β-Alanine and the Hormonal Response to Exercise

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- endocrine
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Abstract

The effect of 30 days of β-alanine supplementation (4.8 g per day) on resistance exercise performance and endocrine changes was examined in eight experienced resistance-trained men. An acute resistance exercise protocol consisting of 6 sets of 12 repetitions of the squat exercise at 70% of one-repetition maximum (1-RM) with 1.5 minutes of rest between sets was performed before and after each supplemental period. Blood draws occurred at baseline (BL), immediate (IP), 15-minutes (15P) and 30-minutes (30P) postexercise for growth hormone, testosterone and cortisol concentrations. A 22% ($p < 0.05$) difference in total number of repetitions performed at the end of 4 weeks of supplementation was seen between β-alanine (BA) and placebo (PL), and Δ mean power was greater in BA (98.4 ± 43.8 w) vs. PL (7.2 ± 29.6 w). Growth hormone concentrations were elevated from BL at IP and 15P for both groups, while cortisol concentrations were greater than BL at all time points for both BA and PL. No group differences were noted. No change from BL was seen in testosterone concentrations for either group. Results indicate that four weeks of β-alanine supplementation can significantly improve muscular endurance during resistance training in experienced resistance-trained athletes. However, these performance gains did not affect the acute endocrine response to the exercise stimulus.

Introduction

An elevation in muscle carnosine concentrations has important implications for athletic performance. This is primarily related to carnosine's ability to enhance muscle buffering capacity during high-intensity exercise [16,22]. Several studies have demonstrated that high-intensity training can elevate the muscle carnosine concentration [17,22,24]. This appears to be an important training adaptation that enhances the anaerobic athlete's ability to delay fatigue. Supplementation with carnosine to maximize muscle carnosine concentrations though does not appear to be effective due to the rapid degradation of carnosine following ingestion by the hydrolyzing enzyme carnosinase [1,18], and the apparent absence of any transport mechanism into muscle. The precursors for carnosine synthesis are the amino acids histidine and β-alanine. Endogenous histidine production is at a sufficient rate to not be a factor in limiting carnosine synthesis [12]. However, β-alanine supplementation has been shown to be effective in increasing muscle carnosine concentrations [4,7,8], and subsequently has been shown to provide a significant ergogenic effect in athletes participating in high-intensity exercise by reducing the rate of fatigue in muscle [2,8,11,20,21].

Improvements in physical work capacity, time to exhaustion and an increase in lactate threshold following β-alanine supplementation have been demonstrated in previously untrained males and females [20,21]. Significant improvements in time to exhaustion and total work performed during high-intensity cycle exercise have also been reported in physically-active males following 10 weeks of β-alanine supplementation [8]. Studies in experienced resistance-trained athletes are limited; however, Hoffman and colleagues [11] have indicated that strength/power athletes supplementing with β-alanine for 10 weeks demonstrated a significantly higher training volume in both the squat and bench press exercises (e.g., number of repetitions performed in a workout) than subjects supplementing with creatine only or a placebo. The greater volume of training suggests that β-alanine supplementation

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may improve the work capacity of the resistance training workout. Previous studies comparing high to low training volume during resistance exercise (total number of sets × repetitions) have indicated that a high volume of training stimulates a greater acute endocrine response (e.g., growth hormone and testosterone) than a low volume of training [10,14]. Considering that β-alanine supplementation in resistance-trained athletes can increase training volume tolerance during an acute resistance exercise session [11], it is of interest to examine if β-alanine supplementation can potentially augment the anabolic hormonal response to the training session. Thus, the purpose of this study was to examine the effect of 30 days of β-alanine supplementation on resistance training volume and on the acute endocrine response to resistance exercise in strength/power athletes.

