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REPORT NO T98-16

**IMPACT OF HYPOHYDRATION AND CREATINE  
SUPPLEMENTATION ON SKELETAL MUSCLE  
PERFORMANCE AND METABOLISM**

**U S ARMY RESEARCH INSTITUTE  
OF  
ENVIRONMENTAL MEDICINE  
Natick, Massachusetts**

**APRIL 1998**



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# REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 1998		3. REPORT TYPE AND DATES COVERED Technical Report; July 1995-Dec 1996	
4. TITLE AND SUBTITLE  IMPACT OF HYPOHYDRATION AND CREATINE SUPPLEMENTATION ON SKELETAL MUSCLE PERFORMANCE AND METABOLISM				5. FUNDING NUMBERS	
6. AUTHOR(S)  Scott J. Montain, Sinclair A. Smith, Ralph P. Mattot, Gary P. Zientara, Ferenc A. Jolesz, Roger A. Fielding, and Michael N. Sawka					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Thermal & Mountain Medicine Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA; Department of Health Sciences, Boston University, Boston, MA; Department of Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115				8. PERFORMING ORGANIZATION REPORT NUMBER  T98-16	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  Same as Block 7				10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release - distribution unlimited				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Two studies were done using $^{31}\text{P}$ magnetic resonance spectroscopy to examine the separate effects of hypohydration and dietary creatine supplementation on muscle metabolism and performance. Volunteers performed supine single-leg knee extension exercise in a 1.5 Tesla whole body magnetic resonance spectroscopy (MRS) system. For the hypohydration study, exercise was performed to exhaustion when euhydrated and 4% hypohydrated. Hypohydration reduced ( $P < 0.05$ ) time to fatigue 15% ( $\text{EU} = 251 \pm 67$ , $\text{HY} = 213 \pm 52$ sec; $\pm \text{sd}$ ). Muscle pH and Pi/BATP were similar during exercise and at exhaustion regardless of hydration state. It was concluded that hypohydration reduces muscle endurance and the effects appear independent of $\text{H}^+$ and Pi concentration. For the creatine studies, 3 repeat bouts of high-intensity exercise were performed before and after 5 days of creatine supplementation ( $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ). Middle-aged persons ( $58 \pm 4$ yr) had lower resting PCr/BATP compared to the young group ( $6.36 \pm 0.94$ vs. $7.18 \pm 0.93$ , $p < 0.05$ ) and a lower mean PCr resynthesis rate for bouts 1 and 2 ( $18.1 \pm 3.5$ vs. $23.2 \pm 6.0 \text{ mmol} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$ , $p < 0.05$ ). After creatine supplementation, resting PCr/BATP increased 15% ( $p < 0.05$ ) in the young group and 29% ( $p < 0.05$ ) in the middle-aged group ( $8.3 \pm 1.3$ vs. $8.3 \pm 1.0$ ) eliminating the difference between groups. Mean PCr resynthesis rate also increased in the middle-aged group ( $p < 0.05$ ) during the creatine trial to a level not different from the young group ( $24.3 \pm 3.8$ vs. $24.2 \pm 3.2 \text{ mmol} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$ ). Time to exhaustion was increased in the young group after creatine supplementation ( $122 \pm 36$ vs. $162 \pm 59 \text{ s}$ , $p < 0.05$ ) but did not improve in the middle-aged group ( $113 \pm 31$ vs. $144 \pm 81 \text{ s}$ , $p = 0.2$ ). It was concluded that creatine supplementation improves PCr availability and resynthesis rate in middle-aged persons to a level similar to young persons, but had variable effects on muscle endurance.					
14. SUBJECT TERMS dehydration, hypohydration, performance, metabolism, creatine, ergogenic aids				15. NUMBER OF PAGES 36	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT		

TECHNICAL REPORT

IMPACT OF HYPOHYDRATION AND CREATINE SUPPLEMENTATION ON  
SKELETAL MUSCLE PERFORMANCE AND METABOLISM

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January 1998

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## EXECUTIVE SUMMARY

This technical report summarizes two experiments done using  $^{31}\text{P}$  magnetic resonance spectroscopy to examine specific nutritional issues of military relevance. The initial experiment was done to determine if increased  $\text{H}^+$  and  $\text{P}_i$  might contribute to physical performance degradations when hypohydrated. The second experiment studied whether several days of oral creatine supplementation would have beneficial effects on muscle metabolism and performance in young and middle-aged persons. For both experiments, volunteers performed supine single-leg knee extension exercise in a 1.5 Tesla whole body magnetic resonance spectroscopy (MRS) system. For the hypohydration study, exercise was performed to exhaustion when euhydrated and 4% hypohydrated. For the creatine studies, 3 repeat bouts of high-intensity exercise were performed before and after 5 days of creatine supplementation ( $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ). Hypohydration reduced ( $P < 0.05$ ) time to fatigue 15% ( $\text{EU} = 251 \pm 67$ ,  $\text{HY} = 213 \pm 52 \text{ sec}$ ;  $\pm \text{sd}$ ). Muscle pH and  $\text{P}_i/\beta\text{-ATP}$  were similar during exercise and at exhaustion regardless of hydration state. It was concluded that hypohydration reduces muscle endurance and the effects appear independent of  $\text{H}^+$  and  $\text{P}_i$  concentration. In the creatine experiment, middle-aged persons ( $58 \pm 4 \text{ yr}$ ) had lower resting  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  compared to the young group ( $6.36 \pm 0.94$  vs.  $7.18 \pm 0.93$ ,  $p < 0.05$ ) and a lower mean PCr resynthesis rate for bouts 1 and 2 ( $18.1 \pm 3.5$  vs.  $23.2 \pm 6.0 \text{ mmol} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$ ,  $p < 0.05$ ). After creatine supplementation, resting  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  increased 15% ( $p < 0.05$ ) in the young group and 29% ( $p < 0.05$ ) in the middle-aged group ( $8.3 \pm 1.3$  vs.  $8.3 \pm 1.0$ ) eliminating the difference between groups. Mean PCr resynthesis rate also increased in the middle-aged group ( $p < 0.05$ ) during the creatine trial to a level not different from the young group ( $24.3 \pm 3.8$  vs.  $24.2 \pm 3.2 \text{ mmol} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$ ). Time to exhaustion was increased in the young group after creatine supplementation ( $122 \pm 36$  vs.  $162 \pm 59 \text{ s}$ ,  $p < 0.05$ ), but did not improve in the middle-aged group ( $113 \pm 31$  vs.  $144 \pm 81 \text{ s}$ ,  $p = 0.2$ ). It was concluded that creatine supplementation improves PCr availability and resynthesis rate in middle-aged persons to a level similar to young persons, but had variable effects on muscle endurance.

## PREFACE

This technical report summarizes two experiments done in collaboration with investigators at Boston University and Brigham and Women's Hospital, Boston, MA. The two experiments specifically addressed 1) the impact of water deficits, or hypohydration on muscle metabolism and endurance and 2) the impact of dietary creatine supplementation on muscle metabolism and endurance in young and older persons. The experiments are presented separately to facilitate readability and cohesiveness.

A major motivation for the experiments was to evaluate the feasibility, efficacy, and constraints of magnetic resonance spectroscopy for evaluating exercising muscle metabolism. The two experiments served as test cases to examine the techniques' usefulness for studying muscle metabolism during dynamic exercise of a rather large muscle group (i.e., vastus medialis and vastus lateralis). Moderate levels of hypohydration (3%-5% body weight loss) had been shown to reduce endurance 13%-44% during both large and small muscle mass activities. Pilot work in our laboratory revealed that hypohydration reduced single-leg knee extension endurance time 18% compared to when euhydrated. The mechanisms by which hypohydration increased skeletal muscle fatigability, however, had been little studied. Hypohydration did not appear to accelerate the rate of glycolysis, as hypohydration did not increase muscle glycogen use during exercise and blood lactate concentration is usually similar or lower during exercise when hypohydrated compared to euhydrated. Hypohydration could, however, accelerate depletion of adenosine triphosphate (ATP) and creatine phosphate (PCr) concentration, or impair the ability to buffer the hydrogen and inorganic phosphate (Pi) ions produced during exercise. No information existed regarding intracellular ATP, ADP, PCr, Pi nor pH during exercise when hypohydrated. While the results of several studies had documented that dietary creatine supplementation could improve exercise performance during repeated bouts of high-intensity exercise, little work had been done to assess what effects creatine supplementation was having on muscle phosphocreatine at rest and during exercise.



