The effects of amino acid supplementation on hormonal responses to resistance training overreaching

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Received 15 June 2006; accepted 14 August 2006

Abstract

The purpose of this investigation was to examine the effects of amino acid supplementation on muscular performance and resting hormone concentrations during resistance training overreaching. Seventeen resistance-trained men were randomly assigned to either an amino acid (AA) or a placebo (P) group and underwent 4 weeks of total-body resistance training designed to induce a state of overreaching. The protocol consisted of two 2-week phases (phase 1, 3 sets of 8 exercises performed for 8-12 repetitions; phase 2, 5 sets of 5 exercises performed for 3-5 repetitions). Muscle strength and resting blood samples were determined before (T1) and at the end of each training week (T2-T5). One-repetition maximum squat and bench press decreased at T2 in the P group but not in the AA group; both groups showed similar increases in strength at T3 to T5. Significant elevations in serum creatine kinase and uric acid were observed at T2 in the P group; the elevation in creatine kinase correlated highly to reductions in 1-repetition maximum squat ($r = -0.67$, $r^2 = 0.45$). Significant elevations in serum sex hormone–binding globulin were observed during overreaching in the P group from T2 to T5; this response was abolished in the AA group. Significant reductions in total testosterone were observed in the P group at T4 compared with T1, and total testosterone values were higher for the AA group than for the P group from T2 to T4. Serum 22-kd growth hormone concentrations were elevated at T2 to T5 in P group only. No differences were observed in resting cortisol and insulinlike growth factor 1. Hemoglobin concentrations were significantly reduced at T2 to T5 in the P group. These results indicate that the initial impact of high-volume resistance training is muscle strength reduction and hormonal/biochemical alterations. It appears that amino acid supplementation is effective for attenuating muscle strength loss during initial high-volume stress, possibly by reducing muscle damage by maintaining an anabolic environment.

1. Introduction

It has been shown that short-term, unaccustomed resistance exercise results in significant muscle damage, as measured directly via muscle biopsies [1] or indirectly via blood markers such as creatine kinase (CK), myoglobin, lactate dehydrogenase, troponin I, and myosin heavy-chain fragments [2,3]. The extent of muscle damage and subsequent decrements in force production appears dependent on the intensity and type of muscle actions used [4,5] and the training status of the individual [2,6-8]. In fact, individuals with at least 6 months, 2 years, and more than 3 years of resistance training experience have shown significantly less elevation in CK and myoglobin concentrations and less muscle damage 12 to 120 hours after a short-term resistance exercise protocol compared with untrained individuals [6,7]. It appears that repeated bouts of resistance exercise impart a protective effect on skeletal muscle, thereby making muscle less susceptible to damage

and accelerating the rate of tissue repair [4,9]. Although the exact mechanisms involved are unclear, adaptations in the skeletal muscle membrane, cytoskeleton, extracellular matrix, and myofibrils are involved.

Amino acid supplementation has been reported to augment recovery during endurance and resistance exercise by mechanisms that are unclear, but appear to involve increased protein synthesis and/or reduced protein degradation [10-13]. Posttranscriptional modifications of protein synthesis have been shown to occur during amino acid supplementation in rats [14]. In addition, reductions in the concentrations of serum CK and lactate dehydrogenase have been reported in response to endurance training with amino acid supplementation, thus suggesting attenuation of muscle damage [15]. Alterations in protein synthesis and degradation are regulated in part by hormonal control [16]. Therefore, it appears that amino acid supplementation may have potential benefits for modulating protein metabolism, which may be useful during resistance training overreaching (ie, a training phase where volume and/or intensity are increased substantially beyond normal training for 2-4 weeks).

Little is known concerning the effects of amino acid supplementation during short-term resistance training overreaching. Considering that athletes frequently compete and/or train with suboptimal recovery, amino acid supplementation may be beneficial if it enables the athlete to maintain performance when adequate recovery between workouts, competitions, practices, and so on is not feasible. Therefore, the purpose of the present study was to investigate the effects of amino acid supplementation during short-term resistance training overreaching. It was our hypothesis that amino acid supplementation, by enhancing recovery between workouts and possibly attenuating muscle damage, would maintain muscle strength during the initial stress of high-volume resistance training overreaching.

2. Methods

2.1. Experimental approach to the problem

A double-blind, placebo-controlled, randomized study design was used to examine the effects of amino acid supplementation on muscle performance, muscle damage, and hormonal factors after 4 weeks of resistance exercise training overreaching. The premise of the resistance training program was to train the entire body on consecutive days to minimize recovery between workouts. This typifies a situation encountered by many athletes where, during the competitive season or off-season training regimen, a substantial increase in training volume occurs (ie, "overreaching"). Resting blood samples and blood pressure were obtained, and muscular strength was assessed before and after each week of training. The experimental design enabled the investigation of the potential ergogenic effects (eg, recovery enhancement) of amino acid supplementation

and corresponding physiological mechanisms during a period where a substantial increase in resistance training volume or intensity was prescribed.

This study examined the blood and hormonal variables related to amino acid supplementation and was part of a larger investigative profile, of which the physical performance data have been published elsewhere [17,18]. We again present the performance data for context and use in regression associations with the blood data to help gain insights as to potential mechanisms of action for the amino acid supplementation.

