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Body Composition, Muscular Performance, and Free Testosterone Following 12-Weeks of Protein Supplementation and Resistance Training in Men Aged 35-55

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Body Composition, Muscular Performance, and Free Testosterone Following 12-Weeks of Protein Supplementation and Resistance Training in Men Aged 35-55

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Abstract

PURPOSE: The purpose of this study was to investigate the effects of 12 weeks of protein supplementation and Autoregulatory Progressive Resistance Exercise (APRE) training on measures of body composition, muscular performance, and free testosterone in middle-aged men. **METHODS**: Untrained males (n = 35; 43.3 ± 6.6 years) participated in this study. Lean mass (LM) and fat mass (FM) were measured via Dual Energy X-ray Absorptiometry (DEXA), while strength was measured utilizing one repetition maximum (1RM), endurance measured using maximum repetitions completed (REPS) at 75% 1RM for the bench and leg press, and free testosterone was measured using changes in serum values. Subjects were randomly placed into one of four groups: protein (PO), APRE, protein plus APRE (PAPRE), or control (CON). Subjects repeated testing for the DEXA, 1RM, REPS, and blood draw every four weeks for 12 weeks. Both PO and PAPRE groups ingested 25g of supplemental protein twice daily. Subjects in the exercise groups completed a resistance training program, 3 days per week, for 12 weeks. **RESULTS**: Analysis revealed a significant time interaction ($p < .01$) and a trend towards significance for group time interaction ($p = .08$) between baseline and week 12 for LM, with the APRE and PAPRE groups showing a significant increase following the 12-weeks. Significant time and group by time interactions ($p < .01$) occurred for BP and LP 1RM. APRE and PAPRE displayed increases at multiple times over the course of the 12-weeks for both BP and LP, while the PRO and CON groups exhibited increases for LP only. While REPS remained unchanged ($p > .05$), volume lifted exhibited a significant time interaction between baseline and week 12 for BP and LP ($p < .01$), and a significant group by time interaction for LP ($p < .01$). PRO increased BP volume, and PAPRE increased both BP and LP volume, while APRE and CON remained relatively the same following the 12-weeks. There were no significant changes in serum free

testosterone over the course of the 12-week study. **CONCLUSION**: APRE, alone and in combination with supplemental protein, provides greater benefits in changes in LM and muscular performance compared to that of supplemental protein alone for middle-aged men following a 12-week intervention.

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your physical, mental, and spiritual investments begin to pay off. I love you, Hayden, Landon, and Maeson more than the four of you will ever know, and I wish this degree can include all of our names because the only way I could complete it is with each of you by my side.

Dedication

This dissertation is dedicated to my beautiful wife Jesi, and our three kids: Hayden, Landon, and Maeson.

Baby, we're going home!

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Chapter 1: Introduction

In 2008, the Physical Activity Guidelines for Americans was released along with the initiative of Healthy People 2020 (Physical Activity Guidelines Advisory Committee, 2008). These guidelines outlined both the benefits and needs for people across all ages to be more physically active in an effort to fight the current obesity epidemic. The goal was set to increase physical activity of Americans, and as of 2015, the initiative had provided an increase from 18.2% to 21.4% of adults over the age of 18 years who met the guidelines for both aerobic and muscle-strengthening activity.

However, despite the overall participation in exercise for adults over the age of 18 increasing, a more detailed breakdown of the middle-age cohort based on sex tells a different story. The current guidelines for physical activity are a modest 150 minutes of moderate intensity exercise per week. The guidelines for muscle-strengthening activities include: moderate to vigorous intensity, such as weightlifting or utilizing resistance bands, involving all major muscle groups on two or more days per week (Physical Activity Guidelines Advisory Committee, 2008). As of 2015, for males between the ages of 18-44 years 37% did not meet the guidelines for both aerobic and muscle strengthening activities, while males between the ages of 45-54 show that 48% did not meet the requirements (National Center for Health Statistics, 2017). Breaking down the percentage of individuals who did not meet these requirements into individuals who did not meet the minimum requirements for muscle-strengthening activities alone is even higher. For males between the ages of 18-44 years and between the ages of 45-54 years it was reported that 65% and 75%, respectively did not meet the minimum requirements for muscle-strengthening activities (National Center for Health Statistics, 2017). The need to find new techniques of muscular strengthening activities should be at the forefront of determining a way to increase the

number of individuals participating and adhering to lifelong involvement in exercise and physical activity. Utilizing aspects of resistance training such as periodized training models, specifically Autoregulatory Progressive Resistance Exercise (APRE), may be the solution in achieving an increase in muscle strengthening activity. The equation to healthy living not only includes participation in physical activity, but also includes proper diet when trying to maximize adaptations.

Diet composition is important in maintaining or increasing lean mass, and the optimal nutrient to consider in this equation is protein. The current recommended dietary allowance (RDA) for adults is 0.8 g/kg·bw/d for adults over the age of 18 years (National Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989). Researchers have stated that while the RDA is 0.8 g/kg·bw/d, this amount is simply the minimum required to fulfill basic nutritional requirements, but individuals who are active and/or partake in exercise routines should strive for a minimum of 1.2 g/kg·bw/d as a requirement for maintenance of lean mass (Antonio, Peacock, Ellerbroek, Fromhoff, & Silver, 2014). Increasing lean mass may require upwards of 1.4-2.0 g/kg·bw/d for individuals participating in exercise training (Jäger et al., 2017). As of 2015, males between the ages of 20-64 years of age consumed only 16% of protein in their total dietary intake, or roughly 1.0 g/kg·bw/d resulting from protein sources (Fulgoni, 2008; National Center for Health Statistics, 2017). With protein intake accounting for less than 20% of their caloric intake that leaves the remaining 80% comprised of carbohydrates and fats (National Center for Health Statistics, 2017). The reasons behind the low intake of protein in the diet are speculative at best and therefore more research is needed on increased dietary protein intake, and therefore shed light on avenues to accomplish this task. The benefits of increased protein intake include the preservation of lean mass (Cermak, Res, de

Groot, Saris, & van Loon, 2012), greater ability to decrease fat mass (Sousa et al., 2012), and even increases in muscular strength when combined with resistance exercise (Antonio et al., 2015). Thus, there is a need to find avenues to incorporate more dietary protein intake on a daily basis. Exercise and diet are important factors when determining overall health, but when investigating males another important factor to take into account is the decrease in testosterone that begins at age 30 and gradually declines with age (Feldman et al., 2002; Harman et al., 2001; Marty, Liu, Samuel, Or, & Lane, 2017).

After the age of 30, men experience a decline in testosterone that gradually continues throughout the aging process (Feldman et al., 2002). Testosterone deficiency in men is known as hypogonadism and is typically diagnosed when testosterone levels are chronically less than 300-350 μg/dl (Haring et al., 2010). Hypogonadism has been seen in individuals as early as the age of 30 (Harman et al., 2001), with prevalence of hypogonadism in men aged 45-54 years reaching 34%, and increases 17% with each decade thereafter (Mulligan, Frick, Zuraw, Stemhagen, & McWhirter, 2008). The maintenance of testosterone levels in men aids in the ability to gain lean mass (Forbes & Reina, 1970; Gallagher et al., 1997), lose fat mass (Couillard et al., 1996; Siervogel et al., 1998), as well as increase muscular performance (Harman et al., 2001). Total testosterone levels increase following an acute bout of resistance exercise (Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2003; Volek, Kraemer, Bush, Incledon, & Boetes, 1997). Individuals who participate in chronic endurance based activity produce a lower basal measure of total testosterone than sedentary individuals (Hackney, Sinning, & Bruot, 1988; Hakkinen, Pakarinen, Alen, Kuahanen, & Komi, 1987; Strauss, Lanese, & Malarkey, 1985; Wheeler, Wall, Belcastro, & Cumming, 1984). What needs to be understood is the possible effects that resistance training has on levels of free testosterone in men. A majority of testosterone (~97%) is bound, while the

remaining approximately 3% is unbound or free. Free testosterone is the form that is available to be taken up by the working tissue. Hence, if resistance training is a natural way of boosting free testosterone, then it potentially opens an avenue to be explored by patients prior to entering therapy for hormone replacement for men suffering from decreased levels of testosterone. Increased free testosterone may lead to increased lean mass, decreased fat mass, and improved muscular performance.

With age, there is a decrease in lean mass (Gallagher et al., 1997), decreased dietary protein intake (Fulgoni, 2008; National Center for Health Statistics, 2017), and men experience a decline in free testosterone values (Feldman et al., 2002; Harman et al., 2001; Marty, Liu, Samuel, Or, & Lane, 2017). Along with these age-related decreases there is a decline in the number of middle-aged individuals participating in muscle-strengthening exercise (National Center for Health Statistics, 2017). To combat the effects on body composition and muscular performance these declines have, there is a need to introduce new avenues of resistance exercise and protein intake that lead to increased lean mass, muscular performance, and testosterone in middle-aged men. By determining the effects of resistance training, increased protein intake, and exercise-induced increases in free testosterone have on middle-aged men the battle against age-related sarcopenia becomes easier, rather than waiting until individuals are unable to maximally benefit from these interventions. Therefore, the purpose of this study is to investigate the effects of protein supplementation, as well as resistance exercise on measurements of body composition, muscular performance, and free testosterone in men aged 35-55 years.

Chapter 2: Literature Review

Introduction

Since 2008, the Physical Activity Guidelines for Americans (Physical Activity Guidelines Advisory Committee, 2008) has set forth guidelines in an effort to increase the number of individuals involved in physical activity. By increasing involvement in physical activity, specifically muscle-strengthening activity, aging adults may have a chance to fight sarcopenia, or the age-related loss in muscle mass. Decreases in muscular performance (Gallagher et al., 1997), as well as changes in hormone levels (Feldman et al., 2002; Harman et al., 2001; Marty, Liu, Samuel, Or, & Lane, 2017), can be accounted for by diminished levels of physical activity and lower dietary intakes of protein (National Center for Health Statistics, 2017) throughout the lifespan. For this review of literature, methodology development, and discussion of results, a search of Medline (PubMed), ProQuest International, and Google Scholar was conducted using the following keywords: periodized resistance training, protein supplementation, testosterone, and all related derivatives. When adequate and related literature was identified, follow-up searches were completed to further examine research design and methodology. This review includes randomized control studies, reviews, and meta-analyses that assessed the following components: a) periodized resistance training, b) autoregulatory progressive resistance exercise c) protein supplementation, d) protein timing, and e) testosterone, on body composition and muscular performance. All articles were published in peer-reviewed journals and when necessary, textbooks were referenced to explain and clarify general concepts. The review of literature is divided into the following major sections: 1) resistance training, 2) protein, and 3) testosterone. Each section is further subdivided.

Resistance Training

Resistance training alters skeletal muscle by increasing muscular strength and size (Abernethy, Jürimäe, Logan, Taylor, & Thayer, 1994; Campos et al., 2002). Resistance training research has focused on the ideas behind the various combinations of sets and repetitions utilized in achieving increased muscular strength and size (McDonagh & Davies, 1984; Tesch, 1988). It is clear that manipulation of acute variables allows muscles to be introduced to new stimuli causing adaptations to occur (Tan, 1999). However, it is still a topic of conversation as to which form of programming maximizes lean mass accretion, fat mass reduction, and muscular performance improvements.

Periodization: Linear vs Undulating. Resistance training is a specialized form of exercise utilizing a wide range of loads and modalities designed to enhance health, fitness, and performance (Faigenbaum et al., 2009). Individuals of all ages benefit from participating in resistance training programs and these benefits include: improved musculoskeletal health (Folland & Williams, 2007), strength (Harries, Lubans, & Callister, 2016), power (Smith, Callister, & Lubans, 2011), endurance (Faigenbaum et al., 2009), cardiovascular (Kraemer et al., 2002), and metabolic health (Liberati et al., 2009). The American College of Sports Medicine (ACSM) recommends periodized programs rather than non-periodized based on superior benefits to body composition measures and muscular performance (Kraemer et al., 2002). Periodization is the methodical planning and configuring of training intensity, volume, frequency, and rest throughout specific timeframes in an effort to maximize training benefits (Hoffman et al., 2009; Mann, Thyfault, Ivey, & Sayers, 2010; Miranda et al., 2011; Monteiro et al., 2009; Peterson, Dodd, Alvar, Rhea, & Favre, 2008; Prestes et al., 2009; Rhea, Ball, Phillips, & Burkett, 2002). Within periodization, there are two program subsets debated upon as to which is most beneficial:

undulating (UP) and linear (LP) periodization (Issurin, 2010; Plisk, & Stone, 2003; Prestes et al., 2009; Rhea et al., 2003). Linear periodized programs consist of phases with pre-designed increases in load and intensity, including initial high volume and low intensity formats that increase gradually (Hartmann, Bob, Wirth, & Schmidtbleicher, 2009; Hoffman et al., 2003, 2009; Kok, Hamer, & Bishop, 2009; Simão et al., 2012). Undulating periodized programs adjust load and intensity for each workout, and this adjustment includes both increasing and decreasing weight used, and the number of repetitions per set (Miranda et al., 2011; Peterson et al., 2008; Prestes, Frollini, de Lima, Donatto, Foschini, de Cássia Marqueti, et al., 2009). Traditional UP programs change the workload with every workout, with some changing on a weekly basis. These manipulations in volume and intensity of stimuli aid in maximizing strength gains.

Both LP and UP programs are utilized in increasing muscular strength, however, a distinction must be made for which program best suits achieving these adaptations. When comparing the effects of LP and UP programs significant increases in one repetition maximum (1RM) for upper- and lower- body were observed over the course of 12 weeks, with training occurring 3 days per week (d/wk). Upper-body muscular strength increased on average by 13% and 23% for the LP and UP groups, respectively (Baker, Wilson, & Carlyon, 1994; Rhea et al., 2002). UP provided greater strength changes in lower-body assessments at 42% above baseline, while both the NP and LP only increased 26% when investigating trained young males (Baker, Wilson, & Carlyon, 1994; Rhea et al., 2002). One of the few studies investigating the differences between LP and UP utilizing untrained subjects observed strength increases for both groups. The UP group increased both upper- and lower-body strength by 6% over that of the LP group over the course of the 9-week study (Kok, Hamer, & Bishop, 2009). These findings illustrate the greater benefit to maximizing strength that UP has on both trained and untrained subjects.

However, most studies utilize younger (20.3 ± 2.5 years) populations (Baker, Wilson, & Carlyon, 1994; Kok, Hamer, & Bishop, 2009; Rhea, Ball, Phillips, & Burkett, 2002), thus creating a need for more periodized resistance training research within the middle-aged and older adult populations. This research may require observing various forms of UP to determine the benefits within these understudied cohorts. Autoregulatory Progressive Resistance Exercise is a variation of UP that has begun to make its way into traditional exercise settings despite being primarily a rehabilitation program.

Autoregulatory Progressive Resistance Exercise. In 1944, Dr. Thomas DeLorme was stationed at Gardiner General Army Hospital in Chicago. This assignment would lead to the merging of modern rehabilitation and the science of strength training. It became his mission to create a faster rehabilitation system for the overload of injured soldiers from the war (Delorme, 1951). In 1945, DeLorme published a seminal article on the heavy weight training program he utilized on many of his patients. DeLorme stated that the most important aspect of rehabilitation was the redevelopment of muscle following healing from an injury, thus restoring normal to near-normal function. His program consisted of multiple sets in which intensity was 10RM. This laid the groundwork for his overarching theory that building endurance within an atrophied or weakened muscle, begins with building muscular strength (DeLorme, 1945). The beauty of his original theories is the overwhelmingly prophetic nature of his conclusion, in that he stated, "Although this program of exercise was developed primarily to expedite the recovery of injured soldiers in the war, there is a definite civilian need for a similar program," (DeLorme, 1945). In 1948, Delorme refined his system into the very first progression-based program, consisting of three progressively heavier sets of 10 repetitions, called the Progressive Resistance Exercise. The first set is at 50% of the 10 RM, followed by 75%, and 100%. DeLorme felt that this progressive

addition of weight would allow for heavier loads to be achieved faster, leading to a more rapid accumulation of muscular hypertrophy (DeLorme & Watkins, 1948).

DeLorme's original program remained the same until 1979 when Kenneth Knight made a slight adjustment creating the Daily Adjustable Progressive Resistance Exercise (DAPRE) program (Knight, 1979). The DAPRE system utilizes a 5-7 RM, consisting of four sets. Set one consists of 10 repetitions at 50%, followed by a set of 6 repetitions at 75%, maximum repetitions at 100%, and finally a set of maximum repetitions utilizing a weight that is adjusted based off of set three. The first two sets are considered to be warm-up sets, while the final two sets are maximal effort working sets. The aspect of maximum repetitions with an adjustable load allows for individuals to work to their fullest potential, while accounting for variations in day-to-day performance.

Coined by Mann and colleagues (2010), Autoregulatory Progressive Resistance Exercise (APRE), is being implemented into resistance training programs outside of rehabilitation. Autoregulation is the process in which individuals adjust to given workloads and the physiological adaptations that ultimately occur from these repeated workload adjustments. Therefore, APRE is a more specialized form of UP with its daily adjustments in workload and intensity (Herrick & Stone, 1996). APRE differs from traditional UP workout regimens by taking into account the individuals' progress with a certain exercise instead of a preset adjustment that has already been scheduled (Herrick & Stone, 1996; Verkhoshansky & Siff, 2009). APRE accounts for the fact that individuals increase strength and respond to different forms of stimuli in training at different rates. APRE permits for decreases in loads while other training programs only allow for increases in loads. This program allows individuals to progress within an exercise when they are ready physiologically, while also accounting for variations in an individual's

abilities on a given day of training. APRE can be broken down into three differing protocols based on a 3, 6, or a 10RM. Among college-age males, APRE is effective at increasing both upper- and lower-body strength and upper-body muscular endurance. Bench press and squat strength increased by 21kg and 20kg, respectively, in the APRE group over the LP group, as well as improving bench press repetitions over the course of the 6-week training period by 3 repetitions (Mann et al., 2010). However, APRE is a new type of UP resistance training program needing more research to determine the applications and usefulness of an APRE training program in older adults. Research shows the benefits of UP (Baker, Wilson, & Carlyon, 1994; Kok, Hamer, & Bishop, 2009; Rhea, Ball, Phillips, & Burkett, 2002) and APRE (Mann et al., 2010) on increasing muscular strength over that of LP, but body composition, specifically lean mass accretion, is an important factor to consider when choosing the most appropriate exercise protocol.

Periodization and Lean Mass. Assessing changes in strength from various periodized programs is essential, however, understanding changes in body composition is equally important. Resistance training elicits muscular growth, whether that is through lean mass accretion (American College of Sports Medicine, 2009; Peterson, Sen, & Gordon, 2011) or increases in cross-sectional area (CSA) (Dons, Bollerup, Bonde-Petersen, & Hancke, 1979; Narici, Roi, Landoni, Minetti, & Cerretelli, 1989; Staron et al., 1994). Illustrating the benefits of resistance training at increasing lean mass, a distinction must be made as to which program is most beneficial at eliciting this increase, as well as the time in which the growth is noticeable. With UP being the superior of the programs for gains in strength (Baker, Wilson, & Carlyon, 1994; Kok, Hamer, & Bishop, 2009; Rhea, Ball, Phillips, & Burkett, 2002), it is still unclear which is more beneficial for changes in lean mass. Overall, both LP and UP are beneficial in gaining lean

mass within younger cohorts. Throughout short durations up to 9-weeks, lean mass increases by 2% and 3% in upper- and lower-body, respectively (Ahmadizad, Ghorbani, Ghasemikaram, & Bahmanzadeh, 2014; Buford, Rossi, Smith, & Warren, 2007), while CSA increases by 0.5% for UP over that of LP groups (Souza et al., 2014). While these studies found no significant differences between UP an LP for changes in CSA, lean mass, and girth, they do illustrate that UP is more beneficial in shorter durations. However, when intervention length is extended LP begins to be more beneficial. When the duration of the training is extended to 12 weeks and beyond, there becomes a clear distinction indicating the benefit of LP with an average increase in lean mass of 1% over that of UP (de Lima et al., 2012; Foschini et al., 2010; Harries, Lubans, & Callister, 2016).. However, when examining CSA, muscle growth is greatest following UP training. CSA increased on average by 3% and 6% more in the arms and thigh, respectively, for UP compared to LP over 12 weeks (Kok, 2006; Kok et al., 2009; Simão et al., 2012; Spineti et al., 2014). The previously mentioned studies (Ahmadizad et al., 2014; Buford, Rossi, Smith, & Warren, 2007; de Lima et al., 2012; Foschini et al., 2010; Harries, Lubans, & Callister, 2016; Kok, 2006; Kok et al., 2009; Simão et al., 2012; Souza et al., 2014; Spineti et al., 2014) were all carried out in young untrained individuals, and indicate that UP is better for lean mass gains in shorter durations and better for CSA increases over longer durations. While research shows the benefits in younger populations, lean mass gains with aging helps combat potential issues with sarcopenia. Surprisingly, in untrained older adults, LP actually causes a decrease of nearly 2%, while UP exhibits an increase of 2% in lean mass following 16 weeks of training (Prestes et al., 2009). While these results indicate UP being a more beneficial program for older adults during long-term interventions, it is the only study to date that compares LP and UP for changes in lean mass (Grgic, Mikulic, Podnar, & Pedisic, 2017). Although the aforementioned studies

(Ahmadizad et al., 2014; Buford , Rossi, Smith, & Warren, 2007; de Lima et al., 2012; Foschini et al., 2010; Harries et al., 2016; Kok, 2006; Kok et al., 2009; Prestes et al., 2009; Simão et al., 2012; Souza et al., 2014; Spineti et al., 2014) show benefits of utilizing UP for young and older individuals, there are no current studies utilizing middle-aged men to assess changes in lean mass following UP. Therefore, not utilizing middle-age men in training studies creates a gap in the understanding of how individuals respond to various exercise programs throughout the aging process. The absence of a middle-age men cohort is only amplified by the simultaneous absence of studies reporting lean mass changes following training with the APRE program.

