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Section: Original Research

Article Title: Protein Supplementation During a 6-Month Concurrent Training Program: Effect on Body Composition and Muscular Strength in Sedentary Individuals

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Running Head: Protein supplementation in sedentary individuals beginning exercise programs

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Protein supplementation during a 6-month concurrent training program: Effect on body composition and muscular strength in sedentary individuals

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Running Head: Protein Supplementation in Sedentary Individuals Beginning Concurrent Training

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ABSTRACT

We examined the effect of a protein supplement on muscular strength and body composition during 6 months of a 5d/wk concurrent strength and endurance training program. Sedentary males (n=26) and females (n=25), 18-25yrs, were randomly assigned to receive a protein (PRO, 42g/serving) or carbohydrate (CON) supplement twice daily. Strength and body composition (DXA) were assessed at baseline, 3(3M) and 6(6M) months. Protein intake was higher in PRO [PRO:2.2g/kg; CON:1.1g/kg, P<0.001]. Females in both groups gained similar strength at 3M and 6M in bench press (BP) and hip sled (HS). Males in PRO gained more BP strength at 3M (PRO:24.6±3.2kg; CON:14.3±3.8kg; p=0.06) and 6M (PRO:34.4±4.3kg; CON:18.7±5.1kg; p=0.03) and HS strength 3M (PRO:67.7±9.2kg; CON:40.8±10.8kg, p=0.07) and 6M (PRO:94.0±10.6kg; CON:65.1±12.4kg, p=0.09) compared to CON. Females in PRO experienced a greater reduction in fat mass over the course of the study (6M) than CON, (PRO:-1.7±0.5kg; CON:0.1±0.5 kg; p=0.06). Changes in lean mass were similar for females in PRO and CON. Loss in fat mass was similar for males in PRO and CON at 3M and 6M. Males in PRO gained more lean mass at 3M compared to CON (PRO:3.2±0.3kg; CON:2.2±0.4kg; p=0.1) but similar gains at 6M (PRO:2.6±0.4kg; CON:2.2±0.5kg; p=0.6). The results of this study demonstrate that PRO used during a concurrent training program may augment positive changes in body composition in young sedentary males and females, and strength gains in males.

Key words: protein intake, gender differences, body composition, body fat, fat-free mass, resistance training, running.

INTRODUCTION

An acute bout of resistance exercise has been reported to result in increased muscle protein synthesis (Biolo, Maggi, Williams, Tipton, & Wolfe, 1995; Biolo, Tipton, Klein, & Wolfe, 1997; Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 1992; Phillips & Van Loon, 2011). When protein or amino acids are consumed following resistance exercise, protein synthesis is increased to a greater degree than when protein is not consumed (Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000; Tarnopolsky et al., 1997). Furthermore, daily protein intake up to 2.5 g/kg/day during prolonged resistance training programs results in greater gains in fat-free mass and muscle strength compared to protein intakes of <1.3 g/kg/day (Cermak, Res, de Groot, Saris, & van Loon, 2012; Morton et al., 2017). When individuals who are unaccustomed to resistance exercise begin a new training program, the influence of protein supplementation may augment their physiological responses, especially over the short-term (Gontzea, Sutzescu, & Dumitrache, 1975; Phillips, Tipton, Ferrando, & Wolfe, 1999; Rennie & Tipton, 2000). In contrast, a recent meta-analysis by Morton et al. (Morton et al., 2017) indicates that protein supplementation is more effective in resistance trained compared to sedentary individuals.

An acute bout of endurance exercise also affects protein metabolism, primarily through an increase in nitrogen excretion and amino acid oxidation (Dohm et al., 1977; Kato, Suzuki, Bannai, & Moore, 2016; Lemon, Dolny, & Yarasheski, 1997; Meredith, Zackin, Frontera, & Evans, 1989). It has been suggested that the increase in amino acid oxidation associated with endurance exercise results in a greater dietary protein requirement of ~16-25% above the recommended 0.8 g/kg/day (Lemon et al., 1997). Based on nitrogen balance data a protein requirement of 0.9 to 1.4 g/kg/d has been suggested for those who are habituated to endurance exercise (Kato et al., 2016; Meredith et al., 1989; Tarnopolsky, MacDougall, & Atkinson, 1988). Unfortunately, there are limited studies that have investigated the impact of higher protein intakes on adaptations to prolonged endurance training (Ferguson-Stegall et al., 2011).

Many individuals involved in habitual exercise combine strength and endurance training (concurrent training). Furthermore, many have opted to supplement their diets with protein, despite the lack of research investigating the impact of increased protein intake during a combined strength and endurance training program. It is assumed that the long-term effect of protein supplementation during a concurrent exercise training program will produce a greater adaptive response to the training stimulus, resulting in greater gains in muscle mass and strength and aerobic capacity.

