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Section: Original Research

Article Title: Effects of High vs. Low Protein Intake on Body Composition and Maximal Strength in Aspiring Female Physique Athletes Engaging in an 8-Week Resistance Training Program

Authors: Bill I. Campbell¹, Danielle Aguilar¹, Laurin Conlin¹, Andres Vargas¹, Brad Jon Schoenfeld², Amey Corson¹, Chris Gai¹, Shiva Best¹, Elfego Galvan², and Kaylee Couvillion¹

Affiliations: ¹Physique and Performance Enhancement Laboratory, University of South Florida, Tampa, FL. ²Lehman College, Bronx, NY. ³University of Texas Medical Branch, Galveston, TX.

Running Head: Protein intake for female physique athletes

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Title Page

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Submission Type: Original Research

Authors: Bill I. Campbell1, Danielle Aguilar1, Laurin Conlin1, Andres Vargas1, Brad Jon Schoenfeld2, Amey Corson1, Chris Gai1, Shiva Best1, Elfego Galvan2, Kaylee Couvillion1.

Author Affiliations:

1Physique and Performance Enhancement Laboratory, University of South Florida, Tampa, FL 33620
2Lehman College, Bronx, NY 10468
3University of Texas Medical Branch, Galveston, TX 77555

Corresponding Author:
Bill I. Campbell, PhD
University of South Florida
PED 206
Tampa, FL, 33620
813-974-4766
email: bcampbell@usf.edu

Running Head: Protein Intake for Female Physique Athletes
Abstract:

Aspiring female physique athletes are often encouraged to ingest relatively high levels of dietary protein in conjunction with their resistance-training programs. However, there is little to no research investigating higher vs. lower protein intakes in this population. This study examined the influence of a high vs. low protein diet in conjunction with an 8-week resistance training program in this population. Seventeen females (21.2±2.1 years; 165.1±5.1 cm; 61±6.1 kg) were randomly assigned to a high protein diet (HP: 2.5g/kg/day; n=8) or a low protein diet (LP: 0.9g/kg/day, n=9) and were assessed for body composition and maximal strength prior to and after the 8-week protein intake and exercise intervention. Fat-free mass (FFM) increased significantly more in the HP group as compared to the LP group (p=0.009), going from 47.1 ± 4.5kg to 49.2 ± 5.4kg (+2.1kg) and from 48.1 ± 2.7kg to 48.7 ± 2 (+0.6kg) in the HP and LP groups, respectively. Fat mass significantly decreased over time in the HP group (14.1 ± 3.6kg to 13.0 ± 3.3kg; p<0.01) but no change was observed in the LP group (13.2 ± 3.7kg to 12.5 ± 3.0kg). While maximal strength significantly increased in both groups, there were no differences in strength improvements between the two groups. In aspiring female physique athletes, a higher protein diet is superior to a lower protein diet in terms of increasing FFM in conjunction with a resistance training program.

Keywords: sports nutrition, bodybuilding, hypertrophy
INTRODUCTION

Dietary protein is an essential component of the human diet. The constituent amino acids (AA) of dietary proteins are used to build body tissues, and thus protein consumption directly influences the accretion of muscle mass (Atherton and Smith, 2012). Acute nitrogen balance studies indicate that individuals involved in regimented resistance training require 1.6 to 1.8 g/kg/day to maximize anabolism – approximately double that of sedentary individuals (Lemon, 2000). More recently, research using the indicator amino acid oxidation technique showed these requirements may be as high as 2.2 g/kg/day in young male bodybuilders (Bandegan et al., 2017). Interestingly, there is some evidence that protein requirements may be attenuated in resistance-trained individuals. Moore et al. (2007) found that consumption of ~1.4 g/kg/day was adequate to maintain a positive nitrogen balance following 12 weeks of regimented resistance training, suggesting that the body becomes more efficient at using AAs for lean tissue synthesis with continued performance of resistive exercise.

