

## Effects of Protein Supplementation on Muscular Performance and Resting Hormonal Changes in Collegiate Athletic

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### Abstract

**Objective:** The effect of protein supplementation on athletic performance and hormonal changes was examined in 32 experienced collegiate strength/power athletes participating in a 12-week resistance training program. **Method:** Subjects were randomly assigned to either a protein supplement (PR; n = 22) or a placebo (PL; n = 10) group. During each testing session subjects were assessed for strength (one repetition maximum [1-RM] bench press and squat), power (Wingate anaerobic power test) and body composition. Resting blood samples were analyzed at weeks 0 (PRE), 6 (MID) and 12 (POST) for total testosterone, cortisol, growth hormone, and IGF-1. **Result:** There was seen no difference in energy intake between PR and PL ( $3034 \pm 209$  kcal and  $3130 \pm 266$  kcal, respectively), but a significant difference in daily protein intake was seen between PR (2.00 g·kg body mass[BM]<sup>-1</sup>·d<sup>-1</sup>) and PL (1.24 g·kgBM<sup>-1</sup>·d<sup>-1</sup>). A greater change ( $p < 0.05$ ) in the  $\Delta$  1-RM squat was seen in PR ( $23.5 \pm 13.6$  kg) compared to PL ( $9.1 \pm 11.9$  kg). No other significant strength or power differences were seen between the groups. Cortisol concentrations were

significantly lower at MID for PL and this difference was significantly different than PR. No significant changes were noted in resting growth hormone or IGF-1 concentrations in either group. **Conclusion:** Although protein supplementation appeared to augment lower body strength development, similar upper body strength, anaerobic power and lean tissue changes do not provide clear evidence supporting the efficacy of a 12-week protein supplementation period in experienced resistance trained athletes.

**Key words:** Sport nutrition, resistance training.

### Introduction

Approximately half of American collegiate athletes are reported to be using nutritional supplements, with protein supplementation being one of the most commonly used (Schenk & Costley., 2002). Although some studies have demonstrated that protein supplementation in previously untrained adults performing resistance exercise does not provide any benefit in regards to increases in lean tissue accretion or strength (Candow et al., 2006), evidence does support a greater protein need for strength and power athletes compared to endurance athletes and the sedentary population (Lemon et al., 1992; Tarnopolsky et al., 1992). Considering that heavy resistance exercise

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results in disruption or damage to the active muscle fibers, a greater protein intake may assist in the repair and remodeling process of these fibers (Tipton et al., 2004). A decrease in muscle damage, attenuation of force decrements, and an enhanced recovery from resistance exercise has been demonstrated in subjects using protein supplements (Kraemer et al., 2006; Ratamess et al., 2003). The combination of resistance training with a greater amino acid pool may result in a positive nitrogen balance and an increase in protein synthesis (Roy et al., 2000). This may have important implications for improvements in both muscle size and strength. Protein intake has also been suggested to have an important role in regulating the anabolic hormones that are involved with muscle remodeling (Chandler et al., 1994; Volek et al., 1997). When a protein supplement was provided to previously untrained men during 12 weeks of resistance training, post-exercise cortisol concentrations were reduced suggesting an attenuation in the rise of post-exercise muscle degradation (Bird et al., 2006). In addition, dietary protein content has also been suggested to influence resting testosterone concentrations, and the hormonal response to an acute resistance exercise session (Kraemer et al., 2006). However, there have only been a few studies that have examined the effect of prolonged protein supplementation on changes in resting hormonal concentrations in experienced resistance trained competitive strength/power athletes. For strength-trained individuals to maintain a positive nitrogen balance it is suggested that they need to consume a protein intake of 1.6 to 1.8 g·kg<sup>-1</sup>·day<sup>-1</sup> (A.D.A., 2000). For many collegiate athletes the ability to achieve adequate protein intake is compromised due to inadequate nutrition attributed to low caloric intake, poor food choices, and irregular meals (Cole et al., 2005; Hinton

et al., 2004). To insure sufficient protein intake many collegiate athletes rely on protein supplementation (Rozenek et al., 2002). However, the evidence supporting the efficacy of protein supplementation to the normal dietary intake of collegiate strength/power athletes is limited. Thus, the purpose of this study was to examine the effect of protein supplementation on strength, power, body composition and resting endocrine concentrations during a 12-week resistance training program in competitive strength/power athletes.