Materials and Methods

Subjects

Eight experienced resistance-trained (≥ 3 years of experience) college-aged males (age: 19.7 ± 1.5 y; height: 176.8 ± 3.7 cm; body mass: 89.0 ± 7.9 kg; body fat: 15.7 ± 2.8 %) volunteered for this study. Following an explanation of all procedures, risks and benefits, each subject gave his informed consent prior to participation in this study. The Institutional Review Board of the College approved the research protocol. Subjects were not permitted to use any additional nutritional supplements and did not consume anabolic steroids or any other anabolic agents known to enhance performance. Screening for supplement and steroid use was accomplished via a health history questionnaire completed during the subject recruitment phase.

Testing protocol

The investigation was performed as a double-blind, randomized cross-over design. Subjects initially reported to the Human Performance Laboratory (HPL) for maximal strength testing (a one-repetition maximum [1-RM] on the free weight squat exercise). Following maximal strength testing (72 hours later), subjects returned to the HPL to begin the experimental protocol. During the first experimental session (PRE), each subject performed 6 sets of 12 repetitions with 70% of their 1-RM on the squat exercise, with a 1.5-minute rest interval between each set. Subjects then began a four-week β-alanine (BA) or placebo (PL) supplementation period and returned to the laboratory at the end of week 4 to perform 1-RM testing on the squat exercise. Subjects returned to the laboratory three days later for the final acute resistance exercise bout (POST). Following a 4-week washout period (no supplementation provided, but subjects maintained their normal training routine), subjects returned to the HPL for 1-RM testing. Subjects returned to the laboratory 3 days later for the start of the second experimental session. Following PRE testing, the subjects began a second 4-week BA or PL supplementation period, receiving the opposite supplement from the previous 4-week period. At the end of the 4-week supplementation period, subjects performed 1-RM testing, and then returned to the HPL 3 days later for the final acute resistance exercise testing session. All testing protocols used 70% of the 1-RM most recently assessed.

Subjects arrived at the HPL following an overnight fast for the acute resistance exercise testing protocols. Following initial baseline blood draw, subjects performed a general and exercise-specific warm-up. The general warm-up consisted of 5 minutes of light stationary cycling at a self-selected cadence and 5 minutes of light stretching. The light stretching consisted of static stretches that each subject self-selected. Subjects were instructed to use the same pre-exercise warm-up routine that they generally employ prior to their normal workouts. This was not standardized within the subject group, but each subject was required to perform the same pre-exercise routine on each testing occasion. The exercise-specific warm-up consisted of performance of 2 – 3 light-to-moderate sets of the squat exercise. Subjects were encouraged to perform up to 12 repetitions per set. However, this was not possible for every set as loading was kept constant. During each repetition, subjects were instructed to squat to the parallel position under control, but to return to the starting position (full extension) as quickly as possible. Repetitions not completed using a full range of motion were discarded. A research assistant (e.g., certified strength and conditioning specialist) was located lateral to each subject to ensure proper range of motion (e.g., upper thighs parallel to the ground) was used for each repetition. Volume of each set was calculated as the number of completed repetitions. All experimental testing sessions occurred at the same time of day.

Throughout the 12-week study, subjects performed the same 4-day per week split routine resistance training program. On days one and three of the week, subjects trained the chest, shoulders and triceps, while on days two and four of the week, subjects trained the legs, back and biceps. During each workout, subjects used an 8 – 10RM per set, with 1.5 – 2.0 minutes of rest between sets. All subjects completed a daily training log, which was collected by study investigators on a weekly basis. Subject compliance during the study was 100%. **Table 1** provides a depiction of the subjects' resistance training program.

Maximal strength testing

The 1-RM squat test was performed using methods previously described by Hoffman [9]. Each subject performed a warm-up set using a resistance that was approximately 40 – 60% of his perceived maximum, and then performed 3 – 4 subsequent trials to determine the 1-RM. A 3- to 5-minute rest period was provided between each trial. The squat exercise required the subject to place an Olympic bar across the trapezius muscle at a self-selected location. Each subject descended to the parallel position which was attained when the greater trochanter of the femur reached the same level as the knee. The subject then ascended until full knee extension. Trials not meeting the range of motion criteria were discarded. Subjects were strength tested on four occasions: before and after each 4-week experimental period.

