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Effects of Twenty-Eight Days of Beta-Alanine and Creatine Monohydrate Supplementation on the Physical Working Capacity at Neuromuscular Fatigue Threshold

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Abstract. Stout, J.R., J.T. Cramer, M. Mielke, J. O'Kroy, D.J. Torok, and R.F. Zoeller. Effects of twenty-eight days of beta-alanine and creatine monohydrate supplementation on the physical working capacity at neuromuscular fatigue threshold. *J. Strength Cond. Res. 20(4):928–931. 2006.*—The purpose of this study was to examine the effects of 28 days of beta-alanine (b-Ala) and creatine monohydrate (CrM) supplementation on the onset of neuromuscular fatigue by using the physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) test in untrained men. Fifty-one men (mean age ± *SD* = 24.5 ± 5.3 years) volunteered to participate in this 28-day, double-blind, placebo-controlled study and were randomly assigned to 1 of 4 groups: placebo (PLA; 34 g dextrose; $n = 13$), CrM (5.25 g CrM plus 34 g dextrose; $n = 12$), b-Ala (1.6 g b-Ala plus 34 g of dextrose; $n = 12$), or b-Ala plus CrM (CrBA; 5.25 g CrM plus 1.6 g b-Ala plus 34 g dextrose; $n = 14$). The supplement was ingested 4 times per day for 6 consecutive days, then twice per day for 22 days before posttesting. Before and after the supplementation, subjects performed a continuous incremental cycle ergometry test while a surface electromyographic signal was recorded from the vastus lateralis muscle to determine PWC_{FT}. The adjusted mean posttest PWC_{FT} values (covaried for pretest PWC_{FT} values) for the b-Ala and CrBA groups were greater than those for the PLA group ($p \leq 0.05$). However, there were no differences between the CrM vs. PLA, CrBA vs. b-Ala, CrM vs. b-Ala, or CrM vs. CrBA groups ($p > 0.05$). These findings suggested that b-Ala supplementation may delay the onset of neuromuscular fatigue. Furthermore, there appeared to be no additive or unique effects of CrM vs. b-Ala alone on PWC_{FT}.

Key Words. carnosine, ergogenic aids, electromyography, cycle ergometry

Introduction

DeVries et al. (3, 4) developed an incremental cycle ergometer test called the physical working capacity at fatigue threshold (PWC_{FT}), which utilizes the relationship between electromyographic (EMG) amplitude and fatigue during submaximal cycle ergometry to identify the power output that corresponds to the onset of neuromuscular fatigue. The PWC_{FT} represents the highest power output that results in a nonsignificant ($p > 0.05$) increase in muscle activation of the vastus lateralis over time. The PWC_{FT} test has been shown to be reliable (2, 4, 17), valid (2), and sensitive to changes in fitness level (2); however, the physiological mechanism responsible for the increase in EMG amplitude during a fatiguing task is unknown. Two potential mechanisms include the accumulation of metabolic by-products (lactate, hydrogen ions [H^+], inorganic phosphate (Pi), and ammonia) and the depletion of stored energy substrates (adenosine triphosphate, phosphocreatine [PCr], and glycogen) (13). It has been suggested that skeletal muscle PCr may serve as a temporal energy buffer as well as a modulator of glycolysis, and that, if increased via creatine supplementation (12), it may influence neuromuscular fatigue (16, 21). In support of this hypothesis, Stout et al. (16) demonstrated that 5 days of creatine monohydrate (CrM) loading (4×5 g·d^{-1}) in trained women's crew team members significantly ($p < 0.05$) increased PWC_{FT}. To date, however, no studies have examined the effects of CrM supplementation on PWC_{FT} in men.

Recent studies by Hill et al. (10) and Harris et al. (8) have demonstrated that 28 days of beta-alanine (b-Ala; 4–6 g·d^{-1}) supplementation increased intramuscular levels of carnosine by approximately 60%. It has been suggested that carnosine serves as a buffer and helps maintain skeletal muscle acid-base homeostasis when a large quantity of H^+ is produced during high-intensity exercise (19). Harris et al. (7) demonstrated improvements in performance during a 4-minute maximal cycle ergometry test in men after supplementing with b-Ala (3.2 g·d^{-1}) for 5 weeks. The authors concluded that the improvements may have been caused by an enhanced H^+ buffering capacity as a result of increased muscle carnosine levels after b-Ala supplementation (7).