## Methods

### Subjects

Thirty-two male strength and power athletes volunteered for this study. Following an explanation of all procedures, risks and benefits each subject gave his informed consent to participate in this study. The Institutional Review Board of the College approved the research protocol. Subjects were not permitted to use any additional nutritional supplementation and did not consume anabolic steroids or any other anabolic agents known to increase performance for the previous year. Screening for anabolic steroid use and additional supplementation was accomplished via a health questionnaire filled out during subject recruitment. Subjects were randomly assigned to either a protein supplement group (PR; n =22: 20.3 ± 1.6y; 1.82 ± 0.06 m; 93.9 ± 7.9 kg) or a placebo group (PL; n =10: 21.0 ± 1.2y; 1.83 ± 0.05 m; 97.7 ± 10.2 kg). All subjects were athletes from the college's basketball team with at least 2 years of resistance training experience. All groups performed the same resistance training program for 12 weeks. The training program was a 3-day per week, split routine (see Table 1) that was supervised by study personnel. All subjects completed a daily training log, which was collected by study investigators on a weekly basis.

**Table 1. 12-week resistance training program.**

	<b>Weeks 1 – 4</b> <b>(Sets x Reps)</b>	<b>Weeks 5 – 8</b> <b>(Sets x Reps)</b>	<b>Weeks 9 – 12</b> <b>(Sets x Reps)</b>
Power Clean			
Bench Press	4 x 8 – 10	4 x 6 – 8	5 x 4 – 6
Push Press	-	4 x 4 – 6	5 x 3 – 5
High Pulls (Snatch grip)	-	3 x 4 – 6	4 x 3 – 5
Incline Bench press	3 x 8 – 10	3 x 6 - 8	4 x 4 – 6
Seated Shoulder Press 10	4 x 8 – 10	-	-
Power dumbbell Shrugs	3 x 6 – 8	-	-
Dumbbell Front Raise	-	3 x 6 - 8	-
Triceps Pushdowns	3 x 8 – 10	3 x 6 – 8	-
Trunk and Abdominal Routine	2 x 10	3 x 10	4 x 10
*Squat	4 x 8 – 10	4 x 6 – 8	5 x 4 – 6
Power snatch	-	-	4 x 3 - 5
Dead Lift	4 x 8 – 10	3 x 6 - 8	4 x 4 – 6
Leg Extensions	3 x 8 – 10	-	-
Leg Curls	3 x 8 – 10	3 x 6 – 8	3 x 6 – 8
Lat Pulldown	4 x 8 – 10	4 x 6 – 8	4 x 4 – 6
Seated Row	4 x 8 – 10	4 x 6 – 8	4 x 4 – 6
Hammer Curls	3 x 8 – 10	3 x 6 – 8	4 x 6 - 8
Dumbbell Biceps Curls	3 x 8 – 10	3 x 6 – 8	-
Trunk and Abdominal Routine	2 x 10	3 x 10	4 x 10

### Testing protocol

Subjects reported to the Human Performance Laboratory on three separate occasions. The first testing session occurred prior to the onset of protein supplementation (PRE), the second testing session occurred during the sixth week of supplementation and training (MID), while the third testing session occurred at the conclusion of the 12-week supplementation program (POST). All testing sessions occurred at the same time of day.

### Blood measurements

Subjects were required to arrive at the laboratory in the early morning following an overnight fast for blood draws. All blood draws occurred at the same time of day for each testing session. Each blood sample was obtained from an antecubital arm vein, with the subject in a seated position. Blood samples were collected into a Serum was allowed to clot at room temperature and subsequently centrifuged

at 1,500 x g for 15 minutes. The resulting serum was placed into separate 1.8-ml microcentrifuge tubes and frozen at -80°C for later analyses.

