The Effects of Acute Creatine Supplementation on Volume of Work and Anaerobic Performance in Vegetarians

by

Kamran Rahpymay-Rad

Honours Bachelor of Kinesiology, Lakehead University 1999
Bachelor of Education, Lakehead University 2001

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In the School of Exercise Science, Physical and Health Education

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Supervisory Committee

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Dr. John Anderson, (Department of Educational Psychology and Leadership Studies)
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Dr. Jason Brandenburg, (University College of Fraser Valley)
External Examiner
Abstract

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The purpose of this study was to examine the acute effects of five days of creatine supplementation on volume of work and anaerobic performance in vegetarian males and females. Twenty recreationally-active non-vegetarians (age 29.2 ± 9.6 yrs) and twelve vegetarians (age 28.0 ± 9.9 yrs) were tested on 5 x 10 RM seated chest press (total work) and 6 x 6 s all-out sprint (anaerobic performance) on a Monark cycle ergometer prior to and after five days of treatment. Treatment consisted of five grams of creatine monohydrate plus one gram of glucose or a placebo consisting of six grams of glucose dissolved in 250 ml of a warm liquid ingested four times per day for five days. Participants were divided in a double blind fashion to one of the four groups: non-vegetarians on creatine NVCr (n = 10); non-vegetarians on placebo NVPla (n = 10); vegetarians on creatine VCr (n = 6); and vegetarians on placebo VPla (n = 6). Significant
improvement was observed (p<0.05) in volume of work and anaerobic performance variables of peak power (PP), mean power (MP), anaerobic capacity (AC), and relative peak power (RPP) in NVCr and VCr. However, there was no significant difference between the vegetarians and non-vegetarians on Cr. Volume of work (total repetitions) also improved significantly in NVPla and VPla but to a lesser extent than the Cr groups. There was no significant change in anaerobic performance in the placebo groups. Furthermore, there was no significant main effect on anaerobic fatigue for any of the four groups. The results of this study indicate that acute creatine supplementation improved total volume of work and anaerobic performance in vegetarian and non-vegetarian participants to the same extent.
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Introduction

Creatine (Cr or phosphocreatine [PCr] or creatine phosphate) is a dietary non-essential nitrogenous based organic compound that has a vital role in energy production for the formation of adenosine triphosphate (ATP) through the Cr kinase reaction. In humans creatine is provided both endogenously and exogenously (Volek & Kraemer, 1996). Endogenously creatine is synthesized by the liver, pancreas, and kidney from the precursor amino acids of glycine, arginine and methionine (Snow & Murphy, 2003). Exogenously creatine is obtained from the diet by the ingestion of red meat or fish (Heymsfield, Arteaga, McManus, Smith, and Moffitt, 1983).

Delanghe et al. (1989) were the first to detect low serum and erythrocyte creatine levels in vegetarian males and females (p<0.01). However, these low creatine and creatinine levels do not necessarily mean decreased tissue content (Williams, Kreider & Branch, 1999). In a 70-kg (154-lb) man, the total creatine pool (free Cr + PCr) is approximately 120-125 mmol/kg dry weight (dw), of which 95% is stored in skeletal muscle and the remaining 5% is found in various tissues (Greenhaff, 1995). In their two vegetarian participants Harris, Soderlund and Hultman (1992) reported a normal range tissue creatine level of 114.6 to 120 mmol/kg (dw). Conversely, Burke et al. (2003) reported a lower tissue creatine in their vegetarian participants compared to the non-vegetarian participants (117 mmol/kg [dw] and 130 mmol/kg [dw], respectively). Despite these equivocal reports both research groups observed higher tissue creatine increases in vegetarian participants after Cr supplementation.

Since 1990 there have been numerous published articles examining the ergogenic effects of creatine on repetitive bouts of high-intensity exercises (Casey et al., 1996;
Dawson et al., 1995; Hoffman, Stout, Flavo, Kang & Ratamess, 2005). Ergogenic effects of creatine supplementation have also been tested on specific populations such as older men (Chrusch et al., 2001), patients with congestive heart failure (Andrews, Greenhaff, Curtis, Perry, Cowley, 1998), and young female athletes (Cox et al., 2002). However, the results of these studies are equivocal (Bemben & Lamont, 2005). The majority of short term studies (5-7 days of 20-30 grams per day) have showed statistically significant enhanced performance whereas other studies have reported no significant gains in performance (Kreider, 2003).

To date, there have been only a few published studies examining the benefits of creatine supplementation for vegetarians (Brees et al., 1994; Clarys, Zinzen, Hebbelinck, & Verlinden 1997; Shomrat, Weinstein, & Katz 2000; Burke et al., 2003) compared to the numerous studies in omnivorous populations. The early studies by Brees et al. (1994) and Clarys et al. (1997) found no significant improvement in performance, whereas, more recent studies by Shomrat et al. (2000) and Burke et al. (2003) demonstrated significant performance improvements with Cr supplementation.

Brees et al. (1994) compared the effects of creatine supplementation on concentric and eccentric power output for isokinetic leg extensions (4 x 15 maximal isokinetic leg extensions). Ten vegetarians and ten meat eaters were tested after five days of supplementation using a crossover design with a three-week washout period. Pooled group analysis of data revealed no statistical difference between the supplementation and placebo groups. Clarys et al. (1997) also used an isokinetic dynamometer (5 x 20 repetitions at 3.14 rad/s) and found no significant differences in torque production between creatine and placebo groups for either vegetarians or non-vegetarians.
Conversely, Shomrat et al. (2000) using a modified Wingate test (3 × 20 s maximal effort) detected increased mean power output to a similar degree in both vegetarians and non-vegetarians following with creatine supplementation. There was no improvement in mean power output for the non-vegetarians on placebo. Burke et al. (2003) assessed the effect of creatine supplementation on one repetition maximum bench press, leg press and isokinetic work after eight weeks of resistance training in vegetarian and non-vegetarian participants. Results indicated that participants on creatine supplementation showed a greater increase in phosphocreatine, total creatine, bench press strength, isokinetic work, type II fiber, and whole body lean tissue compared with the placebo group. Furthermore, vegetarians on creatine showed greater total creatine, phosphocreatine, lean tissue and total work performed compared to non-vegetarians on creatine (p<0.05).

These inconsistencies are likely due to a host of physiological factors, coupled with differences in the experimental designs and some methodological weaknesses. Physiological factors such as initial cellular total creatine level, percentage of type I to type II muscle fibres, and fat free mass could have resulted in participants being categorized as responders, quasi-responders, or non-responders (Syrotuik & Bell, 2004). Weaknesses in the experimental designs and methodology, such as small sample size, lack of muscle biopsies, and short duration cross over designs, could have also attributed to the non-responsiveness of participants (Lemon, 2002).

For the past 10 years, studies have been conducted with regard to the ergogenic effects of Cr supplementation. This body of literature has led to a number of equivocal results with the majority of them supporting the ergogenic effects of Cr supplementation (Williams et al., 1999). A host of factors such as those previously mentioned and others
could have contributed to these equivocal results. Despite the abundance of research on creatine, the effect of Cr supplementation on the vegetarians has been limited to a few equivocal studies. Therefore, there is a need to fully investigate this topic within this particular population.

Statement of the purpose

This study had a two-fold purpose: firstly, to investigate the effect of acute Cr supplementation (5g 4 x per day for 5 days) on the volume of work (measured by 5 sets of a 10 RM seated chest press) and anaerobic performance (determined by 6 x 6 s maximum effort on a Monark cycle ergometer) in vegetarians and non-vegetarians. Secondly, the study examined and compared the results of these performance measures between vegetarians and non-vegetarians.

Research questions

1. Does acute Cr supplementation enhance the volume of work and anaerobic performance in recreationally active vegetarian and non-vegetarian participants?
2. Does Cr supplementation enhance work performance and anaerobic performance for vegetarians and non-vegetarians to the same extent?

Assumptions

1. The participants had not taken creatine monohydrate in the past six months and currently were not taking any other supplements.
2. Both vegetarian and non-vegetarian groups consumed adequate amount of calories to meet their daily energy requirements.
3. Both supplement and placebo groups ingested their substances according to the directions provided to them.
Limitations and delimitations

1. The participants were limited to a small group of vegetarian and non-vegetarian volunteers and not recruited randomly.

2. A true measurement of muscle Cr content through muscle biopsies was not taken.

3. The study was delimited to volunteers from greater Victoria region.
Operational definitions

Non-vegetarians (omnivores): Eating both animal and vegetable food.

Lacto-ovo vegetarians: Vegetarians that consume vegetables, grains, nuts, legumes dairy products (lacto) and eggs (ovo).

Lacto vegetarians: Vegetarians that consume only plant based foods and dairy products but exclude eggs and all animal flesh.

Vegan (also called strict vegetarians): Vegetarians that only consume plant based foods such as vegetables, grains, nuts, seeds, fruits, and legumes.

Creatine (Cr): A compound synthesized from amino acids that is the precursor of phosphocreatine, an important anaerobic energy source for high intensity exercise.

Creatinine: A product of creatine breakdown that is found in the urine.

Anaerobic Performance: Short bursts of repeated sprints on a cycle ergometer.

Repeated Sprints: Consists of 6 bouts of 6 seconds all out cycling against an adjustable resistance. The bouts are interspersed with 24 s of passive rest.