Resistance training has many benefits including increased lean mass and muscular strength (Abernethy et al., 1994; Campos et al., 2002). Of the many training programs, UP is best suited for maximizing results for muscular strength (Baker, Wilson, & Carlyon, 1994; Kok, Hamer, & Bishop, 2009; Rhea, Ball, Phillips, & Burkett, 2002). APRE is new to the traditional exercise setting, but has shown promise as a new variation of UP within younger individuals (Mann et al., 2010). However, APRE needs more research to determine its effectiveness within a middle-aged cohort of exercisers. Maximizing training adaptations also involves dietary interventions, specifically the addition of more protein into the diet as together they act synergistically (Biolo, Tipton, Klein, & Wolfe, 1997; Rennie, 2001; Zawadzki, Yaspelkis, & Ivy, 1992). However, resistance training is only one stimuli needed to alter body composition and muscular performance. Therefore, macronutrient intake specifically that of protein, needs to be addressed.

Protein

In order to elicit muscular hypertrophy outside of the introduction of resistance training, muscle protein synthesis must be balanced with protein degradation (Phillips, Tipton, Aarsland,

Wolf, & Wolfe, 1997). If protein balance is positive then hypertrophy can occur, while if protein balance is negative then protein degradation, or muscle loss, occurs. Stimulation of the mammalian target of Rapamycin (mTOR) pathway, elucidates an increase in the rate of protein synthesis that occurs (McCarthy & Esser, 2010). Through resistance exercise the mTOR pathway activates protein synthesis, however nutritional interventions have the ability to activate this pathway as well. A major participant in protein synthesis through nutritional interventions is the ingestion of protein or amino acids (Cuthbertson et al., 2005; Moore et al., 2009), and more specifically protein with a high concentration of the branched chain amino acid leucine (Biolo, Tipton, Klein & Wolfe, 1997; Norton & Layman, 2006). Therefore, an important aspect of nutritional interventions is to understand the differences in protein sources, quality, quantity, timing, effects on body composition, and influences on muscular performance when combined with resistance exercise.

Types of Protein. The most important aspect when discussing protein is the proper protein source for maximizing results. Milk proteins are extensively studied regarding their role in exercise training adaptations including: accelerated muscle recovery (Cockburn, Stevenson, Hayes, Robson-Ansley, & Howatson, 2010), increased glycogen replenishment (Wojcik, Walberg-Rankin, Smith, & Gwazdauskas, 2001), and creation of a positive protein balance allowing increased muscular strength and hypertrophy (Hartman et al., 2007; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). Milk proteins also contain the greatest amount of leucine at an average of 10%, compared to plant based counterparts containing 6-8% leucine (Norton & Wilson, 2009; Norton, Wilson, Layman, Moulton, & Garlick, 2012). The two types of milk proteins are whey and casein.

Protein Quality. Whey and casein contain the highest amounts of leucine compared to other various protein sources, at 11% and 9%, respectively. However, these sources differ in the

rate at which they are digested, as well as their impact on protein metabolism (Boirie et al., 1997; Dangin et al., 2003; Dangin, Boirie, Guillet, & Beaufrère, 2002). Whey is a fast-digestible protein in that it quickly empties from the stomach into the duodenum, while casein is a slow digesting protein that coagulates within the stomach causing a slower period for gastric emptying (Boirie et al., 1997; Wilson & Wilson, 2006). After understanding the digestion kinetics of whey and casein, the next step is to understand each proteins' role in protein synthesis stimulation. Following feeding, whey stimulates protein synthesis by 68% while casein stimulates by 31% within one hour of ingestion, therefore showcasing whey's ability to stimulate muscle protein synthesis (MPS) more rapidly (Boirie et al., 1997; Dangin, Boirie, Guillet, & Beaufrère, 2002). Trommelen and colleagues (2016) examined the effects of 30g of casein on rates of muscle protein synthesis, with and without exercise. Muscle protein synthesis rates were increased; however, the impact on whole-body protein synthesis was not noticeable. This led to the investigation on the effects of 22g of whey protein versus casein at rest and following exercise. Whey demonstrated a 200% greater increase in leucine concentration as compared to casein, again indicating the fast-acting nature and higher leucine content of whey. It was also noted that whey stimulated a greater increase in muscle protein synthesis at both rest and following the resistance training protocol (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). Indicating that whey protein is more beneficial at acutely stimulating MPS than casein, however the long-term effects of protein supplementation still needs clarification. Examining the effects of 10 weeks of protein supplementation, whey resulted in greater gains in lean mass by 4kg, and strength gains when compared to the casein group (Cribb, Williams, Carey, & Hayes, 2006). Suggesting that a fast-acting protein, such as whey, is a more appropriate protein source than casein when examining muscular adaptations to supplementation. Thus suggesting that the amino

acid content, or the quality, of the protein source is an important element to consider, as leucine is purported to stimulate the mTOR pathway and therefore stimulate protein synthesis (Atherton, Smith, Etheridge, Rankin, & Rennie, 1994.; Buse & Reid, 1975; Kimball, 2007).

Leucine. There are 20 specific amino acids utilized to build proteins utilized in tissue formation (Fluck, 2012), cellular function (Wu, 2009), and growth (Atherton & Smith, 2012). Of these 20, there are essential (EAA) and non-essential (NEAA) amino acids. EAAs are unable to be synthesized or made within the body, thus needing to come from food sources. NEAAs however have the ability to be produced within the body, therefore not needing to be supplemented through dietary interventions. Due to EAAs needing to be introduced via diet, any decreased consumption of these amino acids would impair the body's ability to properly construct cellular proteins that aid in overall function and possible growth (Fluck, 2012). Any increase in plasma amino acids above fasting levels, while transient, renders an increase in protein synthesis. Protein synthesis reaches its peak roughly 2 hours following protein or amino acid ingestion, at which point muscle is most receptive to the anabolic effects (Pasiakos, 2012). Protein's ability to aid in muscle mass accretion is largely based on its profile of EAAs. Animal-based proteins are considered complete proteins as they contain all EAAs, while plant-based proteins lack certain EAAs making them incomplete. When comparing skim milk to soy milk with 18g of protein each, skim milk significantly stimulates MPS by 0.03% more than soy, allowing for a more prolonged and sustained positive protein balance (Wilkinson et al., 2007). This increase in MPS indicates the superior ability of animal-based proteins, specifically milk protein, compared to that of plant-based protein sources. Whey (fast-acting) protein increases mixed muscle protein synthesis to a greater extent compared to soy and casein (slow-acting) proteins at larger doses consisting of 10g of EAAs per protein source (Tang et al., 2009). The fast-acting, or quickly absorbed, nature of whey protein

is responsible for the enhanced anabolic response, allowing for leucine to assimilate more quickly than that of plant proteins or casein (Phillips & Van Loon, 2011). Leucine acts as a signaling molecule within the mTOR pathway, and assists in the increase of skeletal muscle protein synthesis, while suppressing protein degradation (Atherton, Smith, Etheridge, Rankin, & Rennie, 1994.; Buse & Reid, 1975; Kimball, 2007). Loss of muscle mass occurs with age and understanding if this loss is due to a decline in synthesis rates or an increase in degradation is the next step. Multiple human studies support the concept that ingestion of high quality protein (~25g) with 3.5g of leucine maximizes protein synthesis levels in younger individuals (Glynn et al., 2010; West et al., 2011). Thus, supporting the utilization of a protein source with higher leucine content to stimulate and sustain protein synthesis. Increased muscle protein synthesis occurs when there is an increased amount of leucine in a given protein source, and when coupled with exercise, increased leucine prolongs the effect on muscle protein synthesis by 48% (Biolo, Tipton, Klein, & Wolfe, 1997; Norton & Layman, 2006). Leucine alone increases muscle protein synthesis, but when part of a larger whole protein source, such as a whey protein supplement or meal, the effect is greater (Wilson et al., 2011). The benefits of protein containing a higher amount of leucine, especially supplemental protein, in an effort to maximally stimulate muscle protein synthesis drives home the need for the utilization of whey protein when assessing increases in lean mass and loss of fat mass. The determination that whey protein is the superior choice in quality to stimulate muscle protein synthesis, the next aspect to determine is the proper quantity to utilize for lean mass accretion and preservation.

Protein Quantity. Along with the importance of selecting a high-quality protein, the protein quantity to utilize is just as important. MPS is maximally stimulated when net muscle protein accretion is higher. This results from a high-quality protein, such as whey, comprised

with greater amounts of EAAs, especially leucine. Therefore, the quantity of protein ingested is directly associated with its quality. Utilizing a high-quality protein, younger subjects have shown to maximally stimulate MPS following ingestion of 20-30g both prior to and following resistance exercise (Hulmi et al., 2009; Tipton, 2007). Ingestion of 20-30g of protein is important as this is reflective of the ideal quantity required per meal, with 30g/meal more beneficial with age (Paddon-Jones & Rasmussen, 2009).With whey protein providing the best advantage of acutely stimulating MPS over that of casein and soy (Tang et al., 2009; West et al., 2011), the delivery method and quantity was observed. A bolus of 25g whey promoted a greater increase in MPS through higher amino acid concentration in the blood than that of separating the bolus into ten separate 2.5g doses (West et al., 2011). Again, confirming that a single bolus between 20-30g of whey is more beneficial in promoting MPS increases as previously stated (Hulmi et al., 2009; Tipton, 2007). However, with these investigations being conducted on younger populations there is a need to understand proper protein quantity within older cohorts. Cuthbertson et al., (2005) determined that EAAs act in a dose-dependent manner, with 10g offering the maximal increase in MPS, while larger doses between 20-40g offer no added benefit (Cuthbertson et al., 2005). This 10g of EAAs is representative of a high-quality protein equating to 30g of total protein, again serving as a potential threshold amount needed for maximizing MPS. This threshold of 30g was then confirmed when Symons et al., (Symons et al., 2007; Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009) determined that 30g of high-quality protein increased MPS equally in younger- and older-adults, while tripling this amount to 90g showed no added benefit to MPS increases in either group. While the 30g of protein, or 10g of EAAs, threshold was confirmed to maximize MPS (Cuthbertson et al., 2005; Symons et al., 2007, 2009), the idea of timing to maximize the benefits is essential.

Protein Timing. Protein timing is important when attempting to incorporate more daily protein into the diet or in an effort to maximize exercise adaptations. Protein pacing is the concept of spacing total daily dietary protein content into multiple servings throughout the day to increase total protein intake and meal frequency concurrently (Drummond, Crombie, Cursiter, & Kirk, 1998; Fabry, Hejl, Fodor, Braun, & Zvolankova, 1964). Utilizing the concept of separating total protein into multiple smaller boluses rather than fewer large boluses (Areta et al., 2013), and that optimal serving size is a 25g protein bolus (West et al., 2011), protein pacing utilizes 5-6 meals per day consisting of 25g protein per meal to achieve a minimum of 1.4 g/kg·bw/d protein intake (Arciero, Edmonds, He, et al., 2016). This increase in daily dietary protein intake leads to significant reduction in total body fat, abdominal body fat, and slight preservation of lean mass (Arciero, Edmonds, Bunsawat, et al., 2016; Arciero, Edmonds, He, et al., 2016; Arciero et al., 2013). All of the benefits associated with protein pacing have been associated with dietary interventions only, with no exercise associated. Therefore, the need to understand the benefits of timing supplemental protein intake along with exercise is crucial.

Protein and Exercise. The timing benefits of protein ingestion along with exercise is crucial to physiological adaptations that occur. MPS differs between the fed and unfed state. During the unfed state net protein balance remains negative, despite the acute stimuli of a resistance training bout (Biolo, Maggi, Williams, Tipton, & Wolfe, 1995). Tipton's group demonstrated that muscle protein synthesis significantly increased leucine concentration by 190% in the 40g of EAA group compared to the water and artificial sweetener placebo group, respectively (Tipton et al., 1999). Protein synthesis increases from protein ingestion immediately following exercise. However, exercise alone does not create a positive protein balance, therefore relying on the combination of exercise and protein ingestion to maximize benefits. Breen and

colleagues (2011) found that a beverage containing 10.2g of whey protein ingested following exercise, and then again 30 minutes after the first beverage, increased plasma amino acid concentrations. Using the amino acid leucine as a marker of protein synthesis, there was an increase of 130% over that of a carbohydrate only beverage when ingested following exercise (Breen et al., 2011). By doubling the amount of the protein bolus following exercise, 24g of whey with 4.8g of leucine elicits a greater post-exercise MPS rate by 48% over that of a non-caloric placebo following repeated sprint cycling sessions (Coffey et al., 2011). Illustrating the benefit of supplemental whey protein intake on MPS following exercise. Timing of protein ingestion near ±2 hours following exercise provides a greater activation of molecular signaling pathways to regulate protein synthesis (Ivy et al., 2008). The combination of exhaustive exercise and 15g whey protein immediately post-exercise increases the anabolic response more than 24 hours over that of a group that only ingested the whey protein and completed no exercise (Burd et al., 2011). When assessing subsequent exercise performance, time-trial time improved by seven minutes over that of both carbohydrate only and placebo groups following ingestion of a beverage containing 3.67g/100mL protein between exercise bouts separated by four hours (Ferguson-Stegall et al., 2011). Supporting the idea that post-exercise protein consumption improves muscular performance. Previous research (Breen et al., 2011; Burd et al., 2011; Coffey et al., 2011; Ferguson-Stegall et al., 2011; Ivy et al., 2008; Tipton et al., 1999) supports increased amino acids consumption aids in increased muscle protein synthesis, while also allowing for improved exercise performance when consuming protein following an exercise bout. However, the alterations in body composition with increased dietary protein intake and supplemental protein following exercise needs further exploration.

Protein and Body Composition. Many individuals want to alter their body composition. Body composition changes include increasing lean mass and decreasing fat mass, which also allows for increased exercise performance (Jäger et al., 2017). To maximize body composition changes it is important to understand the benefits of protein in combination with exercise, as well as the effects of increased protein intake without exercise.

Protein and Exercise. The synergy of exercise and protein ingestion together, and the ability for an increased anabolic response was established by Rennie (Rennie, 2001), Biolo (Biolo, Tipton, Klein, & Wolfe, 1997) and Zawadzki (Zawadzki et al., 1992). Protein intake has been largely investigated for its role in gains of lean mass in combination with resistance exercise (Cermak, Res, de Groot, Saris & van Loon, 2012; Hartman et al., 2007; Vieillevoye, Poortmans, Duchateau & Carpentier, 2010; Walker et al., 2010). Following a 14-week resistance training intervention, males supplementing with 25g of a protein blend exhibited greater gains in lean mass and muscle cross-sectional area compared to placebo (Andersen et al., 2005). Thus, making a case for the utilization of 25g of protein in combination with a resistance training protocol to maximize results. Cermak and colleagues reported within their meta-analysis that fat-free mass increases by an average of 0.69kg during resistance training protocols over an average of 12 weeks when protein supplementation is involved, versus when a placebo is administered (Cermak et al., 2012). Pertaining to body weight and body fat percentage, protein supplementation augments both in a positive manner, allowing for a reduction in overall weight gain while allowing for greater reductions in fat mass when protein is supplemented twice daily equaling 45-50g (Baer et al., 2011; Pichon et al., 2008; Sousa et al., 2012). While research (Andersen et al., 2005; Cermak et al., 2012; Hartman et al., 2007; Vieillevoye et al., 2010; Walker et al., 2010) demonstrates the benefits of supplemental protein in combination with exercise to induce body composition changes, it is

critical to investigate the benefits of protein supplementation on body composition without exercise.

Increased Dietary Protein. Most of the time increased protein intake is thought to only accompany exercise, however, increased dietary protein alone aids in lean mass preservation, fat loss, and overall weight management (Clifton, 2012; Morenga & Mann, 2012; Westerterp-Plantenga, Lemmens, & Westerterp, 2012). When determining the benefits of an increase in dietary protein, the benefits of high-protein diets must be considered. High-protein diets have many definitions, but the most widely utilized is the consumption of 1.2-1.6 g/kg·bw/d (Leidy et al., 2015), or reaching and/or exceeding 25% of caloric intake (Bray et al., 2012; Makris & Foster, 2011). While diet and exercise are an important combination, the understanding of diet alone and its impact on body composition is equally important. In classical works by Layman (Layman et al., 2005, 2009), lean mass was preserved while fat mass was reduced to greater extents over a 4-month period when dietary protein intake was doubled to 1.6 g/kg·bw/d. By increasing the total dietary protein intake above 1.2 g/kg·bw/d, and resulting in greater lean mass preservations, this aligns perfectly with the idea that 1.2 g/kg·bw/d may actually be the minimum requirement for bodily maintenance (Antonio, Peacock, Ellerbroek, Fromhoff, & Silver, 2014). Confirming the use of high-protein diets even further, multiple studies have supported the consumption of protein between 1.2-1.6 g/kg·bw/d, and nearing 2.0 g/kg·bw/d, for lean mass preservation, fat loss, and overall weight management (Clifton, Condo, & Keogh, 2014; Dong, Zhang, Wang, & Qin, 2013; Santesso et al., 2012; Wycherley, Moran, Clifton, Noakes, & Brinkworth, 2012). As noted previously, the benefits of added protein with and without exercise includes gains in lean mass and reductions in fat mass. Thus, requiring further clarification on the benefits of supplemental protein on aspects of muscular strength.

Protein and Muscular Strength. Supplemental protein's ability to exhibit additive effects for strength are dependent upon factors such as training program, intervention length, training status, and protein quality and quantity (Jäger et al., 2017; Pasiakos, McLellan, & Lieberman, 2015). Thus, the effect supplementation has is varied, with results showing little to no effect on maximal strength outcomes (Andersen et al., 2005; Candow, Burke, Smith-Palmer, & Burke, 2006; Erskine, Fletcher, Hanson, & Folland, 2012; Hulmi et al., 2009; Weisgarber, Candow, & Vogt, 2012; Willoughby, Stout, & Wilborn, 2007). A majority of the studies conducted investigate young, healthy males with few exceptions that observe older males (Bemben et al., 2010; Cribb, Williams, Stathis, Carey, & Hayes, 2007; DeNysschen, Burton, Horvath, Leddy, & Browne, 2009; Kukuljan, Nowson, Sanders, & Daly, 2009). College football athletes taking 42g of a proprietary blend milk protein twice daily for a 12-week period significantly increased maximal squat strength over the placebo group by 8% (Hoffman et al., 2007). While the protein group significantly increased over that of the placebo, the use of a proprietary blend supplement does not help further the understanding of the effects on strength because the exact contents of the proprietary blend are unknown. Therefore, the need to investigate the benefits of utilizing a high-quality protein with known ingredients is imperative. Untrained females supplementing with 36g of protein, in the form of fat-free milk, during a 12-week resistance training intervention increased maximal bench press significantly over that of the carbohydrate placebo group (Josse et al., 2009). The use of milk protein immediately following resistance training increases muscular strength, but what occurs when protein is ingested prior to and immediately post exercise? Taylor and colleagues (2016) found that supplementing with 24g prior to and another 24g immediately post resistance training for an 8-week period increased maximal bench strength by 5kg as compared to 2kg for the placebo group (Taylor et al., 2016). Therefore, supplementing with 24g of whey twice daily, or prior to

and immediately post exercise improves muscular strength two-fold over placebo. Thus, the research (Hoffman et al., 2007; Josse et al., 2009; Taylor et al., 2016) indicates that the combination of resistance exercise and protein consumption aids in strength gains.

Previous research outlines the benefits of utilizing whey protein, especially a whey protein with a higher leucine content, including increased muscle protein synthesis (Breen et al., 2011; Burd et al., 2011; Coffey et al., 2011; Ferguson-Stegall et al., 2011; Ivy et al., 2008; Tipton et al., 1999), improved body composition (Andersen et al., 2005; Cermak et al., 2012; Clifton et al., 2014; Dong et al., 2013; Hartman et al., 2007; Santesso et al., 2012; Vieillevoye et al., 2010; Walker et al., 2010; Wycherley et al., 2012), and overall improvement to muscular performance (Hoffman et al., 2007; Josse et al., 2009; Taylor et al., 2016). However, there is a lack of information comparing the effects of protein to that of a control group, a protein plus exercise group, and a group performing the same exercise program with no supplemental protein. Investigations into these differences will help paint a clearer picture of the synergistic effects of protein plus exercise, as well as its true effects on increases in lean mass and muscular performance.

Testosterone

Testosterone is a major circulating androgen, with approximately 6-7mg per day being produced by the male testes (Coffey, 1988). Testosterone acts by directly binding to receptors found in skeletal muscle stimulating protein synthesis. In the human body, approximately 98% of circulating testosterone is bound to serum proteins, in particular sex hormone-binding globulin (SHBG) and albumin. The remaining 2% is unbound or "free". Testosterone levels peak in men around the age of 20 years, with total testosterone values between 10.4-41.6 nmol/l, and free testosterone values between 0.17-0.73 nmol/l ng/dl (Mayo Clinic: Mayo Medical Laboratories,

2017). Bound testosterone has a high affinity for SHBG and is not readily available to most tissues for activation. As such, testosterone bound to albumin and free testosterone deemed bioavailable. However, with aging the concentration of SHBG increases resulting in a decrease in serum free testosterone and bioavailable testosterone (Matsumoto, 2002). In aging men, a decrease in resting testosterone level occurs and begins at approximately 30 years of age, and continues at a rate of 1% for total testosterone per year, and roughly 2% per year thereafter for free testosterone (Feldman et al., 2002; Harman et al., 2001; Marty, Liu, Samuel, Or, & Lane, 2017). Aging causes sleep patterns to change significantly over time as well, causing more disturbances, negatively effecting resting testosterone values (Kryger, Monjan, Bliwise, & Ancoli-Israel, 2004; Vitiello, Larsen, & Moe, 2004). The observed decreases in resting serum testosterone levels contribute to declines in both muscle mass and muscle strength, and is referred to as "andropause" or hypogonadism (Matsumoto, 2002). As for muscle function, high levels of testosterone have been shown to be positively related to both muscle strength and muscle mass (Harman, 2005). Therefore, it is paramount to understand any potential changes that may result from resistance- or endurance-based training techniques, as well as how sleep patterns effect resting testosterone levels within middle-age men.