### Biochemical and hormonal analyses

Serum total testosterone, growth hormone, IGF-I, and cortisol concentrations were determined using enzyme immunoassays (EIA) and enzyme-linked immunosorbent assays (ELISA). Determinations of serum immunoreactivity values were made using a SpectraMax340 Spectrophotometer (Molecular Devices, Sunnyvale, CA). 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 molar ratio of total testosterone to cortisol (T/C ratio) was determined for each testing session to provide a measure of anabolic/catabolic status of the body.

### **Body composition**

Body composition was determined using whole body-dual energy x-ray absorptiometry (DEXA) scans (Prodigy; Lunar Corporation, Madison, WI). Total body estimates of percent fat, bone mineral density and bodily content of bone, fat and non-bone lean tissue was determined using company's recommended procedures and supplied algorithms. All measures were performed by the same technician. Quality assurance was assessed by daily calibrations and was performed prior to all scans using a calibration block provided by the manufacturer.

### **Strength measures**

During each testing session subjects performed a 1- repetition maximum (1-RM) strength test for the squat and bench press exercises. The 1 RM tests were conducted as described by Hoffman (2006). Each subject performed a warm-up set using a resistance that was approximately 40-60% of his perceived maximum, and then performed three to four subsequent attempts to determine the 1-RM. A 3 – 5 minute rest period was provided between each lift.

### **Anaerobic power measures**

To quantify anaerobic power performance all subjects performed the Wingate anaerobic power test . Following a warm up period of 5-min pedaling at 60 rpm interspersed with three all-out sprints lasting 5 s, the subjects pedaled for 30 s at maximal speed against a constant torque. Peak power, mean power, total work and rate of fatigue were determined. Peak power was defined as the highest mechanical power output elicited during the test. Mean power was defined as the average mechanical power during the 30-s test, and the rate of fatigue was determined by dividing the highest power output from the lowest power output x 100.

### **Dietary recall**

Three-day dietary records were completed every week 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 was used to analyze dietary recalls.

### **Supplement schedule**

The supplement and placebo was in powder form and provided in individual packets. The contents of each packet were mixed with 473 ml of water. Subjects consumed one drink every morning, and a second daily drink following their exercise session. The supplement was comprised of 260 kcal, 42 g of protein, 18 g of carbohydrate and 3 g of fat. Thus, on exercise days subjects in the supplement group would consume 84 g of protein from the supplement source. The protein content of the supplement consisted of a proprietary blend of milk protein concentrate, whey protein concentrate, L- glutamine, and dried egg white. The carbohydrate content of the supplement consisted of maltodextrin. The placebo (maltodextrin) was comprised of 260 kcal, 2 g of protein, 63 g of carbohydrate and 2 g of fat.

### **Statistical analysis**

Statistical evaluation of the data was accomplished by a 2 (group) x 3 (time) or 2 x 2 repeated measures analysis of variance. In the event of a significant F-ratio, LSD posthoc tests were used for pairwise comparisons. In addition,  $\Delta$ PRE – POST comparisons between groups in performance measures were analyzed with independent student's t-tests. Pearson correlation was used to examine selected bivariate correlations. Effect size (ES) calculations were used to determine the magnitude of treatment effect, and are reported to provide a measure of practical significance. A criterion alpha level of  $p \leq 0.05$  was used to determine statistical

significance. All data are reported as mean  $\pm$  SD.