Power measurements

Lower body power during the squat protocols was measured with a Tendo™ Power Output Unit (Tendo Sports Machines, Trencin, Slovak Republic). The Tendo™ unit consists of a transducer that was attached to the end of the barbell which measured linear displacement and time. Subsequently, bar velocity was calculated and power was determined when bar load was manually input. Both peak and mean power output were recorded for each repetition and set and used for subsequent analysis.

Blood measurements

During each experimental session, baseline (BL) blood samples were obtained at pre-exercise. Additional blood samples were also drawn immediately postexercise (IP), 15 (15P), and 30 min-

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Table 1 Resistance training program

Days 1 and 3			Days 2 and 4		
Exercise	**Sets**	**Repetitions**	**Exercise**	**Sets**	**Repetitions**
Squat	1, 4	10 – 12 RM	Bench press	1, 4	8 – 10 RM
Dead lift	1, 4	8 – 10 RM	Incline bench press	1, 4	8 – 10 RM
Leg extensions	3	10 – 12 RM	Incline fly's	3	8 – 10 RM
Leg curls	3	10 – 12 RM	Seated shoulder press	1, 4	8 – 10 RM
Standing calf raises	3	10 – 12 RM	Upright rows	3	8 – 10 RM
Lat pulldown	1, 4	10 – 12 RM	Lateral raises	3	8 – 10 RM
Seated row	1, 4	10 – 12 RM	Triceps pushdown	3	8 – 10 RM
Biceps curls	4	10 – 12 RM	Triceps extensions	3	8 – 10 RM
Trunk and abdominal routine	2	10 – 12	Trunk and abdominal routine	2	10 – 12

RM = repetition maximum: Comma between sets represents warm-up set then number of work sets

utes (30P) postexercise. All blood samples were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a 3-way stopcock with a male luer lock adapter. The cannula was maintained patent using an isotonic saline solution (with 10% heparin). The BL blood samples were drawn following a 15-min equilibration period prior to exercise, and the IP blood samples were taken within 15 seconds of exercise cessation. All blood samples were drawn with a plastic syringe while the subject was in a seated position. Following the resistance exercise protocol, subjects were seated and remained seated for the full 30-min recovery phase. Blood samples were obtained on four occasions: before and following each 4-week experimental period; and each subjects' blood samples were obtained at the same time of day during each session.

Blood samples were collected into two Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA), one containing SST® Gel and Clot Activator and the second containing EDTA. A small aliquot of whole blood was removed from the second tube and used for microcapillary determination of hematocrit. The remaining blood in that tube was used for hemoglobin analysis. The blood in the first tube was allowed to clot at room temperature and subsequently centrifuged at 1500 × g for 15 minutes. The resulting serum was placed into separate 1.8-ml microcentrifuge tubes and frozen at – 80 °C for later analysis.

Biochemical and hormonal analyses

Serum testosterone, growth hormone, and cortisol were determined using enzyme immunoassays (EIA) and enzyme-linked immunosorbent assays (ELISA) (Diagnostic Systems Laboratories, Webster, TX, USA; ALPCO Diagnostics, Salem, NH, USA). Determination of serum immunoreactivity values was made using a SpectraMax340 Spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). To eliminate inter-assay variance, all samples for a particular assay were thawed once and analyzed in the same assay run. All samples were run in duplicate with a mean intraassay variance of < 10%. The detection limits of the testosterone, growth hormone, and cortisol assays were 0.76 nmol·L^{-1}, 0.03 ng·mL^{-1}, and 2.76 nmol·L^{-1}, respectively.