2.2. Participants

Seventeen resistance-trained men were randomly assigned to either an amino acid (AA) or a placebo (P) group. Participants had the following characteristics: AA group (n = 9): age, 19.7 ± 1.4 years; height, 183.6 ± 6.9 cm; body mass, 89.1 ± 19.8 kg; training experience, 4.4 ± 2.2 years; P group (n = 8): age, 21.3 ± 3.0 years; height, 179.4 ± 6.4 cm; body mass, 88.9 ± 11.1 kg; training experience, 5.1 ± 3.0 years. There were no significant differences between groups in physical characteristics. Each of the participants was informed of the benefits and risks of the investigation and subsequently signed an approved consent form in accordance with the guidelines of the university institutional review board for use of human subjects. No participant had any medical or orthopedic problem that would compromise participation and performance in the study. In addition, none of the participants were taking any medications, nutrition supplements, or anabolic drugs that would confound the results of this study.

Participants assigned to the AA group ingested 0.4 g/kg per day TID of essential and conditionally essential amino acids (Big One, Professional Dietetics, Milan, Italy; for amino acid composition, see Table 1); alternately, participants in the P group received identical-looking capsules of powdered cellulose. Each participant was instructed to ingest the supplement separate from meals (ie, 1 hour before a meal and 2 hours after a meal). In relation to the exercise bout, supplement doses were taken 1 to 2 hours pre- and postexercise. Thus, the sequence was (1) morning dose, (2) afternoon dose, (3) afternoon workout, and

Table 1
Composition of the amino acid supplement

Amino acid	Per tablet (mg)	Per 100 g (g)
L-Leucine	250	27.2
L-Lysine	130	14.1
L-Isoleucine	125	13.6
L-Valine	125	13.6
L-Threonine	70	7.6
L-Cysteine	30	3.3
L-Histidine	30	3.3
L-Phenylalanine	20	2.2
L-Methionine	10	1.1
L-Tyrosine	6	0.7
L-Tryptophan	4	0.4

(4) evening dose. All supplemental doses were distributed by a registered dietician who calculated each serving size and distributed the supplements in clearly marked plastic bags. All participants recorded the times of supplementation in accordance with the investigator's instructions.

2.3. Strength testing and resting blood pressure

One-repetition maximum (1 RM) strength was determined for the free-weight squat and bench press exercises according to methods previously described [19] and proceeded as follows: after a 5-minute, general warm-up on a cycle ergometer, participants completed a warm-up set of 5 to 10 repetitions at 40% to 60% of perceived 1 RM. After a 1-minute rest period, a set of 2 to 3 repetitions was performed at 60% to 80% of perceived 1 RM. Subsequently, 3 to 4 maximal trials were performed to determine the 1 RM. This strength testing protocol was performed before the first week (T1) and after each week of training (T2-T5). Strength testing was performed at the same time each session and approximately 24 hours after the last training session. All participants refrained from activity not related to the present investigation for at least 24 hours before testing. Resting blood pressure was assessed at the end of each training week, just before strength testing using a sphygmomanometer and stethoscope.

2.4. Blood sampling

Each participant provided a venous blood sample before (T1) and at the completion of each training week (T2-T5). Venous blood samples were obtained from a superficial forearm vein while participants were in a supine position after a 10-minute equilibration period. Blood samples were obtained in the early morning hours (between 5:00 and 9:30 AM) after a 10-hour overnight fast and abstinence from exercise for at least 12 hours. In addition, blood sampling occurred during a standardized time of day for each participant to minimize the effects of diurnal hormonal variations. Whole blood samples were centrifuged at $1500g$, and resulting serum and/or plasma was harvested and stored at $-80°C$ until analyzed.

2.5. Biochemical analyses

Whole blood was used to determine hemoglobin values in duplicate using the cyanmethemoglobin method at 540 nm (Sigma Diagnostics, St Louis, MO), and hematocrit was analyzed in triplicate via standard microcapillary techniques and microcentrifugation. Serum glucose concentrations were measured in duplicate using standard colorimetric procedures at 450 nm (Sigma Diagnostics). Serum CK and plasma ammonia concentrations were determined in duplicate using standard colorimetric procedures at 340 nm (Sigma Diagnostics). Serum uric acid concentrations were determined in duplicate using standard colorimetric procedures at 520 nm (Sigma Diagnostics).