### Results

Average daily dietary intake is shown in Table 2. No significant difference in daily caloric intake was observed between PR and PL. However, significant differences existed between the groups in protein and carbohydrate intake. No significant changes in body mass, lean body mass or percent body fat were observed from PRE to POST training in either PR or PL, and no between group differences were noted as well (see Table 3). Interestingly, lean body mass was increased by 1.4 kg in PR, but only 0.1 kg in PL. Although these differences did not reach statistical significance ( $p = 0.08$ ,  $ES = 0.78$ ), a trend towards a greater lean tissue accrue ment in PR was evident. Significant increases in strength from PRE occurred for both PR and PL in the 1-RM squat and 1-RM bench press (see Table 3). However, strength comparisons showed that subjects in PR had significantly greater improvement in 1-RM squat strength compared to PL, but no difference in the magnitude of improvement was seen between the groups for the 1-RM bench press. Examination of the subject's

training logs revealed no significant differences between PR and PL in the average weekly training volume ( $6461 \pm 584$  kg and  $6420 \pm 425$  kg, respectively) and weekly training intensity ( $81.5 \pm 6.2$  % and  $81.6 \pm 6.6$ %, respectively) for the bench press. A 6% difference ( $p = 0.09$ ,  $ES = 0.74$ ) was noted between PR and PL in the average weekly training volume ( $9287 \pm 990$  kg and  $8710 \pm 476$  kg, respectively), and a 5.2% difference ( $p = 0.09$ ,  $ES = 0.73$ ) was seen between these groups in the average weekly training intensity ( $86.7 \pm 7.0$  % and  $81.5 \pm 7.3$ %, respectively) for the squat exercise. Wingate anaerobic power test measures are shown in Table 3. No significant PRE to POST changes in peak power, mean power, fatigue rates or total work occurred in either group. In addition, no between group differences were noted as well. Resting total testosterone concentrations are shown in Figure 1. No significant change from PRE was observed in either group, and no between groups differences were noted. Changes in resting cortisol concentrations appear in Figure 2. Cortisol concentrations remained steady during all three measuring time points for PR.

**Table 2. Average daily dietary intake.**

Group	Kcal	CHO (g)	Protein (g)	Total Protein (g·kg <sup>-1</sup> )	Fat (g)	% CHO (g)	% Protein	% Fat
PL (.02)	3139 (300)	435 (39)	120 (12)	1.24 (.12)	102 (15)	55.5 (.02)	15.4 (.01)	29.1
PR (.02)	3072 (241)	350 (38) *	188 (11) *	2.00 (.12) *	102 (10)	45.6 (.02) *	24.5 (.01) *	29.9

\*  $p < 0.05$ , significant difference between groups

**Table 3. Anthropometric results. Data are means ( $\pm$ SD).**

Group	PRE	MID	POST	$\Delta$ PRE – POST
<b>Body Mass (kg)</b>	PL 99.0 (10.2)	99.0 (9.9)	99.3 (10.6)	0.4 (2.0)
	PR 94.7 (7.9)	95.0 (8.2)	95.6 (8.3)	0.9 (1.8)
<b>Lean Body Mass (kg)</b>	PL 76.7 (3.3)	77.5 (3.0)	76.8 (4.3)	0.1 (1.4)
	PR 74.0 (5.8)	75.1 (5.8)	75.4 (6.2)	1.4 (1.9)
<b>Body Fat (%)</b>	PL 21.8 (7.3)	21.1 (7.1)	22.1 (7.1)	0.2 (1.5)
	PR 21.7 (5.5)	20.6 (6.1)	20.9 (5.9)	-0.8 (2.0)

\*  $p < 0.05$ .