Hemoglobin was analyzed in triplicate from whole blood using the cyanmethemoglobin method (Sigma Diagnostics, St. Louis, MO, USA). Hematocrit was analyzed in triplicate from whole blood via microcentrifugation (IEC micro-MB centrifuge, Needham, MA, USA) and microcapillary technique. Plasma volume shifts following the workout were calculated using the formula of Dill and Costill [3]. Serum lactate concentrations were determined with an Analox GM7 enzymatic metabolite analyzer (Analox Instruments USA, Lunenburg, MA, USA).

Dietary recall

Three-day dietary records were completed during each 4-week supplementation or placebo period of the study. Subjects were instructed to record as accurately as possible everything they consumed during the day including supplement (or placebo) and between meal and late evening snacks. FoodWorks Dietary Analysis software (McGraw Hill, New York, NY, USA) was used to analyze dietary recalls.

Supplement composition

The β-alanine supplement (CarnoSyn™) was obtained from Natural Alternatives International (San Marcos, CA, USA). Both the supplement and placebo were in capsule form and were similar in appearance. Subjects were required to consume either the supplement or placebo three times per day (1.6 g per serving) for each 4-week period without food, thereby totaling 4.8 g of β-alanine per day. Subjects received a 7-day supply of supplement or placebo. At the end of each week, subjects were required to report back to the laboratory and return all unused capsules or sign a paper indicating that all capsules were consumed. There was 100% compliance during both experimental sessions.

Statistical analysis

Statistical evaluation of hormonal changes was accomplished using a repeated measures analysis of variance. In the event of a significant F-ratio, LSD post hoc tests were used for pairwise comparisons. Paired *t*-tests were used to analyze the difference between treatment sessions in the mean within-subject response to training in respect of repetitions, 1-RM squat strength, mean and peak power performance, and body mass. Significance was accepted at an alpha level of $p \leq 0.05$. All data are reported as mean ± SD.

Results

No significant difference in average daily caloric intake (2725 ± 723 kcal) or dietary composition was seen between the 4-week experimental periods. Analysis of dietary composition showed that the daily dietary intake was comprised of 41% carbohydrates, 36% fat and 23% protein.

Analysis of variance indicated a significant improvement in the volume of training from PRE to POST in BA, and the training vol-

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Table 2 Performance effects of 4 weeks of β-alanine supplementation

Variable	BA		PL	
	PRE	POST	PRE	POST
Body mass (kg)	89.8 ± 8.5	91.8 ± 8.5	90.0 ± 7.8	89.8 ± 9.1
1-RM squat (kg)	169.8 ± 21.2	175.6 ± 20.1	167.2 ± 20.9	171.1 ± 18.3
Total repetitions performed	41.7 ± 8.5	51.3 ± 9.5	41.7 ± 7.3	42.0 ± 4.1
Total volume (weight × sets × repetitions performed) (kg)	7203 ± 1368	9137 ± 1472*	7303 ± 1365	7448 ± 864**
Peak power (W)	898 ± 194	923 ± 225	962 ± 223	968 ± 231
Mean power (W)	531 ± 63	587 ± 103	570 ± 53	571 ± 53

*= Significantly greater than BA at PRE; **= significantly lower than BA at POST. All data are reported mean ± SD