In theory, increasing skeletal muscle PCr and carnosine concentrations via CrM and b-Ala supplementation, respectively, will work synergistically to delay fatigue by decreasing the reliance on anaerobic glycolysis, reducing intramuscular lactate accumulation, and buffering H^+ during incremental cycle ergometry. However, no previous studies have examined both the unique and the combined effects of CrM and b-Ala supplementation on neuromuscular fatigue. The purpose of this study, therefore, was to examine the effects of 28 days of b-Ala and CrM supplementation on the onset of neuromuscular fatigue as measured by the PWC_{FT} test in untrained men.

Methods

Experimental Approach to the Problem

None of the subjects had ingested creatine, or any other dietary supplements, for a minimum of 12 weeks before

the initiation of the study. During the course of the study, the subjects were asked to maintain their current exercise and dietary patterns and to abstain from other nutritional supplements, nonprescription drugs, and caffeine. After pretesting, the subjects were randomly assigned to 1 of 4 treatment conditions using a double-blind design: (a) placebo (PLA; 34 g dextrose; $n = 13$), (b) creatine (CrM; 5.25 g CrM plus 34 g dextrose; $n = 12$), (c) b-Ala (1.6 g b-Ala plus 34 g dextrose; $n = 12$), or (d) b-Ala plus CrM (CrBA; 5.25 g of CrM plus 1.6 g b-Ala plus 34 g dextrose; $n = 14$). The supplements were identical in taste and appearance, and were dissolved in 16 oz of water and ingested 4 times per day for 6 consecutive days, then twice per day for 22 days before posttesting. Third-party random laboratory testing was conducted on the supplement packets, and the contents were determined to be accurate.

Subjects

Fifty-one men (mean age ± *SD* = 24.5 ± 5.3 years, height = 171.9 ± 27.9 cm, and weight = 82.0 ± 7.1 kg) volunteered for this investigation. All procedures were approved by the Institutional Review Board before the initiation of the study, and each subject was advised of any possible risks before providing informed consent.

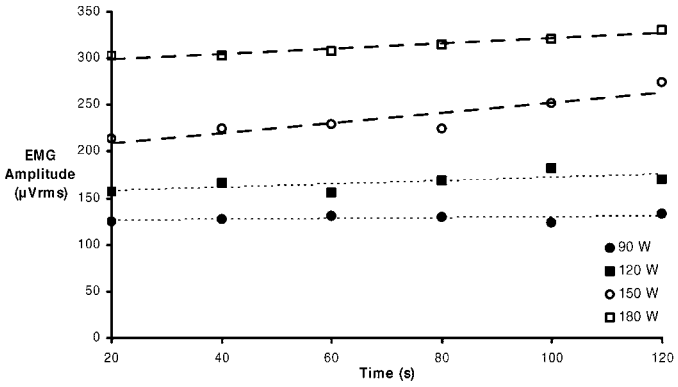
Electromyographic Measurements

A bipolar (2.54-cm center-to-center) surface electrode (Quinton Quick Prep silver–silver chloride; Quinton Instrument Co., Bothell, WA) arrangement was placed on the right thigh over the lateral portion of the vastus lateralis muscle, midway between the greater trochanter and the lateral condyle of the femur. The reference electrode was placed over the iliac crest. Interelectrode impedance was kept below 2,000 Ω by careful abrasion of the skin. The raw EMG signals were preamplified (gain: ×1,000) using a differential amplifier (EMG100C; Biopac Systems, Inc., Santa Barbara, CA), sampled at 1,000 Hz, and stored on a personal computer for off-line analysis. The EMG signals were later band-pass filtered from 10 to 500 Hz (2nd-order Butterworth filter) and expressed as root mean square amplitude values by software (AcqKnowledge version 3.7; Biopac).