Serum total testosterone, human growth hormone (GH), sex hormone–binding globulin (SHBG), insulinlike growth factor 1 (IGF-1), insulin, and cortisol concentrations were determined in duplicate using standard radioimmunoassay (RIA) techniques. Serum total testosterone, cortisol, insulin, and SHBG were measured with iodine 125 (^{125}I) solid-phase RIA (Diagnostic Products, Los Angeles, CA). Serum IGF-1 was measured with ^{125}I solid-phase RIA using an extraction procedure (Diagnostic Products). Serum 22-kd GH was

Table 2
Resistance training program

Week 1		
Monday and Wednesday	Tuesday and Thursday	Friday
Back squat, $3 \times 10\text{-}12^a$	Leg press, $3 \times 10\text{-}12^a$	1 RM squat
Bench press, $3 \times 10\text{-}12^a$	Incline bench press, $3 \times 10\text{-}12^a$	1 RM bench press
Lat pulldown, $3 \times 10\text{-}12^b$	Bent-over row, $3 \times 10\text{-}12^b$	
Lunge, $3 \times 10\text{-}12^b$	Stiff-leg deadlift, $3 \times 10\text{-}12^b$	
Seated shoulder press, $3 \times 10\text{-}12^b$	Upright row, $3 \times 10\text{-}12^b$	
Dumbbell curl, $3 \times 10\text{-}12^b$	Barbell curl, $3 \times 10\text{-}12^b$	
Lying triceps extension, $3 \times 10\text{-}12^b$	Dips, $3 \times 10\text{-}12^b$	
Leg raise, 3×20^c	Sit-ups, 3×20^c	
Week 2		
Monday and Wednesday	Tuesday and Thursday	Friday
Back squat, $3 \times 8\text{-}10^a$	Leg press, $3 \times 8\text{-}10^a$	1 RM squat
Bench press, $3 \times 8\text{-}10^a$	Incline bench press, $3 \times 8\text{-}10^a$	1 RM bench press
Lat pulldown, $3 \times 8\text{-}10^b$	Bent-over row, $3 \times 8\text{-}10^b$	
Lunge, $3 \times 8\text{-}10^b$	Stiff-leg deadlift, $3 \times 8\text{-}10^b$	
Seated shoulder press, $3 \times 8\text{-}10^b$	Upright row, $3 \times 8\text{-}10^b$	
Dumbbell curl, $3 \times 8\text{-}10^b$	Barbell curl, $3 \times 8\text{-}10^b$	
Lying triceps extension, $3 \times 8\text{-}10^b$	Dips, $3 \times 8\text{-}10^b$	
Leg raise, 3×20^c	Sit-ups, 3×20^c	
Week 3		
Monday and Wednesday	Tuesday and Thursday	Friday
Back squat, 5×5^a	Leg press, 5×5^a	1 RM squat
Bench press, 5×5^a	Incline bench press, 5×5^a	1 RM bench press
Deadlift, 5×5^b	High pull, 5×5^b	
Lat pulldown, 5×5^b	Bent-over row, 5×5^b	
Seated shoulder press, 5×5^b	Close-grip bench press, 5×5^b	
Week 4		
Monday and Wednesday	Tuesday and Thursday	Friday
Back squat, 5×3^a	Leg press, 5×3^a	1 RM squat
Bench press, 5×3^a	Incline bench press, 5×3^a	1 RM bench press
Deadlift, 5×3^b	High pull, 5×3^b	
Lat pulldown, 5×3^b	Bent-over row, 5×3^b	
Seated shoulder press, 5×3^b	Close-grip bench press, 5×3^b	

[a] Three minutes of rest between sets.
[b] Two minutes of rest between sets.
[c] One minute of rest between sets.

measured using a ^{125}I liquid-phase RIA with double-antibody technique (Nichols Institute Diagnostics, San Juan Capistrano, CA). All samples for each hormone were determined in the same assay to avoid interassay variance and were thawed only once for each assay procedure. Intra-assay variance was less than 5% for all hormones and SHBG.

2.6. Nutrition assessment

To isolate the independent effects of the supplementation treatments, we attempted to standardize dietary intake at an isoenergetic level for each participant. Before beginning the study, participants were weighed before and after a 7-day period, during which time they recorded all food/beverages consumed according to instructions provided by the same registered dietitian. If body weight fluctuated more than 1 kg during the 7-day period, then participants were provided with nutritional counseling to either increase or decrease food intake to maintain body weight. The 7-day food records were subsequently photocopied and returned to participants. Participants reproduced this 7-day isoenergetic diet throughout the study. Subjects were matched for diet and were approximately at the typical American diet of 55% carbohydrate, 30% fat, and 15% protein for basic composition with no differences between groups.

2.7. Resistance training

Before initiation of the 4-week overreaching program, each participant underwent 4 weeks of base resistance training. This ensured that each subject began the study in a similar, trained state. Base training was 2 days per week, and each workout consisted of 5 exercises (squat, bench press, lat pulldown, leg press, and shoulder press) for 3 sets of 8 to 10 repetitions with 1 to 3 minutes of rest between sets.

Resistance training overreaching was performed on 4 consecutive days using a total-body program (see Table 2). The first 2 weeks consisted of a higher-volume,