## EXPERIMENT #1: INTRODUCTION

The effects that hypohydration (body water deficit) has on increasing heat strain (Montain and Coyle, 1992; Rothstein and Towbin, 1947; Sawka, Pimental, and Pandolf, 1984), cardiovascular strain (Montain and Coyle, 1992; Saltin, 1964b) and reducing/degrading aerobic exercise performance (Armstrong, Costill, and Fink, 1985; Burge, Carey, and Payne, 1993; Saltin, 1964a; Webster, Rutt, and Weltman, 1990) are well documented. Less understood are the effects of hypohydration on skeletal muscle performance and metabolism. While two studies found that hypohydration reduced muscle endurance (Bijlani and Sharma, 1980; Torranin, Smith, and Byrd, 1979) another study found no difference in fatiguability during handgrip exercise (Serfass, Stull, Alexander, and Ewing, 1968). Likewise, anaerobic exercise performance has been reported to be decreased (Webster, Rutt, and Weltman, 1990) or not altered (Fogelholm, Koskinen, Laakso, Rankinen, and Ruokonen, 1993; Houston, Marrin, Green, and Thomson, 1981; Jacobs, 1980) by hypohydration. These previous studies are somewhat confounded, however by not controlling for prior exercise and/or heat exposure and different caloric intake prior to the performance tests. Research is needed that controls for these confounding variables, to determine if hypohydration has direct effects on skeletal muscle that contribute to the well-documented reductions in aerobic performance.

Hypohydration might accelerate depletion of energy stores, accumulation of metabolites (e.g., lactate, hydrogen ions, inorganic phosphate), changes in intracellular electrolyte concentrations, and/or reduce buffering capacity (Costill and Saltin, 1975; Fogelholm, Koskinen, Laakso, Rankinen, and Ruokonen, 1993; Horswill, 1992; Nielsen, Kubica, Bonnesen et al., 1981). Studies examining the effects of hypohydration on muscle glycogen use have found either no effect (Costill and Saltin, 1975) or a small increase in muscle glycogen utilization (Hargreaves, Dillo, Angus, and Febbraio, 1996). Similarly, hypohydration has been reported to not alter (Costill and Saltin, 1975) or increase muscle lactate concentration (Hargreaves, Dillo, Angus, and Febbraio, 1996). The effects of hypohydration on intracellular hydrogen ions ( $H^+$ ) or inorganic phosphate ( $P_i$ ) concentrations in skeletal muscle have not been studied. Elevated  $H^+$  and  $P_i$  concentrations reduce muscle force production during repeated contractions (Fitts, 1994), and intracellular concentrations would be increased by simply reducing intracellular water. These two metabolites can be measured non-invasively and repeatedly during exhaustive exercise using  $^{31}P$  magnetic resonance spectroscopy

(MRS).

The purpose of this study was to determine if hypohydration reduces skeletal muscle performance and to determine whether increased  $H^+$  and  $P_i$  concentrations might contribute to performance degradation. We hypothesized that hypohydration would reduce skeletal muscle endurance and act via increased  $H^+$  and  $P_i$  concentrations. To test these hypotheses,  $^{31}P$  MRS was used to measure high energy phosphates and pH during exhaustive single-leg knee extension exercise when subjects were euhydrated and hypohydrated.

## **EXPERIMENT #1: METHODS**

### **SUBJECTS**

Ten healthy physically active persons (5 men and 5 women), 21 to 40 years of age, participated in this study. The study was approved by the appropriate institutional review boards, and all volunteers gave their voluntary and informed consent prior to participation.

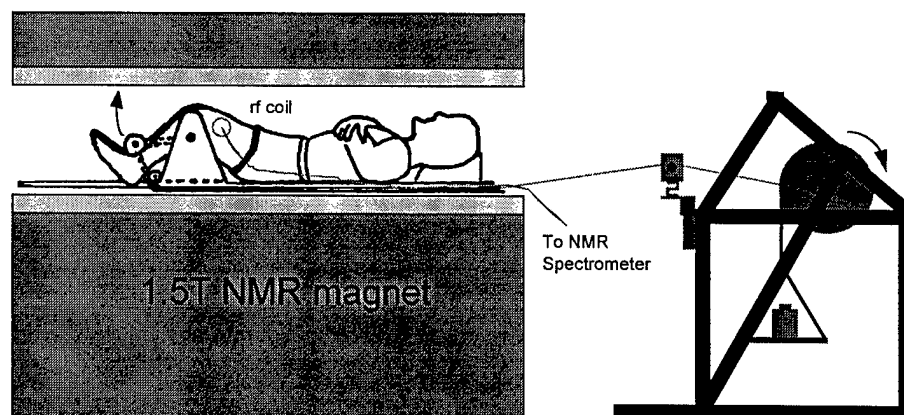
### **EXPERIMENTAL PROCEDURE**

Following several practice sessions to familiarize the volunteers with the experimental procedures and to determine the appropriate exercise intensity for the experimental trials, the volunteers reported to the laboratory on two occasions separated by a minimum of one week. Upon arrival, at 1100 h to 1300 h, an initial nude body weight was obtained to establish baseline body weight. The volunteers then entered a hot room (40°C, 20% rh) to perform 2 to 3 h of moderate intensity treadmill and cycling exercise. For the euhydrated trial (EU), water was available ad libitum during the exercise. For the hypohydration trial (HY), drinking was restricted to produce a 4% body weight loss (BWL). The exercise mode, duration and intensity were held constant for each trial. In the event that the exercise protocol did not elicit the desired body weight loss, supplemental sauna exposure was included. Trial order was randomly assigned and balanced across subjects. After exercise, the volunteers were given a small standardized meal (approximately 400 kcal; 70% carbohydrate) and 200 ml of fruit juice.

Three to eight hours of recovery separated the dehydration sessions and

experimental testing in the magnetic resonance system. During the recovery period, the volunteers were provided ad libitum access to water and other beverages that did not contain sugar or caffeine if they were performing the euhydration trial, but fluids were restricted to maintain the desired water deficit for the hypohydration trial. This recovery period was spent resting in a temperate climate.

For the experimental trials, volunteers performed single-leg knee extension exercise to exhaustion while lying supine inside a whole-body 1.5 Tesla magnetic resonance system (GE SIGNA, GE Medical Systems, Milwaukee, WI). The experimental setup is illustrated in Figure 1-A. The ergometer was made of nonferrous materials and isolated a relatively large muscle mass to enhance the signal-to-noise ratio (Larson, Hesslink, Hrovat, Fishman, and Systrom, 1994). Single leg knee extension exercise was performed at 37 contractions/min through a range of motion of approximately 110 to 140 degrees knee extension. The resistance was determined from practice sessions and set to elicit exhaustion in approximately 4-5 min. The same resistance was used for both trials and was achieved by adding frictional resistance with known quantities of lead weight suspended over a flywheel (set in motion by the knee-extension exercise). An elastic cord returned the lever arm to the starting position after each knee extension motion. Force, range of motion and kick duration were measured by a computer-based data acquisition system (Strawberry Tree, Sunnyvale, CA) interfaced to a force transducer and 360° potentiometer located in-line between the knee extension lever arm and the flywheel. The average power output was  $19 \pm 3$  Watts. The volunteers were instructed to perform the knee-extension task as long as possible. Endurance time was defined as the time when the power generated each kick declined 20% below the average value during the initial minute of exercise.



**Figure 1-A.** Experimental setup for  $^{31}\text{P}$  MRS studies.

The subjects were given verbal encouragement to produce maximal effort. Both legs were tested in each hydration condition and the results treated as independent observations.

Prior to exhaustive exercise and at select times during recovery, measurements of muscle efficiency (left leg) or muscle strength (right leg) were obtained. For the muscle efficiency tests, subjects performed 6 knee-extensions within the 10 sec  $^{31}\text{P}$  MRS sampling periods 100 sec and 50 sec before exhaustive exercise, at 30 sec of recovery, and every min thereafter through 5 min of recovery. Muscle strength was measured by having the subjects perform a maximal voluntary isometric contraction for 5 sec with the knee at  $\sim 110$  degrees extension. The procedure was performed 100 sec and 50 sec before exhaustive exercise, every 30 sec of recovery for 2 min, and every minute thereafter through 5 min of recovery.

$^{31}\text{P}$  spectra were collected at rest and during exercise through an 11 cm  $^1\text{H}/^{31}\text{P}$  dual radio frequency (RF) transmit/receive coil (USAsia, Inc, Columbus, OH) placed over the quadriceps muscles. Data were acquired using a hard pulse 25.85 MHz excitation (pulse width 600  $\mu\text{sec}$ ), TR = 1,000 msec, spectral width 2,000 Hz and 1,024 sampled free induction decay (FID) signals. Prior to exercise a proton MR image was acquired axially using the  $^1\text{H}/^{31}\text{P}$  RF coil to verify coil placement and muscle group participation. A special linear gradient shim procedure was performed to reduce magnetic field inhomogeneity within the sensitive volume. RF coil transmitter and receiver gains for  $^{31}\text{P}$  MRS were set once to maximize phosphocreatine (PCr) signal acquired from the muscle, and kept constant throughout the study. Ten FID signals were averaged producing 1 average spectrum every 10 seconds. Post-processing consisted of apodization of 10 Hz line broadening, zero-filling to 4,096 points and Fourier transformation, followed by zero and first order phasing. Peak areas from the PCr, Pi and  $\beta$ -ATP peaks were used to determine phosphorous ratios. Muscle pH was calculated from the frequency shift between Pi and PCr using the following equation:  $\text{pH} = 6.73 + \log_{10}[(a-3.275)/5.685-a]$  where 'a' is the chemical shift from Pi to PCr (Gadian, Radda, Dawson, and Wilkie, 1982). Monovalent Pi ( $\text{H}_2\text{PO}_4^-$ ) was calculated using the following equation:  $[\text{H}_2\text{PO}_4^-] = ([\text{H}^+][\text{Pi}]) / (K_{\text{Pi}} + [\text{H}^+])$ ; where [Pi] was estimated from Pi/ $\beta$ -ATP and assumed muscle ATP concentration of 5.5 mM. The equilibrium constant,  $K_{\text{Pi}}$ , was taken to be  $1.86 \times 10^7 \text{ M}^{-1}$ . Recovery kinetics for PCr resynthesis were determined by calculating the time constant for the left leg PCr/ $\beta$ -ATP data. Recovery data were fit to a mono-exponential curve and time constant calculated from the derived rate constant. MRS system calibration was periodically verified using known standards.