10 Repetition Maximum (RM) Chest Press: Chest press performed on a Hammer Strength Iso-Lateral Chest/Back (ILCB) machine in an upright seated position. The tenth repetition is the last repetition which can be successfully completed with good form and acceptable cadence. A repetition is considered a failure if one or both arms pause more than two seconds during the concentric part of the contraction (the lift).

Volume of work: The total number of repetitions completed in the exercise session (number of repetitions completed in 5 sets at a 10RM load).
**Peak power output (PP):** The highest power output, observed during the first 6 s exercise interval. Peak power was expressed in watts (1 W = 6.12 kg-m.min⁻¹), and calculated as resistance (kp) x revolutions x 1.62m/rev x 10 ÷ 6.12 kg-m.min⁻¹.

**Mean power (MP):** The sum of power outputs for all bouts of sprints divided by the total number of bouts (which was 6).

**Relative peak power (RPP):** Peak power output relative to body mass: PP ÷ Body mass (kg).

**Anaerobic capacity (AC):** Total work accomplished over 24 seconds; AC was calculated as the sum of each 6 s PP, or Force x Total distance in 24 seconds.

**Anaerobic fatigue (AF):** Percentage decline in power output during test; AF represents the total capacity to produce ATP via the immediate and short term energy systems. AF was calculated as \((\text{Highest 6 s PP} - \text{Lowest 6 s PP}) ÷ \text{Highest 6 s PP} × 100\).
Methods

Participants’ characteristics

Six female and six male habitual vegetarian volunteers and six female and 14 male non-vegetarian volunteers were recruited from the greater Victoria area for the study (Table 1). All 32 participants were recreationally active. Participants were assigned randomly in a double blind manner to either creatine or placebo groups. Volunteers qualified as lacto-ovo vegetarians if they had been consuming only vegetables, grains, nuts, legumes, dairy products and eggs. Participants were considered vegan if they excluded all animal products from their diet, including dairy products and eggs. Only participants who had been vegetarian for at least six months were selected for this study. All procedures were examined and approved by the University Human Research Ethics Committee and written informed consent was obtained from each participant (Appendix B).

Table 1

<table>
<thead>
<tr>
<th>Group (IV)</th>
<th>M-Age (SD)</th>
<th>M-Height (cm) (SD)</th>
<th>M-weight (kg)(SD)</th>
<th>M-BMI(SD)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- VCr</td>
<td>32.1 (±12.3)</td>
<td>175.4 (± 11.2)</td>
<td>75.8 (± 14.5)</td>
<td>24.4 (± 2.6)</td>
<td>6</td>
</tr>
<tr>
<td>2- VPla</td>
<td>23.8 (± 3.1)</td>
<td>171.7 (± 7.6)</td>
<td>65.0 (± 8.1)</td>
<td>21.9 (± 1.0)</td>
<td>6</td>
</tr>
<tr>
<td>3- NVCr</td>
<td>29.4 (± 8.4)</td>
<td>170.9 (± 9.7)</td>
<td>72.7 (± 12.1)</td>
<td>24.7 (± 2.7)</td>
<td>10</td>
</tr>
<tr>
<td>4- NVPla</td>
<td>29.1 (± 11.1)</td>
<td>175.1 (± 8.3)</td>
<td>78.2 (± 14.2)</td>
<td>25.4 (± 4.5)</td>
<td>10</td>
</tr>
<tr>
<td>Total Mean</td>
<td>28.7 (± 9.4)</td>
<td>173.2 (± 9.0)</td>
<td>73.5 (± 12.9)</td>
<td>24.4 (± 3.3)</td>
<td>32</td>
</tr>
</tbody>
</table>

Note. M = mean, 1- vegetarian on creatine (VCr), 2- vegetarian on placebo (VPla), 3- non-vegetarian on creatine (NVCr), 4- non-vegetarian on placebo (NVPla).
Experimental design

The dependent variables were volume of work (determined by a 5 x 10 RM seated chest press) and anaerobic performance (determined by 6 x 6 s all out cycling sprints) prior to and after 5 days of intervention.

The independent variables or groups included the creatine and placebo treatments for either the vegetarians or non-vegetarians males and females. Control variables were the rest periods between each chest press set (2 min between sets), tempo of the lift (2 s up and 2 seconds down) and the rest between the sprint bouts (24 s between each bout).

Seated Chest press protocol

The seated chest press protocol consisted of 5 sets using resistance equal to each participant’s pre-test 10-repetition maximum (10 RM) seated chest press with a two min rest period between each set. It has been demonstrated that with a constant load (i.e. estimated 10RM ) the number of repetitions decreases with subsequent sets, thus the total volume of work is reduced each set (Benson, Docherty & Brandenburg, 2006; Kelly & Jenkins, 1998).

The seated chest press involved lowering simultaneously the iso-lateral bars until the forearm and upper-arm made approximately a ninety degree angle while sitting upright on the Iso-lateral chest/back Hammer Strength Machine (ILCB) (Schiller Park, Illinois, USA), then extending the arms to complete a repetition. During each set, participants executed repetitions until failure. Failure was defined as the last repetition when the participant was unable to move one or both handle bars forward and up or paused for more than two seconds when the arms were in the extended position (Voleck et al., 1997).
Anaerobic performance variables

The following five anaerobic variables were measured and compared prior to and after interventions: Peak Power (PP), Mean Power (MP), Relative Peak Power (RPP), Anaerobic Capacity (AC) and Anaerobic Fatigue (AF). Peak Power was defined as the highest power output observed during the first 6 s exercise interval. Peak power was expressed in watts \( (1 \text{ W} = 6.12 \text{ kg}^{-1} \cdot \text{min}^{-1}) \) and computed as resistance (kp) \( \times \) revolutions \( \times 1.62 \text{m/rev} \times 10 \div 6.12 \text{ kg}^{-1} \cdot \text{min}^{-1} \). Mean Power was defined as the sum of power outputs for all bouts of sprints divided by the total number of bouts (6) and expressed in watts. Relative Peak Power was defined as peak power output relative to body mass: \( \text{PP} \div \text{Body mass (kg)} \) and expressed in watts kg\(^{-1}\). Anaerobic capacity was defined as total work accomplished over 24 s; AC was computed as the sum of each 6 s PP, or Force \( \times \) total distance in 24 s and expressed in kg\(^{-1}\).min\(^{-1}\) and then converted to watts by dividing the value by 6.12 kg\(^{-1}\).min\(^{-1}\) \( (1 \text{ W} = 6.12 \text{ kg}^{-1} \cdot \text{min}^{-1}) \). Anaerobic fatigue (AF) was defined as the percentage decline in power output during the test and represents the total capacity to produce ATP via the immediate and short term energy systems. AF computes as \( (\text{Highest 6 s PP} \, - \, \text{Lowest 6-second PP}) \, \div \, \text{Highest 6 s PP} \, \times \, 100\).
Familiarization sessions

The purpose of the familiarization/practice sessions was to familiarize participants with all the procedures and control for potential learning effects in performing the chest press and cycle ergometer sprints. Each participant took part in five sessions; three practice sessions and two testing sessions (Figures 1 and 2, pp 11,13). Participants arrived at the Behavioural Medicine Laboratory in a fasting state for at least three hours and at the same time of the day each session, predetermined by their availability. In addition, they were advised to avoid caffeinated drinks for at least six hours prior to their appointment. They were also asked to refrain from any strenuous physical activity 24 hrs prior to their exercise session. On day-one age, height, weight and BMI were recorded for each participant as well as an estimate of their 10 RM chest press and highest cycling rpm which were used as a criterion reference point.

Ten repetition maximum warm-up and estimation

Participants started with two sets of 10 repetitions of a light load while sitting on the Iso-lateral chest/back Hammer Strength Machine with 1 min rest between each set. This was followed by static upper-body musculature stretching (pectoralis major, anterior deltoid and triceps brachii) (Volek et al., 1997). After the second rest period a conservative estimate of 10 RM was established and seated chest presses were executed. If the participant exceeded the estimated 10 RM, a 2 min rest was granted and 2.5-5% of the current estimated weight was added for the following trial(s) until the participant could perform only 10 repetitions of seated chest press. All estimations were predicted within a maximum of five testing sets (Baechle & Earle, 2000).
**Cycle ergometer sprinting warm-up and estimation**

Participants started with 5 min cycling at 60-70 rpm against a resistance of 1.0 kg on MONARK 834 Ergomedic (Varberg, Sweden). This was followed by static lower body musculature stretching (quadriceps, iliopsoas, hamstring and gluteus). Immediately after the stretching, a standardized warm-up was performed that involved two 30 s periods of submaximal cycling at 85-115 rpm against a resistance of 1.5 kg. Sixty seconds of rest was allocated between the two 30 s bouts for recovery (Gaitanos, Williams, Boobis & Brooks, 1993). After 4 min rest the appropriate resistance, based on the gender of the individual, was added to the fly wheel of the cycle ergometer. For females 0.085 kp. kg⁻¹ body weight and for males 0.087 kp. kg⁻¹ body weight was added (Dotan & Bar-Or, 1983).

After the standardized warm-up and recovery, individual participants cycled as hard and as fast as possible for 6 s. The all-out cycling was preceded by a few seconds of mental preparation and the three word-start command of “ready, set and go”. The maximum revolution 6 s was achieved and recorded for six trials with four minutes rest between each trial. The highest revolutions within the 6 s was used as the criterion score for the subsequent practices (Fitzsimons, Dawson, Ward & Wilkinson, 1993).