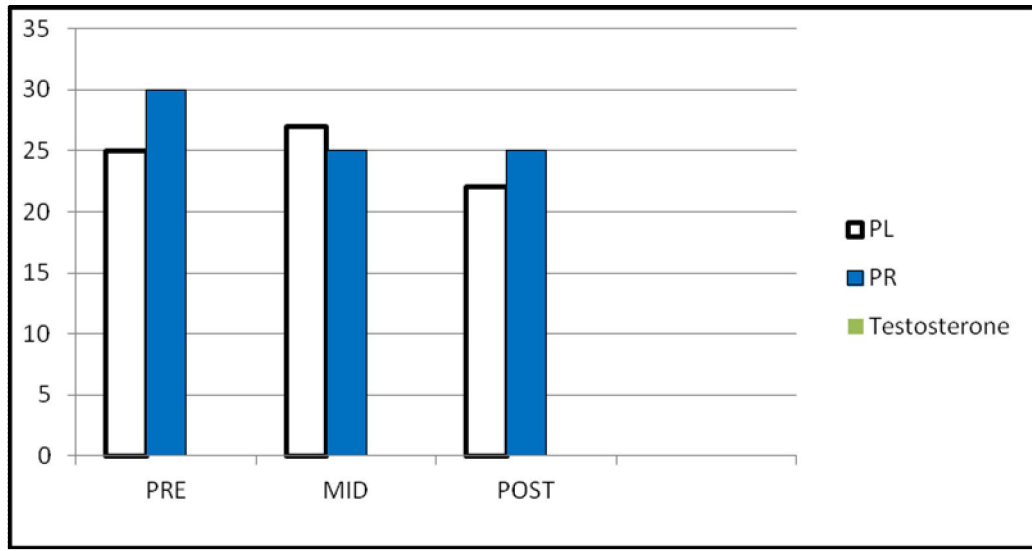


Figure 1. Resting testosterone concentrations (mean ± SD).

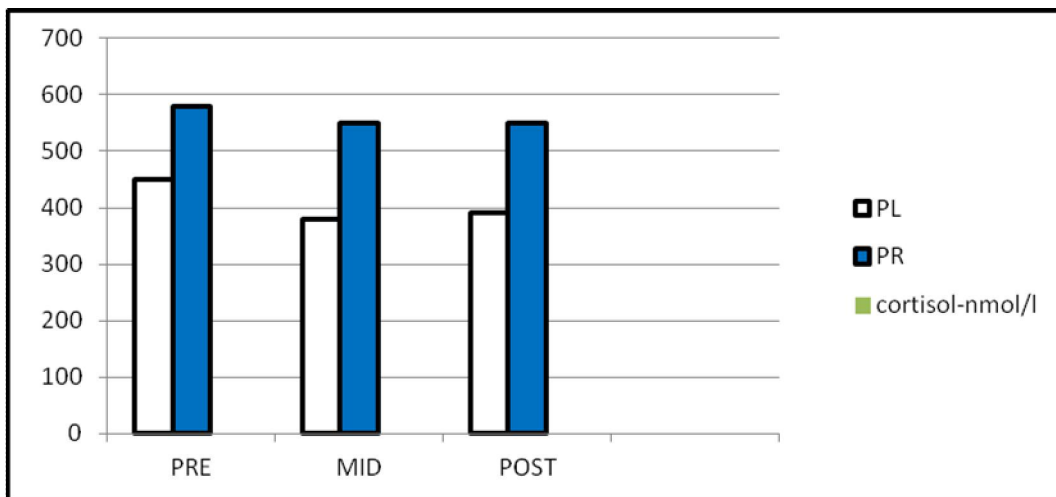


Figure 2. Resting cortisol concentrations (mean ± SD).