## **STATISTICAL ANALYSIS**

The data were analyzed using one- and two-way repeated measures analysis of variance, where appropriate. For all analyses, the data obtained from each leg were treated as an independent set of measurements. During one trial, time to fatigue was not reached due to technical difficulties. Therefore, data from that leg were not included in statistical analysis. On three other trials, collected spectra were uninterpretable and all MR data for those legs were excluded from statistical analysis. Tukey's highly significant difference procedure was used to identify differences between means when statistical significance was achieved. Statistical significance was tested at the  $P < 0.05$  level. Data in the text are reported as means  $\pm$  sd.

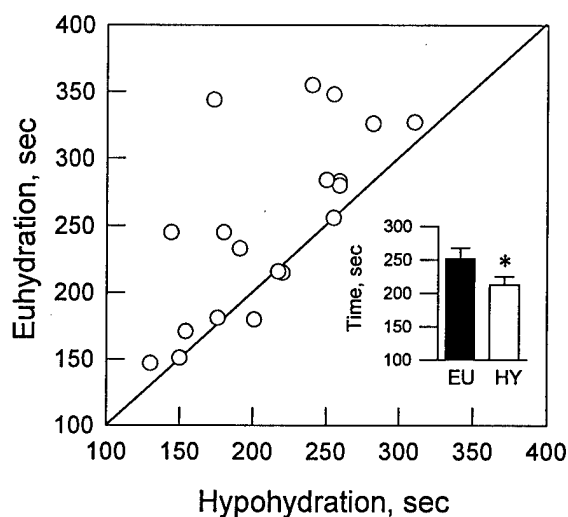
## **EXPERIMENT #1: RESULTS**

### **BODY WEIGHT LOSS**

Prior to performing exercise in the hot room, body weights were  $65.9 \pm 12.8$  kg and  $66.1 \pm 13.0$  kg for euhydration and hypohydration, respectively. The dehydration-rehydration procedures resulted in  $0.6 \pm 0.7\%$  and  $4.0 \pm 0.5\%$  BWL, respectively, prior to the MRS tests.

### **MUSCLE ENDURANCE**

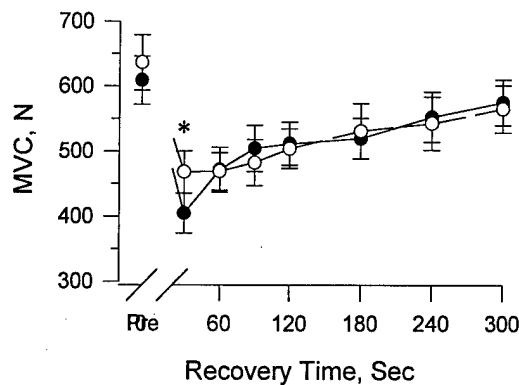
Figure 1-B presents the individual leg and mean endurance times to exhaustion. Hypohydration reduced endurance time  $\geq 8\%$  (coefficient of variation for time to fatigue) in 12 of 19 of the trials performed and mean endurance was reduced ( $P < 0.05$ ) from  $251 \pm 67$  sec to  $213 \pm 52$  sec (15%). Four of ten subjects had reduced endurance time in both legs when hypohydrated, while in three others, only one leg was affected. For these three subjects, the reduced exercise performance occurred in the second leg tested. For one subject, endurance time was reduced in one leg, but not tested in the other leg due to technical problems. These results were similar to our pilot work ( $n=5$  subjects) where 4%-5% BWL reduced endurance in 8 of 10 trials and reduced ( $P < 0.05$ ) mean endurance time from  $230 \pm 108$  to  $192 \pm 101$  seconds (17%). These combined results demonstrate that hypohydration decreased mean endurance time (20 of 29 tests) by 15%-17% using this exercise paradigm.



**Figure 1-B.** Individual and group mean results for time to fatigue when euhydrated and hypohydrated by 4% of initial body weight. \* Hypohydration less than euhydration,  $P < 0.05$ .

## MUSCLE STRENGTH

Figure 1-C presents maximal voluntary contraction (MVC) data. Hypohydration did not alter pre-endurance exercise maximal isometric force. Hypohydrated persons produced a 16% higher ( $P < 0.05$ ) maximal isometric force 30 seconds after exhaustive exercise. No other difference between trials existed during recovery from exhaustive exercise. Furthermore, there was no correlation between the increased MVC at 30 sec post exercise and the reduction in endurance time when hypohydrated.



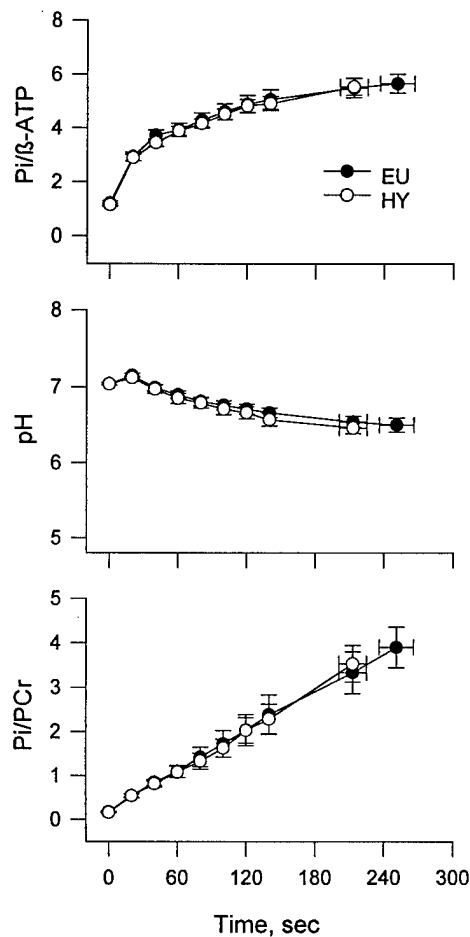
**Figure 1-C.** Maximal isometric force prior to and after exhaustive exercise when eu- and hypohydrated. Data are means $\pm$ se for 9 subjects. \* Hypohydration greater than euhydration,  $P < 0.05$ .

### <sup>31</sup>P MRS

Figure 1-D presents Pi/ $\beta$ -ATP, pH and Pi/PCr collected during all experimental trials, and these variables were not altered by hydration. Pi/ $\beta$ -ATP were similar at rest, averaging  $1.20 \pm 0.33$  and  $1.14 \pm 0.25$  during euhydration and hypohydration trials, respectively. During exhaustive exercise, the Pi/ $\beta$ -ATP rose progressively to peak values of  $5.66 \pm 1.39$  and  $5.55 \pm 1.30$  during euhydration and hypohydration, respectively. Similarly, Pi/PCr rose from resting values averaging  $0.17 \pm 0.05$  to  $3.79 \pm 1.83$  and  $3.46 \pm 1.54$  at exhaustion during euhydration and hypohydration, respectively. The pH fell progressively from  $7.04 \pm 0.07$  at rest to  $6.49 \pm 0.33$  at exhaustion during euhydration and hypohydration, respectively.  $H_2PO_4^-$  levels rose from  $2.2 \pm 0.7$  mM at rest to similar levels (NS) at exhaustion (EU= $19.1 \pm 7.6$  mM, HY= $20.5 \pm 7.1$  mM). Similar to exercise data, hypohydration did not alter ( $P < 0.07$ ) the time constant of PCr synthesis after exhaustive exercise (EU= $63 \pm 19$  sec; HY= $72 \pm 22$  sec).