Thus, the purpose of this paper was to investigate changes in strength, body composition, and peak oxygen consumption (VO_2peak) during a six-month training program that had equal days of strength and endurance training in which subjects were randomized to either a protein supplement (84 g/day) or a carbohydrate supplement of equal caloric value. We hypothesized that the individuals in the protein supplement group would gain more muscle mass and increase strength to a greater degree than individuals in the control group. Because the training program included equal days of aerobic exercise we also investigated the impact of protein supplementation on peak oxygen consumption (VO_2peak). We hypothesized that individuals in the protein supplement group would experience enhanced aerobic adaptions compared to individuals in the control group. This paper is part of a larger study that investigated the effects of protein supplementation on serum insulin-like growth factor-I, insulin-like growth factor binding protein-3, and markers of bone turnover during a concurrent training program, the results of which are presented elsewhere (Ballard, Clapper, Specker, Binkley, & Vukovich, 2005; Ballard, Specker, Binkley, & Vukovich, 2006).

METHODS

Subjects. A pre-participation and health history questionnaire were used to screen individuals for inclusion in the study. Individuals who were participating in an exercise program greater than 2d/wk, had a history of medication use or diseases that may affect bone or calcium metabolism (diabetes, liver or kidney disease, thyroid or parathyroid disease, or gastrointestinal disorders, oral contraceptives) were excluded. Sixty-two individuals [33 males (21 ± 0.5 years); 29 females (21 ± 0.5 years) were enrolled to participate. The protocol was approved by the Human Subjects Committee at South Dakota State University and the Human Subjects Review Board of the U.S. Army Medical Research and Material Command.

Experimental Design. Baseline testing included a 3-day dietary record, fasting blood and urine samples, body composition via dual energy x-ray absorptiometry (DXA), a graded treadmill test to measure peak aerobic capacity (VO_2peak), and a one-repetition maximum (1-RM) test to measure dynamic strength. Following baseline measurements, study participants were matched for strength and randomly assigned to receive and consume a protein supplement (PRO) or a carbohydrate control (CON). Exercise training sessions were conducted five days per week for 6 months. Exercise training sessions were scheduled in the morning (0600 – 0900) and evening (1600-1900). Follow-up testing occurred at 3-months (3M) and 6-months (6M). Incentives (t-shirts, caps, and workout towels) were provided at testing sessions to encourage compliance.

Exercise Testing and Training Protocol. The 1-repetition maximum (1-RM) was defined as the maximal resistance that the individual could move through the full range of motion one time. To ensure familiarization and avoid muscle damage and soreness, the 1-RM was determined after 2 weeks of light lifting for all exercise. Peak oxygen consumption (VO_2peak) and maximal heart rate were measured during a graded treadmill exercise test. The VO_2peak test was completed using a Physio-Dyne Max-1 metabolic cart (Quogue, NY). The test protocol was modified for each individual by allowing them, with help of a graduate research assistants, to determine a comfortable but challenging running speed for the test. The speed of the treadmill remained constant as the grade increased 2% every 2 minutes until volitional exhaustion. The 1-RM and VO_2peak tests were repeated at 3M and 6M and performed at the same time of day for each individual.

Individuals participated in a 5-day per week concurrent training program. Strength training and running were performed on alternate days so that 3-days of strength training and two days of running were performed one week, while the next week consisted of 3-days of running and two days of strength training. Makeup sessions were held on Saturdays for those individuals who missed a session. When more than three sessions were missed without a make-up session, the individual was withdrawn from the study.

Strength training consisted of 2 sets of 10 repetitions and a third set to failure or fatigue all at 70% of their 1-RM. Subjects were instructed to rest 90 seconds between sets. When the individual reached 10 repetitions during the third set, weight was added in 2.5 or 5.0 kg increments depending on the exercise. A new 1-RM was established every four weeks. The following exercises were performed in order: bench press, latissimus pull down (wide grip), hip sled, incline bench press, cable rows, squat, shoulder press, latissimus pull down (narrow grip), calf raises, arm curls, and arm extension. All exercises were performed on Magnum Fitness Systems (South Milwaukee, WI) plate loaded machines. Graduate research assistants supervised each exercise session.

Endurance training consisted of running primarily outside or on an indoor track at 70% of maximum heart rate (HRmax). During each running session, the participant used a Polar heart rate monitor and watch (Polar USA, Lake Success, NY) which was programmed for the individual's heart rate range (65-80% max heart rate). The watch emitted a tone when the individual was outside of their zone. During the initial 3-weeks of the training, a progression was used to increase the duration of running beginning at 25 minutes and increasing by 10 minutes each week until the individuals could run continuously for 45 minutes.

Diet and Dietary Supplements. All individuals were asked to maintain their normal dietary intake during the study. Three-day food records were collected at baseline (before training), and at three and six months. Records were analyzed with Food Processor (v. 8.1, ESHA Research, Salem OR) by the same investigator every time. The protein supplement (Myoplex, EAS, Inc.) contained 280 kcals, 42g protein, 21g carbohydrate, and 1.5g fat per serving, as well as a blend of vitamins and minerals. The control contained a calorically equivalent amount of carbohydrates (70g sucrose and fructose) and an equivalent vitamin and mineral blend. Both the protein and control groups consumed their supplements twice per day, one immediately after the participant's workout, the other dose 8-12 hours later. Supplements were also consumed on non-training days, one serving in the morning and one serving in the evening. Participants were asked to return empty packets for compliance.

Body composition. Body composition was determined by a total body DXA scan (version 12.01; Hologic GDR 4500A, Bedford, MA). Scans were performed following an over-night fast after participants voided. Subjects were provided a t-shirt and hospital pants to wear during the scan. All scans were analyzed by one ISCD-certified technician. Coefficients of variation for total body lean and fat mass and total body percent fat are 0.4%, 1.5%, 1.3%, respectively.