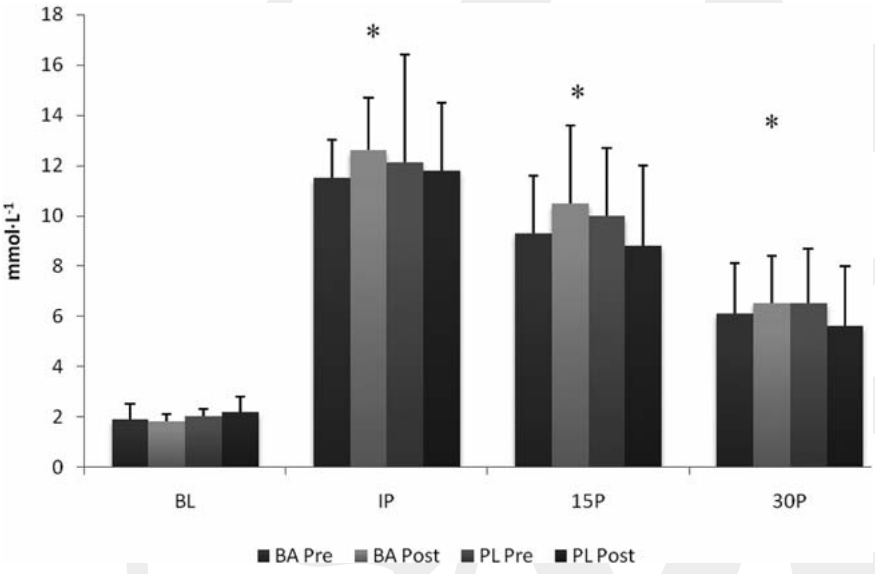

Fig. 1 Comparison of serum lactate concentrations (mean ± SD) for various time points. BA = β-alanine; PL = placebo; * = significantly different than BL.

ume at POST was significantly greater than that seen at the same time point for PL. No other significant performance effects from BA supplementation were seen (see **Table 2**). Examination of PRE to POST differences between BA and PL revealed a significant difference in Δ training volume (1934 ± 834 kg and 145 ± 1491 kg, respectively) and in the total number of repetitions performed per workout (9.0 ± 4.1 and 0.3 ± 7.8, respectively). In addition, a significant difference was seen in Δ mean power between BA (98.4 ± 43.8 W) and PL (7.2 ± 29.6 W). No significant changes between BA and PL were observed in body mass (2.0 ± 2.4 kg vs. 0.1 ± 2.0 kg, respectively), peak power (78.2 ± 75.8 W vs. 24.6 ± 21.1 W, respectively), or in 1-RM squat strength (5.9 ± 4.3 kg vs. 3.9 ± 4.1 kg, respectively).

Lactate responses to the acute resistance exercise protocols are shown in **Fig. 1**. Lactate concentrations were significantly elevated at IP for all exercise sessions, and remained significantly higher than BL at both 15P and 30P. However, no significant differences were observed between trials.

The growth hormone responses to the acute resistance exercise protocols are shown in **Fig. 2**. A significant main effect for time was observed. Significant elevations from BL were seen at both IP and 15P. Growth hormone concentrations returned to BL at 30P. However, no significant trial × time interaction was noted.

The testosterone responses to the acute resistance exercise protocols are shown in **Fig. 3**. No significant group effects were seen. However, a trend ($p = 0.08$) was seen in testosterone elevations from BL to IP across all trials. In addition, no significant changes from BL were noted during any trial, and no significant trial effects were observed.

The cortisol responses to the acute resistance exercise protocols are shown in **Fig. 4**. A significant main effect for time was seen. Cortisol concentrations were significantly elevated from BL at IP, 15P and 30P. No other significant differences were evident.

No significant differences were seen in the change in plasma volume between trials. Average plasma volume shifts were – 21.1 ± 6.5%, – 6.7 ± 10.5% and – 1.6 ± 12.8% at IP, 15P and 30P, respectively. However, blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue level.

Discussion

Results of this study indicated that 4 weeks of β-alanine supplementation was effective in increasing the gain in performance with a resistance training workout, as reflected by the significant improvement in training volume, the number of repetitions performed and the average mean power exhibited during the exercise regimen. However, this elevated training volume did not result in an augmented endocrine response to the exercise stress. In addition, no significant augmentations in body mass, 1-RM strength or peak power performance were noted during 4 weeks of supplementation with β-alanine.