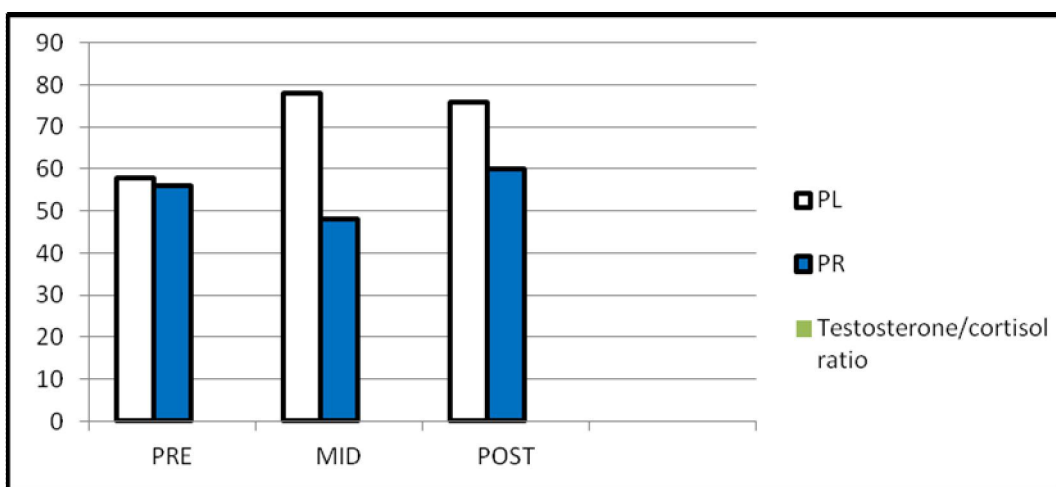
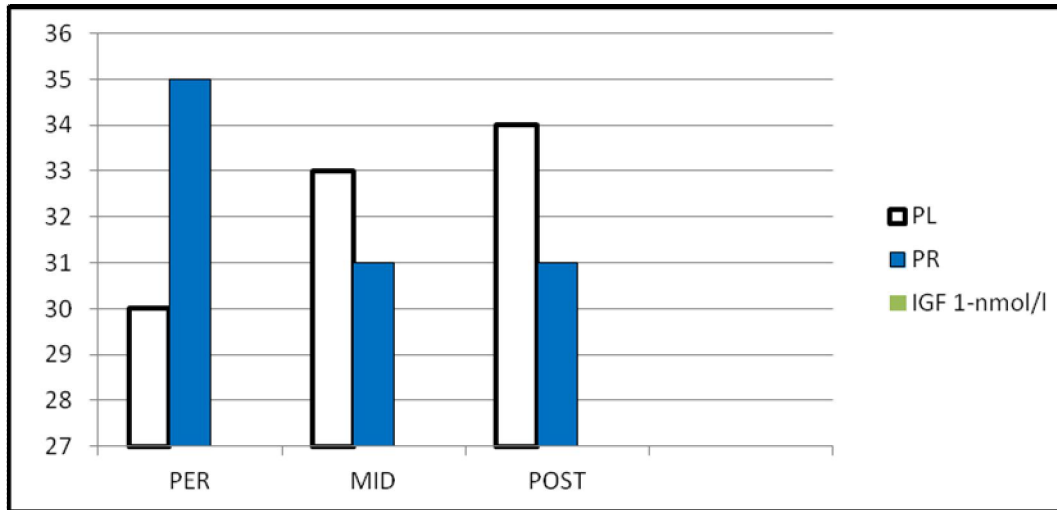
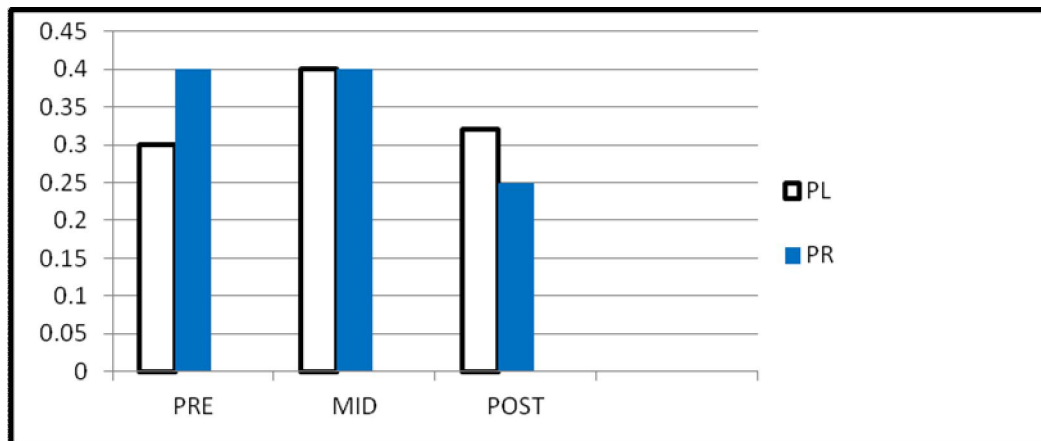


Figure 3. Resting testosterone/cortisol ratio (mean ± SD).