Testosterone and Exercise. Testosterone plays an important role in the adaptations from resistance exercise, allowing for muscular growth and increased strength. Following heavy resistance exercise testosterone is elevated, however, with age there is a decreased acute response in testosterone levels (Vingren et al., 2010). Past research involving endurance-based exercise has focused mainly on female exercisers, while recent studies have observed the effects of endurance exercise on testosterone in males. Endurance exercisers have lower testosterone values at rest when compared to their untrained counterparts. Despite the lower levels at rest,

there is no current evidence suggesting that there is a dysfunction associated with the testosterone's ability to act androgenically when introduced to exercise-based stimuli (Hackney, 1989). Hence the need to further understand the effects various training techniques have on resting testosterone levels in the aging male.

Testosterone and Endurance Training. Endurance training elicits no increase in resting testosterone, both acutely and over prolonged periods, but most past research was performed on women instead of men (Hackney, 1989). Therefore, the effects of endurance training needs clarification within the male population. Previous investigations (Hackney, Sinning, & Bruot, 1988; Hakkinen, Pakarinen, Alen, Kuahanen, & Komi, 1987; Strauss, Lanese, & Malarkey, 1985; Wheeler, Wall, Belcastro, & Cumming, 1984) established that men who partake in high-mileage endurance training exhibit lower testosterone levels at rest. To determine the testosterone profiles of endurance trained individuals compared to that of sedentary individuals, Hackney and colleagues (1998) observed resting values of 53 endurance trained and 35 sedentary individuals between the ages of 20-40. Highlighting that endurance trained individuals exhibit lower resting values of testosterone than that of the sedentary individuals, and free testosterone values between 55-85% of the values assessed in the sedentary group (Hackney et al., 1998). This decreased testosterone level at rest is due to reduced testicular blood flow that occurs as the length of exercise is extended past 60 minutes (Hackney, 1998). Endurance training interventions of young sedentary men found that 6 months of training consisting of running 3 days per week and averaging 35 miles per week lowered serum testosterone levels by 6 nmol/l below baseline values (Wheeler et al., 1991). Following 8 weeks of an intensive training program that consisted of continuous cycling at 70% and intervals of 85-150%, 5 days per week for more than 90 minutes, testosterone measures decreased by 33% by 4 weeks and returned to

baseline values after 8 weeks (Hackney et al., 1989). Thus, indicating that endurance-based training has no effect on raising testosterone like that of resistance training, but may actually cause decreases in resting values over time. The effects of performing a maximum endurance test between endurance trained men versus untrained men demonstrated that the endurance trained individuals began at lower resting values, 137 nmol/l/h compared to 209 nmol/l/h, respectively. Upon test conclusion, the endurance trained individuals post exercise testosterone values were 41 nmol/l/h higher than at rest compared to the 13 nmol/l/h increase of the untrained individuals. These findings demonstrated that the trained individuals had a larger increase in testosterone values from rest compared to the untrained individuals, however, they were still below the values assessed for untrained individuals at rest (Hackney et al., 1997). Indicating that the ability of the testosterone to react to exercise stimuli still remains but the long-term endurance training lowers the overall starting point at rest. Overall, research (Hackney et al., 1997; Hackney et al., 1998, 1989; Wheeler et al., 1991) indicates endurance training does not involve an increase in testosterone values following prolonged interventions, rather causing a decrease in the resting values of individuals both young and old. However not all exercisers participate in endurance training activity, therefore creating a need for clarification on the effects of resistance training on resting testosterone values.

Testosterone and Resistance Training. Endurance training elicits no increases in resting testosterone values (Hackney et al., 1997; Hackney et al., 1998, 1989; Wheeler et al., 1991), however, not all individuals partake in endurance training and opt for resistance-based exercise. Testosterone levels increase in relation to exercise-related variables such as intensity (Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003), volume (Fry, Kraemer, & Ramsey, 1998), acute (Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2003) versus sequential bouts (Volek, Kraemer,

Bush, Incledon, & Boetes, 1997), and type of muscular contractions performed (Bamman et al., 2001; Durand et al., 2003). Volek and colleagues (1997) investigated the effects on testosterone pre- and post-exercise following the bench press and jump squat exercises, separated by one week. Testosterone significantly increased post-exercise by 1.5 and 2.5 nmol/l following the acute bouts of bench press and jump squat, respectively (Volek et al., 1997). Illustrating the acute effects of upper- and lower-body resistance exercise has on testosterone, but the long-term exercise effects need clarification. Investigating the effects of short-term resistance exercise in younger males, total testosterone increased 7 nmol/l above baseline compared to that of a non-exercise based control group following three bouts of resistance exercise separated by 48 hours (Willoughby & Taylor, 2004). Evidence of testosterone levels increasing to a new resting value following short-term repeated bouts of resistance exercise, but the effects of an extended resistance training program is still unclear. Total testosterone and free testosterone exhibited no changes over the course of a 28-day heavy resistance training program when assessed in a group of young trained males (Willoughby et al., 2014). Illustrating that if testosterone values are to change due to length of training then the program period may need to be extended past four weeks. Following 10 weeks of training, back squat 1RM increased 24kg and 11kg, while free testosterone increased 0.01 nmol/l and remained unchanged for a group of younger and older previously untrained men, respectively. However, when assessing all time points, free testosterone was significantly higher for the young 30 year olds over that of the 60 year olds, reaching a difference of 0.02 nmol/l between the groups (Kraemer et al., 1999). Indicating that long-term resistance training is more beneficial at increasing free testosterone levels for younger individuals than that of their older counterparts. Izquiardo and colleagues (2006) investigated the effects of training to failure against that of not training to failure on muscular performance

measures and testosterone in trained young males over the course of 16 weeks. While muscular performance increased for both groups, testosterone changes differed. Testosterone levels increased 12% above initial baseline resting measures following the 16 weeks for subjects not training to failure, while individuals training to failure experienced no change from resting measurements (Izquierdo et al., 2006). Therefore, while resistance training to failure elicits increases in muscular performance it has no effect on resting total testosterone levels, however free testosterone values were not reported. These previous investigations (Izquierdo et al., 2006; Kraemer et al., 1999; Volek et al., 1997; Willoughby et al., 2014; Willoughby & Taylor, 2004) highlight the effects resistance training has on increasing measures of testosterone values within the young, trained population, and has limited investigations on the effects of older populations, or middle-aged untrained men. While the studies were carried out over various lengths of time, it is evident that resistance training is a stimuli appropriate to increase testosterone values over longer periods. However, very few studies report free testosterone values which still makes it unclear as to how free testosterone is truly affected by training stimuli or length of training program. Thus, leaving room to explore the response of free testosterone values to various forms of resistance-based exercise programs such as APRE, especially in middle-aged men. While exercise, specifically resistance-based, increases testosterone values it is equally important to understand how sleep is associated with resting testosterone values.

Testosterone and Sleep. Sleep patterns change significantly over time, causing more disturbances to occur with age (Kryger et al., 2004; Vitiello et al., 2004). Testosterone itself follows a diurnal rhythm, peaking in the morning and reaching its minimum in the evening (Plymate, Tenover, & Bremner, 1989). The nocturnal rise of testosterone is highly associated with deep sleep (Veldhuis, Iranmanesh, Godschalk, & Mulligan, 2000) and rapid eye movement

(REM) cycles (Schiavi, White, & Mandeli, 1992). Axelsson et al (Axelsson, Ingre, Åkerstedt, & Holmbäck, 2005) demonstrated that young men experienced an exponential rise in testosterone following nighttime and daytime sleeping periods. This leads researchers to believe that is not necessarily the time of day the sleep occurs, but rather the ability to reach deep sleep or REM. Sleep deprivation (Åkerstedt, Palmblad, de la Torre, Marana, & Gillberg, 1980; González-Santos, Gajá-Rodíguez, Alonso-uriarte, Sojo-aranda, & Cortés-Gallegos, 1989) and experimentally fragmented (Luboshitzky, Zabari, Shen-Orr, Herer, & Lavie, 2001) sleep causes a decline in circulating androgen levels and disrupts the natural rise of testosterone during sleep. Thus, indicating the potential issues with circulating androgens with the inherent changes in sleep patterns as we age. Middle-aged men (Luboshitzky, Shen-Orr, & Herer, 2003) already exhibit signs of decreased night secretion of testosterone from sleep, and older men (Bremner, Vitiello, & Prinz, 1983) demonstrate lower testosterone levels than that of younger individuals when sleep is disturbed. Therefore, tracking sleep to determine if an individual is suffering from sleep related decreases in testosterone values is imperative.

Testosterone acutely increases following a bout of resistance exercise (Ahtiainen et al., 2003; Volek et al., 1997), and it is known that individuals who engage in chronic endurance training experience lower resting testosterone values than their sedentary peers (Hackney, Sinning, & Bruot, 1988; Hakkinen, Pakarinen, Alen, Kuahanen, & Komi, 1987; Strauss, Lanese, & Malarkey, 1985; Wheeler, Wall, Belcastro, & Cumming, 1984), yet questions remain. Previous research focuses on the acute (Volek et al., 1997)and prolonged effects of resistance training (Izquierdo et al., 2006; Kraemer et al., 1999; Willoughby et al., 2014; Willoughby & Taylor, 2004) on testosterone, but free testosterone is rarely reported. Therefore, a gap in the literature exists focusing on changes in resting free testosterone following extended periods of

resistance training, specifically APRE, and in middle-aged men.

Conclusion

APRE indicates to be a beneficial form of UP within the traditional exercise setting, but more research is still needed in investigating changes in lean mass and muscular performance outside that of the athletic population. The benefits of increased protein intake, especially protein with higher leucine content, on changes in body composition and muscular performance measures are shown in young and older populations yet lack substantial findings within the cohort of middle-aged adults. Total testosterone values increase following both acute and long-term resistance exercise, however free testosterone is rarely reported following any length of intervention. With free testosterone decreasing with age more information is needed to illustrate how free testosterone values change over the course of a resistance training program. Therefore, the purpose of this study is to investigate the effects of protein supplementation and APRE on measurements of body composition, muscular performance, and free testosterone in men aged 35-55 years.

Chapter 3: Methods

Study Design

This study employed a randomized controlled trial design. Each subject reported to the Exercise Science Research Center for a total of four visits. The first visit (week 0; baseline) included demographic measurements, sleep survey, body composition, 6ml blood draw for baseline free testosterone measures, one-repetition maximum testing, and repetitions to failure.

Exclusion Criteria

Subjects were excluded from participation if they had uncontrolled cardiovascular disease, metabolic disease, hypertension, were currently taking testosterone supporting supplements, testosterone replacement therapies, or taking any protein supplementation products. Subjects who had participated in a resistance based exercise program within the previous six months were also excluded in accordance with previous studies utilizing resistance training protocols (Campos et al., 2002; Ivey et al., 2000).

Subjects

Previous research involving the topics of resistance training and/or protein supplementation predominantly observed younger and older populations of both men and women, therefore the subjects for this research study consisted of middle-aged men in an effort to understand the changes in body composition, muscular performance, and free testosterone. Based on power calculations of 80% at alpha level of .05 from pilot data in our own lab, and accounting for an attrition rate of 30%, a total of 48 subjects were recruited to participate in the study (Faul, Erdfelder, Lang, & Buchner, 2007). Inclusion criteria included the following: 35-55 years of age, classified as low-risk individuals as categorized by the American College of Sports Medicine (ACSM) risk stratification (Reibe, Ehrman, Liguori, & Magal, 2017), and have

refrained from resistance training for at least six months in accordance to previous studies utilizing resistance training protocols (Campos et al., 2002; Ivey et al., 2000). Before baseline testing began, each subject completed a signed statement of informed consent, a questionnaire regarding health history, and the Pittsburgh Sleep Quality Index (PSQI; sensitivity of 89.6% and specificity of 86.5%; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) questionnaire previously approved by the University of Arkansas Institutional Review Board. Subjects were recruited from the Northwest Arkansas area.

Estimated Energy Intake/Dietary Inventories

Subjects recorded their dietary intake for 24 hours prior to the first testing session, and then repeated the diet 24 hours before the following three testing sessions on weeks 4, 8, and 12. The subjects' diets were not standardized, but subjects were instructed not to change their dietary habits during the course of the study. The dietary records were analyzed with Nutritionist Pro dietary assessment software (Axxya Systems, LLC. Woodinville, WA) to determine the average total protein composition of their diet. This information was utilized to assess any changes in dietary protein intake for supplement groups and how that affects body composition, muscular performance, and free testosterone measures.

Each subject was instructed to abstain from vigorous exercise, alcohol, and caffeine 24 hours before participation. Food logs were distributed to all subjects to be completed 24 hours prior to each trial and they were instructed to maintain the same dietary habits prior to each testing session. In order to determine any changes in daily protein intake over the course of the study, subjects recorded two random days of diets during week 12 of the protocol. To account for dietary consumption and consistency of blood samples on testing days, subjects fasted three

hours prior to each trial. To ensure consistency in blood sampling, blood was collected ± 1 hour of original sample on weeks 4, 8, and 12 (Gray, Feldman, McKinlay, & Longcope, 1991).

Procedures

Anthropometric and Body Composition Assessment (Weeks 0, 4, 8, 12). Total body mass was determined on a calibrated electronic scale with a precision of ± 0.02 kg (Pelstar, McCook, IL). Body composition was determined using a calibrated Lunar Prodigy dual-energy x-ray absorptiometry (DEXA) (DEXA; General Electric, Fairfield, CT) by personnel with x-ray technology training. The DEXA body composition test involved the participant lying in the supine position in a pair of shorts/t-shirt. A low dose of radiation, 0.4 micro-Gray (μGy) scanned their entire body for approximately 6 minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass (Zack, Nickols-Richardson, Herbert, & Poole, 2002; Rothney et al., 2012). For the purposes of this study we only analyzed changes in fat mass and lean mass over the course of the intervention. DEXA is a highly reliable and valid assessment of body composition (Ball, Altena, & Swan, 2004; Eston, Rowlands, Charlesworth, Davies, & Hoppitt, 2005; Nana, Slater, Stewart, & Burke, 2015; Silva, Minderico, Teixeira, Pietrobelli, & Sardinha, 2006).

Venipuncture. The blood sampling procedure (i.e., venipuncture) and subsequent serum analysis was approved by the Institutional Biosafety Committee (IBC protocol # 13021) at the University of Arkansas. Subjects underwent a single stick blood draw of 6 mL (BD Diagnostics, Franklin Lakes, NJ), by a trained technician, using a 21-gauge needle (BD Diagnostics, Franklin Lakes, NJ). Samples were allowed to sit at room temperature for a minimum of 30 minutes and no longer than one hour. Samples were then centrifuged at 2500 rpm for a time of 15 minutes in

a refrigerated centrifuge (Quest Diagnostics Inc., Madison, NJ). Upon completion of centrifuging, samples were aliquoted into 1.5 ml cryotubes. Samples were then immediately stored in a -80 ˚C freezer until time for analysis. The blood drawn during this assessment was used for measuring free testosterone levels for baseline measures and potential subsequent changes in weeks 4, 8, and 12.

Serum Free Testosterone. Serum free testosterone was determined using an ELISA kit (REF DKO015; Diametra, Immunodiagnostic Systems Ltd., Boldon-UK). The testosterone ELISA kit is based on competitive binding of human free testosterone from serum samples and enzyme-labeled testosterone. Per manufacturer's instructions (Diametra, Immunodiagnostic Systems Ltd., Boldon-UK) prior to analysis, calibrators were conducted at various picograms per milliliter (pg/ml) concentrations of testosterone, and were stable for a period of 6 months at 2-8 ˚C. Samples remained frozen at a minimum of -20 ˚C until analysis occurred and were not repeatedly thawed and refrozen in an effort to maintain sample integrity, therefore all relevant samples that were removed from the freezer and thawed were analyzed on that day. When preparing the wash solution, the contents of the "10X Concentration Wash Solution" vial with distilled water to equate to a final volume of 500 ml prior to use. Reagents sat at room temperature for a minimum of 30 minutes. Coated microwell strips that were unused were released securely from the foil pouch containing the desiccant and subsequently stored 2-8 ˚C. In an effort to avoid contamination, the unused reagents were not transferred back into original vials. For the purposes of accuracy, the determination was performed in duplicates, preparing two wells for each point on the calibration curve, two per control, two per sample, and one for Blank. Samples were then incubated for a total of one hour at 37 ˚C. Contents were then removed and the wells washed 5 times with 500 microliters (µL) of diluted wash solution.

Absorbance was read at 450 nm with reference against a wavelength of 620-630 nm or against Blank within 5 minutes. Mean absorbance (Em) was calculated for each sample at all points on the calibration curve. Mean absorbance concentration were then plotted against that of the calibrators, drawing the curve of best-fit. From the manufacturer, within and between assay variability is ≤ 10% for each, and the assay has a sensitivity of 0.06 pg/ml. Serum free testosterone has been shown to be a valid and reliable method of measuring increases, as well as decreases, in androgen markers in men (Boyle, Compton, Rigg, Menon, & McCann, 1984).

Muscular Strength and Endurance Testing (Weeks 0, 4, 8, 12). Subjects performed 1RM muscular strength tests for the upper- and lower-body using a plate-loaded flat barbell bench press (Hammer Strength, Rosemont, IL) and plate-loaded leg press (Hammer Strength, Rosemont, IL), respectively. Muscular performance values were utilized to determine potential changes that occurred throughout the study resulting from supplementation or exercise intervention. The ACSM (Reibe et al., 2017) recommends the use of the bench- (ICC=.99, coefficient of variance = 6.5%) and leg-press (ICC=.99, coefficient of variance = 3.3%), and both tests have shown to be a valid and reliable method to assess upper- and lower-body strength (Levinger et al., 2009; Reynolds, Gordon, & Robergs, 2006). The 1RM was established using protocol specified by the ACSM. These tests were performed at each of the four testing sessions. For the bench press, subjects lied horizontally on their back, hands placed slightly greater than shoulder width apart, lowering the bar until touching the chest and then returning it to a full arms-length away from the body. A missed repetition consisted of the subject being unable to return the weight to a full arm's length from the body (Desgorces, Berthelot, Dietrich, & Testa, 2010). For the leg press, subjects were required to keep both feet flat on the foot platform and to lower the weight until the knees were at 90°, and to maintain a constant rhythm without resting

after repetitions. A missed repetition consisted of the subject being unable to return the weight to a full leg's length from the body (Wilborn et al., 2013). Subjects began the 1RM bench- and leg-press test by first completing eight to ten repetitions at approximately 50% and 120% of their established bodyweight, respectively. Following a two-minute rest, three to five repetitions were performed with 5-10 kg additional for the bench and 20-40 kg added for leg press. After another 1-2 minute rest, the weight was increased an additional 5-10 kg for bench or 20-40 kg for leg press. Subjects then completed 2-3 repetitions before resting for 1-2 minutes. From this point forward, the weight was increased gradually, until an official 1RM is reached. The rest period between each successful lift was two minutes.

Following completion of the 1RM testing and a 5-minute rest period, subjects performed as many repetitions as possible at 75% of the 1RM to test muscular endurance. Subjects performed as many repetitions as possible until they could no longer perform the lift on their own or proper form was broken (Desgorces, Berthelot, Dietrich, & Testa, 2010; Wilborn et al., 2013). This protocol was in accordance to studies measuring muscular performance by Cooke et al (2014) and Wilborn et al (2013) (Cooke et al., 2014; Wilborn et al., 2013). Volume was then calculated for the bench- and leg-press by multiplying the number of repetitions completed for each exercise by the 75% of the 1RM for the respective exercise.

Group Assignment. Following the initial visit subjects were randomly placed into one of four groups for a 12-week period: whey protein (PRO), autoregulatory progressive resistance exercise (APRE), whey plus autoregulatory progressive resistance exercise (PAPRE), or the control (CON) group.

The PRO group was given supplemental protein consisting of 25g of protein (Elite Whey, Dymatize, Dallas, TX). The PRO group was instructed to take one serving (25g) twice daily, one

serving in the morning between breakfast and lunch, and the other in the afternoon before dinner. They continued living life without any form of resistance-based training but were allowed to maintain any aerobic-based physical activity. PRO group reported to the lab for testing and refill of protein supplementation purposes on week 0, 4, 8, and 12.

The APRE group were prescribed a three day per week APRE resistance training program. Resistance exercises for the bench- and leg-press were utilized for the APRE method. The remainder of the resistance training protocol consisted of the following exercises: biceps curls, triceps extensions, leg extensions, leg curls, and lat pull-downs. For the remaining exercises, individuals performed three sets of 10 repetitions at self-selected weights, and upon successful completion of all three sets of all 10 repetitions were asked to increase weight as needed. Subjects were prescribed the remainder of the exercises in an attempt to complete a comprehensive full body exercise protocol. Subjects reported to the lab for testing on week 0, 4, 8, and 12.

The PAPRE group was given supplemental protein consisting of 25g of protein (Elite Whey, Dymatize, LLC. Dallas, TX) to be taken twice daily, as well as prescribed a three day per week APRE resistance training program. On non-training days, one serving of protein was consumed in the morning between breakfast and lunch, and the other in the afternoon before dinner. On training days, one serving of protein was consumed immediately following the exercise bout, while the second serving was either be consumed in the morning or in the afternoon depending on time of training. Subjects reported to the lab for testing on week 0, 4, 8, and 12.

The CON group had no supplementation or exercise regimen to follow, they were asked to continue to live their normal lives and came in solely for testing purposes on week 0, 4, 8, and 12.

APRE and Resistance Training Protocol. Subjects in the exercise groups completed a supervised resistance training program as prescribed and utilized the facilities on the University of Arkansas campus. Subjects completed three training sessions per week for the duration of the study period (Chrusch et al., 2001). Resistance exercises for the bench- and leg-press utilizing the APRE method. Utilizing each subjects' baseline 1RM, an estimated 6RM, or 85% of the established 1RM (Baechle, Earle, & Wathen, 2000), was calculated for bench- and leg-press only. For bench- and leg-press exercises, subjects performed four total sets per exercise. The first set was comprised of 10 repetitions at 50% of the 6RM value. The second set consisted of six repetitions at 75% of 6RM, with the third set performed "to failure" at 100% 6RM. The fourth set utilized an adjusted load based off of the number of repetitions completed in the third set. After determining the load adjustment, subjects adjusted the load used in the third set, and completed an additional "to failure" set. Based off of the number of repetitions completed during the fourth set, each subject determined their appropriate load adjustment for the next training session.

