by Dr. Buckenmeyer, department chair of the kinesiology department. Your informed consent is requested if you wish to participate as a subject in this research project. Before you consent to participate, please read the following regarding the details of the study so that you fully understand what your involvement as a participant will be and what risks you may experience as a participant. If you have questions about anything related to the study or your involvement in the study, please ask.

Purpose and brief description of the study.

The purpose of this study is to determine the relationship between rapid weight loss and damage sustained to muscle and connective tissue in the days leading up to competition in collegiate wrestlers. This study will measure biological markers of hydration, muscle damage, connective tissue damage, and weight loss to assess the effect weight cutting has on the body when done during periods of intense training. Urine and blood samples will be collected at the beginning of the week leading up to a competition and the morning of the competition. Data will also be collected during the week to monitor weight loss patterns and habits.

Your involvement as a participant.

Should you choose to participate, you will be asked to complete a Par-Q and health questionnaire to determine if you meet the criteria for the study. If you meet the criteria and still wish to participate, your body composition will be measured using 3-site skinfold calculations. You will then be instructed to report to the lab on the morning of day 1 of the study after an overnight fast (no food or drinks after midnight. Water is permitted).

This study will be conducted over six (6) days. On the morning of day 1, you will be measured for height and body weight. You will also be asked to donate a urine sample and 10 mL blood sample. Once completed, you will be asked to keep a diet journal of your daily food/drink consumption, including types and number of servings of foods/beverages for the next 5 days, including that day.

Throughout the week, you will be asked to go about your normal routine and practices. The researcher will attend practice during the week to weigh you in and out of practice to monitor weight fluctuations.

The morning of day 6, the day of the competition, you will once again report to the lab after an overnight fast. You will have your body weight measured. You will once again be asked to donate a urine sample and one 10 mL blood sample.

This completes your requirements as a subject and you will be eligible for the participant incentive.
Blood samples will be drawn out of the vein in the forearm by a registered nurse. There is a total of two (2) blood draws; one on day 1 and one on day 6.

**Elimination Criteria:** Should you sustain an injury that withholds you from practice, miss practice, or do not weigh in for the competition, you will be eliminated from the study and your data will not be presented.

**Before agreeing to participate you should understand the following:**

- **Your participation is completely voluntary**
  
  You are free to withdraw from this study at any time without penalty.

- **Confidentiality**
  
  You will be assigned an identification code. This code will be used instead of your name when documenting data and labeling samples. All files will be kept on the researcher’s personal computer, which is password protected, or locked in the kinesiology office. Upon completion of the study, the key that links your name to your identification code will be destroyed.

  Blood samples will be kept in a freezer in a locked lab. Upon completion of the study, all remaining blood samples will be decontaminated and destroyed.

  The researcher, involved faculty, and research assistants will be the only people to have access to the data and samples.

  Group data will be presented. Any individual data that is highlighted will not include any information that could identify the subject.

- **Duration of participation**
  
  You will be recruited the week before the study begins. The study will last six (6) days.

- **Monetary or other reward for participation**
  
  Two pairs of wrestling shoes will be raffled off to individuals who complete the study. In order to be eligible for the raffle, you must complete all aspects of the study. This includes all blood draws, urine samples, and submitting 5 days of diet journals.

- **Risks**
  
  Risks associated with this study are minimal. There is risk associated with the blood draw, which include bruising, swelling, redness, and in rare cases infection. These risks are being minimized by hiring a registered nurse to perform the blood draws. You will receive a fresh needle for every blood draw.

- **Benefits**
  
  If you complete this study, you will receive the results from the blood analysis, which will indicate the amount of muscle or connective tissue damage you have sustained throughout the season and how the week’s weight cut and training influenced that damage. This information will be presented to you along with normative numbers, which will indicate if you are at a higher risk for sustaining a severe injury.

  On a larger scale, this research may produce evidence to show the detrimental effects of weight cutting in tangent with high intensity training.
• **Contact Information.** If you have any questions concerning the purpose or results of this study, you may contact Matt Martone by phone (585) 734-0842 or by email matthew.martone@cortland.edu. For questions or concerns about your rights as a research participant, contact the SUNY Cortland Institutional Review Board by email at irb@cortland.edu, or by phone 607-753-2511.

I ___________________________ have read the description of the project for which this consent is requested, I understand my rights, and I hereby consent to participate in this study.

_________________________________  ______________________________
Signature                                                      Date
Appendix C

**Subject Eligibility Questionnaire**

Name: __________________________

Email: __________________________

Are you a member of the SUNY Cortland varsity wrestling team: __________

What weight class do you intend to compete at this year: __________

Have you had a muscle or connective tissue injury in the past 6 months (injury that has withheld you from practice or physical activity for at least 1 week): ________________

(If yes, please explain in the space below)

Participation in this study will require you to provide two urine samples and two 10 mL blood samples. Are you comfortable in providing these samples:

__________________________
Appendix D

Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor. Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
2. Do you feel pain in your chest when you do physical activity?
3. In the past month, have you had chest pain when you were not doing physical activity?
4. Do you lose your balance because of dizziness or do you ever lose consciousness?
5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
7. Do you know of any other reason why you should not do physical activity?

If you answered YES to one or more questions:

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

If you answered NO to all questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

Delay becoming much more active:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.
I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

Name _________________________________  
Signature ______________________________  
Date_________________________________  
Signature of Parent ______________________  
Witness ______________________________  
or Guardian (for participants under the age of majority)
Appendix E

Table 6. Creatine Kinase Activity (U/L)

<table>
<thead>
<tr>
<th>CK activity (U/L)</th>
<th>Subject 1:</th>
<th>Subject 3:</th>
<th>Subject 3:</th>
<th>Subject 4:</th>
<th>Subject 4:</th>
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<td></td>
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<td>Pre</td>
<td>Post</td>
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<tr>
<td>Trial 1</td>
<td>118.93</td>
<td>60</td>
<td>114.11</td>
<td>94.82</td>
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<td>Trial 2</td>
<td>128.04</td>
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<td>121.07</td>
<td>95.89</td>
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<tr>
<td>Average (U/L)</td>
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<td>64.11</td>
<td>120</td>
<td>97.5</td>
<td>75.53</td>
<td>32.32</td>
</tr>
</tbody>
</table>

Figure 2. Pre-week creatine kinase activity for subject 1.

Figure 3. Creatine kinase activity for subject 3.
Figure 4. Creatine kinase activity for subject 4.

Figure 5. Creatine kinase activity for subject 5.
Figure 6. Example of blank well for creatine kinase activity.