Calculations and statistics. A three-way repeated measures analysis of variance was used to determine the effect of treatment (CON, PRO) over time (0, 3M, and 6M) among men and women (JMP v.12, SAS Institute, Inc). When a significant F-ratio was obtained a Tukey post-hoc test was used to locate significant differences. A two-tailed t-test was used to determine if the change from baseline to 3M and 6M was significantly different from zero. Furthermore, an analysis of covariance controlling for baseline values was used to determine if the change from baseline to 3M and 6M was different between treatments. Effect sizes (ES) were calculated using Cohen's d. Values presented are means ±SE unless otherwise noted.

RESULTS

Subjects and compliance. Mean ages at baseline were similar between those who were supplemented with protein (21.0 ± 0.6 [n=29]) or control (20.3 ± 0.5 years [n=22]), as well as between males (20.5 ± 0.5 [n=26]) and females (21.5 ± 0.6 years [n=25]). Height was not different between treatments but, as expected the males (PRO: 178.5 ± 1.32 cm, CON: 183.3 ± 1.8 cm) were taller than the females (PRO: 167.8 ± 1.6 cm CON: 169.8 ± 1.82 cm). Fifty-one individuals (26 male; 25 female) completed the study with strength and body composition data available at all three time points. The individuals who did not complete the study (n=11) either withdrew on their own or were withdrawn due to non-compliance with the exercise protocol. Non-compliance was defined as missing more than three exercise sessions throughout the entirety of the study. Supplement compliance was measured by having subjects return empty packets. Non-compliance was also defined as not consuming more than three supplements. We did not need to eliminate any participants due to non-compliance of only supplements. Based on the return of empty packets, subjects consumed all servings.

Dietary intake. Total energy consumption was not different between groups over time (Table 1). When supplement intake was included, there were no differences in energy intake; however, protein intake was greater ($p<0.001$) in protein group and carbohydrate intake was greater ($p<0.01$) in the control group for both males and females. When calculated on a per kilogram basis, the protein group consumed significantly more protein (PRO: ~2.2 g/kg/day; CON: ~1.0 g/kg/day, $p<0.001$) for both males and females.

Peak oxygen consumption. The concurrent training program resulted in increases in peak oxygen consumption (VO_2peak) (Table 2). Females in the protein group had a higher ($P=0.06$) VO_2peak at baseline and the difference remained throughout the study. Both groups increased similarly, ~6-7% during the first 3 months and another 2-3% during the last 3 months, or ~9% during the six-month study period. Men and women in both groups responded similarly. There was no effect of protein supplementation on the change in VO_2peak.

Muscular strength. The concurrent training program lead to gains in muscular strength (Figure 1). At 3M PRO and CON gained similar amounts of strength in the bench press (PRO: 18.4 ± 2.4 kg; CON: 14.7 ± 2.7 kg; ES=0.2) and hip sled (PRO: 52.3 ± 6.8 kg; CON: 50.1± 7.8 kg;

ES=0.1). However, at 6M, the protein-supplemented group experienced a greater gain in strength compared to the control group in the bench press (PRO: 27.4 ± 2.4 kg; CON: 15.9 ± 2.8 kg; p=0.003; ES=0.8), but not the hip sled (PRO: 72.3 ± 7.8 kg; CON: 73.6 ± 9.0 kg).

At 3M, females in both groups had similar strength gains for the bench press (PRO: 12.3 ± 3.4 kg; CON: 14.5 ± 4.2 kg) and hip sled (PRO: 45.6 ± 9.5 kg; CON:51.2 ± 10.2 kg). At 6M the women in PRO gained a slight advantage in strength in the bench press (PRO: 19.0 ± 1.7 kg; CON: 14.3 ± 1.9 kg; p=0.1) but not in the hip sled (PRO: 59.6 ± 9.8 kg; CON: 74.6 ± 11.1 kg; p=0.3) compared to the CON group.

Males in the protein group had greater gains in the bench press at 3M (PRO: 24.6 ± 3.2 kg; CON:14.3 ± 3.8 kg; p=0.06, ES=0.9) and 6M (PRO: 34.4 ± 4.3 kg; CON: 18.7 ± 5.1 kg; p=0.03; ES=1.0) compared to control group. Males in PRO gained more strength in the hip sled at 3M (PRO: 67.7 ± 9.2 kg; CON: 40.8 ± 10.8 kg, p=0.07; ES=0.54) and 6M (PRO: 94.0 ± 10.6 kg; CON: 65.1 ± 12.4 kg, p=0.09; ES=0.41) compared to CON. Males in the PRO group gained more BP strength at 3M and 6M than females in PRO and CON. There was no difference in the amount of BP strength gained between males in the CON group compared to females in both groups at 3M and 6M. Despite males gaining 30% to 40% more strength in the hip sled, the only difference was between males in the PRO group gaining more strength than females in the PRO group.