The remainder of the resistance training protocol consisted of the following exercises: biceps curls, triceps extensions, leg extensions, leg curls, and lat pull-downs. For these exercises, individuals performed three sets of 10 repetitions at self-selected weights, and upon successful completion of all three sets of all 10 repetitions were asked to increase weight as needed. Subjects were prescribed the remainder of the exercises in an attempt to complete a comprehensive full body exercise protocol to maximize positive changes in body composition,

muscular performance. A rest period of 1 minute separated each set. Subjects completed three training sessions per week on non-consecutive days for the duration of the study period (Chrusch et al., 2001).

Supplementation Protocol. Subjects who were randomly assigned to receive protein supplementation were given four weeks' worth of supplementation at week 0, 4, and 8. Subjects were instructed to ingest the supplement for the duration of the 12-week study period. Daily supplementation consisting of 25g of whey protein (Elite Whey, Dymatize, LLC. Dallas, TX) to be ingested twice daily, totaling 50g daily, in an effort to simulate pulse feeding of protein throughout the day as demonstrated by Areta and colleagues (Areta et al., 2013). Subjects were instructed to ingest one scoop in the morning between breakfast and lunch, and the second scoop in the afternoon between lunch and dinner. On training days, subjects placed in the PAPRE group were instructed to consume one scoop immediately following exercise and the other scoop in either the morning or afternoon based on time of training. Compliance monitoring was accomplished by having subjects return empty bags of the supplement or the unused bags of protein at each testing session (Cooke et al., 2014).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS version 24; Chicago, IL.) was used to analyze all data. One-way ANOVAs were utilized to assess differences between the groups at baseline for all demographic variables. To assess the effects of protein supplementation and the APRE protocol on performance and physiological variables within and between groups, investigators utilized repeated measures ANOVAs (group [CON vs. PRO vs. APRE vs. PAPRE] x time [baseline, week 4, week 8, week 12]) for strength and endurance, as well as serum free testosterone variables. Repeated measures ANOVAs (group [CON vs. PRO vs. APRE vs.

PAPRE] x time [baseline, week 12]) were utilized for body composition, volume, and total protein intake variables over the course of the study. Outliers were explored using boxplots. If the outliers cause significance to rise or fall past .05 they were removed, if no changes occurred then they remained within the sample. When significance was observed within the repeated measures, follow-up univariate analyses were conducted to determine where significant differences exist, followed by paired-samples t-tests to examine time and group by time interactions. When model assumptions were violated, Greenhouse-Geisser adjusted p-values were reported. For statistically significant F-scores, simple main effects were analyzed with the inclusion of Bonferonni corrections. An alpha level of $p < .05$ defined significance at the multivariate levels.

Independent variables consisted of the four assigned groups (PRO, APRE, PAPRE, and CON). Dependent variables were lean mass, fat mass, bench- and leg-press 1RM, bench- and leg-press maximum repetitions, bench- and leg-press volume, serum free testosterone and total protein intake.

Chapter 4: Results

A total of 35 (43.3 ± 6.5 years) subjects completed the study. There was a 97.8% compliance rate for the 17 subjects who consumed protein supplementation and an 89.9% compliance rate for the 17 subjects required to complete the APRE training. There were no significant differences at baseline between groups for demographic variables (Table 1).

Table 1
Initial Subject Demographic Information

	PRO (n = 8)	APRE (n = 8)	PAPRE (n = 9)	CON (n = 10)	p-value
Age (years)	43.5 ± 6.5	42.3 ± 5.1	45.11 ± 8.4	42.5 ± 6.5	.81
Height (cm)	177.70 ± 8.25	178.90 ± 7.06	178.39 ± 3.21	182.61 ± 6.29	.36
Weight (kg)	96.74 ± 13.16	93.38 ± 12.89	92.91 ± 14.31	86.97 ± 11.49	.45
Body Fat (%)	35.9 ± 3.5	31.6 ± 7.3	31.3 ± 4.0	29.1 ± 8.4	.17

Note. Data are presented as Mean ± *SD*. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline. Significance was set at $\alpha < 0.05$.

Body Composition

Fat Mass. No significant differences existed between groups at baseline (F [3, 31] = 1.68, p = .19; Table 2). Repeated measures ANOVA revealed no significant group by time interaction between baseline and 12-weeks (F [3, 31] = 1.33; p = .28; Table 2, Figure 1).

Lean Mass. Groups were not significantly different at the baseline time point (F [3, 31] = .56, p = .65). Repeated measures ANOVA indicated a trend for a group by time interaction between baseline and 12-weeks (F [3, 31] = 2.43; p = .08; Table 2). Follow-up paired-samples t-tests showed a significant difference between baseline and 12-week time points for the exercise

groups ($p \leq .05$; Figure 2). The APRE (t (7) = -2.79, $p = .03$) and PAPRE (t (8) = -3.11, $p = .01$) groups significantly increased lean mass, each by 2.7%, between baseline and week 12 (Table 2).

Table 2
Body Composition

	PRO (n = 8)	APRE (n = 8)	PAPRE (n = 9)	CON (n = 10)	p-value T	p-value G x T
Fat Mass					.10	.28
Baseline	33.94 ± 6.40	29.01 ± 9.80	28.22 ± 7.40	24.74 ± 10.25		
Week 12	33.69 ± 4.77	26.85 ± 7.51	27.82 ± 7.17	24.72 ± 10.52		
Total % Δ	~ 0.0	- 7.5	- 1.4	~ 0.0		
Lean Mass					.001	.08
Baseline	59.18 ± 8.00	60.89 ± 4.89	60.83 ± 7.27	57.63 ± 4.72		
Week 12	59.79 ± 8.32	62.52 ± 4.79	62.46 ± 7.74	57.59 ± 5.04		
Total % Δ	+ 1.0	+ 2.7	+ 2.7	~ 0.0		

Note. Data are presented as Mean ± *SD* and in kilograms (kg). Total % Δ = percent change over course of intervention. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline ($p > .05$). T = Time; G x T = Group by time interaction. Significance was set at $\alpha < 0.05$.

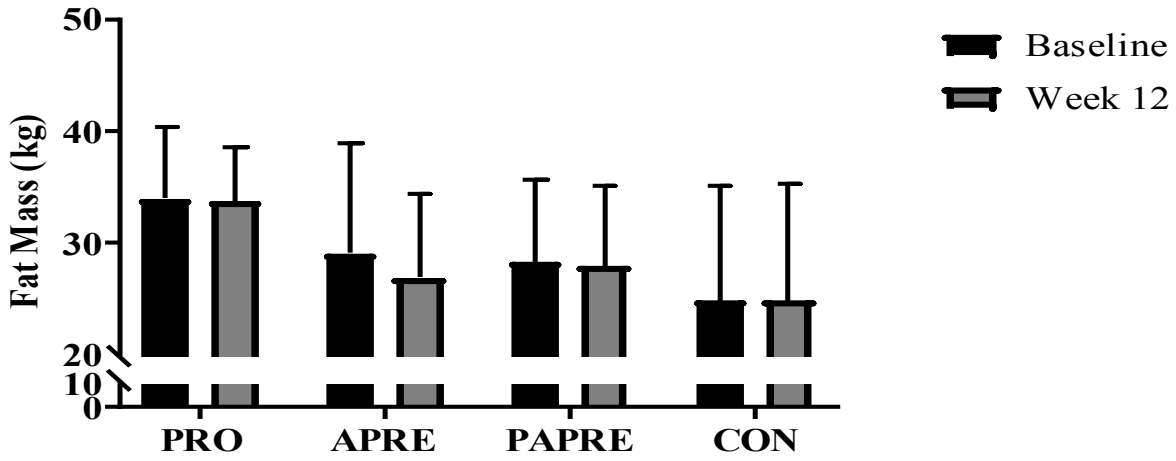

Figure 1. Data are presented as Mean ± *SD* and in kilograms (kg). Fat mass (kg) for baseline and 12-week testing. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control

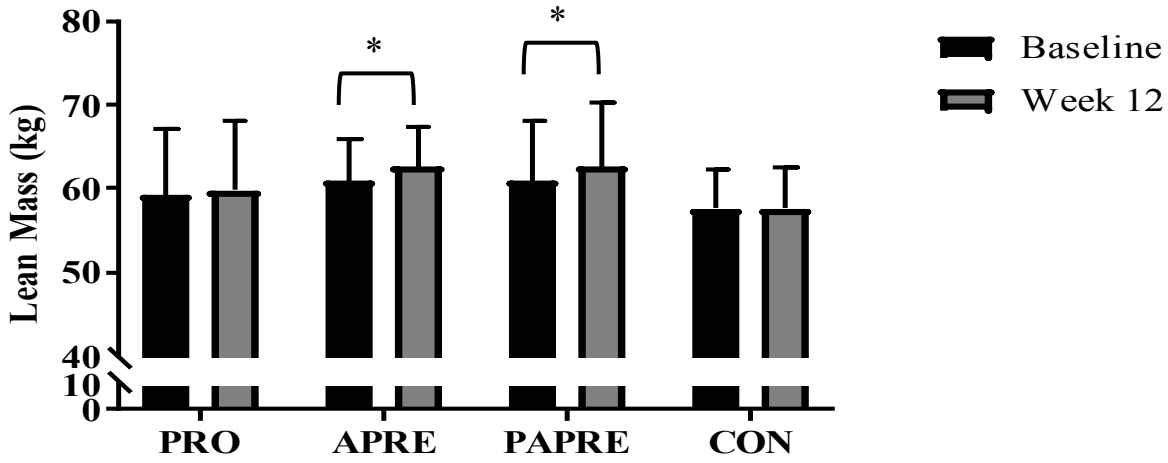

Figure 2. Data are presented as Mean ± *SD* and in kilograms (kg). Lean mass (kg) for baseline and 12-week testing. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. * Indicates significant difference between baseline and 12-week testing ($p < .05$) following paired-samples t-tests.

Muscular Strength

Bench Press 1RM. Baseline analysis found no significant differences between groups (F [3, 31] = 1.62, p = .21). Repeated measures ANOVA determined a significant group-by-time interaction for the 12-week intervention (F [9, 93] = 16.43; p < .001; Table 3). Follow-up paired-samples t-tests revealed a significant difference between time points for the exercise groups while the non-exercise groups exhibited no differences between time points. The APRE group produced significantly different 1RM values between baseline and week 4 (t (7) = -8.79, p < .001), as well as between week 8 and week 12 (t (7) = -4.71, p = .00 (Figure 3). While the PAPRE group significantly increased 1RM values between baseline and week 4 (t (8) = -7.84, p < .001), between week 4 and week 8 (t (8) = -4.54, p = .00), as well as between week 8 and week 12 (t (8) = -3.52, p = .01) (Figure 3). Over the course of the study the APRE and PAPRE groups increased bench press 1RM by 28%, while the PRO and CON groups increased by 9.6% and 2.3%, respectively (Table 3).

Leg Press 1RM. The group differences were non-significant at baseline (F [3, 31] = .51, p = .68). Repeated measures ANOVA indicated a significant group-by-time interaction for the 12-week intervention (F [9, 93] = 20.05, p < .001). Follow-up paired-sample t-tests reported a significant difference between time points for all four groups. The PRO group significantly increased 1RM values between week 4 and week 8 (t (7) = -3.84, p = .01), while the CON group produced significantly increased 1RM values between baseline and week 4 (t (9) = -6.33, p < .001) (Figure 4). The APRE group significantly increased 1RM values between baseline and week 4 (t (7) = -5.27, p = .00), as well as between week 8 and week 12 (t (7) = -9.42, p < .001) (Figure 4). The PAPRE group's 1RM values were significantly greater between baseline and week 4 (t (8) = -7.09, p < .001), between week 4 and week 8 (t (8) = -13.61, p < .001), as well as

between week 8 and week 12 (t (8) = -4.66, p = .00) (Figure 4). Over the course of the study the PRO and CON groups increased leg press 1RM by an average of 16%, while the APRE and PAPRE groups increased by 46% and 67%, respectively (Table 3).

Table 3
Muscular Strength

	PRO	APRE	PAPRE	CON	*p* - value	
	(*n* = 8)	(*n* = 8)	(*n* = 9)	(*n* = 10)	T	G x T
Bench Press					< .001	< .001
Baseline	55.97 ± 17.48	71.31 ± 18.38	63.89 ± 14.97	59.55 ± 7.48		
Week 4	56.82 ± 16.16	80.11 ± 18.69	73.74 ± 17.24	60.23 ± 8.04		
Week 8	60.23 ± 17.01	86.65 ± 20.67	78.29 ± 15.59	61.36 ± 6.52		
Week 12	61.36 ± 14.78	90.91 ± 19.85	81.57 ± 16.60	60.91 ± 19.85		
Total % Δ	+ 9.6	+ 27.5	+ 27.7	+ 2.3		
Leg Press					< .001	< .001
Baseline	271.59 ± 57.52	269.03 ± 73.40	272.98 ± 63.26	243.86 ± 42.87		
Week 4	286.36 ± 51.41	325.28 ± 60.48	355.05 ± 68.50	262.05 ± 43.40		
Week 8	306.25 ± 57.57	351.42 ± 67.96	398.74 ± 73.85	276.36 ± 49.45		
Week 12	313.35 ± 48.42	393.47 ± 70.73	455.05 ± 97.23	286.59 ± 52.14		
Total % Δ	+ 15.4	+ 46.3	+ 66.7	+ 17.5		

Note. Data are presented as Mean ± *SD* and in kilograms (kg). Strength values are presented as one repetition maximum (1RM) in kilograms (kg); Total % Δ = percent change over course of the 12-week intervention. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline ($p > .05$). T = Time; G x T = Group by time interaction. Significance was set at $\alpha < 0.05$.

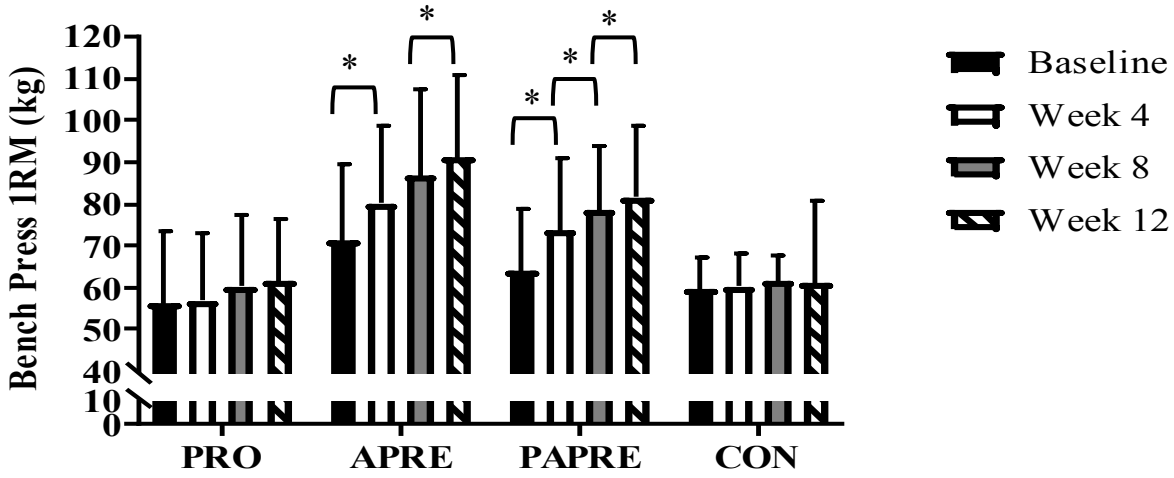

Figure 3. Data are presented as Mean ± *SD* and in kilograms (kg). Bench press 1RM (kg) for baseline, week 4, week 8, and week 12 testing. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. * Indicates significant differences between time points ($p <$ .016) following paired-samples t-tests with Bonferroni correction.

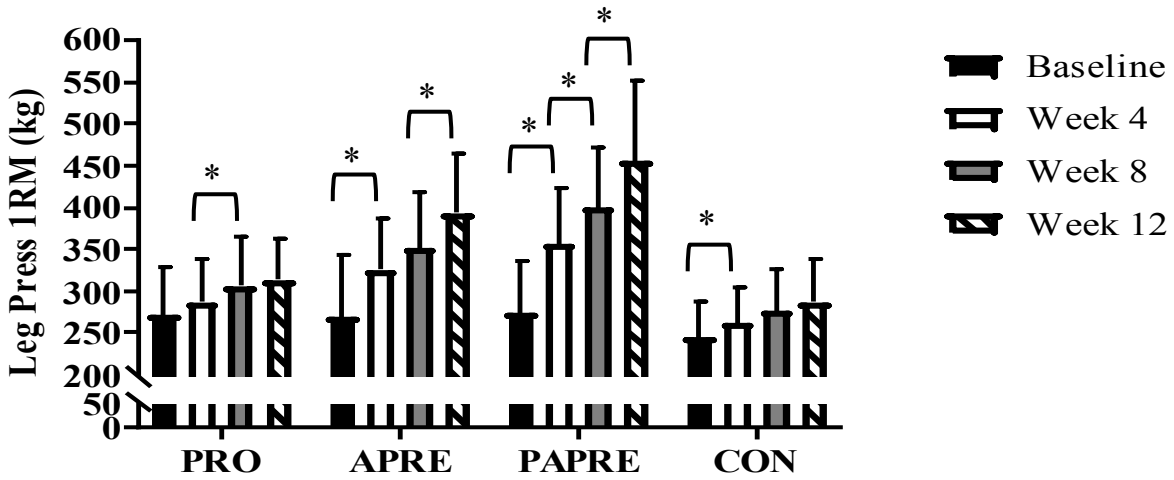

Figure 4: Data are presented as Mean ± *SD* and in kilograms (kg). Leg press 1RM (kg) for baseline, week 4, week 8, and week 12 testing. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. * Indicates significant differences between time points ($p <$ .016) following paired-samples t-tests with Bonferroni correction.

Muscular Endurance

Bench Press Repetitions. Baseline analysis determined no significant differences existed between groups (F [3, 31] = .36, p = .79). Repeated measures ANOVA reported no significant group by time interaction for the 12-week intervention between any of the groups (F [9, 93] = 1.15, p = .43) (Table 4). All groups maintained the same number of repetitions at 75% of their 1RM throughout the course of the 12-week intervention, despite the increase in weight lifted.

Bench Press Volume. Groups produced no significant differences in bench press volume at the baseline testing time point (F [3, 31] = .99, p = .41). Repeated measures ANOVA revealed no significant group-by-time interaction for the 12-week intervention, however there was a trend toward significance (F [3, 31] = 2.45, p = .08) (Table 4). Follow-up paired-sample t-tests revealed a significant difference between baseline and week 12 time points for the PRO and PAPRE groups. The PRO (t (7) = -2.98, p = .02) and PAPRE (t (8) = -3.19, p = .01) groups produced significantly greater bench press volumes between baseline and week 12. Calculation of effect sizes between baseline and week 12 testing determined that PRO exhibited a small effect (d = 0.34), APRE a moderate effect (d = 0.58), PAPRE a moderate effect (d = 0.59), and CON exhibited a very small effect (d = 0.14).

Leg Press Repetitions. Groups exhibited no significant differences at baseline (F [3, 31] = .89, p = .46). Repeated measures ANOVA determined no significant group by time interaction for the 12-week intervention between any of the groups (F [9, 93] = 5.87, p = .67) (Table 4). Despite a large increase in leg press 1RM values for the APRE and PAPRE groups, subjects were able to maintain the same number of repetitions at 75% of 1RM throughout the course of the 12-week intervention, as did the PRO and CON groups.

Leg Press Volume. Groups did not significantly differ at baseline testing for leg press volume (F [3, 31] = .55, p = .65). Repeated measures ANOVA indicated a significant group-by-time interaction between baseline and 12 weeks (F [3, 31] = 2.45, p = .01) (Table 4). Follow-up paired-sample t-tests revealed a significant difference between baseline and week 12 time points for the PAPRE group only. The PAPRE group significantly increased leg press volume by 76.7% between baseline and week 12 (t (8) = -4.19, p = .00). Overall, the PAPRE increased leg press volume by 63% over that of the PRO, APRE, and CON groups over the course of the 12-week intervention (Table 4).

Table 4
Muscular Endurance

	PRO (n = 8)	APRE (n = 8)	PAPRE (n = 9)	CON (n = 10)	p - value T	p - value G x T
Repetitions						
Bench Press					.54	.43
Baseline	8.8 ± 1.8	9.8 ± 2.4	10.0 ± 3.5	9.3 ± 2.5		
Week 4	10.1 ± 2.6	11.0 ± 2.4	10.8 ± 2.0	8.3 ± 3.1		
Week 8	9.9 ± 2.5	10.5 ± 1.3	11.1 ± 2.8	8.4 ± 3.1		
Week 12	9.5 ± 2.8	9.5 ± 1.1	10.6 ± 2.2	9.4 ± 2.4		
Leg Press					.60	.67
Baseline	11.9 ± 3.6	15.8 ± 5.2	13.9 ± 4.9	14.1 ± 5.1		
Week 4	11.4 ± 2.6	14.5 ± 3.5	13.9 ± 2.9	12.7 ± 4.3		
Week 8	11.9 ± 3.1	13.6 ± 2.3	15.1 ± 4.3	12.4 ± 5.4		
Week 12	11.5 ± 2.9	12.5 ± 2.8	14.2 ± 3.8	13.2 ± 6.7		
Volume						
Bench Press					< .001	.08
Baseline	380.39 ± 194.78	529.83 ± 231.34	509.34 ± 270.03	416.82 ± 121.20		
Week 12	454.55 ± 245.58	647.73 ± 166.26	662.88 ± 241.36	433.86 ± 127.14		
Total % Δ	+ 19.5	+ 22.3	+ 30.1	+ 4.1		
Leg Press					< .01	< .01
Baseline	2475.00 ± 1052.59	3191.76 ± 1576.93	2801.77 ± 1023.97	2612.73 ± 1106.65		
Week 12	2674.72 ± 745.01	3627.27 ± 742.67	4952.02 ± 2124.00	2926.59 ± 1702.23		
Total % Δ	+ 8.1	+ 13.6	+ 76.7	+ 12.0		

Note. Data are presented as Mean ± *SD*. Repetitions = maximum repetitions completed at 75% of 1RM; Volume = number of repetitions x 75% of 1RM; Total % Δ = percent change over course of intervention. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline ($p > .05$). T = Time; G x T = Group by time interaction. Significance was set at $\alpha < 0.05$.