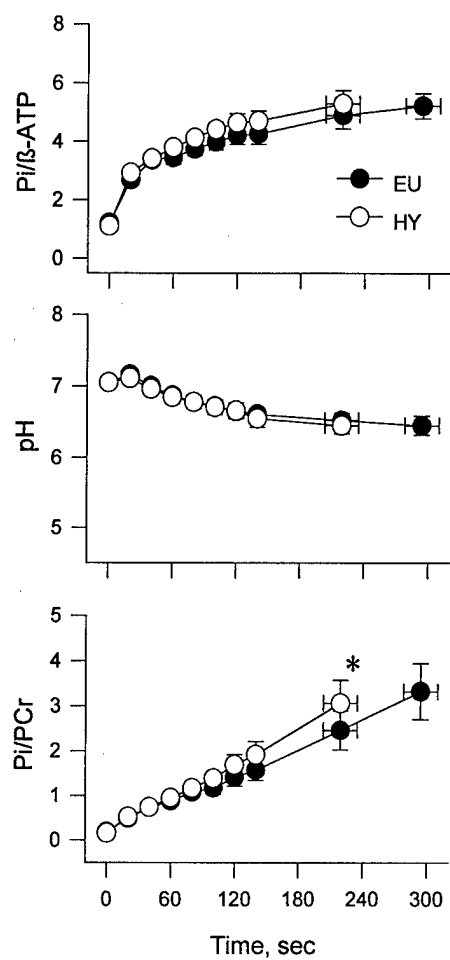
To further investigate whether elevated levels of Pi or  $H^+$  could contribute to reduced endurance time, the data were subdivided to only compare spectra from trials when endurance times were reduced. Figure 1-E presents this subset. Note that Pi/ $\beta$ -

ATP and pH were similar (NS) between euhydration and hypohydration during rest and exercise. In contrast, the Pi/PCr ratio increased more rapidly and to a higher ( $P<0.05$ ) level at the time of hypohydration exhaustion during hypohydration compared to euhydration. Examination of the individual data revealed that the higher Pi/PCr when hypohydrated was largely attributable to 3 of 10 trials, and there was no statistical correlation between the higher Pi/PCr ratios and reduced endurance times.  $\text{H}_2\text{PO}_4^-$  levels were also similar ( $P>0.05$ ) between trials at time of hypohydration exhaustion.



**Figure 2-D.** Muscle Pi/β-ATP, pH and Pi/PCr data for all tests when euhydrated and hypohydrated. Data are means±se for 15 paired comparisons.





**FIGURE 1-E.** Muscle Pi/β-ATP, pH and Pi/PCr for subgroup of tests when hypohydration shortened time to fatigue. Data are means±se for 10 paired comparisons. \*Hypohydration greater than euhydration,  $P < 0.05$ .

## EXPERIMENT #1: DISCUSSION

To our knowledge, this study is the first to simultaneously examine the impact of hypohydration on skeletal muscle performance and muscle metabolism. The level of hypohydration studied is commonly achieved by athletes during competition and training (American College of Sports Medicine, 1996). To minimize the likelihood of hypoglycemia and to replace some of the carbohydrate metabolized during the dehydration procedures, a small meal was given during the recovery period prior to the experimental trials. To isolate the effects of hypohydration on muscle from the potentially confounding effects of elevated body temperature (Nielsen, Kubica, Bonnesen et al., 1981), a minimum of three hours rest separated the heat exposures from experimental testing, and the MRS experiments were conducted in a cool room (~18°C).

We found that hypohydration reduced muscular endurance by 15%, but had no effect on muscle strength. These findings support data from earlier studies which demonstrated that hypohydration can impair muscle endurance (Bijlani and Sharma, 1980; Torranin, Smith, and Byrd, 1979) but had no effect on muscle strength (Bijlani and Sharma, 1980; Greenleaf, Prange, and Averkin, 1967; Saltin, 1964a; Serfass, Stull, Alexander, and Ewing, 1968; Singer and Weiss, 1968). It also agrees with data from studies demonstrating that hypohydration can reduce aerobic endurance (see reference (Sawka, Montain, and Latzka, 1996) for review). Our results extend the findings of these earlier studies by separating the effects of hypohydration from the confounding effects of elevated body temperature, cardiovascular strain, heat exposure, and differing quantities of exercise prior to experimental testing. This study also demonstrated that hypohydration has no effect on recovery of muscle strength after exhausting exercise.

During exercise, we employed  $^{31}\text{P}$  MRS to assess whether hypohydration would accelerate the accumulation of  $\text{H}^+$  or  $\text{Pi}$  during exhaustive exercise. These two variables were chosen as both have been shown to reduce cross-bridge formation and force production, and are the two variables within muscle often considered to be responsible for fatigue during high intensity exercise (Enoka and Stuart, 1992; Fitts, 1994). We hypothesized that if hypohydration had direct effects on muscle metabolism, then the hypohydration trials would likely be associated with elevated exercise  $\text{H}^+$  and/or  $\text{Pi}$  concentrations. The results of this study did not support this hypothesis, however, as  $\text{Pi}/\beta\text{-ATP}$ ,  $\text{pH}$ , and  $\text{H}_2\text{PO}_4^-$  responses to exercise were not affected by 4%

BWL. The lone observation that suggested that hypohydration had an effect on muscle metabolism was the accelerated increase in Pi/PCr in the subgroup of trials with shortened time to fatigue. This would suggest that hypohydration required greater reliance on creatine kinase to sustain muscle ATP in these trials. The fact that Pi/PCr ratio was not consistently elevated even in this subgroup or correlated with maintenance of endurance time, however, would further support the contention that moderate levels of hypohydration had little or no effect on muscle metabolism.

How hypohydration reduces muscle endurance remains an intriguing question. Approximately 50% of the water lost would be expected to come from the intracellular water compartments, and 4% BWL would be expected to reduce intramuscular water 4%-5% (Costill, Coté, and Fink, 1976). It is unlikely that insufficient oxygen delivery was responsible for the shortened time to fatigue. The muscle mass activated during exhaustive exercise was not large enough to limit leg blood flow, and the exercise device was designed to reduce any isometric and eccentric muscle loading during the recovery phase of the contraction - minimizing disruptions in muscle blood flow when the muscle wasn't performing the knee extension movement. Furthermore, any impairment in oxygen delivery would be expected to increase muscle glycolytic flux and formation of lactate and H<sup>+</sup>. We found no difference in muscle pH during exhaustive exercise regardless of whether the exercise was performed when euhydrated or hypohydrated.

Alternative mechanisms within muscle include altered cell depolarization and changes in calcium release and/or uptake by the sarcoplasmic reticulum. Dehydration-induced changes in the ionic status of the T tubular lumen and intracellular compartments could contribute to the development of fatigue by negatively affecting the T tubular charge movement (Fitts, 1994). Similarly, longer calcium transients might reduce calcium flux upon depolarization and reduce force production (Fitts, 1994). The possibility that elevated intracellular magnesium ions play a role appears unlikely as other investigators (Costill and Saltin, 1975) found no difference in intracellular magnesium concentration during exercise when volunteers were tested euhydrated or hypohydrated by 4% of their initial body weight.

An alternative explanation for the detrimental effects of hypohydration on muscle endurance is that hypohydration alters central nervous system function. In the subgroup of trials in which muscle strength was measured by performance of MVC before and after exercise, muscle endurance time was reduced ( $P < 0.05$ ) 14%, yet volunteers were able to generate greater absolute force during the initial period of recovery; suggesting that the subjects were either less willing or unable to sustain

voluntary concentric exercise when hypohydrated, despite having adequate muscle strength. An unwillingness or inability to generate or maintain adequate CNS drive to the working muscle is thought to be responsible for the debilitating fatigue that accompanies many infections and illnesses, recovery from injury, and chronic fatigue syndrome (Davis and Bailey, 1997). Hypohydration may impair performance in a similar manner. These conditions are characterized by an increased perception of effort during physical activity, yet the afflicted are capable of generating maximal force (Davis and Bailey, 1997). Body water loss also increases perception of effort during physical activities (Dengel, Weyand, Black, and Cureton, 1993; Montain and Coyle, 1992), yet has no apparent effects on maximal strength (Bijlani and Sharma, 1980; Saltin, 1964a; Sawka, Montain, and Latzka, 1996). In addition, hypohydration is known to alter neuronal firing of osmoreceptive cells located in the organum vasculosum laminae terminalis and cells near the preoptic/anterior hypothalamic areas of the brain (Boulant, 1997). Neuronal activation mediated by hypohydration might also alter the magnitude of corollary discharge from the motor cortex.

In summary, we found that moderate hypohydration 1) decreases skeletal muscle performance by reducing endurance by 15%; 2) does not alter muscle strength or recovery of muscle strength after exhaustive exercise; and 3) does not alter pH and Pi/ $\beta$ -ATP response to exhaustive exercise. These findings clearly identify another physiologic system by which hypohydration adversely affects human exercise performance; however, the mechanisms for this action are unclear.

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## EXPERIMENT #2: INTRODUCTION

Studies have reported age-related reductions in skeletal muscle size, type II fiber diameter, mitochondrial enzyme activity, and high-energy phosphate metabolism that are associated with the decline in skeletal muscle strength and endurance capacity that occurs with aging (Brooks and Faulkner, 1994; Coggan, Spina, King, et al., 1992; Conley, Cress, Jubrias, Esselman, and Odderson, 1995; McCully, Forciea, Hack, et al., 1991; McCully, Fielding, Evans, Leigh and Posner, 1993; Thompson, Kemp, Sanderson, and Radda, 1995). However, the underlying processes through which these changes occur are not well understood. Reduced levels of resting phosphocreatine (PCr) reported in the elderly may be in part responsible for these declines (McCully, Forciea, Hack, et al., 1991; Moller, Bergstrom, Furst, and Hellstrom, 1980). A decline in PCr availability has been shown to be one of the factors contributing to muscle fatigue during moderate to high intensity exercise (Cady, Jones, Lynn, and Newham, 1989; Hultman, Bergstrom, and Anderson, 1967). In addition, resynthesis rates of PCr after exercise have been reported to decline with age by approximately 8% every 10 years after age 30 (McCully, Fielding, Evans, Leigh, and Posner, 1992). It is believed that PCr resynthesis is regulated by creatine kinase bound to the outer membrane of mitochondria and that the initial rate of PCr recovery is proportional to the rate of mitochondrial oxygen consumption (Kemp, Taylor and Radda, 1993; Mahler, 1985; Meyer, 1988; Taylor, Styles, Mathews, et al., 1986). Strong correlations between PCr resynthesis rate, mitochondrial enzyme activity and oxygen consumption support this relationship (Mahler, 1985; McCully, Fielding, Evans, Leigh, and Posner, 1993; Meyer, 1988; Takahashi, Inaki, Fujimoto, et al., 1995; Thompson, Kemp, Sanderson, and Radda, 1995).