Despite the compelling acute research showing increased protein needs with resistance training, there is a paucity of longitudinal studies investigating optimal daily protein intakes to maximize body composition. In a very short-term study, Lemon et al. (1992) found that protein intake of 1.35 versus 2.62 g/kg/day produced similar increases in lean body mass and thigh muscle cross sectional area in novice lifters following a 4-week intensive resistance training program. The higher protein condition slightly reduced body fat while the lower protein condition showed a small increase, but these changes were not statistically significant. Antonio et al. (2014) reported similar body composition changes in resistance trained men and women consuming 4.4 vs 1.8 g/kg/day over the course of an 8-week RT program. Follow-up work from the same lab showed that resistance-trained individuals lost more body fat with a protein intake of 3.4 versus 2.3
g/kg/day after 8 weeks RT; while lean mass increases were similar between groups (Antonio et al., 2015). The training programs in both of these studies were unsupervised, confounding the ability to draw causality.

Given the limited research on the topic, significant gaps in the literature remain to be addressed. In particular, a dearth of evidence exists on protein requirements for resistance-trained women. This is especially true of female physique athletes, such as those aspiring to compete in bikini and figure contests. Judging for these contests is based in part on a combination of muscle symmetry, shape and definition. Lower body fat levels and ample muscle mass are therefore requisites for success. Accordingly, progressive resistance training is an important component in preparation for competition. The purpose of this study was to investigate the effects of higher versus lower daily protein intakes on body composition changes in aspiring female physique athletes following a supervised daily undulating periodized resistance training program. We hypothesized that a higher daily protein consumption would result in greater improvements in fat-free mass.

**METHODS**

This study utilized a parallel groups, repeated measures design where participants were randomized to ingest either a high protein diet or a low protein diet in conjunction with a supervised resistance training program for 8-weeks. Participants visited the laboratory on two occasions, immediately prior to and after an 8-week supervised resistance-training program. Before each laboratory visit the participants were instructed to fast for 10-hours (an overnight fast) and refrain from physical activity for the previous 36 hours. The primary dependent variable (DV) measured before and after the 8-week resistance training program was body composition (fat-free
mass, fat mass, and body fat percentage). Secondary DVs included maximal strength (back squat and deadlift) and resting metabolic rate (RMR).

Participants

Healthy, young, aspiring female physique athletes volunteered to participate in the study. In order to qualify for participation into the study, all participants were required to have resistance trained for the previous three months or longer and needed to be able to deadlift 1.5x bodyweight. All participants gave written informed consent before enrollment in the study. The study was approved by the University of South Florida Institutional Review Board and is in compliance with the Declaration of Helsinki as revised in 1983. Figure 1 summarizes the participant study flow. There were no differences between groups at pre-training for any dependent variable.

Resting Metabolic Rate, Body Composition, and Maximal Strength

Upon entering the laboratory, participants urinated and then had their body weight measured on a physician beam scale (Health-O-Meter, Model 402KL, McCook, IL, USA). Next, RMR testing procedures were conducted in a manner as previously described (Campbell et al., 2016). Intra and inter-day test-retest correlation calculated for the device used in the present study were as follows: intra-day RMR Pearson correlation was $r = 0.96$ ($p < 0.01$) and the inter-day RMR Pearson correlation was $r = 0.90$ ($p < 0.01$). Intra-day RMR ICC was 0.981 and the inter-day RMR ICC was 0.946.

After RMR assessments were completed, body composition was assessed using the Body-Metrix™ BX-2000 A-mode ultrasound (IntelaMetrix, Livermore, CA) with a standard 2.5 MHz probe according to procedures as previously described (Colquhoun et al. 2017). All body
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Composition assessments were completed by the same technician whose calculated fat-free mass test-retest reliability was: ICC 0.99; SEM 0.37 kg; minimal difference 1.03 kg.