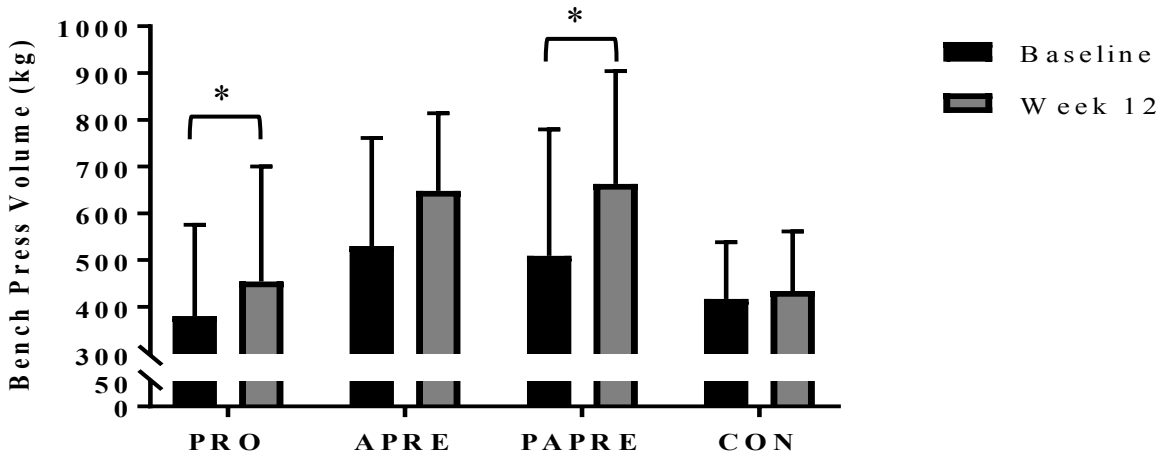

Figure 5. Data are presented as Mean ± *SD*. Bench press volume for baseline and week 12 testing. Volume = numbers of repetitions completed multiplied by 75% of 1RM. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. * Indicates significant differences between baseline and 12-weeks ($p < .05$) following paired-samples t-tests.

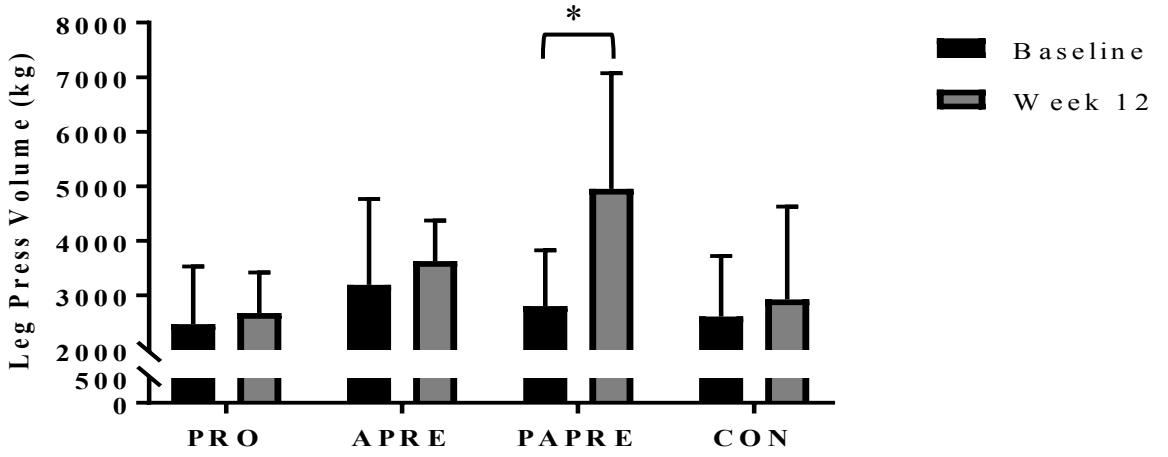

Figure 6: Data are presented as Mean ± *SD.* Leg press volume for baseline and week 12 testing. Volume = numbers of repetitions completed multiplied by 75% of 1RM. PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. * Indicates significant differences between baseline and 12-weeks ($p < .05$) following paired-samples t-tests.

Blood Analysis

Serum free testosterone. Baseline serum free testosterone analysis resulted in no significant differences between groups (F [3, 30] = .79, p = .51) (Table 5, Figure 7*). Blood measures were only obtained at baseline for subject 1 (PRO), and subject 18 (APRE) had no samples collected at any time point due to difficulty in obtaining samples. Subject 29 (APRE) is missing a sample for week 4 due to feeling nauseated during venipuncture attempt, however there were no issues in obtaining samples at baseline, week 8, and week 12. Repeated measures ANOVA indicated a significant main effect for time (F [3, 84] = 4.36, p < .01), but no significant group by time interaction (F [9, 84] = 1.00, p = .36) (Table 5). Paired-samples t-test showed a trend toward significance for the PRO and APRE groups at different time intervals, while the PAPRE and CON displayed no significant differences between any time intervals. PRO showed a positive trend toward significance between week 4 and week 8 (t (6) = - .26, p = .02), while the APRE group positively trended toward significance between baseline and week 4 (t (5) = - 3.03, p = .03).

One-way ANOVA analysis was used to determine any significance between sleep quality as measured by the PSQI and serum free testosterone values. Results indicate there is no significance between the two measures (F [1, 9] = .02, p = .89).

Table 5

Serum Free Testosterone

	PRO (n = 7)	APRE (n = 6)	PAPRE (n = 9)	CON (n = 10)	p - value T	p - value G x T
Free Testosterone					< .01	.36
Baseline	6.78 ± 2.11	8.52 ± 2.84	6.50 ± 2.80	8.22 ± 1.97		
Week 4	6.89 ± 2.43	10.42 ± 3.97	7.06 ± 2.40	9.51 ± 2.87		
Week 8	5.85 ± 2.40	9.26 ± 2.64	7.64 ± 3.54	9.51 ± 2.82		
Week 12	7.14 ± 3.27	10.38 ± 3.26	8.00 ± 2.97	9.53 ± 2.67		

Note. Data are presented as Mean ± *SD*. Serum free testosterone values are presented as picograms per milliliter (pg/mL). PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline. T = Time; G x T = Group by time interaction. Significance was set at $\alpha < .05$.

Dietary Protein Intake

No significant differences existed between groups at the baseline testing time point (F [3, 31] = .38, p = .77) (Table 6). Repeated measures ANOVA determined a significant group by time interaction occurred between baseline and week 12 (F [3, 31] = 6.33, p < .01) (Table 6). Follow-up paired-samples t-tests exhibited a significant difference for the supplement and non-supplementation groups between baseline and week 12. The PRO (t (7) = -2.76, p = .03) and the PAPRE (t (8) = -4.49, p < .01) groups significantly increased their protein intake between baseline and week 12, while the APRE (t (7) = .09, p = .93) and the CON (t (9) = 1.34, p = .21) groups produced similar protein intakes between the two time points (Figure 8). Thus, indicating that a supplementation of 50g of protein per day can significantly increase the total amount of daily protein consumed. Of the 17 subjects given protein supplementation, there was a 97.8% compliance rate over the course of the 12-week study.

Table 6
Dietary Protein Intake

	PRO (n = 8)	APRE (n = 8)	PAPRE (n = 9)	CON (n = 10)	p - value T	p - value G x T
Total					.01	< .01
Baseline	90.16 ± 35.03	98.45 ± 33.84	95.17 ± 40.04	108.81 ± 44.14		
Week 12	142.60 ± 36.67	96.91 ± 19.75	154.91 ± 25.23	87.64 ± 20.15		

Note. Data are presented as Mean ± *SD* and total grams of protein consumed per day (g/d). PRO = Protein Only, APRE = Autoregulatory Progressive Resistance Exercise Only, PAPRE = Protein + Autoregulatory Progressive Resistance Exercise, CON = Control. No significant differences were observed between the groups at baseline ($p > .05$). T = Time; G x T = Group by time interaction. Significance was set at $\alpha < 0.05$.

Chapter 5: Discussion

The purpose of this study was to investigate the effects of protein supplementation, as well as Autoregulatory Progressive Resistance Exercise on measurements of body composition, muscular performance, and free testosterone in men aged 35-55 years. These are the first data, to our knowledge, evaluating the effects of an APRE exercise program in combination with protein supplementation on measures of body composition, muscular performance, and free testosterone in middle-aged men. Over the course of the 12-week intervention it was found that the APRE program was successful at significantly increasing lean mass and muscular performance values when completed by itself as well as when combined with supplemental protein, but failed to alter free testosterone values.

Body Composition

Lean Mass. The results of the current study indicate lean mass remained the same over the course of the 12-week intervention but showed a trend toward statistical significance (Table 2). Follow-up analysis revealed both exercise groups (APRE and PAPRE) significantly increased lean mass by 2.6% and 2.8%, respectively (Figure 2). The increased lean mass for the exercise groups is in line with prior investigations utilizing UP programs (Ahmadizad et al., 2014; Buford , Rossi, Smith, & Warren, 2007; de Lima et al., 2012; Foschini et al., 2010; Harries et al., 2016; Kok, 2006; Kok et al., 2009; Prestes et al., 2009; Simão et al., 2012; Souza et al., 2014; Spineti et al., 2014). This is also the first investigation utilizing the APRE method to assess and report changes in lean mass. The average 2.7% increase in lean mass, while similar to previous investigations, may actually be a key component in bridging the gap in determining the most beneficial length for interventions. In previous investigations, UP programs exhibited greater gains in lean mass over that of LP programs when prescribed over shorter durations (≤ 9 weeks)

utilizing younger populations (Ahmadizad et al., 2014; Buford et al., 2007; Souza et al., 2014), while LP leads to an increase in lean mass of 1% on average in younger cohorts when carried out for 12 weeks or more (de Lima et al., 2012; Foschini et al., 2010; Harries, Lubans, & Callister, 2016). Untrained older adults gained 2% in lean mass when UP programs were extended to 16 or more weeks, while losing an average of 2% when performing an LP program over the same time period (Prestes et al., 2009). This investigation utilized APRE for a 12-week duration, while our cohort was middle-aged men, it may be of importance to continue research to determine if middle-aged individuals respond best to interventions of this length or if it is the APRE program that allows for UP-based programs to increase lean mass.

Fat Mass. The current study found that there were no significant changes in measures of fat mass between the four groups following the 12-week intervention. This is expected considering the study protocol did not include a dietary intervention outside of simply adding the supplemental protein and subjects were told to maintain current dietary lifestyles. While the supplementation groups significantly increased their daily protein intake to 1.5 and 1.7 g/kg•bw for the PRO and APRE groups (Table 6), respectively, there was no significant change in fat mass following the 12-week intervention (Figure 1). In an effort to elicit greater fat loss, a caloric deficit must be accompanied with an exercise program despite increasing an individual's protein intake well above the current RDA standards (Longland et al., 2016). Arciero and colleagues (Arciero, Edmonds, Bunsawat, et al., 2016; Arciero et al., 2013) demonstrated that when administering a dietary change to elicit changes in body composition, that a caloric deficit is needed to induce losses in fat mass even when protein is increased.

Muscular Performance

Strength. The current study is the only study to our knowledge utilizing APRE within a middle-aged male population to determine the potential benefits of increasing muscular strength. Results of the current study show significant gains in strength for all four groups following the 12-week intervention. When observing bench press 1RM both the APRE and PAPRE groups increased by an average of 27.6% following the 12-week intervention (Table 3; Figure 3). This gain in upper-body strength is superior to previous research demonstrating UP-based programs cause an increase of 23% of a period of 12 weeks (Baker, Wilson, & Carlyon, 1994; Rhea et al., 2002). Mann and colleagues (2010) found a 7% increase in bench press strength for those completing the APRE program, while this current study demonstrates using APRE for 12 weeks increases bench press 1RM by an average of 27.6%, nearly four times greater than that of a 6-week program. This present investigation also utilized an untrained population while Mann (2010) utilized a young trained population during their investigation into APRE. Demonstrating that APRE is a beneficial program to be utilized within the middle-age population to increase upper-body strength.

Lower-body strength was also assessed during this investigation utilizing leg press 1RM. The current study found that APRE alone increased lower body strength by 46.3%, while the PAPRE group which included supplemental protein increased lower body strength by 66.7% over the course of the 12-week intervention (Table 3; Figure 4). While Mann and colleagues (2010) utilized an estimated 1RM for squat to assess lower body strength, determining that the APRE group increased by 10% over the course of the 6-week program. Compared to previous investigations (Baker, Wilson, & Carlyon, 1994; Rhea et al., 2002), which demonstrated increases in lower-body strength of 42% for UP programs over the course of a 12-week

intervention, while the LP groups only displayed a 26% increase. These results demonstrate that using an APRE program is consistent with that of a 12-week UP program at increasing lower-body strength in untrained subjects. Current results also indicate greater strength gains when combined with 50g daily of supplemental whey protein as our PAPRE group increased strength by 20% over that of our APRE group. These results are reflective of previous research showing the added benefits of supplemental protein in conjunction with a resistance exercise program compared to a group prescribed exercise only. Previous investigations (Taylor et al., 2016) indicate that both exercise groups increased strength, but the group ingesting 24g prior to and immediately following exercise increased muscular strength two-fold over that of the exercise only group.

Endurance. Our current study observed that all groups within the study maintained similar number of repetitions at 75% 1RM at each testing session. Mann (2010) utilized the 225-pound bench press test which had a constant weight utilized and assessed the change in repetitions. The key difference between the present investigation and Mann (2010) is the protocol utilized to determine muscular endurance. While Mann and colleagues utilized a constant weight performed to failure, we prescribed a weight based on the individual's personal 1RM. This weight changed throughout the intervention (Table 3) as the participant's increased their muscular strength; thus, we calculated the total volume of weight lifted as an indicator of muscular endurance (Table 4). Doing so, we found that the APRE and PAPRE groups increased bench press volume by 22.3% and 30.1% (Table 4; Figure 5), as well as leg press volume by 13.6% and 76.7% (Table 4; Figure 6), respectively. This increase in amount of weight lifted over the course of the intervention gives insight to the APRE program facilitating an increase in muscular endurance. The muscular endurance values are hard to compare to the Mann study

since the baseline number of repetitions was not disclosed and the volume in which the groups lifted cannot be determined and lower-body endurance was not observed either. However, their APRE group displayed a three repetition increase in the 225-pound bench press test over the course of the 6 weeks. Thus, the results of the current investigation highlight the effectiveness of the APRE programming at increasing muscular performance in a cohort outside of an athletic population.

Free Testosterone

The results of the current investigation indicate an overall time component for changes in the free testosterone values of our subjects. With the PRO and APRE groups showing a significant increase at different time points throughout the intervention, but the PAPRE and CON exhibiting no significant changes (Table 5). These results are consistent with previous investigations (Kraemer et al., 1999) that determined there were no changes in free testosterone values following a resistance training protocol for older individuals. Since we had a non-exercise and an exercise based group exhibit increases it could be that the changes we observed are due to daily fluctuations in testosterone values rather than intervention based changes as previously seen in other investigations (Brambilla, Matsumoto, Araujo, & McKinlay, 2009; Brambilla, O'Donnell, Matsumoto, & McKinlay, 2007). Considering most of our subjects displayed free testosterone values that were well within the expected range, it may be of importance to look at individuals with chronically lower testosterone to determine if there are any changes due to resistance training or protein supplementation, or is there a potential ceiling effect. Thus, further research is needed to determine the effects of APRE training on overall total testosterone and other various hormonal changes from the training intervention.

Conclusion

In summary, the results of the current study indicate that daily supplementation with 50g of whey protein and APRE training for 12 weeks provides a significant gain in lean mass and muscular performance, but does not aid in reducing fat mass or augment serum free testosterone values in middle-aged men. This study significantly adds to the literature as it is the only study to date, to our knowledge, that has investigated APRE training in middle-aged men, as well as APRE training in combination with protein supplementation.

Future research with APRE should concentrate on length of intervention, as by the end of the 12-week period we had subjects leg pressing over 1000 pounds. The most common discomfort reported was that of muscle soreness, which typically subsided between weeks 3 and 4. While this is not a typical issue if it was simply looking at 1RM and prescribing a set percentage to lift, but the APRE program has the potential to quickly increase an individual's volume lifted very quickly and it is uncertain how individuals' body will respond to the repeated stresses of the heavy loads. While this concern may not be an issue in younger individuals, it is a component to consider when assessing the benefits of APRE training when utilizing older adults or sedentary/untrained individuals. It should also be noted that the lack of fat mass changes within the current investigation may be due to lack of dietary intervention within the methodology. Future research should consider dietary interventions that would elicit a sustained caloric deficit in an effort maximize body composition changes throughout the course of the intervention. Lastly, to further investigate changes in hormonal changes, researchers should look at how APRE effects acute changes in testosterone levels immediately pre- and post-exercise, as well as investigating changes in other hormones over the course of the study.

In terms of overall practical applications, this current investigation provides insight that the utilization of APRE programming is beneficial at continuously increasing muscular performance at each time point. This incremental increase in strength may be a useful tool when introducing individuals who are new to resistance training as a means of providing results for individuals to grasp and continue with exercise. The increases in strength may also be useful in developing a better foundation of muscular strength to then appropriately prescribe an exercise program moving forward. Overall, the benefits of using APRE to elicit changes in muscular performance within middle-aged men allows for new methods of exercise programming to be utilized that may be more beneficial throughout the lifespan, instead of trying to manipulate exercise programs that only take into account a young or older population.

References

Abernethy, P. J., Jürimäe, J., Logan, P. A., Taylor, A. W., & Thayer, R. E. (1994). Acute and chronic response of skeletal muscle to resistance exercise. *Sports Medicine*, *17*(1), 22–38. https://doi.org/10.2165/00007256-199417010-00003

Ahmadizad, S., Ghorbani, S., Ghasemikaram, M., & Bahmanzadeh, M. (2014). Effects of short-term nonperiodized, linear periodized and daily undulating periodized resistance training on plasma adiponectin, leptin and insulin resistance. *Clinical Biochemistry*, *47*(6), 417–422. https://doi.org/10.1016/J.CLINBIOCHEM.2013.12.019

Ahtiainen, J. P., Pakarinen, A., Kraemer, W. J., & Häkkinen, K. (2003). Acute hormonal and neuromuscular responses and recovery to forced vs. maximum repetitions multiple resistance exercises. *International Journal of Sports Medicine*, *24*(6), 410–418. https://doi.org/10.1055/s-2003-41171

Åkerstedt, T., Palmblad, J., de la Torre, B., Marana, R., & Gillberg, M. (1980). Adrenocortical and gonadal steroids during sleep deprivation. *Sleep*, *3*(1), 23–30. https://doi.org/10.1093/sleep/3.1.23

Allowances, N. R. C. (US) S. on the T. E. of the R. D. (1989). Protein and Amino Acids. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK234922/

American College of Sports Medicine. (2009). Progression models in resistance training for healthy adults. *Medicine & Science in Sports & Exercise*, *41*(3), 687–708. https://doi.org/10.1249/MSS.0b013e3181915670

Andersen, L. L., Tufekovic, G., Zebis, M. K., Crameri, R. M., Verlaan, G., Kjær, M., … Aagaard, P. (2005). The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. *Metabolism*, *54*(2), 151–156. https://doi.org/10.1016/j.metabol.2004.07.012

Antonio, J., Ellerbroek, A., Silver, T., Orris, S., Scheiner, M., Gonzalez, A., & Peacock, C. A. (2015). A high protein diet (3.4 g/kg/d) combined with a heavy resistance training program improves body composition in healthy trained men and women – a follow-up investigation. *Journal of the International Society of Sports Nutrition*, *12*(1), 39. https://doi.org/10.1186/s12970-015-0100-0

Antonio, J., Peacock, C. A., Ellerbroek, A., Fromhoff, B., & Silver, T. (2014). The effects of consuming a high protein diet (4.4 g/kg/d) on body composition in resistance-trained individuals. *Journal of the International Society of Sports Nutrition*, *11*(1), 19. https://doi.org/10.1186/1550-2783-11-19

Arciero, P., Edmonds, R., Bunsawat, K., Gentile, C., Ketcham, C., Darin, C., … Ormsbee, M. (2016). Protein-pacing from food or supplementation improves physical performance in overweight men and women: The PRISE 2 Study. *Nutrients*, *8*(5), 288.

https://doi.org/10.3390/nu8050288

Arciero, P., Edmonds, R., He, F., Ward, E., Gumpricht, E., Mohr, A., … Astrup, A. (2016). Protein-pacing caloric-restriction enhances body composition similarly in obese men and women during weight loss and sustains efficacy during long-term weight maintenance. *Nutrients*, *8*(8), 476. https://doi.org/10.3390/nu8080476

Arciero, P. J., Ormsbee, M. J., Gentile, C. L., Nindl, B. C., Brestoff, J. R., & Ruby, M. (2013a). Increased protein intake and meal frequency reduces abdominal fat during energy balance and energy deficit. *Obesity*, *21*(7), 1357–1366. https://doi.org/10.1002/oby.20296

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., Broad, E. M., … Coffey, V. G. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of Physiology*, *591*(9), 2319–2331. https://doi.org/10.1113/jphysiol.2012.244897

Atherton, P. J., & Smith, K. (2012). Muscle protein synthesis in response to nutrition and exercise. *The Journal of Physiology*, *590*(5), 1049–57. https://doi.org/10.1113/jphysiol.2011.225003

Atherton, P. J., Smith, K., Etheridge, T., Rankin, D., & Rennie, M. J. (2010). Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. *Amino Acids*, *38*(5), 1533–1539. https://doi.org/10.1007/s00726-009-0377-x

Axelsson, J., Ingre, M., Åkerstedt, T., & Holmbäck, U. (2005). Effects of acutely displaced sleep on testosterone. *The Journal of Clinical Endocrinology & Metabolism*, *90*(8), 4530–4535. https://doi.org/10.1210/jc.2005-0520

Baechle, T. R., Earle, R. W., & Wathen, D. (2000). Resistance Training. In T. R. Baechle & R. W. Earle (Eds.), *Essentials of strength and conditioning.* Champaigne, IL: Human Kinetics.