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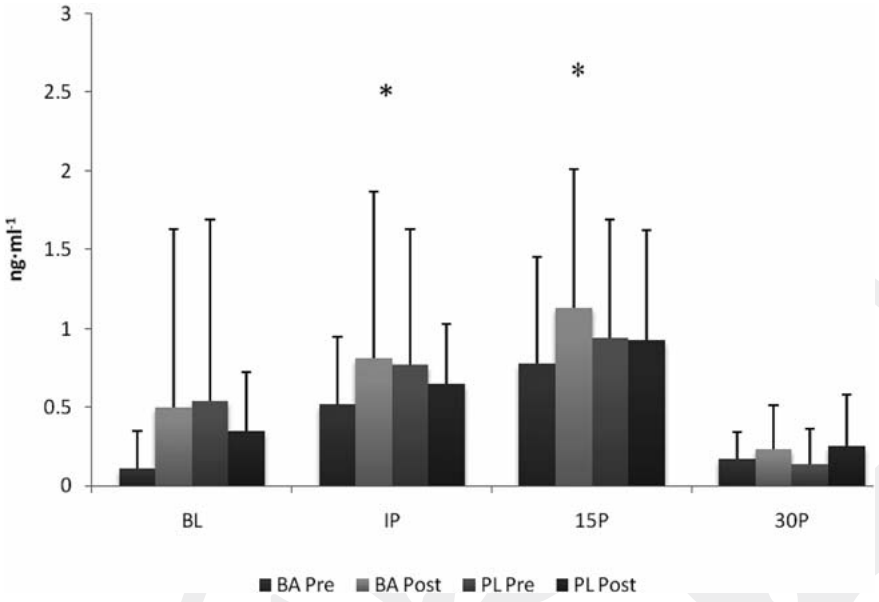

Fig. 2 Comparison of serum growth hormone concentrations (mean ± SD) for various time points. BA = β-alanine; PL = placebo; * = significantly different than BL.

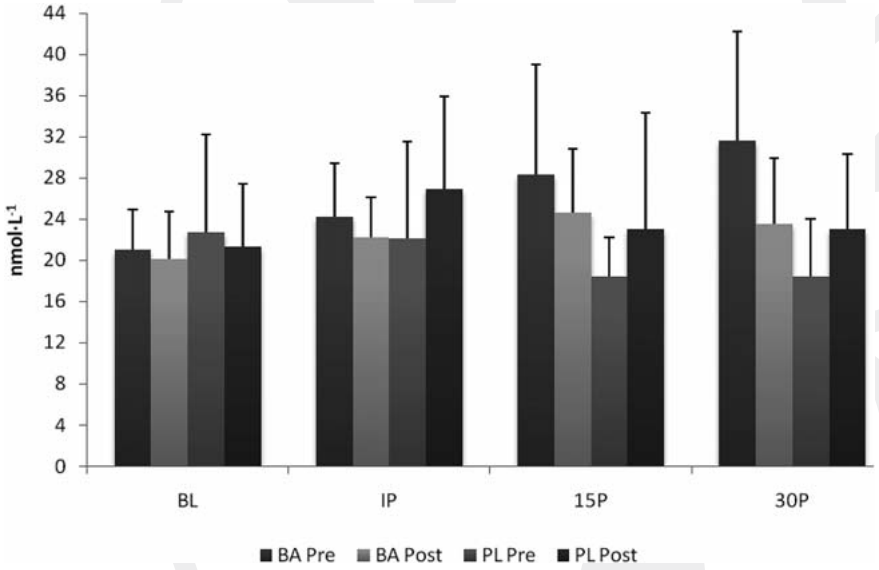

Fig. 3 Comparison of serum testosterone concentrations (mean ± SD) for various time points. BA = β-alanine; PL = placebo.