Body composition. Despite randomization, PRO weighed less (p<0.05) and had less body fat (p<0.05) compared to CON at baseline (Table 3). Total body lean mass was not different between PRO and CON groups at baseline. There were significant changes in body composition over the study period. Both groups experienced an increase in lean mass (3M, PRO: 2.6 ± 0.2 kg; CON: 1.7 ± 0.3 kg; ES=0.53; 6M, PRO: 2.4 ± 0.3 kg; CON: 1.9 ± 0.3 kg; ES= 0.33) and a reduction in body fat (3M, PRO: -0.6 ± 0.2 kg; CON: -0.4 ± 0.4 kg; ES=-0.08; 6M, PRO: -1.0 ± 0.3 kg; CON: -0.3 ± 0.4 kg; ES=-0.19) (Figure 2A). However, individuals in PRO experienced a greater gain in lean mass compared to CON during the first three months only (Figure 2A). At

3M and 6M, the change in weight, fat mass and lean mass, was significantly different from zero for PRO, but only the change in lean mass was significantly different from zero for CON.

Females (Figure 2B) in PRO experienced a trend toward a greater reduction in body fat compared to CON from baseline to 3M (PRO: -1.0 ± 0.3 kg; CON: -0.2 ± 0.3 kg, P=0.1; ES=-0.50). From baseline to 6M PRO lost more fat than CON (PRO: -1.7 ± 0.5kg; CON: 0.1 ± 0.5 kg; p=0.06; ES=-0.36), but there was no difference in the amount of lean mass gained between groups at 3 or 6-months (3M: PRO: 1.6 ± 0.2kg; CON: 1.3 ± 0.3kg; ES=0.19; 6M: PRO: 1.9 ± 0.3kg; CON: 1.8 ± 0.3kg; ES=0.15). At 3M and 6M, the change in fat mass and lean mass was significantly different from zero for females in PRO, but only the change in lean mass was significantly different from zero for females in CON. Despite females in the PRO group losing 40% to 85% more fat mass than men in the PRO and CON groups there was no difference among the groups at 3M or 6M.

There were no differences between PRO and CON in the amount of fat mass lost among males at either 3M (PRO: -0.2 ± 0.3kg; CON: -0.5 ± 0.4 kg; ES=0.04) or 6M (PRO: -0.5 ± 0.2kg; CON: -0.4 ± 0.6 kg; ES=-0.10). Males (Figure 2C) in PRO experienced a non-significant 1-kg greater gain in lean mass different during the first 3 months compared to CON (PRO: 3.2 ± 0.3kg; CON: 2.2 ± 0.4 kg; p=0.1; ES=0.72) but similar gains at 6M (PRO: 2.6 ± 0.4 kg; CON:2.2 ± 0.5 kg; p=0.6; ES=0.38). At 3M and 6M the change in lean mass was significantly different from zero for men in both the PRO and CON groups. While the males gained 15% to 30% more lean mass than the females there was no difference among groups.

DISCUSSION

Previous studies have reported greater gains in lean mass and strength during resistance training programs when subjects consumed higher protein intakes (1.4 to 2.5 g/kg/day) (Arciero et al., 2006; Cermak et al., 2012; Chromiak et al., 2004; Longland, Oikawa, Mitchell, Devries, & Phillips, 2016; Morton et al., 2017; Rozenek, Ward, Long, & Garhammer, 2002). The greater gains in lean mass with protein supplementation during resistance training programs appears to be similar for young and old, as well as younger untrained and trained subjects (Cermak et al., 2012). However, a more recent meta-analysis indicates that protein supplementation during resistance training is more effective at improving lean mass in younger subjects or individuals who are resistance-trained (Morton et al., 2017).

The present study incorporated a concurrent strength and endurance training program, alternating days of strength and endurance, to investigate the impact of increased protein intake on exercise training adaptations in previously untrained individuals. We hypothesized that individuals in the protein group would gain more lean mass and increase strength and VO_2peak to a greater degree than individuals in the control group. These hypotheses are based upon two assertions: 1) that individuals who participate in strength or endurance training on a regular basis have higher protein requirements than non-exercisers (Kato et al., 2016; Lemon et al., 1997; Lemon, Tarnopolsky, MacDougall, & Atkinson, 1992; Meredith et al., 1989; Tarnopolsky et al., 1992, 1988); and 2) immediate post-exercise consumption of protein results in greater adaptations to strength training, including muscle hypertrophy, due to an increased net muscle protein balance from the combined exercise and nutritional stimuli (Borsheim, Tipton, Wolf, & Wolfe, 2002; Phillips & Van Loon, 2011; Rasmussen et al., 2000; Tarnopolsky et al., 1997; Thomas, Erdman, & Burke, 2016; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999).

Previous studies have reported either no benefit or greater gains in lean mass and strength during strength training programs when subjects consumed higher protein intakes (1.4 to 2.5 g/kg/day) (Antonio, Peacock, Ellerbroek, Fromhoff, & Silver, 2014; Cermak et al., 2012; Chromiak et al., 2004; Consolazio, Johnson, Nelson, Dramise, & Skala, 1975; Morton et al., 2017; Reidy et al., 2016; Rozenek et al., 2002). These studies have focused primarily on strength training programs and limited research is available investigating the effect of protein supplementation on adaptations from endurance training. Ferguson-Stegall (Ferguson-Stegall et al., 2011) provided chocolate milk (~20g/d of protein) following 60 min/d of cycling for 4.5 weeks. Subjects consuming chocolate milk experienced improvements in body composition and aerobic power compared to a carbohydrate only group and placebo group.