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 RM &amp; 6 X 6s Estimation</td>
<td>Familiarization I</td>
<td>Rest</td>
<td>Familiarization II</td>
<td>Rest</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 1.** Timeline for familiarization sessions.
Practice sessions

After the standardized warm-up individual participants started with the 5 x 10 RM seated chest press to failure. During the first set if the participant was able to lift her/his estimated weight more than 10 repetitions, extra weight was added for the next practice session. The set was terminated if one or both arms failed to complete that particular repetition within 4 s with good technique. For cycle sprinting, individuals had to accomplish at least 95% of her/his criterion score that was established during the first familiarization session. If not met, the participant was required to rest for four minutes and then repeat the test (Fitzsimons et al., 1993).

Testing sessions

After one day of rest, the participants were tested just prior to treatment (day 6) and again at the end of the treatment period on day 12 (Figure 2). Participants performed the standardized warm-up prior to the start of the testing sessions. Participants performed 5 x 10 RM to failure on each set. They were also required to do the concentric (up) and eccentric (down) actions with a 2 s tempo for each contraction. A clip-style Seiko metronome (Tokyo, Japan) was used in order to maintain the appropriate tempo. After the chest press, 15 min of rest was allocated prior to the sprint test. After the standardized warm-up participants were tested for six all-out 6 s sprints interspersed with 24 s of recovery. After the first bout, there was a 5 s advanced warning prior to the start of the next sprint. Participants assumed the ready position on the command of “get set” and upon the stop watch beeper signal and “go” command they sprinted as fast as possible. In order to standardize the measure, each sprint was initiated from a stationary start and participants remained seated during all sprints as they had practiced. They were
encouraged to produce an all-out effort on each sprint bout. The rest periods between each sprint required the participant to sit quietly on the cycle ergometer. Upon the last sprint the participants performed a 5 min active recovery at 60 rpm with a 1.0 kg load followed by 10 min of passive recovery.

![Timeline](image)

*Figure 2. Timeline for pre- and post-treatment testing.*

**Supplementation**

Participants were assigned to either creatine supplementation or a glucose placebo drink in a double-blind manner. They were asked to consume either 5g of Cr plus 1g of glucose for a total of 6g of Cr plus glucose combined or an equivalent quantity of glucose four times daily (separated by 3-4 hours) for 5 days. The powders were dissolved in 250 ml of warm liquid (Greenhaff et al., 1993). Participants were also cautioned to keep their package in a cool and dry environment away from sun light. In addition, they were told not to mix the powders with coffee (Appendix C).

**Statistical analysis**

All statistical procedures were performed using SPSS for windows version 15 statistical software (SPSS, LEAD technologies INC., USA). A $2 \times 4$ ANOVA (group x time) repeated measures (pre- and post-treatment) analysis of variance was used for analysis of all dependent variables (total repetitions, peak power, mean power, anaerobic capacity, relative peak power and anaerobic fatigue). This involved mean comparisons between groups (Cr and Placebo) as well as within groups (vegetarians and non-
vegetarians). The level of significance for all analyses was set at $\leq .05$ and all data are reported as mean ($\pm$SEM).
Results

A total of 32 participants took part in five sessions; three practice sessions and two testing sessions. The pre-treatment mean comparisons of the performance variables revealed no significant differences. Therefore, the pooled data analysis of variance was used to compare the post-treatment means (Appendix D). Mean values are presented in graphical form, with error bars indicating the SEM.

Total chest press repetitions (work)

The multivariate analysis of variance (repeated measure) revealed an overall significant differences for the total number of repetitions. The significant effect on composite variable at $p<.05$ was $p=.000$ (Wilks' $\Lambda = .259$, $F = 80.19$). Further analysis revealed that the significance was due to the treatment effect $p = .000$ (Wilks' $\Lambda = .518$, $F = 26.68$) (Figure 3). Additional t-test analysis showed that the two placebo groups had also significantly improved their total repetitions (NVPla: $p = .02$ & VPla: $p = .04$). However, the improvement for the Cr groups was more prominent and significant compared to the Pla groups. The vegetarian and non-vegetarian groups on Cr improved their total repetitions by 16.5% and 11.2%, respectively, whereas the vegetarian and non-vegetarian groups on Pla improved their total repetitions by 3.1% and 4.4%, respectively (Figure 3).
Figure 3. Mean (SEM) total repetitions chest press for non-vegetarians (NV) and vegetarians (V) before and after creatine (Cr) and placebo (Pla) treatments. * Significant difference between pre- and post-treatment means (p< .05). † Significant difference between Cr and Pla groups.
Peak Power

The multivariate analysis of variance revealed an overall significant difference for peak power. The significant effect on the composite variable at $p < .05$ was $p = .010$ (Wilks' $\Lambda = .784$, $F = 7.73$). Further analysis revealed that the significance was due to the treatment effect $p = .007$ (Wilks' $\Lambda = .771$, $F = 8.31$). Despite the significant effect of treatment on peak power there was no significant difference in change between the non-vegetarian and vegetarian participants on creatine ($p = .277$). Peak power for non-vegetarians on Cr improved by 3.8% and for vegetarians on Cr by 3.4%. There was no significant change in Pla groups (Figure 4).

![Graph showing peak power for NV and V before and after treatment]

*Figure 4.* Mean (SEM) peak power (W) for non-vegetarians (NV) and vegetarians (V) before and after creatine (Cr) and placebo (Pla) treatments. *Significant difference between pre- and post-treatment means ($p < .05$).
Mean Power

The overall mean power of the groups was significantly different \( p = .034 \) (Wilks’ \( \Lambda = .850, F = 4.94 \)). Further analysis revealed that the significance was due to the treatment effect \( p = .004 \) (Wilks’ \( \Lambda = .739, F = 9.90 \)). Despite the significant effect of treatment on mean power there was no significant difference in change between the non-vegetarian and vegetarian participants on creatine \( (p = .369) \). Mean power for the non-vegetarians improved by 4.0% compared to 2.0% for the vegetarians. There was no significant change in Pla groups (Figure 5).

![Figure 5. Mean (SEM) power (W) for non-vegetarians (NV) and vegetarians (V) before and after creatine (Cr) and placebo (Pla) treatments. *Significant difference between pre- and post-treatment means \((p<.05)\).](image-url)
*Anaerobic capacity*

The overall mean anaerobic capacity of the groups was significantly different \( p = .002 \) (Wilks’ \( \Lambda \) = .696, \( F = 12.21 \)). Further analysis revealed that the significance was due to the treatment effect \( p = .002 \) (Wilks’ \( \Lambda \) = .694, \( F = 12.34 \)). Despite the significant effect of treatment on anaerobic capacity there were no significant difference in change between the non-vegetarian and vegetarian participants on creatine \( (p = .304) \). Anaerobic capacity for the non-vegetarians improved by 3.6% compared to a 2.0% improvement for the vegetarians. There was no significant change in Pla groups (Figure 6).
Relative peak power

The overall mean relative peak power of the groups was significantly different $p = .008$ (Wilks' $\Lambda = .771$, $F = 8.30$). Further analysis revealed that the significance was due to the treatment effect $p = .034$ (Wilks' $\Lambda = .849$, $F = 4.98$). Despite the significant effect of treatment on relative peak power there was no difference in change between the non-vegetarian and the vegetarian participants on creatine ($p = .157$). Relative peak power for non-vegetarians improved by 3.8% and by 3.6% for the vegetarians. There was no significant change in the Pla groups (Figure 6).

![Graph showing relative peak power](image)

**Figure 7.** Mean (SEM) relative peak power (W. Kg$^{-1}$) for none-vegetarians (NV) and vegetarians (V) before and after creatine (Cr) and placebo (Pla) treatments. *Significant difference between pre- and post-treatment means ($p < .05$).
Anaerobic fatigue

The overall multivariate analysis of variance showed no significant difference in anaerobic fatigue for either the non-vegetarian or vegetarian participants ($p = .694$). In addition, there was no difference between the treatment groups ($p = .932$) (Figure 7).

![Bar chart](image)

**Figure 8.** Mean (SEM) anaerobic fatigue (%) for non-vegetarians (NV) and vegetarians (V) before and after creatine (Cr) and placebo (Pla) treatments.
Discussion

The purpose of this study was to examine the effects of five days of acute creatine monohydrate supplementation on the total volume of work performed during 5 x 10 RM seated chest press and on anaerobic performance as measured by 6 x 6 s maximum sprints on a Monark cycle ergometer. In addition, the study investigated the differences in response between the vegetarian and the non-vegetarian participants on the creatine supplement.

Total repetitions for 5 x 10 RM of seated chest press were significantly improved in non-vegetarians and vegetarians on creatine as well as for the placebo groups. However, the percentage improvement for the creatine groups was significantly higher than the placebo groups. Mean total repetitions for vegetarians on creatine improved the most, followed by the non-vegetarians on creatine, then non-vegetarians on placebo, and finally the vegetarians on placebo (16.5%, 11.2 %, 4.4% and 3.1%, respectively).