Baer, D. J., Stote, K. S., Paul, D. R., Harris, G. K., Rumpler, W. V, & Clevidence, B. A. (2011). Whey protein but not soy protein supplementation alters body weight and composition in free-living overweight and obese adults. *The Journal of Nutrition*, *141*(8), 1489–94. https://doi.org/10.3945/jn.111.139840

Baker, D., Wilson, G., & Carlyon, R. (1994). Periodization: The effect on strength of manipulating volume and intensity. *J Strength Cond Res*, *8*(4), 235–242.

Ball, S., Altena, T., & Swan, P. (2004). Comparison of anthropometry to DXA: a new prediction equation for men. *European Journal of Clinical Nutrition*, *58*, 1525–1531. https://doi.org/10.1038/sj.ejcn.1602003

Bamman, M. M., Shipp, J. R., Jiang, J., Gower, B. A., Hunter, G. R., Goodman, A., … Urban, R. J. (2001). Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *American Journal of Physiology-Endocrinology and Metabolism*,

280(3), E383–E390.

Bemben, M. G., Witten, M. S., Carter, J. M., Eliot, K. A., Knehans, A. W., & Bemben, D. A. (2010). The effects of supplementation with creatine and protein on muscle strength following a traditional resistance training program in middle-aged and older men.Â. *The Journal of Nutrition, Health & Aging*, *14*(2), 155–159.

Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D., & Wolfe, R. R. (1995). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *The American Journal of Physiology*, *268*(3 Pt 1), E514-20. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7900797

Biolo, G., Tipton, K. D., Klein, S., & Wolfe, R. R. (1997a). An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *The American Journal of Physiology*, *273*(1 Pt 1), E122-9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9252488

Boirie, Y., Dangin, M., Gachon, P., Vasson, M.-P., Maubois, J.-L., & Beaufrere, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences*, *94*(26), 14930–14935. https://doi.org/10.1073/pnas.94.26.14930

Boyle, W. J., Compton, A. A., Rigg, G., Menon, K. M. J., & Mccann, D. S. (1984). Serum testosterone measurements. *American Journal of Clinical Pathology*, *81*(6), 754–761. https://doi.org/10.1093/ajcp/81.6.754

Brambilla, D. J., Matsumoto, A. M., Araujo, A. B., & McKinlay, J. B. (2009). The Effect of Diurnal Variation on Clinical Measurement of Serum Testosterone and Other Sex Hormone Levels in Men. *The Journal of Clinical Endocrinology & Metabolism*, *94*(3), 907–913. https://doi.org/10.1210/jc.2008-1902

Brambilla, D. J., O'Donnell, A. B., Matsumoto, A. M., & McKinlay, J. B. (2007). Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology*, *67*(6), 853–862. https://doi.org/10.1111/j.1365-2265.2007.02976.x

Bray, G. A., Smith, S. R., de Jonge, L., Xie, H., Rood, J., Martin, C. K., … Redman, L. M. (2012). Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. *JAMA*, *307*(1), 47. https://doi.org/10.1001/jama.2011.1918

Breen, L., Philp, A., Witard, O. C., Jackman, S. R., Selby, A., Smith, K., … Tipton, K. D. (2011). The influence of carbohydrate-protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *The Journal of Physiology*, *589*(16), 4011–4025. https://doi.org/10.1113/jphysiol.2011.211888

Bremner, W. J., Vitiello, M. V., & Prinz, P. N. (1983). Loss of circadian rhythmicity in blood testosterone levels with aging in normal men*. *The Journal of Clinical Endocrinology & Metabolism*, *56*(6), 1278–1281. https://doi.org/10.1210/jcem-56-6-1278

Buford, T. W., Rossi, S. J., Smith, D. B., & Warren, A. J. (2007). A comparison of periodization models during nine weeks with equated volume and intensity for strength. *Journal of Strength and Conditioning Research*, *21*(4), 1245.

Burd, N. A., West, D. W. D., Moore, D. R., Atherton, P. J., Staples, A. W., Prior, T., … Phillips, S. M. (2011). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *The Journal of Nutrition*, *141*(4), 568–73. https://doi.org/10.3945/jn.110.135038

Buse, M. G., & Reid, S. S. (1975). Leucine. A possible regulator of protein turnover in muscle. *The Journal of Clinical Investigation*, *56*(5), 1250–61. https://doi.org/10.1172/JCI108201

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research*, *28*(2), 193–213. https://doi.org/10.1016/0165-1781(89)90047-4

Campos, G., Luecke, T., Wendeln, H., Toma, K., Hagerman, F., Murray, T., … Staron, R. (2002). Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *European Journal of Applied Physiology*, *88*(1–2), 50–60. https://doi.org/10.1007/s00421-002-0681-6

Candow, D. G., Burke, N. C., Smith-Palmer, T., & Burke, D. G. (2006). Effect of whey and soy protein supplementation combined with resistance training in young adults. *International Journal of Sport Nutrition and Exercise Metabolism*, *16*(3), 233–244. https://doi.org/10.1123/ijsnem.16.3.233

Cermak, N. M., Res, P. T., de Groot, L. C., Saris, W. H., & van Loon, L. J. (2012). Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *American Journal of Clinical Nutrition*, *96*(6), 1454–1464. https://doi.org/10.3945/ajcn.112.037556

Chrusch, M., Chilibeck, K., Chad, K., Shawndavison, Burke, Chrusch, M. J., … Davison, K. S. G. B. (2001). Creatine supplementation combined with resistance training in older men. *Med.Sci.SportsExerc*, *33*(12), 2111–2117. https://doi.org/10.1097/00005768-200112000-00021

Clifton, P. (2012). Effects of a high protein diet on body weight and comorbidities associated with obesity. *British Journal of Nutrition*, *108*(S2), S122–S129. https://doi.org/10.1017/S0007114512002322

Clifton, P., Condo, D., & Keogh, J. (2014). Long term weight maintenance after advice to consume low carbohydrate, higher protein diets – A systematic review and meta analysis.

Nutrition, Metabolism and Cardiovascular Diseases, *24*(3), 224–235. https://doi.org/10.1016/J.NUMECD.2013.11.006

Cockburn, E., Stevenson, E., Hayes, P. R., Robson-Ansley, P., & Howatson, G. (2010). Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage. *Applied Physiology, Nutrition, and Metabolism*, *35*(3), 270–277. https://doi.org/10.1139/H10-017

Coffey, D. (1988). Androgen action and the sex accessory tissues. In *The physiology of reproduction* (pp. 1081–1119).

Coffey, V. G., Moore, D. R., Burd, N. A., Rerecich, T., Stellingwerff, T., Garnham, A. P., … Hawley, J. A. (2011). Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints. *European Journal of Applied Physiology*, *111*(7), 1473–1483. https://doi.org/10.1007/s00421-010-1768-0

Cooke, M. B., Brabham, B., Buford, T. W., Shelmadine, B. D., McPheeters, M., Hudson, G. M., … Willoughby, D. S. (2014). Creatine supplementation post-exercise does not enhance training-induced adaptations in middle to older aged males. *European Journal of Applied Physiology*, *114*(6), 1321–1332. https://doi.org/10.1007/s00421-014-2866-1

Couillard, C., Lemieux, S., Moorjani, S., Lupien, P. J., Thériault, G., Prud'homme, D., … Després, J. P. (1996). Associations between 12 year changes in body fatness and lipoprotein-lipid levels in men and women of the Québec Family Study. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity*, *20*(12), 1081–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8968853

Cribb, P. J., Williams, A. D., Carey, M. F., & Hayes, A. (2006). The Effect of Whey Isolate and Resistance Training on Strength, Body Composition, and Plasma Glutamine. *International Journal of Sport Nutrition and Exercise Metabolism*, *16*(5), 494–509. https://doi.org/10.1123/ijsnem.16.5.494

Cribb, P. J., Williams, A. D., Stathis, C. G., Carey, M. F., & Hayes, A. (2007). Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. *Medicine and Science in Sports and Exercise*, *39*(2), 298–307. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=17277594&site=ehost-live&scope=site

Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., … Rennie, M. J. (2005). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *19*(3), 422–4. https://doi.org/10.1096/fj.04-2640fje

Dangin, M., Boirie, Y., Guillet, C., & Beaufrère, B. (2002a). Influence of the protein digestion rate on protein turnover in young and elderly subjects. *The Journal of Nutrition*, *132*(10),

3228S–33S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12368423

Dangin, M., Boirie, Y., Guillet, C., & Beaufrère, B. (2002b). Influence of the protein digestion rate on protein turnover in young and elderly subjects. *The Journal of Nutrition*, *132*(10), 3228S–33S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12368423

Dangin, M., Guillet, C., Garcia-Rodenas, C., Gachon, P., Bouteloup-Demange, C., Reiffers-Magnani, K., … Beaufrère, B. (2003). The rate of protein digestion affects protein gain differently during aging in humans. *The Journal of Physiology*, *549*(2), 635–644. https://doi.org/10.1113/jphysiol.2002.036897

De Lima, C., Boullosa, D., Frollini, A., Donatto, F., Leite, R., Gonelli, P., … Cesar, M. (2012). Linear and daily undulating resistance training periodizations have differential beneficial effects in young sedentary women. *International Journal of Sports Medicine*, *33*(9), 723–727. https://doi.org/10.1055/s-0032-1306324.

DeLorme, T. L., & Watkins, A. L. (1948). Technics of progressive resistance exercise. *Archives of Physical Medicine and Rehabilitation*, *29*(5), 263.

Delorme, T. L. (1951). Progressive Resistance Exercise: Technic and Medical Application. *Journal of the American Medical Association*, *146*(6), 606. https://doi.org/10.1001/jama.1951.03670060082034

DeLorme, T. L. (1945). Restoration of muscle power by heavy-resistance exercises. *JBJS*, *27*(4), 645–667.

DeNysschen, C. A., Burton, H. W., Horvath, P. J., Leddy, J. J., & Browne, R. W. (2009). Resistance training with soy vs whey protein supplements in hyperlipidemic males. *Journal of the International Society of Sports Nutrition*, *6*(1), 8. https://doi.org/10.1186/1550-2783-6-8

Desgorces, F. D., Berthelot, G., Dietrich, G., & Testa, M. S. A. (2010). Local muscular endurance and prediction of 1 repetition maximum for bench in 4 athletic populations. *Journal of Strength and Conditioning Research*, *24*(2), 394–400. https://doi.org/10.1519/JSC.0b013e3181c7c72d

Dong, J.-Y., Zhang, Z.-L., Wang, P.-Y., & Qin, L.-Q. (2013). Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. *British Journal of Nutrition*, *110*(5), 781–789. https://doi.org/10.1017/S0007114513002055

Dons, B., Bollerup, K., Bonde-Petersen, F., & Hancke, S. (1979). The effect of weight-lifting exercise related to muscle fiber composition and muscle cross-sectional area in humans. *European Journal of Applied Physiology and Occupational Physiology*, *40*(2), 95–106. https://doi.org/10.1007/BF00421155

Drummond, S. E., Crombie, N. E., Cursiter, M. C., & Kirk, T. R. (1998). Evidence that eating frequency is inversely related to body weight status in male, but not female, non-obese adults reporting valid dietary intakes. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity*, *22*(2), 105–12. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9504318

Durand, R. J., Castracane, V. D., Hollander, D. B., Tryniecki, J. L., Bamman, M. M., Oneal, S., … Kraemer, R. R. (2003). Hormonal responses from concentric and eccentric muscle contractions. *Medicine & Science in Sports & Exercise*, *35*(6), 937–943. https://doi.org/10.1249/01.MSS.0000069522.38141.0B

Erskine, R. M., Fletcher, G., Hanson, B., & Folland, J. P. (2012). Whey protein does not enhance the adaptations to elbow flexor resistance training. *Medicine & Science in Sports & Exercise*, *44*(9), 1791–1800. https://doi.org/10.1249/MSS.0b013e318256c48d

Eston, R., Rowlands, A., Charlesworth, S., Davies, A., & Hoppitt, T. (2005). Prediction of DXA - determined whole body fat from skinfolds : importance of including skinfolds from the thigh and calf in young , healthy men and women. *European Journal of Clinical Nutrition*, *59*, 695–702. https://doi.org/10.1038/sj.ejcn.1602131

Fabry, P., Hejl, Z., Fodor, J., Braun, T., & Zvolankova, K. (1964). The frequency of meals. Its relation to overweight, hypercholesterolemia, and decreased glucose-tolerance. *Lancet (London, England)*, *2*(7360), 614–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14186149

Faigenbaum, A. D., Kraemer, W. J., Blimkie, C. J. R., Jeffreys, I., Micheli, L. J., Nitka, M., & Rowland, T. W. (2009). Youth resistance training: Updated position statement paper from the National Strength and Conditioning Association. *Journal of Strength and Conditioning Research*, *23*, S60–S79. https://doi.org/10.1519/JSC.0b013e31819df407

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*, 175–191.

Feldman, H. A., Longcope, C., Derby, C. A., Johannes, C. B., Araujo, A. B., Coviello, A. D., … McKinlay, J. B. (2002). Age trends in the level of serum testosterone and other hormones in middle-aged Men: Longitudinal results from the Massachusetts Male Aging Study. *The Journal of Clinical Endocrinology & Metabolism*, *87*(2), 589–598. https://doi.org/10.1210/jcem.87.2.8201

Ferguson-Stegall, L., McCleave, E. L., Ding, Z., Doerner, P. G., Wang, B., Liao, Y.-H., … Ivy, J. L. (2011). Postexercise carbohydrate–protein supplementation improves subsequent exercise performance and intracellular signaling for protein synthesis. *Journal of Strength and Conditioning Research*, *25*(5), 1210–1224. https://doi.org/10.1519/JSC.0b013e318212db21

Fluck, M. (2012). Regulation of protein synthesis in skeletal muscle. *Dtsch Z Sportmed*, *63*, 75–80.

Folland, J. P., & Williams, A. G. (2007). The Adaptations to Strength Training. *Sports Medicine*, *37*(2), 145–168. https://doi.org/10.2165/00007256-200737020-00004

Forbes, G., & Reina, J. (1970). Adult lean body mass declines with age: Some longitudinal observations. *Metabolism*, *19*(9), 653–663. https://doi.org/10.1016/0026-0495(70)90062-4

Foschini, D., Araújo, R. C., Bacurau, R. F. P., De Piano, A., De Almeida, S. S., Carnier, J., … Dâmaso, A. R. (2010). Treatment of obese adolescents: The influence of periodization models and ACE genotype. *Obesity*, *18*(4), 766–772. https://doi.org/10.1038/oby.2009.247

Fry, A. C., Kraemer, W. J., & Ramsey, L. T. (1998). Pituitary-adrenal-gonadal responses to high-intensity resistance exercise overtraining. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *85*(6), 2352–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9843563

Fulgoni, V. L. (2008). Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003-2004. *The American Journal of Clinical Nutrition*, *87*(5), 1554S–1557S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18469286

Gallagher, D., Visser, M., De Meersman, R. E., Sepúlveda, D., Baumgartner, R. N., Pierson, R. N., … Heymsfield, S. B. (1997). Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *83*(1), 229–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9216968

Glynn, E. L., Fry, C. S., Drummond, M. J., Timmerman, K. L., Dhanani, S., Volpi, E., & Rasmussen, B. B. (2010). Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *The Journal of Nutrition*, *140*(11), 1970–6. https://doi.org/10.3945/jn.110.127647

González-Santos, M. R., Gajá-Rodíguez, O. V., Alonso-uriarte, R., Sojo-aranda, I., & Cortés-Gallegos, V. (1989). Sleep deprivation and adaptive hormonal responses of healthy men. *Archives of Andrology*, *22*(3), 203–207. https://doi.org/10.3109/01485018908986773

Gray, A., Feldman, H. A., McKinlay, J. B., & Longcope, C. (1991). Age, disease, and changing sex hormone levels in middle-aged men: Results of the Massachusetts Male Aging Study*. *The Journal of Clinical Endocrinology & Metabolism*, *73*(5), 1016–1025. https://doi.org/10.1210/jcem-73-5-1016

Grgic, J., Mikulic, P., Podnar, H., & Pedisic, Z. (2017). Effects of linear and daily undulating periodized resistance training programs on measures of muscle hypertrophy: a systematic review and meta-analysis. *PeerJ*, *5*, e3695. https://doi.org/10.7717/peerj.3695

Hackney, A. C. (1989). Endurance Training and Testosterone Levels. *Sports Medicine*, *8*(2), 117–127. https://doi.org/10.2165/00007256-198908020-00004

Hackney, A. C. (1998). Testosterone and reproductive dysfunction in endurance-trained men. Retrieved December 10, 2017, from http://www.sportsci.org/encyc/testosterone/testosterone.html

Hackney, A. C., Fahrner, C. L., & Gulledge, T. P. (1998). Basal reproductive hormonal profiles are altered in endurance trained men. *The Journal of Sports Medicine and Physical Fitness*, *38*(2), 138–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9763799

Hackney, A. C., Fahrner, C. L., & Stupnicki, R. (1997). Reproductive hormonal responses to maximal exercise in endurance-trained men with low resting testosterone levels. *Experimental and Clinical Endocrinology & Diabetes*, *105*(5), 291–295.

Hackney, A. C., Sharp, R. L., Runyan, W. S., & Ness, R. J. (1989). Relationship of resting prolactin and testosterone in males during intensive training. *British Journal of Sports Medicine*, *23*(3), 194. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19650255

Hackney, A. C., Sinning, W. E., & Bruot, B. C. (1988). Reproductive hormonal profiles of endurance-trained and untrained males. *Medicine and Science in Sports and Exercise*, *20*(1), 60–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3343919

Hakkinen, K., Pakarinen, A., Alen, M., Kuahanen, H., & Komi, P. V. (1987). Relationships between training volume, physical performance capacity, and serum hormone concentrations during prolonged training in elite weight lifters. *International Journal of Sports Medicine*, *8*, 61–65.

Haring, R., Ittermann, T., Völzke, H., Krebs, A., Zygmunt, M., Felix, S. B., … Wallaschofski, H. (2010). Prevalence, incidence and risk factors of testosterone deficiency in a population-based cohort of men: results from the study of health in Pomerania. *The Aging Male*, *13*(4), 247–257. https://doi.org/10.3109/13685538.2010.487553

Harman, S. (2005). Testosterone in older men after the Institute of Medicine Report: where do we go from here? *Climacteric*, *8*(2), 124–135. https://doi.org/10.1080/13697130500118001

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. *The Journal of Clinical Endocrinology & Metabolism*, *86*(2), 724–731. https://doi.org/10.1210/jcem.86.2.7219

Harries, S., Lubans, D., & Callister, R. (2016). Comparison of resistance training progression models on maximal strength in sub-elite adolescent rugby union players. *Journal of Science and Medicine in Sport*, *19*(2), 163–169. https://doi.org/10.1016/J.JSAMS.2015.01.007

Hartman, J. W., Tang, J. E., Wilkinson, S. B., Tarnopolsky, M. A., Lawrence, R. L., Fullerton, A. V, & Phillips, S. M. (2007). Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in

young, novice, male weightlifters. *The American Journal of Clinical Nutrition*, *86*(2), 373–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17684208

Hartmann, H., Bob, A., Wirth, K., & Schmidtbleicher, D. (2009). Effects of different periodization models on rate of force development and power ability of the upper extremity. *J Strength Cond Res*, *23*(7), 1921–1932. https://doi.org/10.1519/JSC.0b013e3181b73c69

Herrick, A. B., & Stone, W. J. (1996). The effects of periodization versus progressive resistance exercise on upper and lower body strength in women. *Journal of Strength and Conditioning Research*, *10*(2), 72–76. Retrieved from http://uark.summon.serialssolutions.com/2.0.0/link/0/eLvHCXMwjV1NSwMxEA1qL4L4URW1CnPytrqbbHbXk1TboqK0ansu2U2iIG5rtwr-e2ey2xZUxHMuIXnzkZmXeYwJfuJ733xCKjEPTzGUJyn3LWLAT2WoreA2MFxljqpzJ9qt-OYqai3qb9Vtz5yk89x6lFHR_DRIEIwI0fPxm0ciUtRsrRQ1llktQCMnO437j_P3l5Sy5N

Hoffman, J. R., Ratamess, N. A., Kang, J., Falvo, M. J., & Faigenbaum, A. D. (2007). Effects of protein supplementation on muscular performance and resting hormonal changes in college football players. *Journal of Sports Science & Medicine*, *6*(1), 85–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24149229

Hoffman, J. R., Ratamess, N. A., Klatt, M., Faigenbaum, A. D., Ross, R. E., Tranchina, N. M., … Kraemer, W. J. (2009). Comparison between different off-season resistance training programs in Division III American college football players. *Journal of Strength and Conditioning Research*, *23*(1), 11–19. https://doi.org/10.1519/JSC.0b013e3181876a78

Hoffman, J. R., Wendell, M., Cooper, J., Kang, J., Data, R., Hoffman, :, … Kang, J. (2003). Comparison between linear and nonlinear in - season training programs in freshman football players. *Journal of Strength and Conditioning Research National Strength & Conditioning Association J . Strength Cond . Res*, *17*(3), 561–565. https://doi.org/10.1519/00124278-200308000-00023

Hulmi, J. J., Kovanen, V., Selänne, H., Kraemer, W. J., Häkkinen, K., & Mero, A. A. (2009). Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. *Amino Acids*, *37*(2), 297–308. https://doi.org/10.1007/s00726-008-0150-6

Issurin, V. B. (2010). New Horizons for the Methodology and Physiology of Training Periodization. *Sports Medicine*, *40*(3), 189–206. https://doi.org/10.2165/11319770-000000000-00000

Ivey, F. M., Roth, S. M., Ferrell, R. E., Tracy, B. L., Lemmer, J. T., Hurlbut, D. E., … Hurley, B. F. (2000). Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *55*(11), M641–M648. https://doi.org/10.1093/gerona/55.11.M641

Ivy, J. L., Ding, Z., Hwang, H., Cialdella-Kam, L. C., & Morrison, P. J. (2008). Post exercise carbohydrate–protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. *Amino Acids*, *35*(1), 89–97. https://doi.org/10.1007/s00726-007-0620-2

Izquierdo, M., Ibañez, J., González-Badillo, J. J., Häkkinen, K., Ratamess, N. A., Kraemer, W. J., … Gorostiaga, E. M. (2006). Differential effects of strength training leading to failure versus not to failure on hormonal responses, strength, and muscle power gains. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *100*(5), 1647–56. https://doi.org/10.1152/japplphysiol.01400.2005

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., … Kreider, R. B. (2017). International Society of Sports Nutrition Position Stand : protein and exercise, 1–25. https://doi.org/10.1186/s12970-017-0177-8

Josse, A. R., Tang, J. E., Tarnopolsky, M. A., & Phillips, S. M. (2009). Body composition and strength changes in women with milk and resistance exercise. *Medicine & Science in Sports & Exercise*, *42*(6), 1. https://doi.org/10.1249/MSS.0b013e3181c854f6

Kareen Zack, M., Nickols-Richardson, S. M., Chair William Herbert, C. G., & Poole, K. (2002). *Reliability and validity of body composition and bone mineral density measurements by DXA*. Virginia Polytechnic Institute and State University. Retrieved from https://theses.lib.vt.edu/theses/available/etd-04172002-123509/unrestricted/thesis.pdf

Kimball, S. R. (2007). The role of nutrition in stimulating muscle protein accretion at the molecular level. *Biochemical Society Transactions*, *35*(Pt 5), 1298–301. https://doi.org/10.1042/BST0351298

Knight, K. L. (1979). Knee rehabilitation by the daily adjustable progressive resistive exercise technique. *The American Journal of Sports Medicine*, *7*(6), 336–337. https://doi.org/10.1177/036354657900700605

Kok, L.-Y. (2006). *Comparing linear and undulating periodisation for improving and maintaining muscular strength qualities in women*.