Our data show significant increases in lean mass and decreases in fat mass during the first three months of the training program for the combined (males and females) protein group but not the combined control group. There were no differences in lean or fat mass between the groups at six months. Interestingly, when the body composition data are stratified by sex, there appears to be sex-specific responses to higher dietary protein consumption. Females in the protein group experienced a greater reduction in body fat compared to the females in the control group at both 3- and 6-months, while the change in lean mass was similar between the two groups. Additionally, protein supplementation did not appear to augment gains in strength for women, the gains were similar at three and six months for both groups. Similar results were reported by Josse et al. (Josse, Tang, Tarnopolsky, & Phillips, 2010). Untrained women consumed fat-free milk (36g protein) following resistance exercise. During 12-weeks of strength training, women in the milk group experienced a greater loss in body fat (-1.6kg) compared to the control group (-0.3kg). The milk group gained significantly (~0.8 kg) more FFM during that time and gained more strength in one (bench press) of eight exercises compared to the control group (Josse et al., 2010). Campbell et al. (Campbell et al., 2018) reported trained women who consumed a high protein diet (2.4 g/kg/d) experienced a greater gain in FFM (+2.1kg) and greater loss in fat mass (-1.1kg) compared to women who consumed a low protein diet (0.9 g/kg/d) (FFM, 0.6kg; FM, -0.8kg). Despite the high protein diet group gaining more FFM, the gains in strength (squat and deadlift) were identical between the high and low protein diets (Campbell et al., 2018).

In contrast, in the present study, males in the protein group experienced a greater gain in lean mass compared to men in the control group at 3-months but not at 6-months, while the change in body fat was similar between groups over the course of the study for males. Gains in strength for the bench press and hip sled at 3- and 6-months were greater for males in the protein group, with the greatest amount of gain occurring in the first 3-months. Interestingly, the gains in total body lean mass did not continue past three months for men in either group, yet men in the protein group continued to gain strength, representing an increase in force generated per unit cross-sectional area of muscle. However, the results reported are for the total body lean mass for the entire body. Using MRI, Abe et al. (Abe, Kojima, Kearns, Yohena, & Fukuda, 2003) demonstrated that skeletal muscle mass increased more than total body fat free mass during a 3d/wk, 16-week strength training program, resulting in a non-uniform change in muscle hypertrophy. We unfortunately did not segment the body composition data which may provide information as to changes in muscle mass distribution and potentially why strength continued to increase but total body lean mass did not.

While sex differences appear to be negligible in protein metabolism at rest and following exercise (Fujita, Rasmussen, Bell, Cadenas, & Volpi, 2007; West et al., 2012), there may be differences in the activation of muscle signaling proteins (West et al., 2012) and in leucine oxidation during endurance exercise (Lamont, McCullough, & Kalhan, 2001; S M Phillips, Atkinson, Tarnopolsky, & MacDougall, 1993). Our results demonstrate that men and women responded differently to a high protein diet (2.2g/kg/d) during a concurrent training program and the present data appear to be the first to demonstrate that higher-protein intake for an extended period of time may not be associated with greater gains in lean mass or strength in women, similar to what we observed in men.

It is generally accepted that the continual practice of post-exercise protein supplementation will result in greater gains in muscle mass and strength (Andersen et al., 2005; Candow, Burke, Smith-Palmer, & Burke, 2006; Cribb, Williams, Carey, & Hayes, 2006; Longland et al., 2016). However, protein requirements may be greater during the initial phase of a training program as physiological responses to strength training may be expressed more during the initial phase of training (Brook et al., 2015; Gontzea et al., 1975). Gontzea et al.(Gontzea et al., 1975) demonstrated that individuals who initiate an exercise training program while consuming a constant nitrogen intake, experience a negative nitrogen balance at the beginning of the training program which returns to normal after a period of adaptation to the exercise. More recently, Brook et al. (2015) demonstrated that muscle protein synthesis is activated during the early days of a resistance training program, but that response significantly diminishes and is associated with a reduction in exercise induced muscle signaling proteins (mTORC1) (Brook et al., 2015). Phillips et al. (Phillips et al., 1999) also concluded that habitual resistance training attenuated muscle protein turnover after exercise compared to untrained participants. The results of our study tend to support this conclusion as is evidenced by the greater changes in lean mass during the first 3 months. More research is needed to further elucidate this notion and potential mechanisms behind this finding.

An alternative hypothesis to greater changes occurring during the first half of the study compared to the second half may be related to the possible interference between strength and endurance adaptations that have been proposed to occur during concurrent training. Bell et al. (Bell, Petersen, Wessel, Bagnall, & Quinney, 1991; Bell, Syrotuik, Martin, Burnham, & Quinney, 2000) noted that concurrent strength and endurance training programs up to 10 weeks in duration result in similar changes in strength and endurance capacity. However, concurrent programs greater than 10-12 weeks may exhibit an attenuation in strength adaptations.