Table 3
Changes in muscular strength, hemodynamics, uric acid, and ammonia

	T1	T2	T3	T4	T5
Body mass (kg)					
AA	89.1 ± 19.8	90.4 ± 19.8	90.4 ± 20.1	90.9 ± 20.1	90.9 ± 20.0
P	88.9 ± 11.1	90.1 ± 11.1	89.7 ± 10.7	89.9 ± 10.8	89.8 ± 10.5
1 RM squat (kg)					
AA	130.8 ± 33.5	130.3 ± 33.3	135.9 ± 36.0*	137.0 ± 35.2*	142.0 ± 36.0*,†
P	135.1 ± 20.4	129.9 ± 19.4*	136.4 ± 22.4	139.0 ± 21.5*	143.0 ± 21.5*,†
RM bench press (kg)					
AA	108.6 ± 17.6	107.3 ± 18.4	109.6 ± 16.1	112.6 ± 17.6*	116.7 ± 17.8*,†
P	110.5 ± 15.6	107.1 ± 15.1*	110.8 ± 16.6	112.8 ± 15.9*	116.8 ± 17.1*,†
Hematocrit (%)					
AA	43.9 ± 3.9	42.2 ± 2.9	43.5 ± 3.4	42.7 ± 3.0	43.4 ± 2.8
P	44.4 ± 2.0	41.3 ± 2.4	42.0 ± 1.6	42.4 ± 2.0	42.8 ± 1.3
Systolic blood (mm Hg)					
AA	121.3 ± 7.7	126.7 ± 8.5	128.2 ± 8.2	125.1 ± 8.6	122.2 ± 10.7
P	127.8 ± 12.3	125.8 ± 7.7	127.8 ± 7.7	130.8 ± 11.2	127.0 ± 9.1
Diastolic BP (mm Hg)					
AA	81.3 ± 8.2	84.9 ± 4.5	84.2 ± 7.0	83.3 ± 9.6	80.7 ± 8.4
P	83.8 ± 3.6	84.5 ± 5.5	86.5 ± 6.3	83.5 ± 8.0	86.0 ± 6.6
Hemoglobin (g/dL)					
AA	15.4 ± 1.5	14.9 ± 1.3	15.2 ± 1.7	15.0 ± 1.7	15.2 ± 1.1
P	15.7 ± 1.1	14.6 ± 1.0*	15.0 ± 0.6*	14.8 ± 1.0*	14.8 ± 0.6*
Uric acid (mg/dL)					
AA	6.8 ± 1.2	7.3 ± 1.2	6.8 ± 1.1	6.5 ± 1.1	6.6 ± 1.3
P	6.1 ± 1.6	7.2 ± 2.4*	6.6 ± 1.7	6.1 ± 1.7	5.6 ± 1.8
Ammonia (μmol/L)					
AA	31.9 ± 7.0	23.7 ± 10.0#	18.6 ± 12.9*	26.8 ± 23.0	23.9 ± 13.3
P	38.2 ± 18.4	20.5 ± 13.3*	17.1 ± 5.0*	35.5 ± 18.7	37.2 ± 13.2‡

* $P < .05$ from corresponding time point T1.
† $P < .05$ from corresponding time points T3 and T4.
# $P = .06$ from corresponding time point T1.
‡ $P < .05$ between groups.
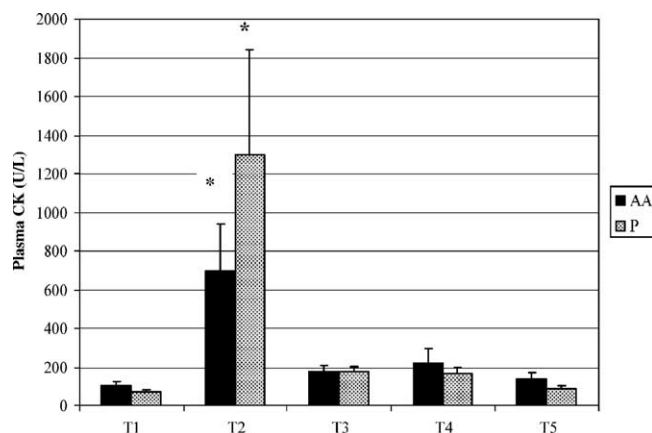
Fig. 1. Resting serum CK concentrations during 4 weeks of resistance training overreaching. Data presented are means ± SEM. *$P < .05$ from corresponding point T1.

moderate-intensity resistance exercise protocol, whereas the last 2 weeks consisted of a high-intensity, lower-volume resistance exercise protocol. All sets were designed to evoke muscular exhaustion. If a participant was able to complete the desired number of repetitions with the current load, weight was added to subsequent sets or during the next workout.

2.8. Statistical analyses

Statistical evaluation of all performance, hormonal, and blood variable data was accomplished using an analysis of variance with repeated measures. Subsequent pairwise differences were determined using a Tukey post hoc test when appropriate. Effect size (ES) for comparisons was calculated. Pearson product-moment correlations were used to determine selected pairwise relationships. Using the nQuery Advisor software (Statistical Solutions, Saugus,
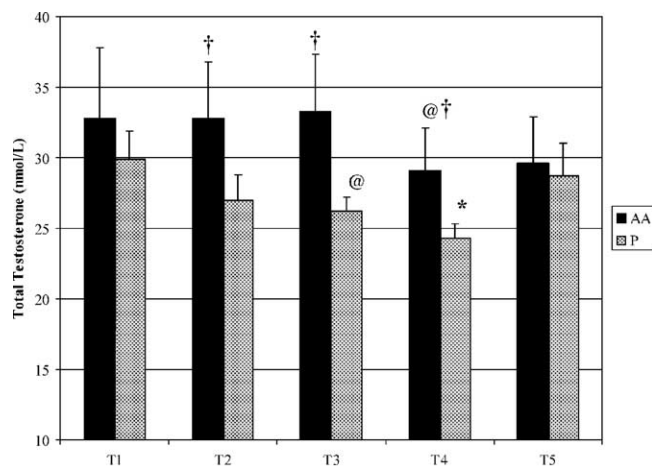
MA), the statistical power for the n size used ranged from 0.80 to 0.92. Significance was set at $P = .05$.