Anaerobic performance as measured by peak power, mean power, anaerobic capacity, and relative peak power also improved significantly in both vegetarians and non-vegetarians on creatine. The average performance improvements for peak power, mean power, anaerobic capacity and relative peak power were 3.6%, 3.0 %, 2.8 %, 3.7%, respectively. There was no significant change in any of these anaerobic indices for the placebo groups. Mean anaerobic fatigue did not change significantly for either the creatine or placebo groups.

Total chest press repetitions (work)

The total five sets of 10 RM showed a significant main effect between pre- and post-supplementation in both creatine and placebo groups. However, the two groups on
creatine supplementation showed a significantly greater improvement than the placebo groups. Vegetarians and non-vegetarians on creatine supplementation increased their total repetitions by 16.5% and 11.2%, respectively, compared to the non-vegetarians and vegetarians on placebo who improved their respective total number of repetitions by 4.4% and 3.1%. These results are in agreement with the previous studies (Voleck et al., 1997; Warber et al., 1998). Voleck et al. (1997) reported a significant improvement in their creatine group compared to the placebo group in a non-vegetarian population. In the current study non-vegetarians and vegetarians on creatine increased their first set of repetitions by 2.8 and 2.0 repetitions, respectively. This is similar to Voleck et al. (1997) who found an increase of 2.3 repetitions in the first set. However, there was a difference between vegetarians and non-vegetarians on creatine in the pattern of the repetition improvement. Improvement for the non-vegetarians was more apparent in the first three sets, whereas the improvement for vegetarians was distributed throughout the five sets. Non-vegetarians and vegetarians on placebo increased their first set repetitions by 2.6 and 0.6 repetitions, respectively. There was an inconsistent increase in the repetitions for consecutive sets for both groups on placebo. The results of the present study are also in agreement with Warber et al. (1998) who reported a 14.4% improvement in a 5 x 10 RM bench press for non-vegetarian participants after consuming 24 g of creatine per day for five days.

The 16.5% increase in total chest press repetitions for vegetarians and 11.2% in non-vegetarians on creatine may be the result of potentially lower endogenous levels of Cr for vegetarians compared to non-vegetarians due their diet. Individuals with lower endogenous levels of muscle creatine have been found to respond to a greater extent to
supplementation than individuals with initially normal or higher creatine levels. Watts, Garnham and Snow (2004) found that muscle Cr, PCr, and TCr content were all lower in vegetarians compared to non-vegetarians. In addition, basal plasma Cr, urinary Cr, and creatinine (Crn) excretion were lower in vegetarians. However, ATP content was similar between the vegetarians and non-vegetarians. After five days of Cr ingestion, muscle TCr content increased by 76.0 % and 36.0 % in vegetarians and non-vegetarians, respectively (Watts et al., 2004). These differences between the two groups in the current study potentially categorises the vegetarians as true responders and non-vegetarians as quasi-responders and non-responders (Syrotuik & Bell, 2004).

Other factors such as muscle fiber types and protein intake could have also contributed to the differences in improvements between vegetarians and non-vegetarians. It has been demonstrated that true responders to creatine supplementation have a greater percentage of type II fibers (63.1%) followed by quasi-responders (51.4%) and finally non-responders (39.5%). In addition, the responders and quasi-responders showed larger initial cross sectional area for type I, IIa and IIb fibers compared to the non-responders (Syrotuik & Bell, 2004). However, in the current study these factors remain unlikely because there is no reason the muscle fibre composition of vegetarians should be different to non-vegetarians.

The second factor that might have contributed to the enhanced muscle Cr upload in vegetarians is the quality of the protein they consume. Creatine is composed of two non-essential and one essential amino acid namely arginine, glycine and methionine. It is known that protein from animal sources is complete and has a higher biological value compared to the protein from vegetable sources. In addition, consumption of a creatine-
free vegetarian diet fully activates glycine amidinotransferase, which is the rate-limiting enzyme for creatine synthesis in the kidney (Heymsfield et al., 1983). This reduced creatine synthesis in vegetarians due to their incomplete protein intake could have contributed to their true responsiveness which in turn translated to a better performance compared to the non-vegetarians. However, because in the present study there was no detailed dietary analysis of protein intake, this is speculative.

The small but significant improvement for the two placebo groups may have been caused by either the placebo or a learning effect. In a qualitative study by Beedie (2007) the majority of participants believed that a placebo effect could produce an influence on sports performance. In addition, 73% of the participants in the study reported experiencing performance enhancement by some form of false-belief. Furthermore, the data revealed a strong relationship between belief and performance, specifically the belief that a substance, special technique, equipment or even the presence of another person will improve performance (Beedie, 2007).

*Anaerobic peak power & mean power*

In the present study mean peak power in non-vegetarians and vegetarians on creatine improved by 3.8% and 3.4%, respectively. These results are in agreement with Dawson et al. (1995) who reported a 4.6% increase in peak power in non-vegetarian participants. Shomrat et al. (2000) found that vegetarian and non-vegetarian participants using a Cr supplement improved their mean power on a modified Wingate test (20 s x 3) to the same extend (5% each). In the current study, non-vegetarians and vegetarians improved their mean power by 4.0% and 2.0%, respectively. However, Shomrat et al. (2000) found that when peak power was compared between the groups only non-
vegetarians improved (approximately by 5%) which is contrary to the present study. The differences in results between these studies could be due to several factors such as the testing protocol, psychological differences (motivation), training status, and hydration level (Inbar, Bar-or & Skinner, 1996). In addition, individual differences such as muscle fiber types (% type I vs. % type II) and muscle fiber cross sectional area differences could have also affected performance (Syrotuik & Bell, 2004).

*Anaerobic capacity (work)*

Previously it has been demonstrated that acute Cr loading increases anaerobic capacity by an average of 5-15% during sets of maximal-effort repetitive sprint performance (Brich et al., 1994; Casey et al., 1996; Volek et al., 1997). In the present study the anaerobic capacity of the non-vegetarians and vegetarians improved by 3.6% and 2.0%, respectively. Although these improvements appeared to be below the average increases they are close or similar to other studies. Dawson et al. (1995) and Kamber et al. (1999) found that non-vegetarians improved their anaerobic capacity by 4.5% and 3.5%, respectively. Recently a meta-analysis of 38 studies revealed a 3.2 % ± 1.1% improvement in anaerobic capacity with an ES of 0.29 ± 0.08 (Branch 2003). The results of the current study are, therefore, within an acceptable range.

The improvement of anaerobic performance could be due to Cr supplementation and which could effect many contractile mechanisms, such as accelerated ATP turnover, delayed PCr depletion, increased PCr resynthesis, decreased lactate and [H+] levels, diminished dependence on anaerobic glycolysis and facilitated muscle relaxation and recovery from intense repeated bouts (Volek & Kraemer, 1996). However, the
mechanisms underlying the ergogenic effect of Cr supplementation have yet to be identified.

The lack of greater improvement in vegetarians in the present study could be attributed to several factors including gender and individual differences. Compared to the non-vegetarian group on Cr (n=10, seven males, three females) the vegetarian group on Cr was composed only of six individuals, of which half were females. It is known that large gender differences exist in anaerobic power capacity when comparing test scores on an absolute basis (Saavedra, Lagasse, Bouchard & Simoneau, 1991). The disproportionate ratio of males to females in the non-vegetarian group on Cr could possibly have contributed to the differences and consequently higher anaerobic performance for non-vegetarians on Cr. This disproportionate improvement is reduced when the relative peak power is compared between the two Cr groups. When expressed relative to body weight, the power out-put for the non-vegetarians and vegetarians on Cr showed similar levels of improvement (3.8% and 3.6%, respectively) (Figure 6).

It has been demonstrated in general, that females have higher serum and erythrocyte Cr concentration, regardless of their diet (Delanghe et al., 1989). In addition, Forsberg, Nilsson, Werneman, Bergstrom and Hultman (1991) reported a 10% greater muscle TCr content in non-vegetarian women compared to men. These potential gender differences could possibly categorize females as either non-responders, quasi-responders or slow responders. Currently the results for gender specific responsiveness to Cr supplementation are equivocal, with some studies showing no change in performance for females when compared to a placebo or males (Eckerson et al., 2005; Ledford & Branch, 1999), whereas other studies found similar improvements in performance for both
genders when compared to placebo groups (Eckerson et al., 2004; Tarnopolsky & Maclellan, 2000).

In regard to individual differences, the training status of the participants, their buffering of acid metabolites and their motivation level could also affect anaerobic energy-transfer. Karlsson, Diamant and Saltin (1971) found that following maximal short-term cycle ergometer sprints, trained participants exhibited higher levels of muscle glycogen, ATP and PCr depletion. In addition, muscle lactic acid and lactate were also higher in the trained participants. In the present study anaerobic training experience of the participants varied from recreationally active to highly active.

Buffering capacity is an integral part of anaerobic energy production. The capability of individuals to increase free hydrogen ions to prevent a decrease in pH may play an important role in anaerobic performance. When the anaerobic system is predominating for the production of energy, excess lactate accumulates which in turn increases blood acidity that negatively influences intramuscular environment and the contractile capacity of active muscles. It is theorized that anaerobic training could possibly enhance short-term capacity by improving the alkaline reserve for buffering (Fitts, 1994). In the current study differences between individuals buffering capacities could have resulted in variations in performance.