Kok, L.-Y., Hamer, P., & Bishop, D. (2009). Enhancing muscular qualities in untrained women: linear versus undulating periodization. *Medicine & Science in Sports & Exercise*, *41*(9), 1797–1807.

Kraemer, W. J., Adams, K., Cafarelli, E., Dudley, G. A., Dooly, C., Feigenbaum, M. S., … American College of Sports Medicine. (2002). American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Medicine and Science in Sports and Exercise*, *34*(2), 364–80. https://doi.org/10.1097/00005768-200202000-00027

Kraemer, W. J., Häkkinen, K., Newton, R. U., Nindl, B. C., Volek, J. S., McCormick, M., …

Evans, W. J. (1999). Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *87*(3), 982–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10484567

Kryger, M., Monjan, A., Bliwise, D., & Ancoli-Israel, S. (2004). Sleep, health, and aging. Bridging the gap between science and clinical practice. *Geriatrics*, *59*(1), 24–6, 29–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14755865

Kukuljan, S., Nowson, C. A., Sanders, K., & Daly, R. M. (2009). Effects of resistance exercise and fortified milk on skeletal muscle mass, muscle size, and functional performance in middle-aged and older men: an 18-mo randomized controlled trial. *Journal of Applied Physiology*, *107*(6). Retrieved from http://jap.physiology.org/content/107/6/1864.short

Layman, D. K., Evans, E., Baum, J. I., Seyler, J., Erickson, D. J., & Boileau, R. A. (2005). Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *The Journal of Nutrition*, *135*(8), 1903–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16046715

Layman, D. K., Evans, E. M., Erickson, D., Seyler, J., Weber, J., Bagshaw, D., … Kris-Etherton, P. (2009). A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *The Journal of Nutrition*, *139*(3), 514–21. https://doi.org/10.3945/jn.108.099440

Leidy, H. J., Clifton, P. M., Astrup, A., Wycherley, T. P., Westerterp-Plantenga, M. S., Luscombe-Marsh, N. D., … Mattes, R. D. (2015). The role of protein in weight loss and maintenance. *The American Journal of Clinical Nutrition*, *101*(6), 1320S–1329S. https://doi.org/10.3945/ajcn.114.084038

Levinger, I., Goodman, C., Hare, D. L., Jerums, G., Toia, D., & Selig, S. (2009). The reliability of the 1RM strength test for untrained middle-aged individuals. *Journal of Science and Medicine in Sport*, *12*(2), 310–316. https://doi.org/10.1016/j.jsams.2007.10.007

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., … Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*, *6*(7), e1000100. https://doi.org/10.1371/journal.pmed.1000100

Longland, T. M., Oikawa, S. Y., Mitchell, C. J., Devries, M. C., Phillips, S. M., Helms, E., … Phillips, S. M. (2016). Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial. *The American Journal of Clinical Nutrition*, *103*(3), 738–46. https://doi.org/10.3945/ajcn.115.119339

Luboshitzky, R., Shen-Orr, Z., & Herer, P. (2003). Middle-aged men secrete less testosterone at night than young healthy men. *The Journal of Clinical Endocrinology & Metabolism*, *88*(7), 3160–3166. https://doi.org/10.1210/jc.2002-021920

Luboshitzky, R., Zabari, Z., Shen-Orr, Z., Herer, P., & Lavie, P. (2001). Disruption of the nocturnal testosterone rhythm by sleep fragmentation in normal men. *The Journal of Clinical Endocrinology & Metabolism*, *86*(3), 1134–1139. https://doi.org/10.1210/jcem.86.3.7296

Makris, A., & Foster, G. D. (2011). Dietary approaches to the treatment of obesity. *The Psychiatric Clinics of North America*, *34*(4), 813–27. https://doi.org/10.1016/j.psc.2011.08.004

Mann, B., Thyfault, J. P., Ivey, P. A., & Sayers, S. P. (2010). The effect of autoregulatory progressive resistance exercise vs. linear periodization on strength improvement in college athletes. *The Journal of Strength & Conditioning Research*, *24*(7), 1718–1723.

Marty, E., Liu, Y., Samuel, A., Or, O., & Lane, J. (2017). A review of sarcopenia: Enhancing awareness of an increasingly prevalent disease. *Bone*, *105*, 276–286. https://doi.org/10.1016/J.BONE.2017.09.008

Matsumoto, A. M. (2002). Andropause: Clinical implications of the decline in serum testosterone levels with aging in men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *57*(2), M76–M99. https://doi.org/10.1093/gerona/57.2.M76

Mayo Clinic: Mayo Medical Laboratories. (2017). Clinical: Testosterone, Total, Bioavailable, and Free, Serum. Retrieved October 20, 2017, from https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/83686

McCarthy, J. J., & Esser, K. A. (2010). Anabolic and catabolic pathways regulating skeletal muscle mass. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(3), 230–5. https://doi.org/10.1097/MCO.0b013e32833781b5

McDonagh, M. J. N., & Davies, C. T. M. (1984). Adaptive response of mammalian skeletal muscle to exercise with high loads. *European Journal of Applied Physiology and Occupational Physiology*, *52*(2), 139–155. https://doi.org/10.1007/BF00433384

Miranda, F., SimÃ£o, R., Rhea, M., Bunker, D., Prestes, J., Leite, R. D., … Novaes, J. (2011). Effects of linear vs. daily undulatory periodized resistance training on maximal and submaximal strength gains.Â. *TheÂ Journal of Strength & Conditioning Research*, *25*(7), 1824–1830.

Monteiro, A. G., Aoki, M. S., Evangelista, A. L., Alveno, D. A., Monteiro, G. A., Piçarro, I. da C., & Ugrinowitsch, C. (2009). Nonlinear periodization maximizes strength gains in split resistance training routines. *Journal of Strength and Conditioning Research*, *23*(4), 1321–1326. https://doi.org/10.1519/JSC.0b013e3181a00f96

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., … Phillips, S. M. (2009). Ingested protein dose response of muscle and albumin protein synthesis after

resistance exercise in young men. *The American Journal of Clinical Nutrition*, *89*(1), 161–8. https://doi.org/10.3945/ajcn.2008.26401

Morenga, L. Te, & Mann, J. (2012). The role of high-protein diets in body weight management and health. *British Journal of Nutrition*, *108*, S130–S138. https://doi.org/10.1017/S0007114512002437

Mulligan, T., Frick, M. F., Zuraw, Q. C., Stemhagen, A., & McWhirter, C. (2008). Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *International Journal of Clinical Practice*, *60*(7), 762–769. https://doi.org/10.1111/j.1742-1241.2006.00992.x

Nana, A., Slater, G. J., Stewart, A. D., & Burke, L. M. (2015). Methodology Review: Using Dual-Energy X-Ray Absorptiometry (DXA) for the assessment of body composition in athletes and active people. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(2), 198–215. https://doi.org/10.1123/ijsnem.2013-0228

Narici, M. V., Roi, G. S., Landoni, L., Minetti, A. E., & Cerretelli, P. (1989). Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps. *European Journal of Applied Physiology and Occupational Physiology*, *59*(4), 310–319. https://doi.org/10.1007/BF02388334

National Center for Health Statistics, N. C. for H. (2017). *Health, United States, 2016: With Chartbook on Long-term Trends in Health*. Hyattsville, MD.

Norton, L. E., & Layman, D. K. (2006). Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *The Journal of Nutrition*, *136*(2), 533S–537S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16424142

Norton, L. E., & Wilson, G. J. (2009). Optimal protein intake to maximize muscle protein synthesis Examinations of optimal meal protein intake and frequency for athletes. *AGRO FOOD INDUSTRY HI-TECH*, *20*(2), 54–57. Retrieved from http://uark.summon.serialssolutions.com/2.0.0/link/0/eLvHCXMwtV1Ni9RAEG1EL4KIouL6AX0QL0Mk6WSS9MGD7GRndtUNaDzoZelPGJbMiBNh9egvtyrd08lGD-PBSxiaTjPkVaqrOq9eEZKyV3E08Ql2brK5RTG71M55LFlWCq2sUEwyHvfSesv3abUozlb5YjjbGcb-K_AwBtBjIe0_gB8WhQH4DSYAVzACuB5kBjW4hBarrV

Norton, L. E., Wilson, G. J., Layman, D. K., Moulton, C. J., & Garlick, P. J. (2012). Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. *Nutrition & Metabolism*, *9*(1), 67. https://doi.org/10.1186/1743-7075-9-67

Paddon-Jones, D., & Rasmussen, B. B. (2009). Dietary protein recommendations and the prevention of sarcopenia. *Current Opinion in Clinical Nutrition and Metabolic Care*, *12*(1), 86–90. https://doi.org/10.1097/MCO.0b013e32831cef8b

Pasiakos, S. M., & M., S. (2012). Exercise and amino acid anabolic cell signaling and the regulation of skeletal muscle mass. *Nutrients*, *4*(12), 740–758.

https://doi.org/10.3390/nu4070740

Pasiakos, S. M., McLellan, T. M., & Lieberman, H. R. (2015). The Effects of Protein Supplements on Muscle Mass, Strength, and Aerobic and Anaerobic Power in Healthy Adults: A Systematic Review. *Sports Medicine*, *45*(1), 111–131. https://doi.org/10.1007/s40279-014-0242-2

Peterson, M. D., Dodd, D. J., Alvar, B. A., Rhea, M. R., & Favre, M. (2008). Undulation training for development of hierarchical fitness and improved firefighter job performance. *Journal of Strength and Conditioning Research*, *22*(5), 1683–1695. https://doi.org/10.1519/JSC.0b013e31818215f4

Peterson, M. D., Sen, A., & Gordon, P. M. (2011). Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. *Medicine and Science in Sports and Exercise*, *43*(2), 249–58. https://doi.org/10.1249/MSS.0b013e3181eb6265

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., & Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology - Endocrinology and Metabolism*, *273*(1). Retrieved from http://ajpendo.physiology.org/content/273/1/E99.short

Phillips, S. M., & Van Loon, L. J. C. (2011). Dietary protein for athletes: From requirements to optimum adaptation. *Journal of Sports Sciences*, *29*(sup1), S29–S38. https://doi.org/10.1080/02640414.2011.619204

Physical Activity Guidelines Advisory Committee, P. A. G. A. (2008). *Physical activity guidelines advisory committee report, 2008*. Washington, DC.

Pichon, L., Potier, M., Tome, D., Mikogami, T., Laplaize, B., Martin-Rouas, C., & Fromentin, G. (2008). High-protein diets containing different milk protein fractions differently influence energy intake and adiposity in the rat. *British Journal of Nutrition*, *99*(4), 739–48. https://doi.org/10.1017/S0007114507831709

Plisk, S. S., & Stone, M. H. (2003). Periodization Strategies. Retrieved from http://www.treinamentoesportivo.com/wp-content/uploads/2012/10/Periodization_Strategies.pdf

Plymate, S. R., Tenover, J. S., & Bremner, W. J. (1989). Circadian variation in testosterone, sex hormone-binding globulin, and calculated non-sex hormone-binding globulin bound testosterone in healthy young and elderly men. *Journal of Andrology*, *10*(5), 366–371. https://doi.org/10.1002/j.1939-4640.1989.tb00120.x

Prestes, J., Frollini, A. B., de Lima, C., Donatto, F. F., Foschini, D., de Cássia Marqueti, R., … Fleck, S. J. (2009). Comparison between linear and daily undulating periodized resistance training to increase strength. *Journal of Strength and Conditioning Research*, *23*(9), 2437–2442. https://doi.org/10.1519/JSC.0b013e3181c03548

Reibe, D. (Senior E., Ehrman, J. K. (Associate E., Liguori, G. (Associate E., & Magal, M. (Associate E. (Eds.). (2017). *ACSM's Guideliens for Exercise Testing and Prescritpion* (Tenth). Philadelphia: Wolters Kluwer Health.

Rennie, M. J. (2001). Control of muscle protein synthesis as a result of contractile activity and amino acid availability: implications for protein requirements. *International Journal of Sport Nutrition and Exercise Metabolism*, *11*(s1), S170–S176. https://doi.org/10.1123/ijsnem.11.s1.s170

Reynolds, J. M., Gordon, T. J., & Robergs, R. A. (2006). Prediction of one repetition maximum strength from multiple repetition maximum testing and anthropometry. *The Journal of Strength and Conditioning Research*, *20*(3), 584–592. Retrieved from https://search.proquest.com/docview/213105296?pq-origsite=gscholar

Rhea, M. R., Ball, S. D., Phillips, W. T., & Burkett, L. N. (2002). A comparison of linear and daily undulating periodized programs with equated volume and intensity for strength. *J . Strength Cond . Res*, *16*(2), 250–255. https://doi.org/10.1519/1533-4287(2002)016<0250:ACOLAD>2.0.CO;2

Rhea, M. R., Phillips, W. T., Burkett, L. N., Stone, W. J., Ball, S. D., Alvar, B. A., & Thomas, A. B. (2003). A comparison of linear and daily undulating periodized programs with equated volume and intensity for local muscular endurance. *J. Strength Cond. Res*, *17*(171), 82–8782. Retrieved from https://s3.amazonaws.com/academia.edu.documents/6854837/linear_vs_daily_undulating_periodization_for_muscular_endurance.pdf?AWSAccessKeyId=AKIAIWOWYYGZ2Y53UL3A&Expires=1505155706&Signature=mFkw79on%2BoWxTCz7oXDwjAhGVW0%3D&response-content-disposition=inli

Rothney, M. P., Martin, F. P., Xia, Y., Beaumont, M., Davis, C., Ergun, D., … Rezzi, S. (2012). Precision of GE Lunar iDXA for the measurement of total and regional body composition in nonobese adults. *Journal of Clinical Densitometry*, *15*(4), 399–404. https://doi.org/10.1016/J.JOCD.2012.02.009

Santesso, N., Akl, E. A., Bianchi, M., Mente, A., Mustafa, R., Heels-Ansdell, D., & Schünemann, H. J. (2012). Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. *European Journal of Clinical Nutrition*, *66*(7), 780–788. https://doi.org/10.1038/ejcn.2012.37

Schiavi, R. C., White, D., & Mandeli, J. (1992). Pituitary-gonadal function during sleep in healthy aging men. *Psychoneuroendocrinology*, *17*(6), 599–609. https://doi.org/10.1016/0306-4530(92)90018-3

Siervogel, R. M., Wisemandle, W., Maynard, L. M., Guo, S. S., Roche, A. F., Chumlea, W. C., & Towne, B. (1998). Serial changes in body composition throughout adulthood and their relationships to changes in lipid and lipoprotein levels. The Fels Longitudinal Study.

Arteriosclerosis, Thrombosis, and Vascular Biology, *18*(11), 1759–64. https://doi.org/10.1161/01.ATV.18.11.1759

Silva, A., Minderico, C., Teixeira, P., Pietrobelli, A., & Sardinha, L. (2006). Body fat measurement in adolescent athletes: multicompartment molecular model comparison. *European Journal of Clinical Nutrition*, *60*, 955–964. https://doi.org/10.1038/sj.ejcn.1602405

Simão, R., Spineti, J., de Salles, B. F., Matta, T., Fernandes, L., Fleck, S. J., … Strom-Olsen, H. E. (2012). Comparison between nonlinear and linear periodized resistance training: hypertrophic and strength effects. *Journal of Strength and Conditioning Research*, *26*(5), 1389–1395. https://doi.org/10.1519/JSC.0b013e318231a659

Smilios, I., Pilianidis, T., Karamouzis, M., & Tokmakidis, S. P. (2003). Hormonal responses after various resistance exercise protocols. *Medicine and Science in Sports and Exercise*, *35*(4), 644–654.

Smith, C. J., Callister, R., & Lubans, D. R. (2011). A systematic review of strength and conditioning programmes designed to improve fitness characteristics in golfers. *Journal of Sports Sciences*, *29*(9), 933–943. https://doi.org/10.1080/02640414.2011.571273

Sousa, G. T. D., Lira, F. S., Rosa, J. C., de Oliveira, E. P., Oyama, L. M., Santos, R. V, & Pimentel, G. D. (2012). Dietary whey protein lessens several risk factors for metabolic diseases: a review. *Lipids in Health and Disease*, *11*(1), 67. https://doi.org/10.1186/1476-511X-11-67

Souza, E. O., Ugrinowitsch, C., Tricoli, V., Roschel, H., Lowery, R. P., Aihara, A. Y., … Wilson, J. M. (2014). Early adaptations to six weeks of non-periodized and periodized strength training regimens in recreational males. *Journal of Sports Science & Medicine*, *13*(3), 604–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25177188

Spineti, J., Figueiredo, T., Miranda, H., De Salles, B., Fernandes, L., & Simão, R. (2014). The effects of exercise order and periodized resistance training on maximum strength and muscle thickness. *International SportMed Journal*, *15*(4), 374–390. https://doi.org/DOI 10.1007/BF00572189

Staron, R. S., Karapondo, D. L., Kraemer, W. J., Fry, A. C., Gordon, S. E., Falkel, J. E., … Hikida, R. S. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *Journal of Applied Physiology*, *76*(3), 1247–1255. https://doi.org/10.1152/JAPPL.1994.76.3.1247

Strauss, R. H., Lanese, R. R., & Malarkey, W. B. (1985). Weight loss in amateur wrestlers and its effect on serum testosterone levels. *JAMA: The Journal of the American Medical Association*, *254*(23), 3337. https://doi.org/10.1001/jama.1985.03360230069025

Symons, T. B., Schutzler, S. E., Cocke, T. L., Chinkes, D. L., Wolfe, R. R., & Paddon-Jones, D.