3. Results

Changes in muscular strength, hemodynamic variables, uric acid, and ammonia are presented in Table 3. In response to high-volume overreaching, 1 RM squat and bench press decreased significantly at T2 in the P group (ES = 0.3 and 0.3, respectively); alternately, no change was observed in the AA group at this time point. At T3, 1 RM squat and bench press returned to baseline (T1) values in the P group, whereas a significant increase in 1 RM squat was observed in the AA group (ES = 0.2). Both 1 RM squat and bench press increased significantly at T4 and T5 for both groups, with no differences observed between groups. There were no significant differences in systolic and/or diastolic pressure or hematocrit at any time point throughout the study.
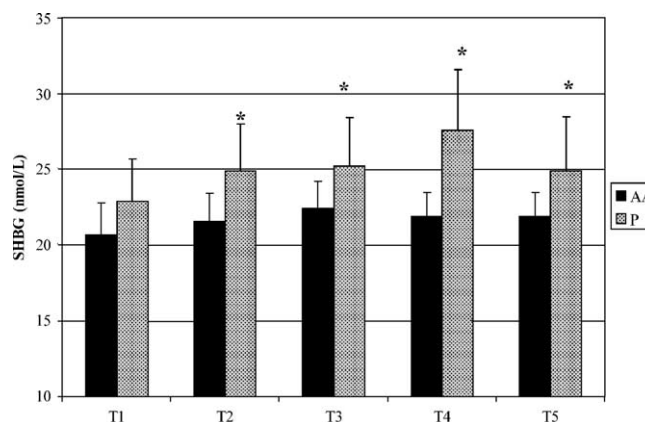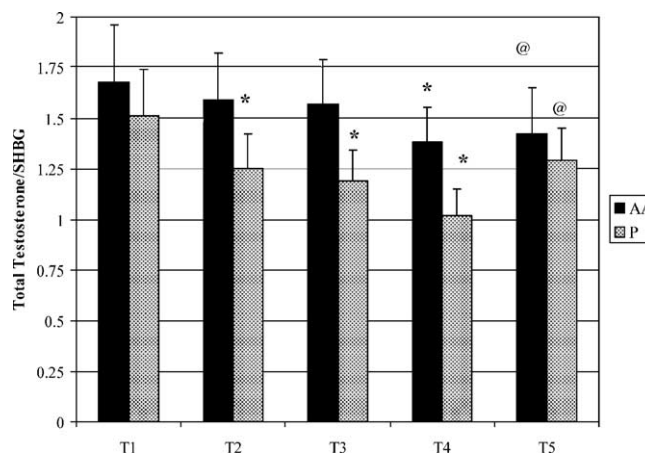

Fig. 3. Resting serum SHBG concentrations during 4 weeks of resistance training overreaching. Data presented are means ± SEM. *$P < .05$ from corresponding point T1.

Fig. 2. Resting serum total testosterone concentrations during 4 weeks of resistance training overreaching. $^{†}P < .05$ from corresponding value for P group. Data presented are means ± SEM. *$P < .05$ from corresponding point T1. $^{@}P = .08$ from corresponding time point T1.

Fig. 4. Resting ratio of total testosterone to SHBG concentrations during 4 weeks of resistance training overreaching. Data presented are means ± SEM. *$P < .05$ from corresponding point T1. $^{@}P = .07$ from corresponding time point T1.
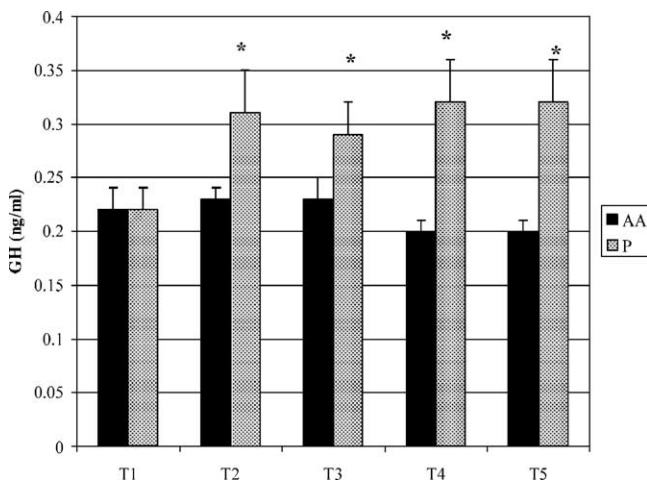
Fig. 5. Resting serum 22-kd GH concentrations during 4 weeks of resistance training overreaching. Data presented are means ± SEM. *$P < .05$ from corresponding point T1.