The third major factor that could have possibly influenced anaerobic performance in the present study was motivation. Previously, controlled scientific investigations have demonstrated that both strength and endurance capacities can be exceeded when participants are subjected to various forms of motivation (Hellebrandt & Waterland, 1962; Ikai & Steinhäus, 1961). Wilmore (1968) examined the influence of motivation on
physical work capacity and performance in three groups of participants (two control
groups and one experimental). He suggested that the supermaximal performances elicited
under experimental conditions (motivational level) resulted from an increased anaerobic
capacity, which was possibly due to reduced psychological inhibitions and tolerance to
increased levels of anaerobic metabolites. It is difficult to classify motivational factors;
however, they need to be considered when measuring maximal physical efforts. Although
in the present study the motivational levels were consistent across all conditions, it is
possible some participants were intrinsically less motivated than others.

*Anaerobic fatigue*  

Anaerobic fatigue is defined as the percent decline in power output during the
high-intensity exercise (Fitts, 1994). In the present study, percent anaerobic fatigue
changes were inconsistent and insignificant. The anaerobic fatigue percentage of non-
vegetarians on Cr showed a small increase from 16.5% to 17.6%; conversely the
anaerobic fatigue for the vegetarian group decreased from 22.2% to 21.8%. There was
no change for the placebo groups (Figure 7). The lack of significant change in anaerobic
fatigue is in most part in agreement with Shomrat et al. (2000), with the exception of an
improvement after the second exercise bout in vegetarians.

It has been suggested that metabolite accumulation could possibly contribute to
fatigue. During short term, high intensity exercise lactic acid production exceeds its
removal. The disassociation of lactic acid to protons (H\(^+\)) causes the pH to decrease and it
is the un-buffered protons that contribute to fatigue (Gevers, 1977). Theoretically Cr
supplementation should decrease lactate and the associate (H\(^+\)); however, the results of
previous studies have been equivocal. Some studies reported increased lactate levels
following Cr supplementation (Dawson et al. 1995; Volek et al. 1997), whereas other studies have reported decreased levels (Balsom, Soderlund & Ekblom, 1994; Kamber et al. 1999) or even no change for both non-vegetarians and vegetarians (Birch et al., 1994; Shomrat et al., 2000). The non-significant and inconsistent changes in anaerobic fatigue in the present study could be partly attributed to participants pacing themselves to conserve energy for the last bouts or psychological factors (i.e., motivation). The other possibility could be that Cr supplementation does not affect anaerobic fatigue. However, the unchanged anaerobic fatigue in this study could be interpreted as being beneficial to performance in that most of the measures of anaerobic performance improved significantly without any accompanying increase in anaerobic fatigue.

Conclusions

The results of this study support the ergogenic effects of Cr on anaerobic performance. Both the creatine and placebo groups improved their total work volume (5 x 10 RM). However, the percent improvements in the creatine groups were significantly more pronounced. Anaerobic performance measures, such as peak power, mean power, anaerobic capacity, and relative peak power were also significantly improved in both Cr groups with no significant differences between non-vegetarians and vegetarians. There were no significant changes in any of the anaerobic performance variables in the placebo groups. Factors such as age, gender, training status, diet, muscle composition, and motivation may all have influenced the results of this study. However, the findings of the present study support the ergogenic benefits of creatine supplementation but, contrary to the initial expectations, the vegetarian group did not respond more than the non-vegetarian group. Possible reasons for this finding are the small sample size of
vegetarians and the disproportionate number of females in the vegetarian group compared to the non-vegetarian group. Future research should match the groups for gender distribution or use separate male and female groups. In addition, biological markers could be used, along with a dietary analysis of the participants, in order to help explain the possibly different responses of the vegetarians and non-vegetarians to creatine supplementation.
References


capacity and body weight after two and six days of loading in men and women. 


Appendix A: Review of the literature
This review of the literature explores the following topics: Introduction, biochemistry and biosynthesis of creatine, factors that affect creatine loading into human skeletal muscle, ergogenic effects of creatine, and some explanations for reported inconsistencies in findings from various studies.

Introduction

Creatine monohydrate (CrH$_2$O) is perhaps one the most popular supplements that elite competitors, amateur and recreational athletes have used to gain a competitive edge. Annual Cr sales in the United States have risen to $400 million (Grande & Graves, 2005) and according to Jeukendrup and Gleeson (2004), the world wide annual Cr consumption by athletes is currently estimated to be approximately three million kg.

Creatine was first discovered in 1832 by the French scientist Michel Eugene Chevreul (as cited in Williams, Kreider & Branch, 1999). In 1847, Justus von Liebig confirmed that creatine was a regular constituent of animal flesh. He also reported a greater amount of creatine in wild animals as compared to captive animals, which may have been less active (as cited in Williams et al., 1999). Due to the expense of extracting creatine from fresh meat, and lack of funding early research was very limited.

Nonetheless, in the early 1900s creatine supplementation in animal models showed increased muscle creatine content. The phosphorylated form of creatine, phosphocreatine (PCr), was discovered in 1927 and determined shortly afterward to be an important constituent in anaerobic energy production (Williams et al., 1999). According to Kreider (1999), creatine was used as an ergogenic aid by eastern block countries as early as the 1960s. The popularity of creatine supplementation increased markedly, however, after the 1992 Olympics in Barcelona. Apparently, Linford Christie, the men’s 100 meter gold
medalist, and Sally Gunnell, the women’s 400-meter hurdles champion, both used creatine supplements (Jeukendrup & Gleeson, 2004).

**Biochemistry and biosynthesis of creatine**

Creatine (or phosphocreatine [PCr] or creatine phosphate) is a non essential nitrogenous based compound (Figure A1) that participates in the production and maintenance of adenosine triphosphate (ATP) (Reents, 2000). Creatine is synthesized primarily in the liver and pancreas from two non-essential amino acids, arginine and glycine, and an essential amino acid, S-adenosyl-methionine (Snow & Murphy, 2003). In a two step reaction, guanidinoacetate is first formed from arginine and glycine in a reaction catalyzed by arginine-glycine amidinotransferase. In the second step, a methyl
group from S-adenosyl-methionine is transferred to guanidinoacetate, which creates creatine (Figure A2) (Jeukendrup & Gleeson, 2004). Muscle tissue does not synthesize Cr; instead, Cr uptake is obtained from the circulating blood by a sodium dependent transporter in the muscle membrane. Creatine becomes phosphorylated once it moves into the muscle cell. The phosphorylation of Cr is accomplished with the aid of creatine kinase (Tijungga et al., 2000). Creatine can also be obtained exogenously via a diet rich in animal flesh, such as fish and red meat (Table A1). In a 70-kg (154-lb) man, the total creatine pool (free Cr + PCr) is approximately 120g, of which 95% is stored in skeletal muscle and the remaining 5% is found in various tissues such as the brain, liver, kidneys, and testes (Jeukendrup & Gleeson, 2004).

Figure A2. Creatine synthesis and transport in the body (Adapted from Jeukendrup & Gleeson, 2004).
According to Wyss and Kaddurah (2000), the total muscle Cr gradient is 30 mmol/kg to 40 mmol/kg wet weight or 120 mmol/kg to 160 mmol/kg dry weight. In skeletal muscle, 40% of the total Cr is in the free form and the remaining 60% is stored in the phosphorylated form (Heymsfield et al. 1983). The Cr degradation rate is approximately 1.6% or 2 grams per day (Snow & Murphy, 2003). Thus, to maintain Cr stores in the body, approximately 2 g of Cr must be synthesized or consumed daily. Usually, fifty percent of this requirement is satisfied via a mixed omnivorous diet (Table A1), and the remainder is synthesized endogenously by the liver, kidney, and pancreas (MacArdle, Katch & Katch, 2005). The endogenous Cr synthesis by the aforementioned organs depends on adequate amount of macronutrient consumption. This is due to the fact that the three amino acids glycine, arginine and methionine involved in Cr production must be provided via diet. The degradation of Cr and PCr is a spontaneous and a non-enzymatic process (Figure A1b) in which the Cr pool is converted to creatinine which subsequently is excreted in the urine via the kidneys (Wyss & Kaddurah, 2000).

Table A1
Sources of Dietary Creatine
(Adapted from Jeukendrup & Gleeson, 2004).

<table>
<thead>
<tr>
<th>Food type</th>
<th>Creatine content (g/100mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH</td>
<td></td>
</tr>
<tr>
<td>Shrimp</td>
<td>Trace</td>
</tr>
<tr>
<td>Cod</td>
<td>0.3</td>
</tr>
<tr>
<td>Herring</td>
<td>0.65-0.1</td>
</tr>
<tr>
<td>Plaice</td>
<td>0.2</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.45</td>
</tr>
<tr>
<td>Tuna</td>
<td>0.4</td>
</tr>
<tr>
<td>MEAT</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>0.45</td>
</tr>
<tr>
<td>Pork</td>
<td>0.4</td>
</tr>
<tr>
<td>OTHER</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>0.01</td>
</tr>
<tr>
<td>Cranberries</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Factors affecting creatine loading into human skeletal muscle

The actual maximum Cr concentration attained by body is unknown (Snow & Murphy, 2003). The average total creatine (TCr) content in skeletal muscle is approximately 120-130 mmol/kg dry weight; however, normal distribution ranges from approximately 90-160 mmol-kg dry weight. While dietary Cr supplementation can raise the TCr content in some individuals as high as 180 mmol-kg dry weight, the muscle TCr content in some healthy individuals is unaffected with supplementation (Harris et al., 1992; Snow & Murphy 2003; Syrotuik & Bell, 2004). Currently it is unclear what factors contribute to this large individual variability; however, the following factors have been investigated or postulated: the co-ingestion of Cr and carbohydrates, the effect of exercise and training on Cr supplementation, muscle fibre type, gender, and chronically reduced dietary Cr intake (Vegetarianism) (Snow & Murphy 2003; Syrotuik & Bell, 2004).