Dietary creatine supplementation (20 to 30 grams/day for 4 to 6 days) has been reported to increase muscle creatine concentration by as much as 50% and enhance muscle performance during intermittent high-intensity exercise bouts (Balsom, Ekblom, Soderlund, Sjodin, and Hultman, 1993; Balsom, Soderlund, Sjodin, and Ekblom, 1995; Birch, Noble, and Greenhaff, 1994; Earnest, Snell, Rodriguez, Almada, and Mitchell, 1995; Greenhaff, Casey, Short, Harris, Soderlund, and Hultman, 1993; Greenhaff, bodin, Soderlund, Hultman, 1994; Harris, Soderlund, and Hultman, 1992). The performance enhancing effect of creatine may result from increased muscle creatine availability which sustains the initially rapid rate of PCr resynthesis further into recovery



and increases available PCr during later exercise bouts (Balsom, Soderlund, Sjodin, and Ekblom, 1995; Greenhaff, Bodin, Soderlund, and Hultman, 1994; Harris, Soderlund, and Hultman, 1992). No studies have investigated the effects of creatine in older persons who, because of intrinsic deficits in muscle energy metabolism, may benefit from creatine supplementation.

The purpose of this study was to determine the effects of creatine supplementation and age on muscle energy metabolism and physical performance using  $^{31}\text{P}$  MRS.  $^{31}\text{P}$  MRS provides frequent serial non-invasive measurements of intramuscular phosphorus compounds and greatly improves measurement resolution over muscle biopsy techniques (Meyer, Kushmerick, and Brown, 1982), the most frequently used method of assessing the effects of creatine supplementation on muscle metabolism to date. We hypothesized that middle-aged persons would have lower resting muscle PCr concentrations and slower PCr resynthesis rates than younger adults and that creatine supplementation would have greater effects on muscle energy metabolism and physical performance in middle-aged persons.

## **EXPERIMENT #2: METHODS**

### **SUBJECTS**

Middle-aged (> 50 yr) and young (< 40 yr) subjects were recruited for participation in the study. The younger group consisted of 4 men and 1 woman and the middle-aged group of 3 men and 1 woman. The physical characteristics of the subjects are presented in Table 2-A. All subjects were free from chronic disease and on no regular medications as determined by a medical history questionnaire. The study was approved by the appropriate institutional review boards, and all subjects gave their voluntary and informed consent prior to participation.

The level of habitual physical activity was not different between the young and middle-aged groups as indicated by the Harvard Alumni Questionnaire (Paffenbarger, Wing, and Hyde, 1978). In addition, due to a potential effect of diet on skeletal muscle creatine, the subjects were questioned on their normal dietary habits. There were no vegetarians in the study, and all the subjects reported consuming at least 5 servings of meat per week.

TABLE 2-A. Physical characteristics of the young and middle-aged subjects.

Group	n	Age (yrs)	Height (cm)	Placebo	Creatine
				Weight (kg)	Weight (kg)
Young	5	31 ± 5.2	174 ± 12.8	67.9 ± 11.7	68.1 ± 12.1
Middle Aged	4	58 ± 4.5	179 ± 3.11	83.3 ± 12.8	83.9 ± 12.7

## CREATINE SUPPLEMENTATION

Two single-blind exercise trials were performed, consisting of a placebo trial followed by a creatine trial 7 days later. The trials were not randomized, as skeletal muscle creatine levels can remain elevated above basal levels for 4 to 5 weeks after stopping supplementation (Hultman, Bergstrom, and Anderson, 1996). Five days prior to each trial, the subjects began consuming  $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  of either a placebo (granulated sugar) or  $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  of creatine monohydrate (*Phosphagen*, Experimental and Applied Sciences, Pacific Grove, CA) combined with  $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  of a flavored powder drink mix. The mixture was dissolved in water and consumed 4 times per day.

## EXERCISE

Both groups performed single leg knee extension exercise while lying supine inside a whole-body 1.5 tesla MR system (General Electric SIGNA, General Electric Medical Systems, Milwaukee, WI). The exercise apparatus provided concentric resistance via a lever arm and pulley system integrated with a flywheel and resistance strap. An elastic cord returned the lever arm to the starting position after each knee extension. Knee extensions were performed from ~110 to ~145 degrees of knee extension at 37 contractions per minute set by an audible metronome. Power output during exercise was determined by measuring the tension and displacement applied to an in-line pulley and by estimating leg mass (Winter, 1979).

Prior to the experimental trials, 2 to 3 exercise practice sessions were performed to familiarize the subjects with the experimental procedures and to determine the appropriate exercise intensity. During the experimental trials, 3 single leg exercise bouts were performed separated by 3 minutes of recovery. Bouts 1 and 2 were 2

minutes each in duration and bout 3 was continued to exhaustion. Exhaustion was defined as the time when the rate and/or range of motion could not be maintained by the subject after being given verbal encouragement by the investigators. Flywheel resistance was determined from practice sessions and set to elicit exhaustion in 1 to 2 minutes during bout 3. The resistance was kept constant across experimental trials. Both legs were tested in each experimental condition and the results treated as independent observations.

### **<sup>31</sup>P MR SPECTROSCOPY**

<sup>31</sup>P spectra were collected during exercise through a <sup>1</sup>H/<sup>31</sup>P dual radio frequency transmit/receive 11 cm surface coil (USAsia Inc., Columbus, OH) placed over the quadriceps muscles. <sup>31</sup>P data were acquired using a hard pulse 25.85 MHz excitation (pulse width 600 usec), TR = 1,000 msec, spectral width 2,000 Hz and 1,024 sampled free induction decay (FID) points. Prior to exercise, a proton MR image was acquired axially using the <sup>1</sup>H/<sup>31</sup>P surface coil to verify coil placement and muscle group participation. A linear gradient shim procedure was performed to reduce field inhomogeneity within the sensitive volume. Surface coil transmitter and receiver gains for <sup>31</sup>P MRS were set once to maximize PCr signal acquired from the muscle and kept constant throughout the study. Ten FID signals were averaged producing 1 spectra every 10 seconds. Care was taken to ensure that exercise began and ended at the onset of a FID cycle. The MR system was calibrated using known standards on each testing day.

FID processing consisted of apodization of 10 Hz line broadening, zero-filling to 4,096 points and Fourier transformation, followed by zero and first order phasing. Relative concentrations of inorganic phosphate (Pi), phosphocreatine (PCr) and  $\beta$ ATP were determined from spectral peak areas and presented as  $PCr/\beta ATP_{rest}$ ,  $Pi/\beta ATP_{rest}$  and  $\beta ATP/\beta ATP_{rest}$  ratios, where  $\beta ATP_{rest}$  is the mean area of six initial resting  $\beta ATP$  peaks collected prior to exercise. The relative quantity of PCr hydrolysis during exercise was represented as the change in  $PCr/\beta ATP_{rest}$  during each exercise bout. Lastly, pH was calculated using the chemical shift between the Pi and PCr frequency (Taylor, Styles, Mathews, et al., 1986).

The rate of PCr resynthesis and a time constant (Tc) indicating half time of recovery were determined from a mono-exponential curve fit to the  $PCr/\beta ATP_{rest}$  recovery data (Kemp, Taylor, and Radda, 1993; Mahler, 1985; Meyer, 1988; Thompson, Kemp, Sanderson, and Radda, 1995). The following equation was used: y

=  $a[1-\exp(bx)]+c$ , where  $y$  represents the PCr value at any given time  $x$ ,  $a$  is the change in PCr during recovery,  $b$  is the rate constant ( $1/b = T_c$ ) and  $c$  is the initial PCr value at the onset of recovery. The values for PCr resynthesis rate were determined from the slope of the initial 10 seconds of the mono-exponential curve fit (Mahler, 1985; Thompson, Kemp, Sanderson, and Radda, 1995) and converted to  $\text{mmol}^{-1} \cdot \text{min}^{-1} \cdot \text{kg wet weight}^{-1}$  by assuming the area of  $\beta\text{ATP}_{\text{rest}}$  is equivalent to  $5.5 \text{ mmol}^{-1} \cdot \text{kg wet weight}^{-1}$  (Taylor, Styles, Mathews et al., 1986).