Maximal strength testing took place approximately 24 hours after the body composition assessment. After completing a body mass warm-up, participants followed the National Strength and Conditioning Association’s 1RM testing protocol (Sheppard and Triplett, 2016) for the back squat and deadlift. For both lifts, the same research personnel observed each maximal repetition attempt.

Dietary Intervention

In the week prior to initiating the resistance-training program, each participant met with a nutrition counselor to receive instructions on how to track their food intake using a smartphone app (MyFitness Pal®). After a three-day food tracking familiarization and baseline assessments were completed, participants were matched according to total fat mass and randomized to the high protein group (HP; n = 8) or the low protein group (LP; n = 9). Participants in the high protein group were instructed to ingest at least 2.4 grams of protein/kg body mass per day and participants in the low protein group were instructed to ingest no more than 1.2 grams of protein/kg body mass. While the participants were instructed to track all food intake, there were no restrictions or guidelines placed on dietary carbohydrate or fat intake during the study intervention for either group. Pre and post-workout protein intake was standardized throughout the study. Participants in the HP group consumed 25 grams of whey protein isolate (Dymatize ISO-100) immediately before and another 25 grams immediately after each resistance exercise bout in the presence of research personnel. Participants in the LP group consumed 5 grams of whey protein isolate (Dymatize ISO-100) immediately before and another 5 grams immediately after each resistance exercise bout in the presence of research personnel. Also, in addition to meeting with a nutrition coach at the
beginning of the study, each participant had access to their individual nutrition coach throughout the study duration to answer any nutrition questions related to selection of food choices and adhering to the assigned low or high protein diet.

Resistance and High Intensity Interval Training Program

After 1RM strength testing, participants began an 8-week resistance-training program. Twenty-eight workouts were scheduled to be completed over the 8-week period, with four workouts per week during weeks 1-3 and 5-7, and two workouts per week during the midpoint of the training program (week 4) and last week of the training program (week 8). The reduced training frequency during weeks 4 and 8 was a pre-planned reduction in volume and served as a taper. In order to maintain compliance with the training program, participants had to attend at least 85% of all scheduled supervised workouts.

The resistance-training program consisted of two upper-body focused days and two lower-body focused days per week. The lower-body workouts consisted of five exercises per session and required that each participant complete back squats, deadlifts, and hip thrusts and then choose from a list of other lower-body exercises to complete the required number of exercises for the workouts. Upper-body workouts consisted of six exercises per session and required that the participants complete barbell rows, overhead press, and assisted pull-ups and then choose from a list of other upper-body exercises to complete the required number of exercises for the workouts. The set and repetition ranges varied throughout the program, including five sets of 3-5 repetitions, four sets of 9-11 repetitions, and three sets of 14-16 repetitions. Participants self-selected the load that would allow them to complete the appropriate number of repetitions within the specified repetition ranges while allowing for approximately one additional repetition with good form. Each workout was supervised by two to three research assistants in the Performance and Physique
Enhancement Laboratory at the University of South Florida, equating to a supervisor:participant ratio of approximately 1:6.

The high intensity interval training program consisted of a progressive increase in the number of sets of 30-second, maximal intensity sprints. For the first two weeks of the intervention, participants engaged in four sets of 30-second high intensity interval exercise sets. The number of sets increased to five sets for the third and fourth weeks, to six sets for the fifth and sixth weeks, and to seven sets for the final two weeks of the intervention. Participants could choose their mode of exercise (treadmill, outdoor sprinting, cycle ergometer, rowing machine, etc.) and were instructed to rest two minutes between each set.

Statistics

Descriptive statistics (mean ± sd) for all DVs were calculated. The distribution of each body composition, strength, and resting metabolic rate measure was examined with the Shapiro-Wilk test (Razali and Wah, 2011; Shapiro and Wilk, 1965). Data for nutrition intake was analyzed via an independent samples t-test. Data for all other DVs was analyzed via a 2 group (high protein vs. moderate protein) × 2 time (pre- and post-training) between-within factorial ANOVA with repeated measures on the second factor. For each outcome, an effect size (ES) was calculated as the pretest-posttest change, divided by the pooled pretest SD. All analyses were completed using SPSS (Version 22, IBM. Armonk, NY) software and the alpha criterion for significance was set at 0.05.