Creatine and carbohydrates co-ingestion

Green, Hultman, Macdonald, Sewell, and Greenhaff (1996) demonstrated that five grams of Cr followed 30 minutes later by 93 grams of simple carbohydrates (CHO) four times daily resulted in 60% more TCr increase in the muscle as compared to Cr ingestion alone (P<0.01). In addition, there was a decrease in urinary Cr excretion in Cr+ CHO group (p<0.001). Sole Cr supplementation did not effect serum insulin, while Cr + CHO dramatically elevated insulin level (P<0.001). This substantial muscle Cr storage with co-ingestion of CHO results from insulin-mediated glucose absorption by skeletal muscle, which in turn facilitates transport of creatine into muscle fibres (MacArdle et al., 2005). Steenge, Simpson and Greenhaff (2000) also found that the ingestion of 50 g of
protein in conjunction with 50 g of CHO was as effective in stimulating pancreatic insulin release and whole body Cr retention as ingesting 100 g of CHO.

*Creatine supplementation exercise and training*

It has been shown that Cr supplementation in conjunction with prolonged, submaximal exercise results in an augmented Cr loading in the active muscles (Harris et al., 1992; Robinson, Sewell, Hultman, & Greenhaff, 1999). Robinson et al. (1999) examined the effect of glycogen -depleting exercise on subsequent muscle TCr accumulation and glycogen re-synthesis during post-exercise periods when the diet was supplemented with CHO or Cr + CHO. Fourteen men cycled with one leg to exhaustion, and the non-exercised leg was used as a control. Muscle biopsies were taken from the exhausted (Ex) and non-exhausted (Nex) limbs after exercise, after 6 hours, and after 5 days of recovery. Results revelled that muscle TCr was unchanged in both groups 6 hours after supplementation but had increased in Ex (p<0.001) and Nex limbs (p<0.05) of the Cr + CHO group after 5 days. In addition, greater TCr accumulation was achieved in the Ex limbs (p<0.01) of this group. Robinson et al. (1999) suggested that muscular work may have altered the mechanism responsible for transporting Cr across the cell membrane, thus resulting in a greater influx of Cr into the muscle. Furthermore, Zange et al. (2002) using P-magnetic resonance spectroscopy discovered that physical education students who had used Cr supplementation increased their muscle PCr: ATP ratio to a greater extent compared to sedentary volunteers. They speculated that individuals involved in regular training may have a better ability to uptake Cr.
**Muscle fiber type**

Measurements on both human single skeletal muscle fibres and pools of fibres have shown that the resting PCr content is 5-15% higher in Type II vs Type I fibres (Greenhaff et al., 1994a). Tesch, Thorsson, and Fujitsuka (1989) found PCr content at rest in Type II and Type I fibres was 82.7 +/- 11.2 and 73.1 +/- 9.5 mmol/kg dry weight, respectively. They also reported that following 30 seconds of maximal exercise, PCr content in fast twitch fibres was lower than slow twitch fibres; however, after 60 seconds of rest slow twitch fibres had significantly higher PCr concentrations. This suggests, Type I fibres initially re-synthesis PCr at a slightly faster rate than Type II fibres during recovery.

**Gender**

Delanghe et al. (1989) were the first group of researchers that measured and compared creatine, creatinine, and carnitine levels between vegetarian and omnivore populations. In their comparison they demonstrated that, in general, females have higher serum and erythrocyte Cr concentration, regardless of their diet (Table A2).
Table A2

Reference Values for Creatine, Creatinine, and Carnitine in Vegetarians (Adapted from Delanghe et al., 1989).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Vegetarians</th>
<th>Reference population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=55)</td>
<td>Females (n=44)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine, $\mu$mol/L</td>
<td>25.1 (9.1)</td>
<td>32.4 (21.4)</td>
</tr>
<tr>
<td>Creatinine, $\mu$mol/L</td>
<td>7.7 (0.6)</td>
<td>7.8 (0.8)</td>
</tr>
<tr>
<td>Carnitine, $\mu$mol/L</td>
<td>44.9 (17.4)</td>
<td>42.3 (19.4)</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>96.2 (23.5)</td>
<td>74.6 (18.1)</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine, $\mu$mol/L</td>
<td>270 (41)</td>
<td>281 (47)</td>
</tr>
</tbody>
</table>

Despite the aforementioned findings most studies report that muscle TCr content is similar between the sexes (Parise, Mihic, Maclellan, Yarasheski, & Tamopolsky, 2001; Murphy et al., 2003). Moreover, it has been demonstrated that the magnitude of skeletal muscle Cr loading after oral Cr supplementation is very similar for both males and females (Harris et al., 1992; Paris et al., 2001). In addition, according to Murphy et al. (2003) creatine transport (CreaT) mRNA expression in skeletal muscle is also similar in males and females.
Chronically reduced dietary Cr intake (vegetarianism)

Individuals who refrain from meat and meat products exclusively rely on endogenous Cr production by the liver, kidneys, and pancreas (Snow & Murphy, 2003). Vegetarians are generally divided into two major groups, lacto-ovo vegetarians and vegans. Lacto-ovo vegetarians consume eggs and other dairy products but exclude any kind of animal flesh such as fish, sea food, chicken, and any red meat. Vegans strictly exclude all animal products from their diet and rely only on plant based foods (i.e., vegetables, grains, nuts, seeds, fruits and legumes) (Thompson & Manore, 2005).

Delanghe et al. (1989) reported that the daily urinary creatinine excretions rates of vegetarians were 30% lower than omnivores. This finding provided the evidence that vegetarians may have a reduced muscle TCr level and that endogenous Cr production is unable to completely compensate for the lack of dietary Cr (Delanghe et al., 1989). In addition, Lukazuk et al. (2002) demonstrated that skeletal muscle TCr content was 13% lower in omnivorous men whom consumed a lacto-ovo vegetarian diet for 26 days. Surprisingly, however, Cr supplementation on the 22nd day did not increase muscle TCr in these uncategorized vegetarian men. More recently Burke et al. (2003) compared the effect of 8 weeks of Cr supplementation between vegetarians (15 lacto-ovo and 3 Vegan), omnivors, and placebo groups. Muscle biopsy indicated that TCr was significantly higher in groups on Cr supplementation; thus, vegetarians on Cr supplementation had a greater increase in TCr, lean tissue, and total work performed as compared to omnivors on Cr supplementation (Burke et al., 2003).
Ergogenic properties of creatine

There have been several proposed possible mechanisms on how elevated levels of Cr and PCr can enhance muscular performance (Figure A3) (Volek & Kraemer, 1996). Nevertheless, the precise mechanisms underlying the ergogenic effectiveness of Cr supplementation remain poorly understood (McArdle et al., 2005). Creatine acts as the “energy reservoir” for the cells, providing phosphate-bond energy to resynthesize ATP in

![Diagram showing mechanisms involving creatine and phosphocreatine](image)

*Figure A3. Possible mechanisms by which elevated levels of Cr and PCr in muscle may act to enhance muscular performance (Adapted from Volek & Kraemer, 1996).*

A reversible creatine kinase reaction:

$$PCr + ADP + H^+ \underset{\text{creatine kinase}}{\rightleftharpoons} (\text{creatine kinase}) \underset{\text{creatine kinase}}{\rightleftharpoons} Cr + ATP$$

[1]
Phosphocreatine may transport intramuscular high-energy phosphate between the mitochondria and the cross bridge sites that initiate muscle contraction. In all-out activities such as sprinting and explosive lifts (up to 10 seconds), maintaining a high sarcoplasmic ATP/ADP ratio from PCr becomes crucial (McArdale et al., 2005). Such short-duration activities place stress on the rate of ATP resynthesis. It is at this level that the energy requirements surpass the energy production from the intracellular macronutrients (Bagdanis, Nevill, Boobis, & Lakomy, 1996). Enhanced PCr energy transfer ability reduces the reliance on energy transfer from anaerobic glycolysis which in turn can increase intramuscular H+ and decrease pH from lactate accumulation (McArdale et al., 2005). Therefore, due to the limited amount of intramuscular PCr, it can be hypothesized that an increase in PCr availability could have the following ergogenic effects (Bogandis et al., 1996; Greenhaff, Bodin, Soderlund, & Hultman, 1994; Volek & Kraemer, 1996):

1. accelerating ATP turnover rate to maintain power output during short-term muscular effort;
2. delaying PCr depletion;
3. diminishing dependence on anaerobic glycolysis with subsequent lactate formation; and
4. facilitating muscle relaxation and recovery from repeated bouts of an intense, brief effort (<10s) through increased rate of ATP and PCr resynthesis; quick recovery permits continued high-level power output.
Some individuals working in the field of strength and conditioning believe that Cr supplementation at the recommended dosage (1) improves repetitive performance in muscular strength and short-term power activities, (2) augments short bursts of muscular endurance, and (3) provides for greater muscular overload to enhance training quality (McArdle et al., 2005). In addition to weight lifters and bodybuilders who traditionally benefited from Cr supplementation, researchers have also demonstrated that Cr supplementation improves anaerobic power output and work in events such as sprint running, cycling, swimming, jumping, football and soccer (Aaserud, Gramvik, Olsen & Jensen, 1998; Brich, Noble, & Greenhaff, 1994; Selesby, Beckett, Kern, & Devort, 2003; Stone et al., 1999; Stout, Echerson, Noonan, Moore & Cullen, 1999; Thorensen, McMillan, Guion & Joynet, 1998).