## ANALYSIS

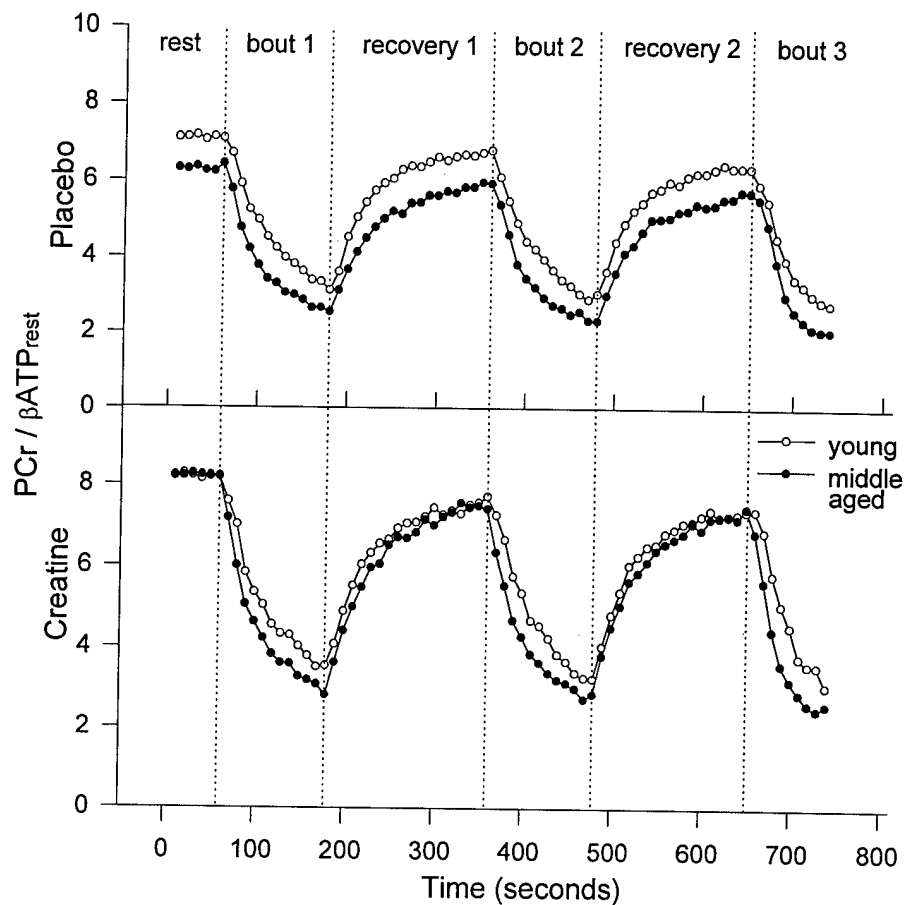
Data obtained from each individual leg were treated as independent observations. Phosphorus ratio, pH, PCr hydrolysis, PCr resynthesis rate,  $T_c$  and exercise power output data were analyzed using analysis of variance and subsequent Newman-Keuls post hoc test to determine mean differences between the young and middle-aged groups. Mean differences between placebo and creatine trials were determined by a repeated measures analysis of variance and subsequent Newman-Keuls post hoc test. Paired t-tests were used to determine mean differences in body weight and time to exhaustion between the placebo and creatine trials for the young and middle-aged groups. The significance level was set at  $p < 0.05$  and results are presented as means and standard deviations.

## EXPERIMENT #2: RESULTS

### AGE

During the placebo trial, resting  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  was lower in the middle-aged group,  $6.36 \pm 0.94$ , compared to the young group,  $7.18 \pm 0.93$  ( $p < 0.05$ ). The difference in  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  between the young and middle-aged groups persisted throughout the exercise and recovery bouts as illustrated in figure 2-A. PCr hydrolysis ( $\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$ ; Table 2-B) during the exercise bouts tended to be lower in the middle-aged group ( $p = 0.06$ ), while power output during exercise was not different between groups (Table 2-B). As indicated by the practice trials, the exercise resistance was increased in bout 3 to ensure that exhaustion occurred in 1 to 2 minutes (Table 2-B). One young subject was excluded from all analysis of PCr hydrolysis, PCr resynthesis rate and time to

exhaustion due to experimental difficulties during exercise in the placebo trial. The young group for these measures consisted of 3 men and 1 woman, age  $30 \pm 5$  years.



**FIGURE 2-A.**  $PCr/\beta ATP_{rest}$  vs. time (seconds) during repeated bouts of exercise for the creatine and placebo trials for both the young and middle-aged groups. Open symbols = young subjects and solid symbols = middle-aged subjects.

TABLE 2-B. Mean power output (Watts), PCr hydrolysis ( $\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$ ), time to exhaustion (seconds), and PCr resynthesis time constant after exercise ( $T_c$ , seconds) for the young and middle-aged subjects' placebo and creatine trials. The  $\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$  for exercise bout 3 represents the PCr hydrolysis that occurred from the onset of bout 3 to exhaustion, or after completion of 2 minutes of exercise, whichever occurred first. \*Indicates a significant difference from the placebo trial ( $p < 0.05$ ).

Trial	Group		Bout 1	Bout 2	Bout 3
Placebo	Young	Mean Power (W)	$19.4 \pm 1.5$	$19.3 \pm 1.5$	$22.4 \pm 3.2$
		$\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$	$-4.48 \pm 1.29$	$-4.50 \pm 1.09$	$-5.33 \pm 1.36$
		Time to Exh. (s)	--	--	$122 \pm 36$
		$T_c$ (s)	$40.1 \pm 12.5$	$40.0 \pm 11.9$	--
	Middle-Aged	Mean Power (W)	$18.4 \pm 5.4$	$18.6 \pm 5.6$	$21.7 \pm 7.1$
		$\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$	$-4.17 \pm 0.84$	$-3.97 \pm 0.99$	$-4.19 \pm 1.35$
		Time to Exh. (s)	--	--	$113 \pm 31$
		$T_c$ (s)	$50.0 \pm 10.2$	$42.8 \pm 11.1$	--
Creatine	Young	Mean Power (W)	$19.9 \pm 2.8$	$19.8 \pm 3.3$	$22.3 \pm 4.5$
		$\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$	$-5.47 \pm 1.78$	$-5.01 \pm 0.86$	$-6.10 \pm 0.94$
		Time to Exh. (s)	--	--	$162 \pm 59^*$
		$T_c$ (s)	$45.8 \pm 14.3$	$43.4 \pm 14.1$	--
	Middle-Aged	Mean Power (W)	$18.5 \pm 4.8$	$18.7 \pm 4.8$	$22.1 \pm 6.1$
		$\Delta\text{PCr}/\beta\text{ATP}_{\text{rest}}$	$-5.68 \pm 1.56^*$	$-5.03 \pm 1.34^*$	$-5.44 \pm 1.16^*$
		Time to Exh. (s)	--	--	$144 \pm 81$
		$T_c$ (s)	$48.7 \pm 10.9$	$45.1 \pm 7.2$	--

Following exercise, the initial PCr resynthesis rate was slower in the middle-aged group compared to the young group ( $p < 0.05$ , Figure 2-B). Figure 2-C shows the mono-exponential curve fit to the  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  data from the first recovery period for a representative young and middle-aged subject. The  $T_c$  obtained from the mono-exponential equation was similar for the young and middle-aged groups (Table 2-B). In addition,  $\text{Pi}/\beta\text{ATP}_{\text{rest}}$  (Figure 2-D) and pH (Table 2-C) were not different between the groups during rest, exercise or recovery.

TABLE 2-C. Muscle pH values for the young and middle-aged subjects during rest and at the end of bout 1, recovery 1 and bout 2 for the placebo and creatine trials.

Trial	Group	Rest	End Bout 1	End Recovery 1	End Bout 2
Placebo	Young	$7.12 \pm .04$	$6.75 \pm .24$	$7.00 \pm .12$	$6.91 \pm .15$
	Middle-Aged	$7.12 \pm .04$	$6.60 \pm .29$	$6.93 \pm .28$	$6.66 \pm .18$
Creatine	Young	$7.12 \pm .03$	$6.76 \pm .26$	$7.03 \pm .15$	$6.76 \pm .33$
	Middle-Aged	$7.11 \pm .02$	$6.66 \pm .11$	$6.95 \pm .12$	$6.77 \pm .14$

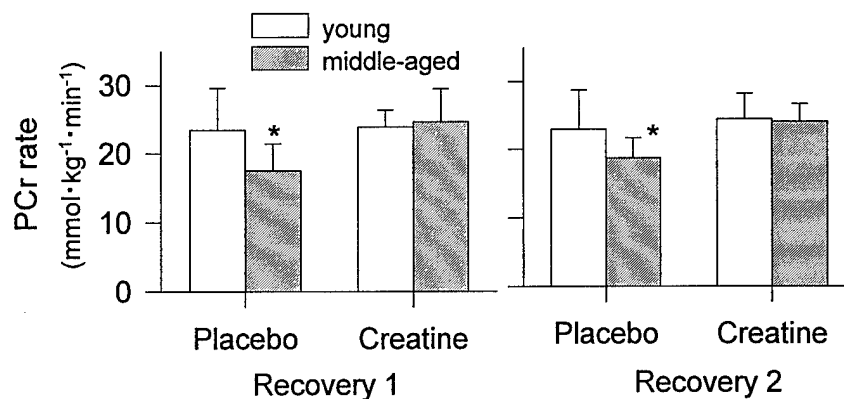
## CREATINE SUPPLEMENTATION

Following creatine supplementation, resting  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  was increased in the young group by 15% ( $p < 0.05$ ) and in the middle-aged group by 30% ( $p < 0.05$ ), eliminating the difference in resting  $\text{PCr}/\beta\text{ATP}_{\text{rest}}$  between the young and middle-aged groups,  $8.3 \pm 1.3$  and  $8.3 \pm 1.0$ , respectively (Figure 2-A). PCr hydrolysis during exercise increased in the middle-aged group ( $p < 0.05$ ) following creatine supplementation and tended to increase in the young group (Table 2-B,  $p = 0.1$ ). Power output remained constant across the placebo and creatine trials for both groups (Table 2-B).