Hemoglobin concentrations were significantly reduced in the P group at T2 to T5 compared with T1 (ES = 0.7-1.0), whereas no changes were observed in the AA group. However, there were no differences in hematocrit or percentage of change in plasma volume compared with corresponding T1 value or with corresponding P values. No significant differences in serum uric acid were observed at any time point in the AA group. However, a significant elevation at T2 was observed in the P group. For both groups, there was no correlation observed between uric acid and any performance variable. In the P group, plasma ammonia was significantly reduced at T2 and T3 in comparison to T1 (ES = 1.0 and 1.1, respectively). Plasma ammonia concentration was significantly higher in the P group compared with the AA group at T5 (ES = 1.0).

Resting serum CK concentrations are presented in Fig. 1. CK concentration was significantly elevated at T2 in the P group and the AA group, but was significantly lower in the AA group at T2. CK concentration was negatively correlated to 1 RM squat ($r = -0.67$, $r^2 = 0.45$, $P < .05$), but not 1 RM bench press ($r = -0.21$, $r^2 = 0.04$, $P > .05$) performance. No other significant differences in CK were observed at any other time point in either group.

Changes in resting serum total testosterone, SHBG, the ratio of total testosterone to SHBG, and GH concentrations are presented in Figs. 2–5. Resting serum total testosterone concentrations (Fig. 2) did not change significantly at T2 and T3 in the AA group, whereas a trend for a reduction was observed in the P group at T3 ($P = .08$). A significant reduction in resting serum total testosterone at T4 was observed in the P group, whereas only a tendency for a reduction was observed in the AA group ($P = .08$). Values for testosterone were significantly higher for the AA group compared with the P group at T2, T3, and T4. In addition, AA values at T4 for the AA group were significantly lower than T1 to T3. No significant correlations ($r = 0.03$-0.18) were observed between total testosterone and 1 RM squat and bench press performance at any time point.

The overreaching protocol stimulated a significant increase in resting serum SHBG concentrations (Fig. 3) in the P group at T2, T3, T4, and T5; however, no alterations in SHBG were noted in the AA group. The ratio of total testosterone to SHBG (ie, free androgen index; Fig. 4) was significantly lower at T2, T3, and T4 in the P group, but only at T4 in the AA group. At T5, there was a tendency for this ratio to be reduced compared with T1 in both groups ($P = .07$). A significant correlation was observed in the P group between the ratio of total testosterone to SHBG and 1 RM squat performance at T4 and T5 ($r^2 = 0.29$ and 0.50, respectively). Significant elevations in resting serum GH concentrations (Fig. 5) were observed in the P group from T2 to T5, whereas no significant differences were observed in the AA group at any time point.

Resting concentrations of serum IGF-1, insulin, cortisol, and glucose are presented in Table 4. No differences were

Table 4
Resting concentrations of serum IGF-1, insulin, cortisol, and glucose

	T1	T2	T3	T4	T5
IGF-1 (nmol/L)					
AA	48.7 ± 16.9		46.0 ± 11.0		45.4 ± 13.7
P	43.8 ± 9.9		47.0 ± 13.0		45.0 ± 10.8
Insulin (μIU/mL)					
AA	11.6 ± 7.2	7.6 ± 4.2*	7.8 ± 4.3*	9.3 ± 8.2[†]	9.3 ± 4.7[†]
P	10.7 ± 8.2	8.2 ± 5.7*	9.4 ± 7.9	8.6 ± 6.9	9.5 ± 9.0
Glucose (mg/dL)					
AA	104.1 ± 14.4	87.6 ± 8.4*	94.8 ± 17.3*	92.5 ± 18.0*	90.5 ± 16.7*
P	97.9 ± 8.9	90.0 ± 10.0*	92.8 ± 9.3	88.9 ± 8.6*	88.2 ± 4.5*
Cortisol (nmol/L)					
AA	590.7 ± 237.9	686.7 ± 198.3	599.0 ± 288.2	595.6 ± 154.1	545.8 ± 226.9
P	590.1 ± 157.2	464.8 ± 137.8	483.5 ± 158.6	454.0 ± 145.4	467.5 ± 162.3

* $P < .05$ from corresponding time point T1.
[†] $P = .07$ from corresponding time point T1.

observed in resting serum IGF-1 and cortisol concentrations at any time point. However, positive correlations between IGF-1 and 1 RM squat were observed at T1 ($r^2 = 0.25$), T3 ($r^2 = 0.25$), and T5 ($r^2 = 0.40$), whereas negative correlations were observed between cortisol and 1 RM squat performance at T3 ($r = -0.78$, $r^2 = 0.61$) and T4 ($r = -0.61$, $r^2 = 0.37$) in the P group and at T2 and T3 in the AA group ($r = -0.55$, $r^2 = 0.30$). Changes in resting serum concentrations of insulin paralleled changes in glucose. Reductions in insulin were observed at T2 and T4 in both groups. Serum glucose was decreased at T5 in both groups (ES = 1.1 for the P group; ES = 0.9 for the AA group); a decrease in both insulin and glucose was only observed in the AA group at T3 (ES = 0.5 and 0.6, respectively).