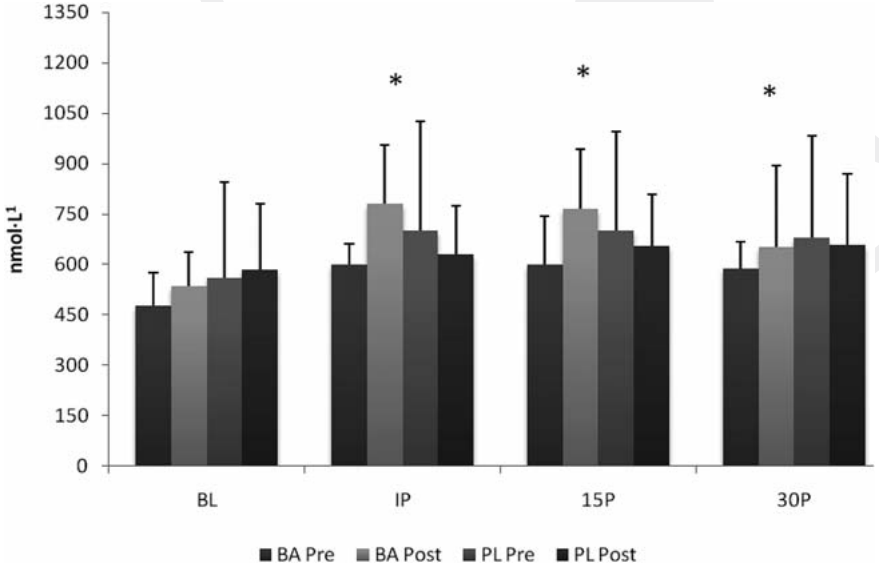

Fig. 4 Comparison of serum cortisol concentrations (mean ± SD) for various time points. BA = β-alanine; PL = placebo; * = significantly different than BL.

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A concern of the 4-week washout period was that it may have been too short to allow muscle carnosine to return to the pre-supplementation level in subjects 1 – 4, given BA first. However, it was notable that whilst during the first session the number of repetitions in these subjects increased from 42.8 ± 10.7 to 53.3 ± 12.0, this had decreased at the start of the second trial back to 45.5 ± 4.0. Further, the subsequent response in these subjects to 4-weeks training (Δ = – 2.3 ± 7.5 repetitions) was very similar to that with PL (first session) in subjects 5 – 8 (i.e. Δ = 2.8 ± 8.4 repetitions). This suggests that any carry-over effect by the end of the 4-week washout period was minimal in terms of its effect on performance. We believe that this provides strong evidence that BA was effective in increasing the response to 4-weeks training, although we acknowledge that this does not totally eliminate the possibility of an effect on the data of a too-short a washout period in subjects 1 – 4 between the first and second sessions.

The performance results seen in this investigation confirm the previous study from our laboratory that demonstrated the efficacy of β-alanine supplementation on improving the response to resistance training sessions [11]. Similar to that previous study, the effect of supplementation was an increase in the volume of training (i.e., a greater number of repetitions performed in the squat exercise in BA compared to PL). In addition, β-alanine supplementation significantly enhanced the average mean power performance during the acute resistance exercise protocol. The mechanism(s) responsible for these improvements is likely related to an enhanced buffering capacity resulting from an increase in muscle carnosine concentrations [16,22]. Although muscle carnosine concentrations were not assessed in this study, previous studies examining trained athletes have shown that the combination of 4 weeks of β-alanine supplementation (3.2 to 6.4 $g \cdot d^{-1}$) similar to the current study, and exercise have yielded significant increases in muscle carnosine levels [2, 7,8].