Creatine supplementation increased ( $p < 0.05$ ) the mean time to exhaustion during exercise bout 3 by 30% in the young and middle-aged groups combined. There were no significant interactions or differences in time to exhaustion with regard to age.

All the subjects were encouraged by the investigators during exercise and appeared to give a maximal effort during the time to exhaustion bout.

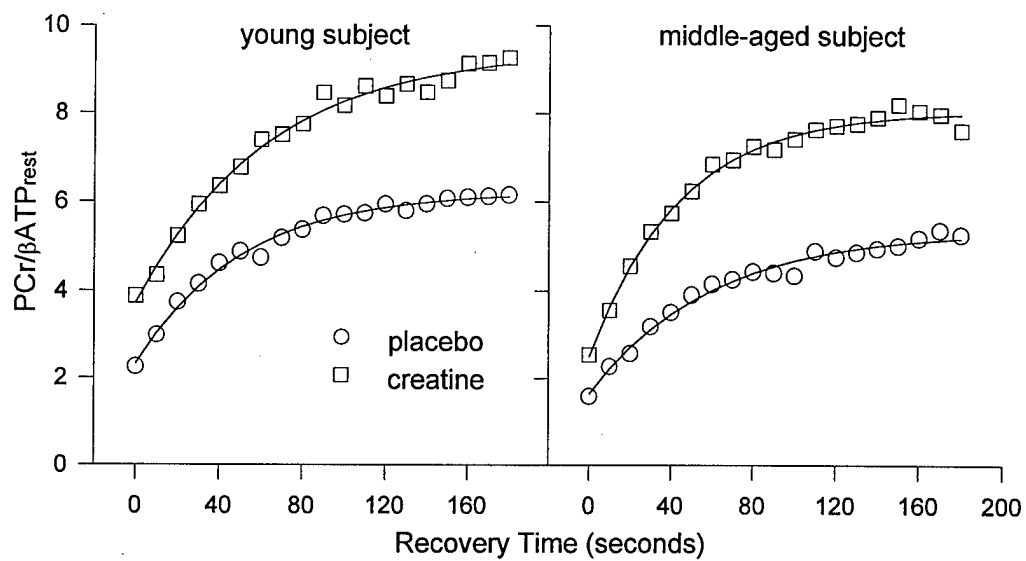
The initial PCr resynthesis rate increased in the middle-aged group ( $p < 0.05$ ) to a level not different from the young group after creatine supplementation (Figure 2-B). In addition, the initial rapid rate of PCr resynthesis appeared to continue further into recovery, maintaining the elevated PCr concentrations in both groups over time (Figures 2-A and 2-C). Creatine supplementation did not significantly change the PCr resynthesis Tc in either group (Table 2-B).



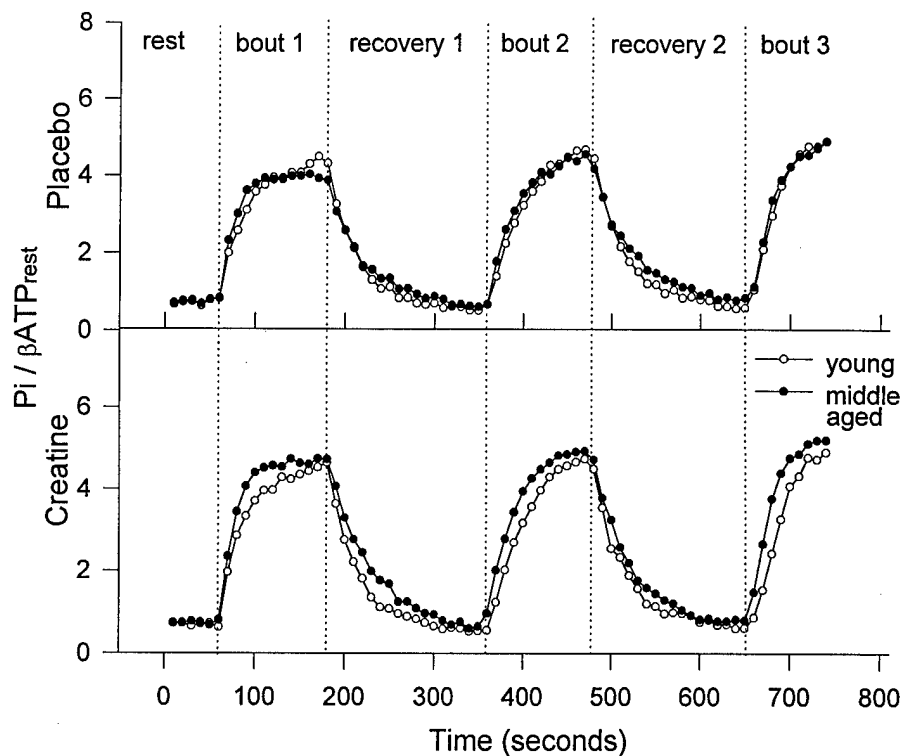
**FIGURE 2-B.** PCr resynthesis rates (PCr rate;  $\text{mmol} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$ ) during recovery 1 and 2 for the placebo and creatine trials. Open bars = young subjects and solid bars = middle-aged subjects. \*Indicates a significant differences from the middle-aged group creatine trial ( $p < 0.05$ ) and a significant difference from the young group placebo trial ( $p < 0.05$ ) during recovery 1 and 2.

The resting  $\text{Pi}/\beta\text{ATP}_{\text{rest}}$  ratio was unaffected by creatine supplementation in the young and middle aged groups (Figure 2-D).  $\text{Pi}/\beta\text{ATP}_{\text{rest}}$  production during exercise tended to increase following creatine supplementation and was similar to the changes observed in PCr hydrolysis (Table 2-B, Figure 2-D). The  $\beta\text{ATP}/\beta\text{ATP}_{\text{rest}}$  ratio (not shown) remained constant from rest throughout exercise and recovery, and there were no differences in  $\beta\text{ATP}/\beta\text{ATP}_{\text{rest}}$  between groups or experimental trials. Additionally, pH declined during the exercise ( $p < 0.05$ ); however, pH was not significantly altered by creatine supplementation in either group (Table 2-C).





**FIGURE 2-C.** Individual  $PCr/\beta ATP_{rest}$  vs. time (seconds) results during recovery 1 for a representative young and middle-aged subject. Circles = the placebo trial and squares = the creatine trial.



**FIGURE 2-D.**  $Pi/\beta ATP_{rest}$  vs. time (seconds) during repeated bouts of exercise during placebo and creatine for both the young and middle-aged groups. Open symbols = young subjects and solid symbols = middle-aged subjects.

## EXPERIMENT #2: DISCUSSION

This study investigated the effects of age on muscle high-energy phosphate metabolism during exercise by comparing young and middle-aged subjects (Table 2-A). In addition, the effects of oral creatine supplementation on muscle performance and metabolism were examined. In order to collect performance and metabolic data simultaneously, knee extension exercise was performed inside a MR system during which the exercise cadence and resistance were held constant and time to exhaustion was used as a measure of quadriceps muscle endurance capacity. Non-invasive  $^{31}P$  MRS was employed to measure quadriceps muscle PCr, Pi, ATP and pH throughout

exercise and recovery during a repeated measures placebo and creatine exercise trial. We hypothesized that older persons would have lower resting PCr concentrations and slower PCr resynthesis rates than younger persons and that oral creatine supplementation would elicit greater improvements in muscle high-energy phosphate metabolism in older persons.