RESULTS

In both protein intake groups for all dependent variables, the skewness and kurtosis coefficients were within a range of ±1.5 and a Shapiro-Wilk’s test (p > 0.05) and a visual inspection
of their histograms, normal Q-Q plots, and box plots showed that data were normally distributed. There were no differences in any dietary intake variable between the two groups at baseline. Macronutrient intake and diet composition is summarized in table 1. Body composition, maximal strength, and resting metabolic rate data are summarized in table 2 and figure 2. There were no differences between the two groups for upper body training volume, lower body training volume, or total body training volume.

DISCUSSION

To the authors’ knowledge, this is the first study to assess the effects of different levels of protein intake on body composition in resistance-trained women in conjunction with a supervised resistance training program. A primary and novel finding of our study is that a high protein diet (2.5 g/kg/day) significantly increased FFM compared to a lower protein diet (0.9 g/kg/day) in the cohort of aspiring female physique athletes. Our results are somewhat in contrast with those of Lemon et al. (1992), who found that novice male lifters realized similar resistance training-induced increases in lean mass with protein intakes of 1.35 versus 2.62 g/kg/day, although non-significantly greater changes were seen with the higher protein consumption. Given that the study lasted just 4 weeks, it is plausible that differences might have reached significance with a longer study period, as with the 8-week duration employed in our protocol. In addition, the lower protein intake of 1.35 g/kg/day used in this comparison study (Lemon et al., 1992) was still greater than the 0.9 g/kg/day ingested in our investigation. This level of protein may have been enough protein to elicit a positive change in fat-free mass.

A recent large-scale meta-analysis encompassing 49 studies with 1,863 participants found that protein supplementation in conjunction with prolonged resistance training significantly increased measures of muscle hypertrophy; however, beneficial effects reached a threshold when
total protein intakes exceeded ~1.6 g/kg/day (Morton et al. 2017). Therefore, our findings of greater increases in lean mass with the higher protein condition may be due to suboptimal protein intake in those consuming lower daily amounts of protein. Recent evidence shows when resistance-trained individuals consume at least 2 grams of protein/kg body mass during periods of unsupervised resistance training, additional intake does not enhance lean mass gains (Antonio et al., 2014; Antonio et al., 2015).

Another important finding from the study was the high protein group lost a significant amount of fat mass whereas reductions in the low-protein group were not statistically significant. These results held true despite the fact that those the higher protein group ingested significantly more kilocalories (approximately 400 kcals) in the form of protein. These results are consistent with those of Antonio et al. (2015), who reported a loss of 2.4% body fat with consumption of 3.4 g/kg/day versus only a 0.7% decrease when consuming 2.3 g/kg/day. Considering that weight loss is a function of energy balance (Thomas et al., 2009), these findings may seem counterintuitive. However, dietary protein has been shown to have a much higher thermic effect (25-30% of total calories) compared to less than 10% for carbohydrate or lipid (Halton and Hu, 2004). Thus, a substantial portion of protein calories consumed are lost as heat. Moreover, increases in non-exercise activity thermogenesis also have been observed following overfeeding (Levine et al., 1999), and it is conceivable that higher protein intakes may enhance this effect. Therefore, differences in fat loss may be explained by a greater portion of energy from the additional protein to be used for lean tissue building as opposed to adipose storage, as well an ability for higher levels of protein intake to positively influence the energy expenditure side of the energy balance equation.
Both training groups experienced a body recomposition (the gaining of fat-free mass while simultaneously losing fat mass). While this finding has been repeatedly observed in overweight and obese populations undergoing a resistance training program (Longland et al., 2016; Josse et al. 2011), this outcome is not typically observed in well-trained individuals that are not classified as overweight/obese. Garthe and coworkers (2011) recruited elite male and female athletes to follow a slow vs. fast weight loss program in which both groups ingested approximately 1.5 grams of protein/kg body mass and resistance trained four days per week. Athletes in the slower weight loss group realized significant increases in lean body mass (~1kg) and significant reductions in fat mass (~4.9kg) during the 8.5-week study intervention. When taken together, the Garthe study (2011) and the present investigation indicate that body recomposition is possible during both a hypocaloric and hypercaloric state provided that a structured exercise and nutritional protocol is followed.