**Figure 4. Resting IGF-1 concentrations (mean  $\pm$  SD).**



**Figure 5. Resting Growth Hormone concentrations (mean  $\pm$  SD).**

However, a significant decrease from PRE was observed at MID for PL. In addition, cortisol concentrations at MID for PL were significantly lower than PR. No other between group differences was observed. The T/C ratio is shown in Figure 3. No significant change from PRE occurred in either PR or PL, nor were any between group differences observed at any time point. Resting IGF-I and growth hormone concentrations during the 12-week study are shown in Figures 4 and 5, respectively. No significant changes from PRE in either PR or PL were seen in the resting

concentrations of these hormones. In addition, no significant differences in the resting concentrations of these hormones were observed between the groups at any time point measured.

### Discussion

The results of this study indicate that protein supplementation in collegiate strength/power athletes may augment lower body strength development compared to a placebo. Changes in resting hormonal concentrations did not appear to support previous research that rotein supplementation may augment anabolic hormonal responses. The energy intakes

reported in this study are in agreement with previous investigations that suggest that collegiate athletes do not consume adequate quantities of macronutrients including meeting desired protein intakes (Cole et al., 2005; Hinton et al., 2004). However, when subjects are provided a protein supplement they do appear to meet or exceed the recommended rotein intake for strength/power athletes. Despite a greater protein intake by PR no significant differences in body mass, lean body mass or fat mass were seen between the groups. Although higher protein intakes were associated with a trend ( $p = 0.08$ ,  $ES = 0.78$ ) towards an increase in lean body mass, it is possible that the relatively low caloric intake by the subjects negatively impacted the ability to make significant gains in lean tissue accrument. Without consuming a sufficient caloric intake the ability of subjects to significantly increase body mass or lean body mass may be compromised. Previous studies have shown that the combination of resistance training with nutritional intervention results in significant increases in body mass (Roy et al., 2000, Rozenek et al., 2002). However, it has also been recommended that caloric intakes of strength/power athletes should exceed 44 - 50 kcal·kgBM· d-1 (A.D.A., 2000), and that the energy intakes of these athletes may exceed 5000 kcal per day (Short & Short., 1983). The energy intakes seen in this study were relatively low in comparison to what is recommended for strength/power athletes, possibly contributing in part to the inability to achieve significant increases in lean tissue accrument and body mass gains. Strength gains were seen in both groups for the 1-RM squat and 1-RM bench press exercises. However, the magnitude of strength improvement in the 1-RM squat was significantly greater in PR. This may be attributed to the higher ( $p = 0.09$ ,  $ES > 0.70$ ) average weekly training volume and intensity seen during the 12 week study for the squat exercise in PR compared to PL.

Interestingly, Kraemer et al. (1998) reported no differences in training volume or intensity in experienced resistance-trained men during several days of protein supplementation. However, they suggested that supplementation for a longer period of time may have resulted in more favorable outcomes. It is thought that protein supplementation can stimulate muscle protein synthesis to counteract the deleterious effects of muscle degradation seen following bouts of resistance exercise (Tipton et al., 2004). If protein degradation is reduced with a concomitant increase in protein accretion the resulting effect would generate a greater stimulus for muscle growth and enhanced recovery, potentially resulting in greater strength gains (Ratamess et al., 2003; Roy et al., 2000). Studies examining the effect of protein supplementation on strength enhancement are limited and results have been inconclusive. Although some investigators have shown augmented strength gains from protein supplementation (Bird et al., 2006), others have reported no effect (Chromiak et al., 2004; Rankin et al., 2004). This present study appears to be the first to examine the effects of protein supplementation on performance gains in experienced resistance-trained college strength/power athletes. Interestingly, the magnitude of improvements was not similar between the squat and bench press exercises in PR and no difference in  $\Delta$  1-RM bench press between PR and PL was seen. Previous research has demonstrated a greater potential for lower body strength improvement in collegiate strength/power athletes (Hoffman et al., 2006) and the results seen in this study likely reflect a greater window of adaptation that exists in these athletes for gains in lower body strength. Mechanisms underlying this greater window of adaptation may be related to a reduced experience in lower body compared to upper body strength training (Hoffman et al., 2006). No significant changes were seen during the 12 week training program in any of the