4. Discussion

The major findings of this study are the following: (1) the initial (first week) response to high-volume resistance training overreaching is a reduction in muscle strength; however, this phenomenon is attenuated by amino acid supplementation; (2) CK and uric acid were elevated only in the placebo group after the first week of overreaching; (3) the total testosterone–SHBG ratio was maintained with amino acid supplementation during overreaching; and (4) amino acid supplementation provided no further benefit to resistance training performance once training volume was reduced (after T3) and participants adapted to the training stress.

Amino acid supplementation has been shown to enhance recovery from exercise by stimulating protein synthesis and/or reducing degradation [13,16,20] and by enhancing glycogen resynthesis when ingested with glucose [20]. Recovery from consecutive total-body workouts is critical to performance during overreaching. Therefore, amino acid requirements to maintain optimal repair and recovery of skeletal muscle may be greater during high-volume and/or high-intensity resistance training [21,22]. In the present study, muscle strength was reduced initially only in the P group, thereby demonstrating the importance of increased amino acid intake during the initial phase of overreaching. Conversely, after an increase in intensity and partial reduction in volume (weeks 3 and 4), no performance reduction was observed. This demonstrated that optimal adaptation to overreaching is mediated by reduction in training volume and/or the ability of resistance-trained men to rapidly adapt to a new training stimulus (via enhanced amino acid intake).

The second major finding of this investigation was that the significant reduction in muscle strength after the first week of overreaching in the P group occurred concomitantly with a significant elevation in serum CK. CK values were inversely correlated ($r = -0.67$, $r^2 = 0.45$) to 1 RM squat performance (but not 1 RM bench press performance), perhaps indicating an underlying relationship between muscle damage and performance. The mechanism for this beneficial influence of amino acid supplementation on muscle damage is unclear; limited data are available examining this concept during

resistance training. Coombes and McNaughton [15] reported significantly less serum concentrations of CK and lactate dehydrogenase during aerobic exercise in those supplementing with branched-chain amino acids (12 g/d). Amino acid supplementation or infusion increases amino acid availability and has been shown to increase protein synthesis [11,23,24]. In fact, supplementation with only 6 g of essential amino acids has been shown to elevate protein synthesis [23]. In addition, amino acid (ie, leucine) infusion has been shown to elevate protein synthesis via posttranscriptional mechanisms (phosphorylation of p70^{S6} and eIF4E-binding protein 1) [25]. Greater protein synthetic rates and amino acid availability potentially could lead to a reduction in damage to myofibrillar and cytoskeletal proteins, thereby helping to maintain force production.

Branched-chain amino acids have been shown to reduce proteolysis in rat skeletal muscle [26,27]. The mechanism is less clear but appears, in part, to inhibit local proteases. Three major proteolytic pathways involve lysosomal, calcium-activated (calpain), and adenosine triphosphate–ubiquitin proteases. Busquets et al [26,27] have shown that branched-chain amino acids significantly reduced lysosomal proteolysis, reduced expression of ubiquitin genes, and showed a tendency to reduce calcium-activated proteolysis as well. Thus, it appears that amino acid supplementation can reduce proteolysis and potentially muscle damage. Our data show that amino acid supplementation attenuates muscle damage during the initial high stress of overreaching, and this may partially explain the enhanced ability to maintain muscle strength.

Similar to CK, serum concentrations of uric acid were also significantly elevated 1 week after the initial stress of overreaching in the P group. It has been suggested that elevated blood concentrations of uric acid may reflect an intracellular energy deficit (via greater stimulation of the purine nucleotide cycle) and may be a possible indicator of training stress [28]. This suggestion was based on endurance training in which uric acid was inversely correlated to endurance performance [28]. To our knowledge, this was the first study to examine serum uric acid concentrations in the context of a resistance training overreaching protocol.

Short-term elevations in testosterone, GH, and cortisol have been reported during resistance exercise [29,30], which have been shown to be influenced by nutritional intake [31]. Large increases in the volume and/or intensity of resistance training may alter circulating hormonal concentrations [32]. However, less is known concerning the effects of amino acid supplementation on resting hormonal concentrations during resistance training overreaching. It has been shown that resting total testosterone concentrations may decrease during high-volume or high-intensity resistance training overreaching [33,34]. In the present study, resting concentrations of testosterone were reduced at T3 and T4 in the P group, whereas the AA group showed a decrease only at T4. Moreover, a large increase in training volume appeared to be a critical variable leading to the reduction observed in

the P group after the second week, whereas amino acid supplementation appeared to maintain resting serum testosterone concentrations during this time. Beyond the initial phase, when volume was reduced and intensity increased, the reductions in resting serum testosterone concentrations observed were independent of supplement status. Therefore, our data demonstrate that amino acid supplementation only affected resting testosterone concentrations during the initial high stress of high-volume overreaching.

SHBG concentrations increased throughout 4 weeks of overreaching; however, this response was abolished by amino acid supplementation. Increased SHBG in the P group may have been because of the need to increase the carrying capacity of testosterone. The increase in SHBG affected the free androgen index (total testosterone–SHBG ratio), which was significantly reduced during the first 2 weeks of overreaching in the P group, although during the last 2 weeks, reductions in the free androgen index were observed in both groups. It has been theorized that it is the free, unbound fraction of testosterone that is biologically active at the level of the receptor, such that changes in free testosterone significantly affect the bioavailability of this hormone. Previous investigations have shown that free testosterone may decrease when the volume and/or intensity are significantly increased [35,36]. Our data indicate that amino acid supplementation was effective in maintaining the free androgen index during the initial, high-volume phase of overreaching. In addition, our data support previous research demonstrating that large increases in training intensity with limited recovery between workout sessions reduces resting concentrations of free testosterone [35,36].