The present study utilized a concurrent training program similar to previous protocols (Arciero et al., 2006; Bell et al., 1991; Cantrell, Schilling, Paquette, & Murlasits, 2014; Glowacki et al., 2004; Schumann, Yli-Peltola, Abbiss, & Hakkinen, 2015). Our results showing gains in strength, positive changes in body composition and increases in VO_2peak are in agreement with these studies which utilized 12-week concurrent training programs. A concern of concurrent training programs is the interference between endurance and strength adaptations stimulated by either type individually and whether the nutritional requirements are greater during concurrent training (Perez-Schindler, Hamilton, Moore, Baar, & Philp, 2015). However, provided energy intake is adequate, current recommendations of protein intake (1.2 to 1.8 g/kg/d) should meet the metabolic demands for strength and endurance athletes (Morton et al., 2017; Perez-Schindler et al., 2015; Phillips & Van Loon, 2011). The consumption of ~2.2 g/kg/d did not appear to augment cardiovascular adaptations associated with endurance training as the increase in VO_2peak was similar between the protein and control groups for both men and women. It is unlikely that strength training interfered with adaptations associated with endurance training as the majority of research supports a performance benefit of incorporating a strength training program for endurance athletes (Beattie, Kenny, Lyons, & Carson, 2014).

Our study is unique in terms of investigating the effect of a protein supplement during a concurrent training program on strength, VO_2peak and body composition in young men and women. Furthermore, the intervention is one of the longest documented, 6 months (24 weeks). The consumption of a protein supplement during a concurrent training program improved lean mass and strength in men to a greater degree than a calorically equivalent carbohydrate supplement. The augmented effect of protein supplementation during a strength and endurance training program on fat free mass and strength was not observed in women, however there was an improvement in fat mass. Overall, protein supplementation is likely beneficial for sedentary individuals beginning a concurrent strength and endurance training program, but the outcomes appear to be different for men and women.

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The study was designed by MDV and BLS; data were collected and analyzed by MDV, TM, TLB, BLS, and MJO; data interpretation and manuscript preparation were undertaken by MDV, TM, TLB, BLS, BDW, and MJO. All authors approved the final version of the paper

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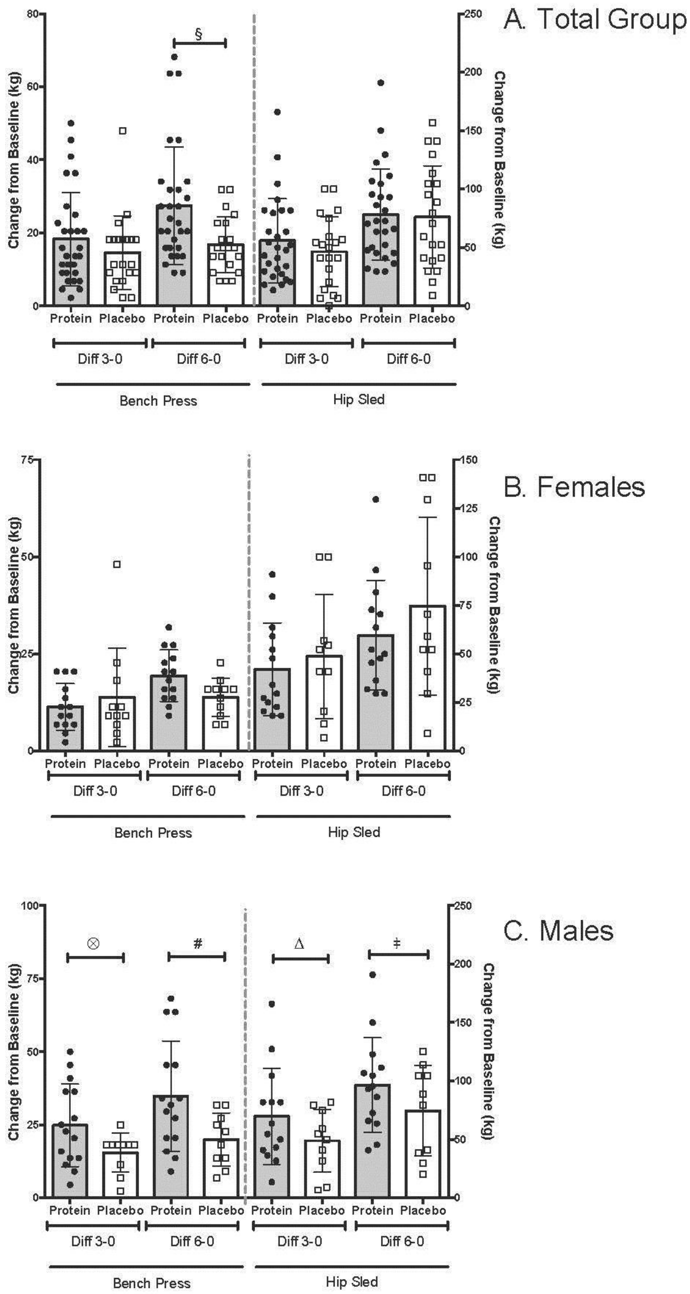

Figure 1. Change in bench press (kg, open bars) and hip sled (kg, closed bars) strength from baseline to three months (Diff 3-0), and baseline to six months (Diff 6-0). Values are least square means ± SE. Bars with similar symbols are different from each other.
§, p=0.003, ⊗,p=0.06, #, p=0.03, ∆, p=0.07; ‡, p=0.09