Why inconsistent results?

Lemon (2000) and more recently Syrotuik and Bell (2004) examined some of the underlying principles behind inconsistent results with regard to the ergogenic effects of Cr supplementation. They listed individual responsiveness to Cr supplementation as the most prominent factor. Other factors such as insufficient knowledge about CreaT protein mRNA, small sample size, gender and age differences, wash out period, and exercise type are also attributed for equivocal results (Lemon 2000).

*Individual responsiveness to supplementation*

Despite the fact that Cr loading (20 g/d) can increase muscle TCr concentration by approximately 20% in as little as 3-6 days, not everyone responds to this protocol to the same extent (Lemon, 2000). Greenhaff et al., (1994) estimated that approximately 20-30 % of individuals either do not respond to Cr loading or respond minimally.
Recently Syrotuik and Bell (2004) categorized their subjects into three groups of responders, quasi-responders, and non-responders with mean changes in resting TCr of 29.5 mmol.kg\(^{-1}\) dry weight (dw), 14.9 mmol.kg\(^{-1}\) dw, and 5.1 mmol.kg\(^{-1}\) dw, respectively. It appears that low initial muscle TCr level is the key factor for responsiveness. Therefore, vegetarian males with the low serum and erythrocyte Cr (mean of 25.1 \text{umol/L} and 270 \text{umol/L}, respectively) would show the greatest responsiveness to Cr supplementation (Delanghe et al., 1989; Green et al., 1996; Harris et al., 1992).

**Insufficient knowledge about CreaT protein mRNA expression**

To date there has been only one study that attempted to examine the regulation of CreaT mRNA expression (Rajab et al., 1999). Elevated TCr content in rat myocardium was induced by Cr supplementation for 16 days; the results indicated that the myocardial CreaT mRNA expression increased along with the enhanced TCr content (Rajab et al., 1999). Recently Murphy et al. (2003) suggested that perhaps CreaT gene transcription rates are elevated and/or transporter mRNA degradation rates are attenuated with an increased TCr content. Presently it is not known if skeletal muscle CreaT mRNA content would respond in a similar fashion to Cr supplementation. However, it has been demonstrated that 3-6 months of Cr supplementation in rats decreased CreaT protein levels in skeletal muscle (Murphy et al., 2003).

**Small sample size**

Another possibility that can potentially contribute to inconsistency results is the use of small sample sizes (Lemon, 2000). As it was mentioned earlier, not everyone responds to Cr loading the same way; therefore, having a small sample size with many non-responders can lead into type II error (inability to observe a real difference due to
response variability). Tarnopolsky and MacLennan (2000) suggested that with an
independent group study design (which is often used in the Cr studies) a sample of ≥ 15
per group might be necessary to detect a real statistical difference. As a result of this
weak research design, some published Cr studies have very low statistical power, which
in turn conveys inaccurate results with respect to any effects of Cr loading (Lemon,
2000).

Gender and age differences

While the majority of studies examined the effect of Cr supplementation on
males, some studies used both male and female participants. It is possible that the latter
studies confounded the data because it is not known if both genders respond to Cr
supplementation in the same manner. Zingelius et al. (1998) demonstrated that with
respect to changes in body fat free mass with Cr supplementation, females respond to a
lesser degree than their male counterparts. There are also data indicating that females
have a higher baseline muscle Cr (Forsberg, Nilsson, Werneman, Bergstrom & Hultman,
1991). On the other hand, Parise et al. (2001) found no significant gender differences in
baseline muscle Cr or in response to Cr supplementation. More recently Murphy et al.
(2003) found no difference in CreaT m RNA and protein expression between male and
female participants. To what extent gender differences affect Cr loading into the skeletal
muscle is currently unclear and requires more attention.

Rawson and Clarkson (1999) and Smith et al. (1998) demonstrated that Cr effect
will reduce with age. However, this non-responsiveness is more prominent in older
women (61-81 years) with regard to increase muscle size and function (muscle
strength/fatigue or timed speed events) (Shulte, Martin, Petrella, Bartha & Lemon, 2000).
Insulin insensitivity could be a possibility for this none or slow responsiveness to Cr supplementation; however, the real mechanisms responsible for any effect of ageing or ageing/gender interaction remain unclear (Lemon, 2002).

Washout period

In cross over research designs in which subjects act as both controls and placebos, a short period of Cr washout can contribute to no significant results between the control and placebo groups (Lemon 2002). The minimum washout period for Cr is reported to be 28 days (Febbraio, Flanagan, Snow, Zhao & Carey, 1995). Lemon et al. (1995) studied the effect of CrH2O supplementation on total integrated force in 20 X 30 seconds maximal isometric ankle extensions with 16 seconds recovery between contractions. A crossover design was used, which included a 5 week washout period. Creatine supplementation in this study increased the total integrated muscle force by 11%. In another crossover design, Maganaris and Maughan (1998) examined the effect of Cr and placebo supplementation on isometric knee extension strength in 10 healthy males. The 5 day supplementation period in this study, unlike the Lemon et al. study, was separated only by 3 days. Further examination of the data revealed that the group that received Cr supplementation first retained their improvement during placebo treatment. These studies demonstrate that after supplementation Cr is retained in the skeletal muscle for a period of time (approximately 28 days) (Febbraio et al., 1995). Therefore, in order to achieve consistency in studies that use a cross over design, an adequate washout period is also necessary.
Exercise type

When examining the ergogenic effects of Cr it is important to consider the type of exercise being performed. It has been demonstrated that Cr supplementation enhances the muscle phosphagen concentration (Burke et al., 2003; Harris et al., 1992). Therefore, brief, intense activities that rely on the ATP-PCr energy system demonstrate the greatest performance benefits (Lemon, 2002). These activities include repetitive cycling, isokinetic torque production, and isotonic force production (Williams et al., 1999). Lemon et al. (1995) (via repeated measures) suggested that the ergogenic effect would be greatest during very brief maximal efforts, while the ATP synthesis rate from the creatine kinase reaction with Cr-loading is decreased significantly by 8 seconds, 2 seconds, and is basically nonexistent by 29 seconds of maximal muscle contraction. Therefore, one would expect that endurance activities are usually unaffected (Engelhardt, Neumann, Berbalk & Reuter, 1998). However, this is not the case and some endurance activities as long as 80 minutes in duration may still benefit from Cr supplementation. Such endurance activities are generally repetitive, intermittent, and intense in nature (Preen et al., 2001). This observation is probably due to either the cumulative ergogenic effects of enhanced PCr resynthesis between activity bouts, or due to an enhanced activity economy, as a result of Cr-induced changes in oxygen uptake kinetics (Jones, Carter, Pringle & Campbell, 2002; Preen et al., 2001).
Summary

It is theorised that creatine supplementation enhances short bursts of explosive athletic performance. To date there have been a plethora of published research articles on the ergogenic effects of creatine. The majority of these studies support the ergogenic benefits of creatine. Conversely, numerous others do not show any ergogenic effects. Early studies were confounded with the lack of knowledge about the nature of creatine and methodological weaknesses. Therefore, it is essential to consider factors such as sample size, gender, age, wash out period, exercise type, creatine transport mRNA and most importantly individual responsiveness to creatine supplementation. This review of the literature has attempted to explore these factors and illustrate that despite the large body of knowledge the precise mechanisms underlying the ergogenic effectiveness of creatine supplementation remain poorly understood and further investigation is required.
References


Burke, G. D., Chilibeck, D. P., Parise, G., Candow, G. D., Mahoney, D., & Tarnopolsky,


Murphy, M., Tunstall, J. R., Mehan, A. K., Cameron-smith, D., Mckenna, J. M.,


Appendix B: Informed Consent
Title of the study: The Effects of acute creatine supplementation on work and anaerobic power in lacto-ovo vegetarians.


Purpose of the study: The purpose of the study is to determine the acute (5 days) effects of creatine supplementation on work and anaerobic power in lacto-ovo vegetarian and non-vegetarian males and females.

Possible benefits of the study: Possible benefits include increased muscle strength, enhanced work performance and anaerobic power capacity. In addition, the outcome of this research will add important knowledge to the field of sport nutrition with particular significance for lacto-ovo vegetarian individuals.

Exercise sessions: You will be required to report to the Behavioural Medicine lab five times within a 12 day period. The first three sessions are familiarization/practice sessions and the last two are the actual testing sessions. Each session takes just under 60 minutes and will involve performing 5 sets of 10 repetition maximum chest press on the Iso-lateral Hammer Strength machine (with 2 minutes rest between each set) followed by 6 bouts of 6 seconds maximal effort sprint (with 24 seconds rest between each bout) on a Monark cycle ergometer. The Chest press and cycling sprints will be separated by 15 minutes of rest.