No significant differences were observed in resting serum cortisol concentrations or the testosterone/cortisol ratio (T/C ratio) at any time point in the present investigation. Resting cortisol concentrations have been shown to be highly variable over the course of resistance training programs [32]. Generally, significant increases in volume or intensity have resulted in higher resting concentrations of cortisol [35,36]. Overreaching in elite weightlifters has been shown to decrease the T/C ratio [36], but to a lesser extent when the individuals have previous exposure to the overreaching stimulus [37]. It has been suggested by some investigators that resting cortisol changes reflect only long-term (>1 month) training stresses [36]. Therefore, it is possible that the experimental protocol used in this study was not of sufficient duration to elicit long-term elevations in resting cortisol.

Resting concentrations of serum 22-kd GH were elevated in the P group, but did not change in the AA group. Although the pulsatilty of GH secretion needs to be considered when interpreting resting measures, resting GH concentrations have not been shown to change significantly during resistance training [38]. This was supported by the lack of change in GH concentrations in the AA group throughout the experimental period. However, our data show that the short-term overreaching protocol posed a significant physiological stress in the P group. Thus, the

elevation in resting GH concentrations observed in the P group may have been a compensatory response to maintain tissue anabolism, considering that the free androgen concentrations were reduced. In addition, IGF-1 concentrations did not change in the present study despite elevated GH in the P group. This finding has been reported previously where the IGF-1 response to increased GH secretion was delayed by at least 24 hours [39]. It is likely that this delayed response may partially explain the lack of change in IGF-1 and indicate that circulating IGF-1 may not be a meaningful marker of the implicit activity of the GH-IGF-1 axis, particularly because IGF-1 has autocrine/paracrine activity within skeletal muscle. In addition, the participants in the present study initiated the overreaching period with high initial resting concentrations of IGF-1. Therefore, the potential for further increase may have been limited.

Interestingly, resting serum glucose and insulin concentrations were reduced throughout the experimental period in both groups at several time points. These findings are unique, as reductions in resting serum glucose have not consistently been observed during resistance training. However, basal concentrations of insulin are not regulated by normal basal serum glucose concentrations (eg, 80-100 mg/dL) and have been shown to be lower during strength training [40] and in bodybuilders with large muscle mass [41]. Although insulin secretion is pulsatile and a basal value may not be indicative of a positive training adaptation, our data support previous investigations demonstrating greater insulin sensitivity during overreaching.

It has been suggested that greater amino acid intake may be beneficial to high-volume exercise by reducing the potential risk of sports anemia [12]. Plasma proteins and erythrocytes may be broken down to support protein anabolism during stressful training. Hemolysis and subsequent reductions in blood hemoglobin (and haptoglobin) have been shown to occur in endurance athletes and during strength training [42]. Schobersberger et al [42] attributed this to the mechanical stress of large muscle mass contractions on red blood cells and alterations of membrane integrity, possibly due to higher concentrations of epinephrine during exercise. Unique in this study was the finding that hemoglobin concentrations were significantly reduced throughout the 4-week overreaching protocol in the P group, yet were maintained in the AA group. The results of the present study demonstrate that amino acid supplementation has a positive impact on preserving blood hemoglobin concentrations during resistance training overreaching.

Ammonia increases during exercise, and this response has been shown to be exaggerated [43] or blunted [44] during endurance exercise overtraining. Although elevated resting ammonia values have been suggested as a metabolic marker of overreaching, studies examining its responses are equivocal [45]. We had hypothesized that elevations in ammonia concentrations would occur in response to resistance training overreaching and that this response would be attenuated by amino acid supplementation.

However, we noted significant decreases in ammonia in both groups at T2 and T3, although ammonia concentrations at T5 were lower in the AA group than the P group. These findings are difficult to rectify because (1) ammonia concentrations during exercise in overreached subjects are equivocal; (2) few well-designed studies have examined resting ammonia concentrations in response to overreaching; and (3) no studies have examined ammonia concentrations in response to resistance exercise overreaching. Clearly, additional research on the responses of ammonia concentrations to overreaching is necessary.

In conclusion, a temporary loss of muscle strength observed during the initial phase of resistance training overreaching was attenuated with amino acid supplementation. The mechanism(s) for the ergogenic effect of amino acid supplementation is unclear, but may involve elevated protein synthesis and/or reduced proteolysis, thereby reducing muscle damage associated with the stress of unaccustomed resistance training. Amino acid supplementation maintained serum concentrations of biologically active free testosterone and of hemoglobin during overreaching. The overreaching protocol lowered serum glucose and insulin concentrations and had no effect on resting serum concentrations of IGF-1 and cortisol. This investigation demonstrated a potential benefit of amino acid supplementation for maintaining performance during stressful training (ie, initial phase of high-volume overreaching) when recovery time is limited.

Acknowledgments

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