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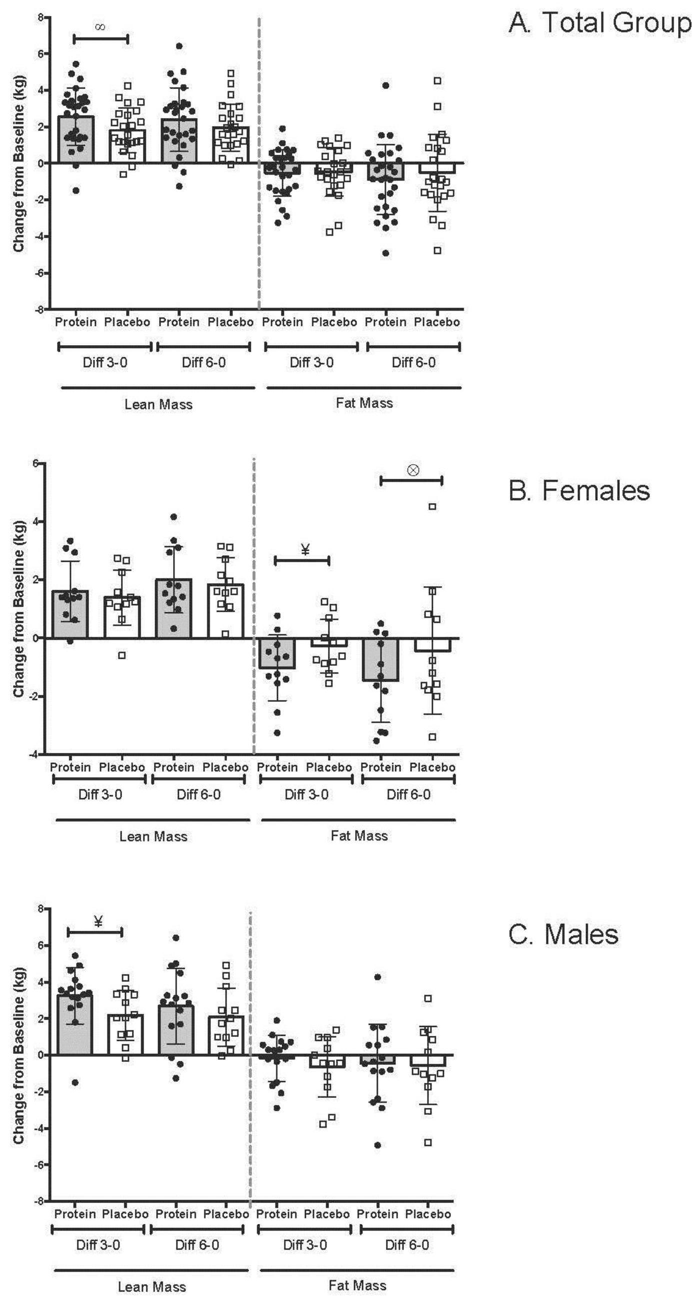

Figure 2. Change in lean mass (kg, filled bars and symbols) and total body fat (open bars and symbols) from baseline to three months (Diff 3-0), and baseline to six months (Diff 6-0) as measured by DXA in all subjects (A), in women (B), and in men (C). Symbols represent individual changes. Bars represent mean ± SD changes of groups. Bars with similar symbols are different from each other. ¥,p=0.10, ⊗,p=0.06; ∞, p=0.02;

Table 1. Dietary analysis at baseline, 3-and 6-months of a concurrent training program in individuals assigned to either a protein or carbohydrate-control supplement.

	Baseline		3 Months		6 Months	
	Control	Protein	Control	Protein	Control	Protein
Energy Intake, kcals						
Total	2130 ± 204	2095 ± 182	2348 ± 204	1949 ± 182	2194 ± 216	2244 ± 186
Female	1904 ± 202	1711 ± 160	1792 ± 202	1735 ± 171	1670 ± 213	1838 ± 184
Male	2472 ± 275	2371 ± 253	2862 ± 288	2093 ± 244	2654 ± 304	2447 ± 235
Energy Intake+S, kcals						
Total			2908 ± 204	2509 ± 182	2754 ± 216	2804 ± 186
Female			2352 ± 202	2295 ± 171	2230 ± 213	2398 ± 184
Male			3422 ± 288	2653 ± 244	3214 ± 304	3007 ± 235
Carbohydrate, g						
Total	281 ± 25	290 ± 22	293 ± 25	253 ± 22	279 ± 26	302 ± 23
Female	250 ± 27	253 ± 22	232 ± 27	235 ± 23	227 ± 29	250 ± 25
Male	322 ± 34	318 ± 31	353 ± 35	268 ± 30	325 ± 37	321 ± 29
Carbohydrate+S, g						
Total			433 ± 25	295 ± 22*	419 ± 26	348 ± 23*
Female			372 ± 27	277 ± 23*	367 ± 29	292 ± 25*
Male			493 ± 36	310 ± 30*	465 ± 38	369 ± 29*
Fat, g						
Total	78 ± 9	70 ± 8	88 ± 9	69 ± 8	73 ± 9	80 ± 8
Female	65 ± 10	52 ± 8	65 ± 10	59 ± 9	53 ± 11	65 ± 9
Male	96 ± 12	95 ± 12	108 ± 13	75 ± 11*	92 ± 14	89 ± 10
Protein, g						
Total	79 ± 7	82 ± 8	87 ± 8	79 ± 7	86 ± 9	79 ± 7
Female	68 ± 6	62 ± 5	67 ± 6	62 ± 5	69 ± 7	59 ± 6
Male	100 ± 12	90 ± 11	104 ± 12	88 ± 11	100 ± 13	92 ± 10
Protein +S, g						
Total			87 ± 8	163 ± 7*	86 ± 9	163 ± 8*
Female			67 ± 7	145 ± 5*	69 ± 7	143 ± 6*
Male			104 ± 13	172 ± 11	109 ± 14	176 ± 11*
Protein, g/kg						
Total	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.1
Female	0.9 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
Male	1.3 ± 0.5	1.2 ± 0.4	1.2 ± 0.5	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.4
Protein +S, g/kg						
Total			1.1 ± 0.1	2.2 ± 0.1*	1.0 ± 0.1	2.3 ± 0.1*
Female			0.9 ± 0.3	2.3 ± 0.4*	0.9 ± 0.3	2.2 ± 0.4*
Male			1.2 ± 0.5	2.2 ± 0.5*	1.1 ± 0.5	2.2 ± 0.5*