Determination of Physical Working Capacity at Fatigue Threshold

The PWC_{FT} values were determined from the vastus lateralis muscle using the methods described by deVries et al. (3, 4) (Figure 1). The subjects began pedaling (with toe clips) at 60 W (70 rpm) on a calibrated, electronically-braked cycle ergometer (Lode Excalibur Sport Cycle Ergometer, Groningen, The Netherlands). The power output was then increased by 30 W every 2 minutes until the subject could no longer maintain 70 rpm. During each 2-minute interval, six 10-second EMG samples were recorded from the vastus lateralis. The PWC_{FT} was determined by averaging the highest power output that resulted in a nonsignificant ($p > 0.05$; single-tailed *t*-test) slope value for the EMG amplitude vs. time relationship with the lowest power output that resulted in a significant ($p \leq 0.05$) slope value (Figure 1).

Test-retest reliability for the PWC_{FT} test was determined by using a separate sample of 12 subjects measured 28 days apart. Using the recommendations of Weir (22), the intraclass correlation coefficient (*r*) was 0.948



Sequence	Power Output (W)	Slope Coefficient ($\mu Vrms^{-1}$)	Statistical Significance
#1	90	0.05	NS
#2	120	0.18	NS
$PWC_{I\text{-}T} = (150 + 120) \div 2 = 135$ W			
#3	150	0.55	≤ 0.05
#4	180	0.29	≤ 0.05

FIGURE 1. Illustration of the method used to determine the physical working capacity at fatigue threshold (PWC_{FT}) for 1 subject. EMG μVrms = electromyographic amplitude in root mean squared microvolts; NS = not significant.

(*SEM* = 22.79 W), which was similar to values reported by Stout et al. (16) and deVries et al. (2, 3) in young athletic women ($r = 0.94$), young men ($r = 0.947$), and older men ($r = 0.976$). In addition, there was no significant difference ($p = 0.721$) between the mean PWC_{FT} values from test 1 (mean ± *SEM* = 204.2 ± 18.1 W) to test 2 (201.9 ± 20.9 W).

Statistical Analyses

Two separate 1-way analyses of covariance (ANCOVA) were used to analyze the PWC_{FT} data based on the recommendations of Huck and McLean (11). The independent variable, group, included 4 levels: PLA, b-Ala, CrM, and CrBA. The pretest and posttest values were used as the covariate and dependent variable, respectively. Preliminary least squares regression analyses were conducted to examine the linearity of the relationships between the covariate and the dependent variable within all groups, and the interaction between the covariate and group was used to test for homogeneity of slopes (6). When appropriate, Bonferroni-corrected post hoc pairwise comparisons were used to examine the differences among the groups. For effect size, the partial eta squared (η^2) statistic was calculated, and according to Green et al. (6), η^2 of 0.01, 0.06, and 0.14 represents small, medium, and large effect sizes, respectively. An alpha of $p \leq 0.05$ was established a priori. SPSS (version 11.5, SPSS, Inc., Chicago, IL) was used for all statistical analyses.

RESULTS

Table 1 contains the mean and *SEM* values for the pretesting and posttesting PWC_{FT} results. There were sig-

TABLE 1. Mean and *SEM* values for PWC_{FT} (W) at pretesting and posttesting for each group.*

		Placebo	Beta-alanine	Creatine	Creatine + beta-alanine
Pretest	Mean	215.8	170.0	172.5	190.7
	SEM	19.0	15.9	11.6	18.6
Posttest	Mean	211.2	198.8	183.8	214.3
	SEM	23.7	19.9	14.8	17.1