Both protein intake groups experienced significant increases in maximal strength. However, the influence of higher protein intake did not improve maximal strength in comparison to the lower protein intake. This finding was consistent with the data reported by Josse and coworkers (2010) in which non-resistance trained females did not increase lower-body strength when additional protein was ingested (in the form of milk) in conjunction with a 12-week resistance training program. The finding was also consistent with data reported by Hida et al. (2012) in which collegiate female athletes did not increase maximal strength when additional protein was ingested (in the form of egg white protein) in conjunction with an 8-week training regimen. Also, non-resistance trained overweight females increasing protein intake (in the form of yogurt supplementation) did not improve maximal strength as compared to a lower protein ingestion group (Thomas et al., 2011). Despite the difference in FFM between the two protein
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intake groups, there was no associated difference in resting metabolic rate. While males tend to realize an increase in resting metabolic rate with an increase in resistance-training induced fat-free mass, females are void of such elevations in resting metabolic rate (Lemmer et al., 2001; Bonganha et al. 2011). The present study reinforces this observation.

Although our study had several notable strengths (supervised workouts, high adherence to daily food tracking, and personalized nutritional counseling), there nevertheless are some limitations that must be considered when drawing practical inferences. For one, there was a large discrepancy between the two protein intakes investigated. Prudent follow-up research would compare protein intakes of approximately 1.6 to 1.8 g/kg/day (a likely zone of optimality) to a super-optimal daily intake of approximately 2.4 g/kg/day as was investigated in the present study. Also, we did not attempt to control for the subjects’ menstrual cycles when testing. This may have influenced body water and thus assessment of lean mass (Stachoń, 2016). While it can be speculated that random distribution in the cohort would render any observed differences small, we nevertheless cannot rule out confounding effects on body composition. Another potential confounding issue was that the high protein group consumed 25 g of whey protein immediately before and after the workouts while the low protein group consumed only 5 grams during these periods. It has been suggested that there is an “anabolic window of opportunity” whereby pre- and post-workout protein consumption heightens the accretion of muscle proteins, and that intake of at least 20 grams of high quality protein is needed to maximize this response (Ivy and Ferguson-Stegall, 2014; Macnaughton et al., 2016). This raises the possibility that the timing of consumption may have been at least partly attributable to results. However, recent meta-analytic data indicates that total protein intake, not precise peri-workout timing, is the determining factor in exercise-induced muscular adaptations (Schoenfeld et al., 2013). Further, given that the intent of the
methodological design was to implement dietary changes to only protein (while keeping dietary carbohydrate and fat consistent between the two groups), this also resulted in a difference in total caloric intake between the two groups, with the high protein group ingesting significantly more kcals per day as compared to the low protein group. It is possible that the changes in lean body mass observed in both groups were, at least in part, due to the elevated caloric intake in the high protein group and the decreased caloric intake in the low protein group during the 8-week dietary intervention. Finally, our findings are specific to young, resistance-trained women; results cannot necessarily be extrapolated to other populations.

**SUMMARY AND PRACTICAL APPLICATIONS**

This is the first study to demonstrate that an under-represented population (female physique athletes) engaging in resistance training benefit from higher protein intakes. Specifically, the findings suggest that higher protein intakes are advisable for these types of athletes seeking to optimize body composition. As there is a large discrepancy between 0.9g/kg/day and 2.5g/kg/day, additional research is required to determine the necessity of intakes as high as 2.5g/kg/day in order to achieve the body recompositiion observed in the present study.