* $P<0.05$ compared to control at same time point.

S = supplement. Data are presented as mean ± SE.

Table 2. Peak oxygen consumption (VO_2peak) measurements for protein and control groups by sex at baseline, 3 months and 6 months of a concurrent training program.

	Baseline		3 Months		6 Months	
	Control	**Protein**	**Control**	**Protein**	**Control**	**Protein**
VO_2Peak (ml/kg/min)						
Total	39.3 ± 0.6	43.2 ± 0.5	41.9 ± 0.7†	46.0 ± 0.6†	42.2 ± 0.7†	46.6 ± 0.7†
Female	33.9 ± 0.7	41.4 ± 0.8⊗	35.8 ± 0.9†	43.2 ± 0.7†⊗	36.5 ± 0.9†	44.1 ± 0.8†⊗
Male	44.2 ± 0.9	44.6 ± 0.8	47.4 ± 1.0†	48.6 ± 1.0†	48.1 ± 1.0†	48.9 ± 0.9†

Values are reported as mean ± SE. ⊗, $p=0.06$ between control and protein for females.

† $P<0.05$ compared to baseline.

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Table 3. Body composition measurements during a concurrent strength and endurance training program for protein and control groups by sex at baseline, 3-months and 6-months.

	Baseline		3 Months		6 Months	
	Control	**Protein**	**Control**	**Protein**	**Control**	**Protein**
Weight, kg						
Total	81.7 ± 0.3	73.1 ± 0.3	82.6 ± 0.3	74.6 ± 0.3	82.8 ± 0.3	74.2 ± 0.3
Female	74.5 ± 0.4	65.0 ± 0.4	75.4 ± 0.4	65.5 ± 0.4	75.6 ± 0.4	65.5 ± 0.4
Male	88.3 ± 0.5	79.1 ± 0.4	89.3 ± 0.4	81.4 ± 0.4	89.4 ± 0.5	80.7 ± 0.4
Lean Mass,						
kg	61.8 ± 0.2	57.8 ± 0.1	63.6 ± 0.2	60.4 ± 0.2	63.7 ± 0.2	60.2 ± 0.2
Total	50.7 ± 0.2	48.5 ± 0.2	52.1 ± 0.2	50.1 ± 0.2	52.5 ± 0.2	50.5 ± 0.2
Female	71.9 ± 0.3	64.8 ± 0.2	74.1 ± 0.3	68.1 ± 0.2	74.0 ± 0.3	67.5 ± 0.3
Male						
Fat Mass, kg						
Total	21.2 ± 0.2	16.2 ± 0.2	20.8 ± 0.2	15.6 ± 0.2	20.7 ± 0.2	15.3 ± 0.2
Female	25.0 ± 0.3	17.7 ± 0.3	24.8 ± 0.3	16.7 ± 0.3	24.6 ± 0.3	16.2 ± 0.3
Male	17.7 ± 0.3	15.1 ± 0.3	17.1 ± 0.3	14.9 ± 0.3	17.2 ± 0.3	14.6 ± 0.3
Body Fat, %						
Total	25.6 ± 0.2	21.8 ± 0.2	24.7 ± 0.2	20.6 ± 0.2	24.6 ± 0.2	20.3 ± 0.2
Female	32.2 ± 0.2	26.4 ± 0.2	31.3 ± 0.2	24.7 ± 0.2	31.1 ± 0.2	24.1 ± 0.2
Male	19.5 ± 0.3	18.4 ± 0.3	18.6 ± 0.3	17.5 ± 0.3	18.7 ± 0.3	17.4 ± 0.3

Data are presented as mean ± SE.

Time effect for weight ($P=0.0002$), lean mass ($P<0.0001$), fat mass ($P=0.007$), and body fat ($P<0.0001$) with 3-months and 6-months different from baseline.

Treatment effect for weight ($P<0.0001$), lean mass ($P<0.0001$), fat mass ($P<0.0001$), and body fat ($P<0.0001$) with Protein different from Control.