power performance measures for either group. Although protein supplementation has been shown to significantly enhance power performance (Andersen et al., 2005), others have shown no significant differences between subjects consuming a protein supplement compared to placebo (Chromiak et al., 2004). However, neither of those studies used experienced strength/power athletes. It is likely that the lack of specificity between the training program and exercises used to assess power performance in this study was the primary factor that negated any potential effects of the supplement on power assessments. An additional purpose of this study was to examine whether resting hormonal concentrations can be influenced by protein supplementation. A significantly lower cortisol concentration was seen at MID for PL compared to PR. These results contrast slightly with those found in other studies that demonstrated that resting cortisol concentrations tend to remain the same or decrease in subjects supplementing with protein (Roy et al., 2000; Bird et al., 2006). The results seen in this study may reflect the higher (6%) training volume in the squat exercise experienced by PR. This is supported by previous studies demonstrating elevations in training volume, despite higher daily protein intake, can result in significant elevations in resting cortisol concentrations (Volek et al., 1997). It is possible that the higher training volume may have impacted the results seen in this study as well. Previous research has shown that high protein diets are associated with low resting levels of testosterone (Andersen et al., 2005), while others have reported a negative relationship between the protein-to-carbohydrate ratio and resting testosterone concentrations (Volek et al., 1997). In this study 24% of the total energy consumed by PR was from protein, and only 15% of the total energy consumed by PL was from protein. Although the protein-to-carbohydrate ratio was lower than that reported by Anderson

and colleagues (1987) (44% of total energy from protein in high protein group versus 10% of total energy in low protein group), this difference likely contributed to the results observed in this study. A negative correlation ( $-0.64$ ,  $p < 0.05$ ) was observed between testosterone concentrations at MID and the protein content of the diet. This trend continued, but the correlation between testosterone concentrations at POST and protein content did not reach significance ( $r = -0.37$ ,  $p = 0.10$ ). This is similar to previous results reported by Volek and colleagues (1997). The data of this study appear to support the importance of macronutrient composition on resting testosterone homeostasis. No significant changes from PRE were seen in either resting growth hormone or IGF-I concentrations. Resting growth hormone concentrations appear to be responsive to amino acid supplementation (Bratusch-Marrain & Waldäusi., 1979), however others have reported no effect of protein supplementation on resting growth hormone or IGF-I concentrations (Roy et al., 2000). It does appear that changes in IGF-I concentrations are dependent upon energy intake, with caloric restriction being associated with decreases in IGF-I concentrations, while increases in caloric intake tends to elevate IGF-I (Forbes et al., 1989; Thissen et al., 1994).

### **Conclusion**

In conclusion, the results of this investigation confirm previous studies that have demonstrated that collegiate strength/power athletes may not meet daily recommended energy or protein needs. When athletes are provided a protein supplement they do appear to meet the recommended daily protein intake for strength/power athletes. Protein supplementation did appear to augment lower body strength development in experienced strength/power athletes. However, results of upper body strength, anaerobic power and lean tissue changes

do not provide clear evidence supporting the efficacy of a 12-week protein supplementation period in experienced resistance trained athletes. Further examination appears warranted on protein supplementation in athletes that are consuming a diet meeting recommended energy intakes for strength/power athletes.

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