Procedures: You will receive either Cr monohydrate (CrH2O) or glucose (placebo) for 5 days four times per day. The Cr group will consume 5g of CrH2O + 1g of Glucose 4 X per day = total of 24g per day. The placebo group consume 6g of glucose 4 X per day = total of 24g per day. You will be required to dissolve the content of the package in warm to hot liquid of your choice prior to ingestion. You will be tested prior to and after the end of the loading phase for 5 sets of 10 repetition maximum on Hammer Strength chest press machine and 6 bouts of 6 seconds maximal effort cycle ergometer sprints under a well supervised condition.

Possible risks: Possible risks with repetition maximum and sprint performance may include muscle pulls or strain, light headedness and nausea respectively. In order to minimize these risks adequate warm-up prior to the exercise session and adequate cool down at the end of each session will be allowed. In addition, you are advised to refrain from food and cafffeinated drinks three hours prior to your session.

Possible unlikely risks with creatine monohydrate ingestion include muscle cramping and gastrointestinal discomfort to minimize these you are advised to refrain from strenuous physical activities 48 hours prior to your session. Adequate water intake (8 glasses per day) will also help avoid most of potential side effects.
There may be unforeseen risks during this study or after it is completed. You will be free to withdraw from the study at anytime without any obligation.
**Ongoing consent:** To make sure that you continue to consent to participate in this research, prior to each session I will remind you of the purposes of the study and will ask you that if you wish to continue with the procedures.

If you have any questions or concerns regarding this study please do not hesitate to contact Kamran Rad @ 250-721-2215, Dr. David Docherty @ 250-721-8375 or the Associate Vice-President, research at the University of Victoria @ 250-472-4545.

We will advise you of any new information that will have bearing on your decision to continue in the study.

We will give you feedback on your individual results and the results of the study at the completion of the research.

I acknowledge that the study and contents of the consent have been explained to me, that I understand the contents, and that I have received a copy of the consent for my own records.

Participant’s full name: ________________________________

Participant’s signature: _______________________________ Date: __________

Researcher’s signature: _______________________________ Date: __________
Appendix C: Creatine Direction
Caution: Open packets carefully, content may spill. Keep this package away from sunlight, heat and humidity. Store your package in a dark and cool area.

Instruction: Mix one packet with warm liquid of your choice (i.e., tea but **NOT** coffee) four times per day (separated by 3-4 hours) for the next 5 days. If you decide to withdraw from the study please return the remaining of your package. For any questions please contact me at 721-2215.
Appendix D: Statistical Analysis
Table D1

Between groups pre-treatment mean comparisons

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td>MPPBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
<td>25849.088</td>
<td>1</td>
<td>25849.088</td>
<td>0.833</td>
<td>0.369</td>
</tr>
<tr>
<td>Within groups</td>
<td>930868.192</td>
<td>30</td>
<td>31028.940</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MACBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
<td>12409.698</td>
<td>1</td>
<td>12409.698</td>
<td>1.656</td>
<td>0.208</td>
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<tr>
<td>Within groups</td>
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<td>30</td>
<td>7496.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
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<td>33903.249</td>
<td>1.611</td>
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<td>Within groups</td>
<td>631194.421</td>
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<td>21039.814</td>
<td></td>
<td></td>
</tr>
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<td>Total</td>
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</tr>
<tr>
<td>MRPPBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
<td>0.915</td>
<td>1</td>
<td>0.915</td>
<td>0.368</td>
<td>0.549</td>
</tr>
<tr>
<td>Within groups</td>
<td>74.673</td>
<td>30</td>
<td>2.489</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75.588</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAFBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
<td>44.295</td>
<td>1</td>
<td>44.295</td>
<td>1.044</td>
<td>0.315</td>
</tr>
<tr>
<td>Within groups</td>
<td>1272.861</td>
<td>30</td>
<td>42.429</td>
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</tr>
<tr>
<td>Total</td>
<td>1317.156</td>
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<tr>
<td>MTRPBT*P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between groups</td>
<td>0.352</td>
<td>1</td>
<td>0.52</td>
<td>0.020</td>
<td>0.889</td>
</tr>
<tr>
<td>Within groups</td>
<td>528.617</td>
<td>30</td>
<td>17.621</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>528.969</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Mean peak power before treatment (MPPBT), Mean anaerobic capacity before treatment (MACBT), Mean power before treatment (MPBT), Mean relative peak power before treatment (MRPPBT), Mean anaerobic fatigue before treatment (MAFBT), Mean total repetitions before treatment (MTREPBT). Participants (P).
Table D2

Multivariate analysis of variance for mean peak power pre and post treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lambda</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPP</td>
<td>0.784</td>
<td>7.734</td>
<td>1.0</td>
<td>28.0</td>
<td>0.010</td>
<td>0.126</td>
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<td>MPP*P</td>
<td>0.958</td>
<td>1.228</td>
<td>1.0</td>
<td>28.0</td>
<td>0.277</td>
<td>0.042</td>
</tr>
<tr>
<td>MPP*T</td>
<td>0.771</td>
<td>8.314</td>
<td>1.0</td>
<td>28.0</td>
<td>0.007</td>
<td>0.229</td>
</tr>
<tr>
<td>MPP<em>P</em>T</td>
<td>0.971</td>
<td>0.831</td>
<td>1.0</td>
<td>28.0</td>
<td>0.370</td>
<td>0.029</td>
</tr>
</tbody>
</table>

*Note.* Mean peak power (MPP), Participants (P), Treatment (T), Interactions (*)
Table D3

Multivariate analysis of variance for mean anaerobic capacity pre and post treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lambda</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC</td>
<td>0.696</td>
<td>12.212</td>
<td>1.0</td>
<td>28.0</td>
<td>0.002</td>
<td>0.304</td>
</tr>
<tr>
<td>MAC*P</td>
<td>0.916</td>
<td>2.569</td>
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<td>0.120</td>
<td>0.084</td>
</tr>
<tr>
<td>MAC*T</td>
<td>0.694</td>
<td>12340</td>
<td>1.0</td>
<td>28.0</td>
<td>0.002</td>
<td>0.306</td>
</tr>
<tr>
<td>MAC<em>P</em>T</td>
<td>0.971</td>
<td>0.994</td>
<td>1.0</td>
<td>28.0</td>
<td>0.673</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Note. Mean anaerobic capacity (MAC), Participants (P), Treatment (T). Interactions (*)
Table D4

Multivariate analysis of variance for mean power pre and post treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lambda</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>0.850</td>
<td>4.949</td>
<td>1.0</td>
<td>28.0</td>
<td>0.034</td>
<td>0.150</td>
</tr>
<tr>
<td>MP*P</td>
<td>0.971</td>
<td>0.833</td>
<td>1.0</td>
<td>28.0</td>
<td>0.369</td>
<td>0.029</td>
</tr>
<tr>
<td>MP*T</td>
<td>0.739</td>
<td>9.909</td>
<td>1.0</td>
<td>28.0</td>
<td>0.004</td>
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</tr>
<tr>
<td>MP<em>P</em>T</td>
<td>0.969</td>
<td>0.898</td>
<td>1.0</td>
<td>28.0</td>
<td>0.352</td>
<td>0.031</td>
</tr>
</tbody>
</table>

*Note.* Mean power (MP), Participants (P), Treatment (T). Interactions (*)
Table D5

Multivariate analysis of variance for mean relative peak power pre and post treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lambda</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPP</td>
<td>0.771</td>
<td>8.302</td>
<td>1.0</td>
<td>28.0</td>
<td>0.008</td>
<td>0.229</td>
</tr>
<tr>
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<td>2.114</td>
<td>1.0</td>
<td>28.0</td>
<td>0.157</td>
<td>0.070</td>
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<tr>
<td>MRPP*T</td>
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<td>4.985</td>
<td>1.0</td>
<td>28.0</td>
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<td>28.0</td>
<td>0.241</td>
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</table>

*Note:* Mean relative peak power (MRPP), Participants (P), Treatment (T). Interactions (*)
Table D6

Multivariate analysis of variance for mean anaerobic fatigue pre and post treatment

<table>
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<tr>
<th>Effect</th>
<th>Lambda</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAF</td>
<td>0.998</td>
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<td>28.0</td>
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</tr>
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<td>0.159</td>
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<td>28.0</td>
<td>0.694</td>
<td>0.006</td>
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<td>MAF*T</td>
<td>1.000</td>
<td>0.007</td>
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<td>28.0</td>
<td>0.932</td>
<td>0.000</td>
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<td>0.114</td>
<td>1.0</td>
<td>28.0</td>
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</tr>
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</table>

*Note.* Mean anaerobic fatigue (MAF), Participants (P), Treatment (T). Interactions (*)
Table D7

Multivariate analysis of variance for mean total repetitions pre and post treatment

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<th>Effect</th>
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<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
<th>Par Eta^2</th>
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</thead>
<tbody>
<tr>
<td>MTREP</td>
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<td>80.193</td>
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<td>28.0</td>
<td>0.000</td>
<td>0.741</td>
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<td>0.875</td>
<td>1.0</td>
<td>28.0</td>
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<td>0.030</td>
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<td>26.068</td>
<td>1.0</td>
<td>28.0</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>MTREP<em>P</em>T</td>
<td>0.918</td>
<td>2.487</td>
<td>1.0</td>
<td>28.0</td>
<td>0.126</td>
<td>0.082</td>
</tr>
</tbody>
</table>

*Note.* Mean total repetitions (MTREP), Participants (P), Treatment (T). Interactions (*)