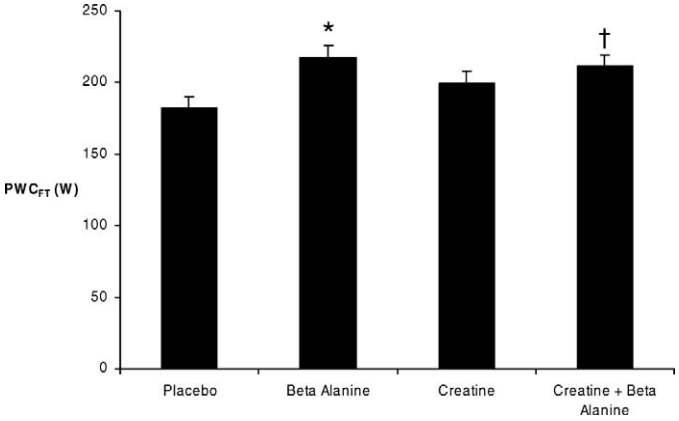


FIGURE 2. Means values (± *SEM*) for posttest PWC_{FT} scores adjusted for the initial differences in pretest PWC_{FT} (covariate). *The increase in PWC_{FT} from pretesting to posttesting was greater for the beta-alanine group than for the placebo group ($p = 0.004$, $\eta^2 = 0.17$). †The increase in PWC_{FT} from pretesting to posttesting was greater for the creatine + beta-alanine group than for the placebo group ($p = 0.011$, $\eta^2 = 0.13$).

nificant linear relationships between pretest PWC_{FT} and PLA ($p < 0.001$, $r = 0.95$, slope = 1.18), b-Ala ($p < 0.001$, $r = 0.91$, slope = 1.14), CrM ($p < 0.001$, $r = 0.92$, slope = 1.17), and CrBA ($p < 0.001$, $r = 0.82$, slope = 0.83) groups. There was no interaction ($p = 0.123$, $\eta^2 = 0.12$) between pretest PWC_{FT} and group, which supported the homogeneity-of-slopes assumption. The posttest PWC_{FT} means were adjusted during the ANCOVA procedure based on the pretest PWC_{FT} differences for the PLA (−29.0 W), b-Ala (+18.6 W), CrM (+16.0 W), and CrBA (−2.9 W) groups. The ANCOVA indicated a significant difference ($p = 0.019$, $\eta^2 = 0.19$) among the group means for the posttest PWC_{FT} values after adjusting for the pretest differences. The strength of association (i.e., effect size, η^2) indicated that the treatment groups (PLA, b-Ala, CrM, and CrBA) accounted for 19% of the variance of the posttest PWC_{FT} values, holding constant the pretest PWC_{FT} scores. Bonferroni-corrected pairwise comparisons indicated that the increase in PWC_{FT} from pretesting to posttesting was greater for the b-Ala group than for the PLA group ($p = 0.004$, $\eta^2 = 0.17$), and the increase from pretesting to posttesting was greater for the CrBA group than for the placebo group ($p = 0.011$, $\eta^2 = 0.13$). There were no differences between the CrM and PLA groups ($p = 0.136$, $\eta^2 = 0.05$), b-Ala and CrM groups ($p = 0.132$, $\eta^2 = 0.05$), b-Ala and CrBA groups ($p = 0.591$, $\eta^2 = 0.01$), or CrM and CrBA groups ($p = 0.305$, $\eta^2 = 0.02$). Figure 2 shows the group means (± *SEM*) for the posttest PWC_{FT} scores adjusted for the initial differences in pretest PWC_{FT}.

DISCUSSION

The primary and original findings of this study suggested that b-Ala supplementation may delay the onset of neuromuscular fatigue during incremental cycle ergometry. Furthermore, there appeared to be no unique or additive effects of CrM on PWC_{FT} compared to b-Ala alone. In agreement, Hill et al. (10) examined the effects of CrM and/or b-Ala supplementation on work completed during cycling to exhaustion at 110% of estimated power maximum in men. The authors reported that 28 days of supplementing b-Ala or CrM increased the amount of work completed; however, there appeared to be no additive effect when both were supplemented simultaneously (10).

McClaren et al. (13) have suggested that a decrease in muscle pH, as a result of the accumulation of H^+ or intracellular and extracellular ammonia, may be responsible for fatigue-induced increases in muscle activation and the corresponding increase in EMG amplitude. Taylor et al. (20) also found that, for incremental cycle ergometry, the accumulation of plasma lactate and ammonia was associated with an increase in EMG amplitude measured from the rectus femoris muscle. Therefore, evidence has suggested that a reliance on anaerobic glycolysis and the subsequent H^+ accumulation that ensues leads to an increase in EMG amplitude from the working muscles because of elevated lactate concentractions and decreases in pH.