## AGE

Our results confirm reports that older persons have a lower resting muscle PCr concentration as compared to younger persons (McCully, Forcica, Hack et al., 1991; Moller, Bergstrom, Furst, and Hellstrom, 1980) (Figure 2-A). Resting PCr availability was 11% lower in the middle-aged group ( $p < 0.05$ ) and the quantity of PCr hydrolyzed during exercise tended to be lower ( $p = 0.06$ , Table 2-B). An increase in the percentage of type I fibers within muscle has been reported to occur with age and may explain the reduced resting PCr concentration found in older subjects due to the characteristically lower PCr concentrations of type I fibers (Coggan, Spina, King, et al., 1992). However, it should be noted that not all studies investigating age-related changes in muscle metabolism report reductions in resting PCr and increases in the percentage of type I fibers (McCully, Forcica, Hack, et al., 1991; McCully, Fielding, Evans, Leigh and Posner, 1993; Taylor, Crowe, Bore, et al., 1984). McCully et al. (1991) reported resting PCr/Pi levels to be  $\sim 38\%$  lower in middle-aged ( $66.8 \pm 1.9$  yr) and elderly ( $80.0 \pm 5.1$  yr) subjects compared to young ( $24.6 \pm 4.7$  yr) subjects. In a subsequent study, McCully et al. (1993) found no difference in resting PCr/Pi values and no difference in fiber type distribution in older ( $66.0 \pm 6.0$  yr) versus young ( $28.2 \pm 6.8$  yr) subjects. Given these findings, the affect of aging on resting PCr concentration and fiber type distribution requires further investigation.

The results of this study also agree with reports that older individuals have a reduced PCr resynthesis rate after exercise (Conley et al., 1995; McCully et al., 1991a; McCully et al., 1991; McCully et al., 1992a; McCully et al., 1993) (Figure 2-C). PCr resynthesis rate was 22% slower in the middle-aged subjects and may be associated with their reduced resting PCr concentration (Figure 2-B). As PCr is hydrolyzed to resynthesize ATP during exercise, the free creatine concentration in the muscle increases. During recovery, the elevated muscle creatine concentration drives the creatine kinase reaction toward the production of PCr (Meyer et al., 1984). A low muscle PCr concentration is indicative of a low total creatine availability and it has been suggested that a reduced availability of creatine during recovery from exercise limits

creatine kinase resynthesis of PCr (Balsom et al., 1995; Greenhaff, et al, 1994; Harris et al., 1992). Furthermore, an increase in muscle creatine availability elicited by oral creatine supplementation has been reported to prolong the initial rapid rate of muscle PCr resynthesis during recovery (Greenhaff, et al, 1994).

There was no difference in pH between groups or trials (Table 3) and it is assumed that muscle oxidative capacity did not change in the seven days between trials. Both the PCr recovery Tc and initial PCR resynthesis rate have been reported to be independent of pH and exercise intensity when the variation in pH is similar to that observed between the young and middle-aged groups in this study (Meyer, 1988). Although there were differences in the initial PCR resynthesis rate between groups, this appears to be the phase of recovery least affected by pH and dependent more on the muscle ATP and creatine concentration (Arnold, Matthews and Radda, 1984; Meyer, 1988; Taylor, Kemp, Sanderson, et al., 1986). Therefore, it is unlikely that pH significantly influenced the differences in initial PCR resynthesis rate observed between the young and middle-aged groups.

## **CREATINE SUPPLEMENTATION**

Following oral creatine supplementation, resting PCr concentration increased in the young group ( $p < 0.05$ ) and, to a greater extent, in the middle-aged group ( $p < 0.05$ ); eliminating the difference in resting PCr between the young and middle-aged subjects (Figure 2-A). The variation in response to creatine supplementation by the young and middle-aged groups is consistent with reports that persons having relatively low resting muscle PCr concentrations tend to have a larger increase in resting muscle PCr after creatine supplementation (Greenhaff, et al, 1994; Harris et al., 1992).

In addition to the increase in resting PCr, there was an increase in PCr hydrolysis during exercise in the middle-aged group without a change in exercise power output (Table 2-B) suggesting that a greater proportion of ATP supplied to the working muscles was derived from PCr following creatine supplementation. Other studies have reported an increase in PCr use and a reduction in glycolytic and aerobic activity during exercise after creatine supplementation (Balsom et al., 1995; Greenhaff, et al, 1993). The young group tended to use more PCr after creatine supplementation ( $p = 0.1$ ).

The slower PCr resynthesis rate observed in the middle-aged subjects was increased ( $p < 0.05$ ) to a rate similar to that of the young group following creatine supplementation (Figure 2-B). These results support the concept presented previously that muscle creatine availability may affect creatine kinase activity during recovery and

consequently, affect PCr resynthesis rate independent of oxidative capacity and pH (Balsom, Soderlund, Sjodin, and Ekblom, 1995; Greenhaff, Bodin, Soderlund and Hultman, 1994; Harris, Soderlund, and Hultman, 1992). Similar increases in PCr resynthesis rate have been reported to occur after intense physical training (Kent-Braun, McCully, and Chance, 1990) and similar differences exist when trained and untrained populations are compared (Guthrie, Frostick, Goodman, Mikulis, Plyley, and Marshall, 1996; McCully, Vandenborne, DeMeirleir, Posner, and Leigh, 1992; Takahashi, Inaki, Fujimoto, et al., 1995). However, the increase in PCr resynthesis rate in these studies was attributed to improvements in mitochondrial oxidative capacity associated with training which enable the mitochondria to supply ATP to the creatine kinase system at a faster rate. Assuming that mitochondrial oxidative capacity was not affected by creatine supplementation in the present study, the increase in PCr resynthesis rate observed in the middle-aged group most likely resulted from the increase in muscle creatine availability. These results suggest that the availability of muscle creatine may significantly influence the PCr resynthesis rate after exercise in healthy middle-aged persons.

The mono-exponential  $T_c$  is a function of the quantity of PCr hydrolyzed and the PCr resynthesis rate (Kemp, Taylor, and Radda, 1993; Mahler, 1985; Meyer, 1988; Thompson, Kemp, Sanderson, and Radda, 1995). Due to the combined increase in PCr hydrolysis and resynthesis rate ( $p < 0.05$ ) in the middle-aged group after creatine supplementation, the  $T_c$  remained relatively constant (Table 2-B). Likewise, the lack of a significant difference between the young and middle-aged mean  $T_c$  during the placebo trial (Table 2-B) may be explained by the lower PCr hydrolysis ( $p = 0.06$ ) and slower PCr resynthesis rate ( $p < 0.05$ ) observed in the middle-aged group. These results suggest that the  $T_c$  alone may not adequately describe differences in muscle energy metabolism that may exist between experimental groups and conditions.

The initial rapid rate of PCr resynthesis in the young and middle-aged groups appears to proceed for a longer duration after creatine supplementation (Figure 2-C). This maintains an elevated PCr level during recovery (Figure 2-A) despite the increased PCr hydrolysis during exercise observed after creatine supplementation in the middle-aged group (Table 2-B). The prolonged high rate of PCr resynthesis observed in this study agrees with results reported by Greenhaff et al. (1994) that show a sustained initial PCr resynthesis rate in subjects responding to creatine supplementation during the second minute of recovery from exercise. An increase in PCr availability and utilization during exercise and an improved PCr resynthesis capacity during recovery have been proposed as the means through which creatine supplementation improves

muscle performance during intermittent exercise bouts (Balsom, Ekblom, Soderlund, Sjodin, and Hultman, 1995; Greenhaff, Bodin, Soderlund, Hultman, 1994; Harris, Soderlund, Hultman, 1992).

Time to exhaustion in bout 3 of the exercise trial was increased after creatine supplementation in the young group (Table 2-B) indicating that creatine supplementation improves resistance to fatigue in younger persons. This is consistent with the results of several other creatine performance studies involving high-intensity intermittent exercise and relatively young subjects (Balsom, Ekblom, Soderlund, Sjodin, and Hultman, 1993; Balsom, Soderlund, Sjodin, and Ekblom, 1995; Birch, Noble, and Greenhaff, 1994; Earnest, Snell, Rodriguez, Almada, and Mitchell, 1995; Greenhaff, Casey, Short, Harris, Soderlund and Hultman, 1993). Fewer studies have reported that creatine supplementation did not significantly affect exercise performance (Cook, Grandjean, and Barnes, 1995; Febbraio, Flanagan, Snow, Zhao, and Carey, 1995). A greater number of subjects is required to determine if there is a difference in the magnitude of change in time to exhaustion between young and middle-aged persons. In control trials performed by two young subjects, there were no differences in time to exhaustion,  $99 \pm 27$  and  $101 \pm 37$  seconds, respectively, or  $^{31}\text{P}$  MRS measurements between trials, suggesting that time to exhaustion was a reliable measure of muscle performance.

## CONCLUSION

Data from studies have reported that resting PCr concentration and PCr recovery rate are reduced in elderly persons (Conley, Cress, Jubrias, Esselman, and Odderson, 1995; McCully, Forciea, Hack, and Donlon, 1991; McCully, Kakihiira, Vandenborne, and Kent-Braun, 1991; McCully and Posner, 1992; McCully, Fielding, Evans, Leigh, and Posner, 1993; Moller, Bergstrom, Furst, and Hellstrom, 1980). We hypothesized that creatine supplementation would improve PCr availability and resynthesis rate in middle-aged persons more so than young persons. The results of this study indicated that middle-aged persons had a greater improvement in muscle PCr availability and initial PCr resynthesis rate after creatine supplementation as compared to younger persons and that creatine supplementation improved exercise endurance capacity in both groups combined. Whether creatine supplementation improves muscle performance to a greater magnitude in older versus younger individuals requires further study.

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