**Acknowledgement, authorships, declarations:**

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References


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Figure 1. CONSORT Participant Flow
Figure 2. Individual Fat-Free Mass Responses
**Table 1. Macronutrient Intake at Baseline and During the 8-Week Dietary Intervention**

<table>
<thead>
<tr>
<th></th>
<th>High Protein (n=8)</th>
<th>Low Protein (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8-Week Average</td>
</tr>
<tr>
<td>Kcals</td>
<td>1,588 ± 438</td>
<td>1,839 ± 316*</td>
</tr>
<tr>
<td>CHO (grams)</td>
<td>157 ± 61</td>
<td>156 ± 47</td>
</tr>
<tr>
<td>PRO (grams)</td>
<td>89 ± 23</td>
<td>157 ± 18*</td>
</tr>
<tr>
<td>Fat (grams)</td>
<td>67 ± 24</td>
<td>65 ± 21</td>
</tr>
<tr>
<td>Kcal/kg body mass</td>
<td>27 ± 10</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>CHO (g/kg/day)</td>
<td>2.7 ± 1.3</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>PRO (g/kg/day)</td>
<td>1.5 ± 0.5</td>
<td>2.5 ± 0.2*</td>
</tr>
<tr>
<td>Fat (g/kg/day)</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>CHO/PRO/Fat (%)</td>
<td>40-22-38</td>
<td>34-34-32</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; PRO = protein; g/kg/day = grams/kilogram body mass/day.

Significant difference (independent samples t-test); * = p < 0.001; # = p < 0.05
Table 2. Body Composition, Maximal Strength, and Resting Metabolic Rate (mean ± sd)

<table>
<thead>
<tr>
<th></th>
<th>High Protein (n=8)</th>
<th></th>
<th></th>
<th>Low Protein (n=9)</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
<td>Cohen’s d</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>61.2 ± 7.9</td>
<td>62.2 ± 8.2</td>
<td>+1.0</td>
<td>0.12</td>
<td>61.4 ± 4.4</td>
<td>61.2 ± 4.6</td>
</tr>
<tr>
<td>Fat-Free Mass (kg)</td>
<td>47.1 ± 4.5</td>
<td>49.2 ± 5.4</td>
<td>^+2.1</td>
<td>0.42</td>
<td>48.1 ± 2.7</td>
<td>48.7 ± 2.0^</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>14.1 ± 3.6</td>
<td>13.0 ± 3.3^</td>
<td>-1.1</td>
<td>0.32</td>
<td>13.3 ± 3.7</td>
<td>12.5 ± 3.0</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>22.7 ± 3.0</td>
<td>20.7 ± 3.1^</td>
<td>-2.0</td>
<td>0.66</td>
<td>21.4 ± 5.2</td>
<td>20.3 ± 3.9</td>
</tr>
<tr>
<td>1RM Squat (kg)</td>
<td>69.3 ± 18.4</td>
<td>78.7 ± 16.0^</td>
<td>+9.4</td>
<td>0.55</td>
<td>72.0 ± 15.1</td>
<td>81.8 ± 20.1^</td>
</tr>
<tr>
<td>1RM Deadlift (kg)</td>
<td>86.9 ± 14.8</td>
<td>102.8 ± 18.5^</td>
<td>+15.9</td>
<td>0.95</td>
<td>97.2 ± 16.7</td>
<td>111.4 ± 17.6^</td>
</tr>
<tr>
<td>RMR (kcals/day)</td>
<td>1,466 ± 152</td>
<td>1,446 ± 151^</td>
<td>-20</td>
<td>0.13</td>
<td>1,451 ± 104</td>
<td>1,510 ± 196</td>
</tr>
</tbody>
</table>

Note. 1RM = one-repetition-maximum; RMR = resting metabolic rate

*p < 0.05 significantly different from pre; ^p < 0.01 significantly different from pre; ^p = 0.009 group x time interaction