In the present study, 28 days of b-Ala supplementation resulted in a significant increase in PWC_{FT} (b-Ala = 14.5%; CrBA = 11%); this may have been caused by an increase in carnosine concentrations, which may have enhanced intramuscular H^+ buffering capacity (7, 8, 10, 19). Harris et al. (7, 8) and Hill et al. (10) have hypothesized that increasing muscle carnosine through b-Ala supplementation will help maintain the intramuscular environment during intensive exercise by countering the accumulation of H^+. The results of the present study supported this hypothesis and suggested that b-Ala supplementation may delay the fatigue-induced increase in EMG amplitude during submaximal cycle ergometry, which may occur as a result of carnosine-induced increases in H^+ buffering capacity. Future studies should measure muscle carnosine and lactate concentrations during submaximal fatiguing exercise with and without prior b-Ala supplementation to verify this hypothesis.

Few studies have been conducted to determine the effect of CrM supplementation on submaximal exercise performance. Nelson et al. (14) reported that CrM loading in athletic men and women (age range 21–27 years) resulted in a 12% increase in ventilatory threshold as well as a decrease in blood lactate and ammonia concentrations during incremental cycle ergometry. In addition, Stout et al. (17) reported that CrM loading in women's crew athletes (age range 18–21 years) resulted in a significant ($p \leq 0.05$) increase (13%) in PWC_{FT}. Several investigators (5, 14, 15, 16, 21) have hypothesized that increasing muscle PCr content by CrM supplementation may decrease the reliance on anaerobic glycolysis, reduce intramuscular lactate accumulation, and consequently delay the onset of fatigue.

In the present study, 28 days of CrM supplementation resulted in an 11.3% increase in PWC_{FT}, which was similar to the 13% increase observed in our previous study (17). However, the increase in PWC_{FT} for the CrM group

reported in the present study was not statistically different ($p > 0.05$) from the change reported in the PLA group. In support of these findings, Stroud et al. (18) reported that CrM supplementation had no effect on respiratory gas exchange or blood lactate accumulation during an incremental treadmill test in physically active men. It is possible that the discrepancies regarding the effects of CrM supplementation on performance are related to the variability in muscle creatine retention as a result of CrM loading (1, 9). Casey et al. (1) demonstrated a positive relationship ($r = 0.71$, $p \leq 0.05$) between anaerobic exercise performance during cycle ergometry and the magnitude of muscle creatine retention as a result of CrM supplementation. It was concluded that the improvements in anaerobic performance were dependent on the magnitude of muscle creatine retention following CrM supplementation.

In summary, the b-Ala and CrBA groups had significantly greater PWC_{FT} values when compared to the PLA group, which indicated that 28 days of b-Ala supplementation (with or without CrM) may delay the onset of neuromuscular fatigue during incremental cycle ergometry in men. This delay in neuromuscular fatigue may have been caused by the augmented muscle carnosine levels, which may have resulted in a greater capacity to buffer H^+ during exercise. However, there appeared to be no unique or additive effects of CrM on PWC_{FT} vs. b-Ala alone. To test this hypothesis, future studies should examine muscle carnosine and lactate concentrations during submaximal fatiguing exercise with and without b-Ala and/or CrM supplementation.

Practical Applications

The primary results of this study suggested that b-Ala supplementation (3.2 $g \cdot d^{-1}$) for 28 days may delay the onset of neuromuscular fatigue and improve physical working capacity during cycle ergometry. Although recommendations must await further clinical trials, these findings may be useful for nutritionists, strength and conditioning professionals, and athletes contemplating the use of supplemental b-Ala. In addition, these findings may provide a foundation for future studies to test the hypothesis that b-Ala supplementation may increase muscle carnosine concentrations, which consequently may enhance the H^+ buffering capacity within skeletal muscle.

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