It was hypothesized that the greater volume of resistance training resulting from β-alanine supplementation may augment the endocrine response to resistance exercise. This was based on several studies that have demonstrated that alterations in acute program variables, such as rest interval length, training intensity and training volume can alter the acute hormonal response to exercise [6,10,13,14]. A high-volume, moderate-intensity (i.e., 10-RM per set) exercise regimen appears to stimulate a greater anabolic hormonal response (e.g., growth hormone and testosterone) than a low-volume, high-intensity (i.e., < 5 RM per set) exercise regimen [13,14]. These types of training protocols are representative of vastly different training paradigms (e.g., muscle hypertrophy vs. muscle strength/power, respectively), that are associated with a 2- to 5-fold difference in training volume. Although the training volume in this study was significantly higher in BA than PL, the magnitude of improvement (22%) in the number of repetitions performed between BA and PL was not sufficient to augment the hormonal response to the exercise protocol. These results were similar to a previous study from our laboratory that demonstrated that significant elevations in training volume resulting from a nutritional supplement did not augment the anabolic hormonal response to the training session [19].

The elevation in growth hormone at IP is consistent with its response reported in the literature following acute resistance exercise [6,14]. Although these previous studies have shown training volume to be a potent stimulus in the growth hormone response to resistance exercise, the difference in training volume seen in this study did not approach the magnitude of change (2- to 5-fold difference) seen in these previous studies. Growth hormone secretion patterns have also been shown to be quite responsive to changes in the acid-base balance of muscle [5]. Considering that serum lactate was significantly elevated in both BA and PL, with no significant differences between the two treatment groups, it was not surprising to see no difference between BA and PL in growth hormone concentrations postexercise.

Changes in cortisol were also consistent with its response seen following an acute resistance exercise session [6,15]. Cortisol concentrations are generally seen to elevate postexercise in response to metabolic or psychological stress associated with exercises [6,15]. However, it appears that the magnitude of change of training volume in this study (9 repetition difference between BA and PL) did not result in alteration in cortisol secretion patterns.

The testosterone response to the exercise stimulus was not consistent with what is typically seen during an acute bout of resistance exercise. Most studies have generally shown that testosterone concentrations to be significantly elevated postexercise, and to be sensitive to changes in training volume [13,14]. Although a trend ($p = 0.08$) towards an elevation was seen between BL and IP, no difference was noted between BA and PL. Similar to what was discussed previously, it is likely that the 22% increase in training volume of a single exercise routine was not of significant magnitude to result in acute endocrine changes. It is possible that a similar magnitude of difference during an entire training regimen (multi-set, multi-exercise) may result in a significantly different endocrine response. Further research will be needed to provide additional insight into this hypothesis.

No anthropometric, strength or peak power changes were observed following 4 weeks of β-alanine supplementation. This was similar to results previously reported by our laboratory following 10 weeks of β-alanine supplementation in experienced strength/power athletes, despite a significantly higher volume of training seen in the group supplementing with β-alanine [11]. The rate and magnitude of strength and power improvements in experienced resistance-trained athletes are generally quite small, and likely requires a greater duration of training. Although β-alanine supplementation appears to be effective in enhancing the response to resistance exercise workout, it appears that maximal strength and power performance improvements in experienced resistance-trained athletes may require a greater duration of training and supplementation to observe significant performance improvements. However, the ability to improve local muscular endurance does appear to occur within 4 weeks of supplementation as reflected by the significantly higher average mean power outputs and the greater number of repetitions performed.

A potential limitation in this study is that the double-blind cross-over design may have resulted in muscle carnosine concentrations to be maintained at elevated levels during the second phase of the study in those subjects that supplemented with β-alanine in the first trial. Although a 4-week washout period was used, no research is known that has examined the time necessary for muscle carnosine concentration to return to baseline levels following a 4-week supplementation regimen. Considering that the number of repetitions performed in those subjects that supplemented with β-alanine in the first trial declined from the end of the first trial to the beginning of the second trial, and no significant difference was seen in repetitions performed be-

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tween the PRE trials, although speculative, it does suggest that muscle carnosine concentrations returned to baseline levels.

In conclusion, the results of this study suggest that 4 weeks of β-alanine supplementation may significantly improve the response to resistance training sessions in experienced resistance-trained athletes. However, these performance improvements did not affect the acute endocrine response to the resistance exercise stimulus.

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