(2007). Aging does not impair the anabolic response to a protein-rich meal. *The American Journal of Clinical Nutrition*, *86*(2), 451–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17684218

Symons, T. B., Sheffield-Moore, M., Wolfe, R. R., & Paddon-Jones, D. (2009). A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. *Journal of the American Dietetic Association*, *109*(9), 1582–6. https://doi.org/10.1016/j.jada.2009.06.369

Tan, B. (1999). Manipulating resistance training program variables to optimize maximum strength in men: A Review. *J. Strength Cond. Res*, *13*(133). Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.588.6042&rep=rep1&type=pdf

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of Applied Physiology*, *107*(3). Retrieved from http://jap.physiology.org/content/107/3/987.short

Taylor, L. W., Wilborn, C., Roberts, M. D., White, A., & Dugan, K. (2016). Eight weeks of pre- and postexercise whey protein supplementation increases lean body mass and improves performance in Division III collegiate female basketball players. *Applied Physiology, Nutrition, And Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *41*(3), 249–254. https://doi.org/10.1139/apnm-2015-0463

Tesch, P. A. (1988). Skeletal muscle adaptations consequent to long-term heavy resistance exercise. *Medicine and Science in Sports and Exercise*, *20*(5 Suppl), S132-4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3057312

Tipton, K. D. (2007). Role of Protein and Hydrolysates before Exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, *17*(s1), S77–S86. https://doi.org/10.1123/ijsnem.17.s1.s77

Tipton, K. D., Ferrando, A. A., Phillips, S. M., Doyle, D., & Wolfe, R. R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *The American Journal of Physiology*, *276*(4 Pt 1), E628-34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10198297

Trommelen, J., Holwerda, A. M., Kouw, I. W. K., Langer, H., Halson, S. L., Rollo, I., … Van Loon, L. J. C. (2016). Resistance exercise augments postprandial overnight muscle protein synthesis rates. *Medicine and Science in Sports and Exercise*, *48*(12), 2517–2525. https://doi.org/10.1249/MSS.0000000000001045

Veldhuis, J. D., Iranmanesh, A., Godschalk, M., & Mulligan, T. (2000). Older men manifest multifold synchrony disruption of reproductive neurohormone outflow. *The Journal of Clinical Endocrinology & Metabolism*, *85*(4), 1477–1486. https://doi.org/10.1210/jcem.85.4.6546

Verkhoshansky, Y., & Siff, M. C. (2009). *Supertraining :Sixth Edition-Expanded Version*. Retrieved from http://nuke.verkhoshansky.com/Portals/0/Book/Supertraining index.pdf

Vieillevoye, S., Poortmans, J. R., Duchateau, J., & Carpentier, A. (2010). Effects of a combined essential amino acids/carbohydrate supplementation on muscle mass, architecture and maximal strength following heavy-load training. *European Journal of Applied Physiology*, *110*(3), 479–488. https://doi.org/10.1007/s00421-010-1520-9

Vingren, J. L., Kraemer, W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S., & Maresh, C. M. (2010). Testosterone Physiology in Resistance Exercise and Training. *Sports Medicine*, *40*(12), 1037–1053. https://doi.org/10.2165/11536910-000000000-00000

Vitiello, M. V, Larsen, L. H., & Moe, K. E. (2004). Age-related sleep change: Gender and estrogen effects on the subjective–objective sleep quality relationships of healthy, noncomplaining older men and women. *Journal of Psychosomatic Research*, *56*(5), 503–510. https://doi.org/10.1016/S0022-3999(04)00023-6

Volek, J. S., Kraemer, W. J., Bush, J. A., Incledon, T., & Boetes, M. (1997). Testosterone and cortisol in relationship to dietary nutrients and resistance exercise. *J. Appl. Physiol.*, *82*, 49–54. Retrieved from https://uark.illiad.oclc.org/illiad/AFU/illiad.dll?Action=10&Form=75&Value=1133876

Walker, T. B., Smith, J., Herrera, M., Lebegue, B., Pinchak, A., & Fischer, J. (2010). The influence of 8 weeks of whey-protein and leucine supplementation on physical and cognitive performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(5), 409–417. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=20975109&site=ehost-live&scope=site

Weisgarber, K. D., Candow, D. G., & Vogt, E. S. M. (2012). Whey protein before and during resistance exercise has no effect on muscle mass and strength in untrained young adults. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(6), 463–469. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=22806076&site=ehost-live&scope=site

West, D. W., Burd, N. A., Coffey, V. G., Baker, S. K., Burke, L. M., Hawley, J. A., … Phillips, S. M. (2011). Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *American Journal of Clinical Nutrition*, *94*(3), 795–803. https://doi.org/10.3945/ajcn.111.013722

Westerterp-Plantenga, M. S., Lemmens, S. G., & Westerterp, K. R. (2012). Dietary protein – its role in satiety, energetics, weight loss and health. *British Journal of Nutrition*, *108*(S2), S105–S112. https://doi.org/10.1017/S0007114512002589

Wheeler, G. D., Singh, M., Pierce, W. D., Epling, W. F., & Cumming, D. C. (1991). Endurance training decreases serum testosterone levels in men without change in luteinizing hormone pulsatile release. *The Journal of Clinical Endocrinology & Metabolism*, *72*(2), 422–425. https://doi.org/10.1210/jcem-72-2-422

Wheeler, G. D., Wall, S. R., Belcastro, A. N., & Cumming, D. C. (1984). Reduced serum testosterone and prolactin levels in male distance runners. *JAMA: The Journal of the American Medical Association*, *252*(4), 514. https://doi.org/10.1001/jama.1984.03350040044020

Wilborn, C. D., Taylor, L. W., Outlaw, J., Williams, L., Campbell, B., Foster, C. A., … Hayward, S. (2013). The effects of pre-and post-exercise whey vs. casein protein consumption on body composition and performance measures in collegiate female athletes. *Journal of Sports Science & Medicine*, *12*(1), 74–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24149728

Wilkinson, S. B., Tarnopolsky, M. A., Macdonald, M. J., Macdonald, J. R., Armstrong, D., & Phillips, S. M. (2007). Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *The American Journal of Clinical Nutrition*, *85*(4), 1031–40. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17413102

Willoughby, D. S., Spillane, M., & Schwarz, N. (2014). Heavy Resistance Training and Supplementation With the Alleged Testosterone Booster Nmda has No Effect on Body Composition, Muscle Performance, and Serum Hormones Associated With the Hypothalamo-Pituitary-Gonadal Axis in Resistance-Trained Males. *Journal Of Sports Science & Medicine*, *13*(1), 192–199. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=24570624&site=ehost-live&scope=site

Willoughby, D. S., Stout, J. R., & Wilborn, C. D. (2007). Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass, and strength. *Amino Acids*, *32*(4), 467–477. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=16988909&site=ehost-live&scope=site

Willoughby, D. S., & Taylor, L. (2004). Effects of sequential bouts of resistance exercise on androgen receptor expression. *Medicine and Science in Sports and Exercise*, *36*(9), 1499–1506. Retrieved from http://0-search.ebscohost.com.library.uark.edu/login.aspx?direct=true&db=mdc&AN=15354030&site=ehost-live&scope=site

Wilson, G. J., Layman, D. K., Moulton, C. J., Norton, L. E., Anthony, T. G., Proud, C. G., … Garlick, P. J. (2011). Leucine or carbohydrate supplementation reduces AMPK and eEF2 phosphorylation and extends postprandial muscle protein synthesis in rats. *American Journal of Physiology. Endocrinology and Metabolism*, *301*(6), E1236-42.

https://doi.org/10.1152/ajpendo.00242.2011

Wilson, J., & Wilson, G. J. (2006). Contemporary issues in protein requirements and consumption for resistance trained athletes. *Journal of the International Society of Sports Nutrition*, *3*(1), 7. https://doi.org/10.1186/1550-2783-3-1-7

Wojcik, J. R., Walberg-Rankin, J., Smith, L. L., & Gwazdauskas, F. C. (2001). Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, *11*(4), 406–419. https://doi.org/10.1123/ijsnem.11.4.406

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids*, *37*(1), 1–17. https://doi.org/10.1007/s00726-009-0269-0

Wycherley, T. P., Moran, L. J., Clifton, P. M., Noakes, M., & Brinkworth, G. D. (2012). Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*, *96*(6), 1281–98. https://doi.org/10.3945/ajcn.112.044321

Zawadzki, K. M., Yaspelkis, B. B., & Ivy, J. L. (1992). Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *72*(5), 1854–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1601794

Appendix A

Institutional Review Board Approval Document

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

January 31, 2017

MEMORANDUM

TO:	Matthew Stone Ashley Binns Michelle Gray
FROM:	Ro Windwalker IRB Coordinator
RE:	New Protocol Approval
IRB Protocol #:	17-01-382
Protocol Title:	*Effects on Body Composition, Performance Measures, and Free Testosterone with Protein Supplementation in Combination with an Autoregulatory Progressive Resistance Exercise (APRE) Program in Males (35-55 Years)*
Review Type:	☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB
Approved Project Period:	Start Date: 01/31/2017 Expiration Date: 01/19/2018

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (https://vpred.uark.edu/units/rscp/index.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 48 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

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Appendix B

Informed Consent

INFORMED CONSENT

Title: **Effects on body composition, performance measures, and free testosterone with protein supplementation in combination with an Autoregulatory Progressive Resistance Exercise (APRE) program in males (35-55 years).**

Investigator(s): Matthew S. Stone, Ashley Binns & Michelle Gray
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Description: The purpose of this study is to examine the effects of whey protein supplementation versus whey protein plus autoregulatory progressive resistance exercise (APRE) on body composition, performance measures, and changes in free testosterone in non-resistance trained males over a 12-week period. The study involves four visits to the Exercise Science Research Center. The first visit (week 0; baseline) will include demographic measurements, food log with instructions, sleep scale, body composition, blood draw for baseline free testosterone measures, one-repetition maximum testing, and repetitions to failure. Following the initial visit you will be randomly placed into one of three groups for a 12-week period: control, whey protein, autoregulatory progressive resistance exercise, or whey plus autoregulatory progressive resistance exercise (APRE).

The first potential group, control group (CON), will have no supplementation or exercise regimen to follow, you will simply continue to live your normal life and come in solely for testing purposes.

The second potential group, the whey protein only group (PRO), will be given supplemental protein consisting of 25g of protein (Dymatize, Dallas, TX). The PRO group will be instructed to take 1 serving (25g) twice daily, once in the morning between breakfast and lunch, and the other in the afternoon prior to dinner. You will continue living life without any form of resistance based training, but will be allowed to maintain any aerobic based exercise (cycling, running, etc…).

The third potential group will be autoregulatory progressive resistance exercise group (APRE). The APRE group will be as prescribed a three day per week resistance training program. Utilizing the subjects' baseline 1 RM, an estimated six repetition maximum (6 RM) will be calculated for leg press and chest press only. For chest and leg press exercises, subjects will perform four total sets per exercise. The first set will be comprised of 10 repetitions at 50% of the 6 RM value. The second set will consist of 6 repetitions at 75% of 6 RM, with the third set

performed “to failure” at 100% 6 RM. The fourth set utilizes an adjusted load based off of the number of repetitions completed in the third set. After determining the load adjustment, subjects will adjust the load used in the third set, and complete an additional “to failure” set. Based off of the number of repetitions completed during the fourth set, each subject will determine their appropriate load adjustment for the next training day. For the remaining exercises, individuals will perform three sets of 10 repetitions. Subjects will be prescribed the remainder of the exercises in an attempt to complete a comprehensive full body exercise protocol to maximize positive changes in body composition, muscular performance.

The fourth potential group, the protein plus autoregulatory progressive resistance exercise group (APRE). The APRE group will be given supplemental protein consisting of 25g of protein (Dymatize, Dallas, TX) to be taken twice daily, as well as prescribed a resistance training program. On training days, one serving of protein will be taken directly following exercise training, with the other serving taken in the morning between breakfast and lunch, or in the afternoon before dinner, depending on time of training. On non-training days servings will be broken into once in the morning between breakfast and lunch, and the other in the afternoon prior to dinner.

In the next three visits you will perform the 1RM, repetitions to failure, blood draw, DXA, and receive a refill of four weeks’ worth of protein if placed in the PRO or APRE groups.

Exclusion Criteria: You will be excluded from participation if you have uncontrolled cardiovascular disease, metabolic disease, hypertension, or are currently taking testosterone supporting supplements, or testosterone replacement therapies.

Risks and Benefits*:* The risks associated with participation in this study are those involved in traditional exercise. Risks associated with exercise include, but are not limited to, muscle or joint injury, changes in blood pressure, and increased heart rate. Risk will be minimized by having trained personnel present during all testing and familiarization sessions. During the DXA scan you will be exposed to a small amount of ionizing radiation. The amount received during a DXA test is about the same as four (4) days of normal background radiation in Northwest Arkansas. The venipuncture may result in slight bruising, infection, soreness at the site of the blood draw, and/or feeling of lightheadedness/fainting. These risks will be minimized by using a standard operating procedure previously approved by the Institutional Biosafety Committee (IBC protocol # 13021) and only enrolling participants that are otherwise healthy. Blood draws will only be performed by those trained in the procedure. Universal precautions will be utilized to minimize any chance of infection. The subjects will be in a semi-reclined position during the blood draw (minimizing any injury should you feel lightheaded). Also, pressure will be applied to the blood draw site to minimize soreness. Risk will be minimized by having individuals trained in CPR/First aid present during all testing, as well as properly trained individuals during training sessions. You will complete a health history exam before participating and anyone with uncontrolled cardiovascular or metabolic disease will not qualify to participate. Benefits include a free water bottle and a free DXA scan offered to you during the last testing session along with understanding if there is an effect of whey protein and/or APRE on body composition, exercise performance, and free testosterone in males which could help with future training modalities and prescription.

***Voluntary Participation**:* Testing will take place on four separate days for about 90 minutes each session. Permission for you to engage in the testing and exercise protocol is voluntary. You are free to deny or withdraw from testing at any time if you so desire. The IRB Coordinator can be contacted at irb@uark.edu if participants have any questions about the research or their rights as a participant.

Confidentiality: All information will be kept confidential to the extent allowed by law and University policy. Data will be kept in a locking filing cabinet and analyzed in aggregate for participant confidentiality. The only individuals with access to the data will be those directly involved in the research process.

Right to Withdraw: You are free to refuse to participate and to withdraw at any time. Your decision to withdraw will bring no penalty to you.

Informed Consent: I, _____, have read the description of this program, including the purpose of the program, the procedures to be used, the potential risks and side effects, the confidentiality, as well as the option to withdraw from the program at any time. The investigator has explained each of these items to me. The investigator has answered all of my questions regarding the program, and I understand what is involved. My signature below indicates that I freely agree to participate in this study and that I have received a copy of this agreement from the investigator.

I understand that participation in all activities related to this project is voluntary on behalf of all participants. I acknowledge and agree that the University of Arkansas does not provide insurance for any of its activities and shall not be liable for any injuries that occur at any time during participation.

Participant:_____

Date:_____

Witness:_____

Date:_____

Appendix C

Health History Questionnaire

Please answer the following questions. Today's Date: _____

Date of Birth _____ What is your current age? _____

Currently use any form of testosterone support or replacement therapies? _____

Have you ever had any of the following conditions? Check yes or no. <u>If yes, please explain.</u>

Heart Disease	☐ Yes ☐ No
Heart Attack	☐ Yes ☐ No
Angina (Chest Pain)	☐ Yes ☐ No
Peripheral Artery Disease	☐ Yes ☐ No
Stroke	☐ Yes ☐ No
High Cholesterol (>200)	☐ Yes ☐ No
High Blood Pressure (≥140/90)	☐ Yes ☐ No
Diabetes	☐ Yes ☐ No
Rheumatic Fever	☐ Yes ☐ No
Aneurysm	☐ Yes ☐ No
Osteoarthritis	☐ Yes ☐ No
Joint Replacement	☐ Yes ☐ No

No history of hospitalization within the past year that would prohibit physical activity

Have you ever had any of the following conditions? Check yes or no. <u>If yes, please explain.</u>

Severe Illness (in the last year)	☐ Yes ☐ No
Operations (in the last year)	☐ Yes ☐ No
Broken bone/fracture (in the last year)	☐ Yes ☐ No

History of a fall within the preceding one year

Have you fallen in the past 12 months? ☐ Yes ☐ No **<u>If yes, please explain.</u>.**

Additional information

Have you experienced any of these symptoms? Check yes or no. <u>If yes, please explain.</u>

Pain and/or discomfort in the chest, neck, jaw, or arms	☐ Yes ☐ No
Shortness of breath at rest or with mild exertion	☐ Yes ☐ No

Dizziness ☐ Yes ☐ No

Ankle edema (swelling) ☐ Yes ☐ No

Rapid or irregular beating heart ☐ Yes ☐ No

Leg pain, cramping, or tightness during exercise ☐ Yes ☐ No

Heart murmur ☐ Yes ☐ No

Fatigue or shortness of breath during the day ☐ Yes ☐ No

Do you smoke? ☐ Yes ☐ No ☐ Quit

Please attach a list of all medication (prescription or over-the-counter) you are currently taking or use the form below.

Medication	Reason Prescribed	When do you take this medication? (check all that apply)
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

Please list all supplements (NOT medications, ex. vitamins, minerals, protein, caffeine, etc.) you are currently taking in the form below.

Supplement	Reason for taking	When do you take this supplement? (check all that apply)
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime
____________________	___________	☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

_____ _____ ☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

_____ _____ ☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

_____ _____ ☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

_____ _____ ☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

_____ _____ ☐ Morning ☐ Mid-Day ☐ Evening ☐ Bedtime

Appendix D

Pittsburgh Sleep Quality Index (PSQI)

Name_____ Date_____

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1 _____
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2 _____
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3)	C3 _____
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=!, 65%-74%=2, <65%=3	C4 _____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5 _____
Component 6	#6 Score	C6 _____
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7 _____

Add the seven component scores together _____ *Global PSQI* _____

A total score of "5" or greater is indicative of poor sleep quality.
If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

Appendix E

Data Collection Sheet

Data Collection Sheet

ID #: _____

Date: _____
Age: _____
Informed Consent: _____
HHQ: _____
Weight (lbs): _____
Weight (kg): _____
Height (in): _____
Height (cm): _____
DXA: _____
Blood Draw (6mL): _____

Bench Press: 1-RM Protocol		**Leg Press: 1-RM Protocol**	
50% Body weight (8-10 reps):	_____	120% Body weight (8-10 reps):	_____
2 minutes rest		2 minutes rest	
5-10kg increase (3-5 reps):	_____	5-10kg increase (3-5 reps):	_____
2 minutes rest		2 minutes rest	
5-10kg increase (2-3 reps):	_____	5-10kg increase (2-3 reps):	_____
2 minutes rest		2 minutes rest	
5-10kg increase (1-2 rep):	_____	5-10kg increase (1-2 rep):	_____
IF NECESSARY		IF NECESSARY	
2 minutes rest		2 minutes rest	
5-10kg increase (1-2 rep):	_____	5-10kg increase (1-2 rep):	_____
2 minutes rest		2 minutes rest	
5-10kg increase (1-2 rep):	_____	5-10kg increase (1-2 rep):	_____
FINAL 1RM: BENCH	_____	**FINAL 1RM LEG PRESS**	_____
Estimated 6RM	_____	**Estimated 6RM**	_____
75% of 1RM	_____	**75% of 1RM**	_____
Reps @ 75%	_____	**Reps @ 75%**	_____

Appendix F

Daily Training Log

APRE Workout

For the chest press and leg press you will utilize the APRE Workout Protocol. The weight used will be based on your 6 repetition maximum. Adjustments for each set will be made according to the chart below. For all other exercises, you will complete 3 sets of 10 repetitions.

Week:_____ Workout:_____

Exercise	Set	Weight Utilized	Reps Completed
Bench Press (Plate Loaded)	1—10 repetitions 2 —6 repetitions 3--------- to failure 4----------to failure	50% of 6 RM _____ 75% of 6 RM _____ 100% of 6 RM_____ Adjusted load _____	_____ _____ _____
Leg Press (Plate Loaded)	1—10 repetitions 2 —6 repetitions 3--------- to failure 4----------to failure	50% of 6 RM _____ 75% of 6 RM _____ 100% of 6 RM_____ Adjusted load _____	_____ _____ _____
Bicep Curl (3 x 10) (Dumbbell)	1 2 3	_____ _____ _____	_____ _____ _____
Tricep Pushdown (3 x 10) (Cable)	1 2 3	_____ _____ _____	_____ _____ _____
Quad Extension (3 x 10) (Machine)	1 2 3	_____ _____ _____	_____ _____ _____
Hamstring Curls (3 x 10) (Machine)	1 2 3	_____ _____ _____	_____ _____ _____
Lat Pulldowns (3 x 10) (Cable)	1 2 3	_____ _____ _____	_____ _____ _____

APRE Adjustment Protocol for 3rd and 4th Sets

Set 3 Repetitions	Set 4 Load Adjustments
0-2	-10 lbs.
3-4	-5 lbs.
5-7	No change
8-12	+5 lbs
13+	+10 lbs

****Adjusted amount after 4th set becomes new 6 RM for your next workout**

Appendix G

24-Hour Dietary Log and Instructions

DIETARY FOOD RECORD INSTRUCTIONS

- **ALL foods and beverages (INCLUDING WATER)** that are consumed should be recorded.
- Be very specific in your description of the type, the preparation method, and the amount of each food/beverage you consume.
- Use the label on foods to help you determine portion sizes.
- Save labels from packages and return them with your food record forms (this will greatly assist and enhance our analysis of your true nutrient intake).
- Use nutrient descriptors (e.g., low-fat, fat-free, light, reduced calorie, etc.) and brand names (e.g., Kraft, Nabisco, Planters, etc.) to describe foods.
- Record food/beverage consumption after each meal/snack instead of waiting until the end of the day.

MEATS/CHEESES

Description: Include description of the type, cut, and preparation method.

Portion Sizes:

List cooked (not raw) amounts of meats.
Determine amounts by weighing when possible.
Three ounces of cooked meat is equivalent to approximately a deck of cards or the palm of your hand.

Listed below are examples of how to document foods

3oz. Skinless, boneless, chicken breast-roasted
3oz. Ground beef round-fried
3oz. Deli turkey breast slices
3oz. Atlantic cod-baked
3oz. Sirloin steak-grilled
1/2 cup cubed beef stew meat
1 slice ham, 3" x 4" x 1/4"
1 oz colby cheese
1 piece cheddar cheese, 3" x 2" x 1"

STARCH/BREAD
(CEREALS, BREADS, PASTAS, RICES, BEANS)

Description: Include a complete description of the starch/bread including preparation method and brand name if applicable.

Portion Sizes:

List cooked (not raw) amounts of starch/bread products.
Generally, a measuring cup will suffice for cereals, rices, pastas, and beans.

½ cup brown rice (Uncle Bens)
2 slices rye bread-toasted
2 cups spaghetti noodles-boiled
1½ cups dry cereal (Cheerios)
1 cup oatmeal (Quaker Oats)-microwaved
8 animal crackers
Blueberry muffin, small
½ cup canned baked beans
1 corn tortilla, 6" across

FRUITS/VEGETABLES

Description: Include description of fruit/vegetable and whether it was fresh, frozen, or canned.

Include preparation method (e.g., steamed, fried, etc.)

Portion Sizes:

For whole pieces of fruit or vegetables, you may use small, medium, or large.
For many fruits/vegetables, cups may be used also.

1 medium Granny Smith apple
½ of a large tomato-fresh
5 small strawberries-fresh
½ cup canned pineapple-canned in water
1 cup frozen peas-steamed
¾ cup frozen mixed vegetables
3 spears steamed broccoli
2 medium raw carrots

COMBINATION DISHES

For standard mixed dishes, it is generally acceptable to list the type of dish without trying to list the ingredients separately. If the food is modified (e.g., low-fat), indicate this and try to describe how the food was modified. Provide enough detail to explain the composition of the dish. For tossed salad, list the individual ingredients paying careful attention to salad dressings and other caloric-dense toppings (bacon bits, cheese, ham, chopped egg, etc.).

1 cup bean chili w/o meat
3 slices thin crust large cheese pizza with pepperoni-frozen
½ cup potato salad
1 cup tuna casserole
1 cup macaroni and cheese (Kraft)
1 slice angel food cake
2 cups tossed salad
    2 cups lettuce greens
    3 slices cucumber
    3 slices tomato
    1 T shredded cheddar cheese
    1 T shredded carrots
    2 T fat-free Italian dressing

BEVERAGES/FLUIDS

Description: Include **ALL BEVERAGES INCLUDING WATER** complete description of the beverage

Portion Sizes: Use fluid ounces, liters, cups, or tablespoons.

6 oz regular coffee, brewed
12 oz Diet Pepsi
1 cup 2% milk
16 oz unsweetened iced tea
4 oz red table wine
6 oz orange juice (from concentrate)
2 T light olive oil

MISCELLANEOUS

Remember to list all condiments and additions to foods and beverages, such as cream, sugar, butter, jelly, lemon, salad dressing, artificial sweeteners, catsup, etc.

3 T low-fat french salad dressing
2 tsp black raspberry jam
1 packet Sweet'n Low
1 T cream (half and half)
2 tsp margarine spread (Country Crock)
3 T fat-free ranch salad dressing (Kraft)

Day of Week/Date:_____ **Name:** _____

Time	**FOOD/BEVERAGE DESCRIPTION**	**AMOUNT**	Total kcal ***(from label)***

Comments: