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SAFETY AND EFFICACY OF WHEY PROTEIN SUPPLEMENTATION IN TEENAGE ATHLETES

Andrew Stranieri
University of Rhode Island, andrewstranieri@gmail.com

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SAFETY AND EFFICACY OF WHEY PROTEIN
SUPPLEMENTATION IN TEENAGE ATHLETES

BY

ANDREW STRANIERI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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MASTER OF SCIENCE THESIS
OF
ANDREW STRANIERI

APPROVED:

Thesis Committee:

Major Professor	Disa L. Hatfield
	Jacob E. Earp
	Kathleen J. Melanson

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
2019

ABSTRACT

In addition to the physical aspects of training, young athletes are also mirroring the nutritional and supplementation practices of adult athletes. While research on the use of supplements among adult athletes is well known, few studies on supplement use by teenage athletes exist. Research has reported that between 22.3% to 71% of high school athletes use some form of supplementation, with whey protein being the most common. Despite the popularity of whey protein among teenage athletes, knowledge regarding its effects on health and performance in teenage athletes is currently insufficient. Therefore, the purpose of this study was to assess the safety of whey protein supplementation in relation to biomarkers of kidney functioning. A secondary purpose of this study was to investigate the effects of whey protein supplementation on body composition and athletic performance in teenage athletes. Ten healthy teenage participants (five boys and five girls) were matched according to body mass, height, tanner stage, age, and strength, and separated and randomly assigned in a double blind manner to either a 24g/d whey protein group (WP) ($n = 5$; age: 16 ± 1 y; Tanner: 4.2 ± 0.8 ; Height: 1.7 ± 0.1 m; Mass: 78.6 ± 23.4 kg; BF: 23.3 ± 11.2 %) or a 27 g/d carbohydrate control group (CG) ($n = 5$; age: 15.2 ± 1.6 y; Tanner: 4.6 ± 0.9 ; Height: 1.7 ± 0.1 m; Mass: 69.8 ± 15.4 kg; BF: 20.5 ± 10.1 %). Participants consumed their given supplement daily for four weeks. Biomarkers of kidney health, assessed via urinalysis, were collected at pre and post. At baseline and post, participants underwent athletic performance tests consisting of 1RM squat and bench press, vertical jump, and 5-10-5 agility run, and body composition testing. One-day diet logs were completed at baseline and post. Diets were analyzed for mean calorie, carbohydrate, protein, and fat

intake. Participants were considered free-living and continued with their individual sport and resistance training programs. Baseline dependent variable differences between groups were compared using independent samples t-tests. Differences in athletic performance measures, body composition, and urinalysis measures were analyzed by a two-way mixed factorial (ANOVA). Significance was set at $p \leq 0.05$ and values are presented as mean $\pm$ SD. There were no baseline differences ($p \geq 0.05$) in age, height, tanner stage, body mass, vertical jump, 5-10-5 pro agility run, squat 1RM, bench press 1RM, PBF, LBM, BMD, or any urinalysis measure. Additionally, no baseline differences ($p \geq 0.05$) were observed for mean calorie, carbohydrate, protein, or fat intake. No main effect ($p \geq 0.05$) for time was revealed for VJ, 5-10-5 pro agility run, squat 1RM, or any body composition or urinalysis measure, but was revealed for bench press 1RM ($p = 0.003$). However, there was no group X time interaction was observed for any athletic performance, body composition, or urinalysis variable ($p \geq 0.05$). To the best of our knowledge, this study is the first of its kind investigating the effects of whey protein on health and performance in teenage athletes. Results of this study suggest that short-term whey protein supplementation in a healthy teenage athlete population has no negative effect on biomarkers of kidney health. Therefore, researchers should now feel safe conducting whey protein supplementation protocols in a teenage population, utilizing longer intervention periods (i.e. ≥ 8 weeks), with the goal of eliciting positive changes in body composition and athletic performance.

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Lastly, I would like to thank my family, for their love and support. Mom, Dad, and Mark, you have stood by me and supported every decision I have made. I dedicate this thesis to them.

PREFACE

This thesis was written to comply with the University of Rhode Island Graduate School manuscript format. The thesis document contains one manuscript: *Safety and efficacy of whey protein supplementation in teenage athletes*. The manuscript has been written in a form formatted for publication in *The Journal of Strength and Conditioning Research*.

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MANUSCRIPT

Title:

Safety and efficacy of whey protein supplementation in teenage athletes

Authors:

Andrew M. Stranieri ^a, Ariana I. Cambio ^a, Andrew M. Procopio ^a, Kathleen J. Melanson ^b, PhD, Jacob E. Earp ^a, PhD, Disa L. Hatfield ^a, PhD.

Affiliations:

^a University of Rhode Island, Department of Kinesiology, Kingston, Rhode Island, 02881, USA. ^b Department of Nutrition and Food Sciences, University of Rhode Island, Kingston, Rhode Island, 02881, USA

Address correspondence to:

Dr. Disa L. Hatfield
Department of Kinesiology
University of Rhode Island
25 West Independence Way, Suite P
Kingston, RI 02881
doch@uri.edu
401-874-5183

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ABSTRACT

The purpose of this study was to investigate the effects of whey protein supplementation on biomarkers of kidney health, body composition, and athletic performance in teenage athletes. Ten healthy participants were matched according to body mass, height, tanner stage, age, sport, and strength, and separated and randomly assigned in a double-blind manner to a 24g/d whey protein group ($n = 5$; age: 16 ± 1 y; Tanner: 4.2 ± 0.8 ; Height: 1.7 ± 0.1 m; Mass: 78.6 ± 23.4 kg; body fat: 23.3 ± 11.2 %) or a 27 g/d carbohydrate control group ($n = 5$; age: 15.2 ± 1.6 y; Tanner: 4.6 ± 0.9 ; Height: 1.7 ± 0.1 m; Mass: 69.8 ± 15.4 kg; body fat: 20.5 ± 10.1 %). Participants consumed their given supplement daily for four weeks. Biomarkers of kidney health were assessed at baseline and post. At baseline and post, participants underwent 1RM squat and bench press, vertical jump (VJ), 5-10-5 pro agility run, and body composition testing. Significance was set at $p \leq 0.05$. No main effect ($p \geq 0.05$) for time was revealed for any urinalysis measure, VJ, 5-10-5 pro agility run, squat 1RM, or any body composition measure, but was revealed for bench press 1RM ($p = 0.003$). No group X time interaction was observed for any aforementioned variable ($p \geq 0.05$). Results suggest that short term whey protein supplementation has no negative impact on kidney health. Therefore, researchers should now feel safe investigating its effects on performance and body composition over a longer intervention period.

Keywords: youth, body composition, athletic performance, children, ergogenic aid

INTRODUCTION

In order to set themselves apart from their peers, young athletes are willing to pursue almost anything they believe will give them a competitive advantage (23). This often leads them to replicating not only the training of the professional athletes they admire, but their nutritional and supplementation practices as well (34). Additionally, pressure from peers and the mass media can cause young athletes to turn to illegal, dangerous means of improving performance and physical appearance (12). While research on the use of supplements among adult athletes is well known (13,19), studies on supplement use by teenage athletes are much more limited, with research reporting that between 22.3% to 71% of high school athletes were using some form of supplementation (30).

Common reasons cited for supplement use in youth athletes is similar to that in adults: improvement of athletic performance and recovery from fatigue (48). Many young athletes decide on their supplementation use without advice from others, occasionally seeking help from a parent, trainer, or coach, or in rare circumstances, doctors and dieticians (11,35). One of the most common supplements taken by young athletes is whey protein. Indeed, a study of young elite Japanese athletes participating in the 2010 Youth Olympic Games reported the most popular supplement used was protein powder, with 21.3% of athletes reported using it (38). A survey of elite young UK athletes reported that athletes were taking whey protein with the goal of maintaining strength and increasing their ability to train longer (36). Additionally, usage of protein powders and shakes by adolescents in an effort to improve appearance and strength have been reported (15).

In adults, whey protein has been well documented to be an excellent stimulator of muscle protein synthesis (9), which is essential for increasing muscle mass. Whey protein has also been well documented for its ability to improve muscular strength, athletic performance, and maintain lean body mass and reduce fat mass (32). Research has also reported that diets higher in protein and lower in carbohydrate are most advantageous for promoting weight loss and preserving lean body mass (18,50).

Despite these known benefits, the use of whey protein and/or high protein diets have been criticized for supposedly negatively impacting kidney (16) and bone (4) health. A recent systematic review examining high protein intake ($\geq 20\%$ but $< 35\%$ of total energy intake) on glomerular filtration rate reported that a high protein intake over a short term period (< 6 months) had no adverse effect on kidney functioning in an adult population (44). In addition, a recent meta-analysis suggested that compared to low or normal protein intakes, a high protein intake (≥ 1.5 g/kg body weight or $\geq 20\%$ energy intake or ≥ 100 g protein/d) does not negatively impact kidney functioning (10). Similar findings have been reported for the effects of protein on bone health, with multiple studies reporting no adverse effects of high protein intake on bone mineral density (BMD) and bone mineral content in a healthy adult population (8,39).

When combined with resistance training, the ergogenic effects of whey protein appear to be enhanced (33). A primary benefit of resistance training is increased muscle strength, and whey protein is often added to resistance training protocols in an effort augment these effects (33). Prior research suggests that the growth spurt observed during puberty is associated with a marked increase in the efficiency of

dietary protein utilization for growth (5). Research also suggests that active youth demonstrate greater overall anabolic sensitivity to dietary protein and amino acids than active adults due to their bodies' need to support more rapid increases in lean tissue due to growth and maturation (31). In addition, active youth require more protein per kg of body mass than adults to support growth (31). This suggests that active, post-pubertal children are more efficient at utilizing protein and therefore whey protein is a potential ergogenic aid for teenage athletes.

There have been limited studies investigating supplemental protein use in teens, with most investigating its effect on whole body protein balance (46). Given the popularity of whey protein supplementation in teenage athletes and the known benefits it can provide for an adult population, research needs to be done examining how this supplement impacts a teenage population. To the best of our knowledge, there have been no studies done at this point investigating the safety of whey protein in teenage athletes. Therefore, the purpose of this study was to assess the safety of whey protein supplementation in relation to kidney functioning. A secondary purpose was to investigate the short term effectiveness of whey protein supplementation, independent of training, on athletic performance and body composition in teenage athletes.

METHODS

Experimental Approach to the Problem

A randomized, maturity-matched control group, double-blind study design was used to assess the effects of whey protein supplementation before and after four weeks of continued resistance and sport-specific training. Body composition, athletic

performance, and biomarkers of kidney health were assessed one week prior (baseline) to the intervention and again after the final week of the intervention (post). All participants were considered free-living and continued with their normal sport-specific and resistance training programs. Participants and their parents received information about the study and during initial finalization sessions, the methods and procedures of the study were explained, participants and subject's parents were allowed to ask questions, and an in depth medical, training, and nutritional history questionnaire was completed. Each participant signed a child assent form and each parent or legal guardian signed a parental permission form. The Institutional Review Board of the University of Rhode Island approved the research protocol.

Participants

Ten healthy teenage boy and girl athletes across a variety of sports (primarily lacrosse, track and field, and football) from a local gym volunteered to participate in the study. To be eligible to participate in the study, all participants had to be post-pubertal (determined via Tanner Stages), have a previously established history of sport participation and resistance training experience (≥ 6 months), and maintain complete training status (without injury) throughout the duration of the study. Detailed medical, nutritional, and training histories were used to assess ability to participate.

Individuals were excluded if they did not meet any of the aforementioned criteria or if they were using any medication that affected exercise capacity, had any cardiopulmonary or metabolic disease, had any orthopedic limitation, or impaired motor development. In addition, individuals were excluded if they identified using any

performance enhancing supplements or were currently taking whey protein on a regular basis.

Procedures

Participants were matched according to body mass, height, maturity, age, sport, and strength, and separated and randomly assigned in a double-blind manner a 24g/d whey protein group (WP) (n=5; three girls, two boys, age: 16 ± 1 y; Tanner: 4.2 ± 0.8 ; Height: 1.7 ± 0.1 m; Mass: 78.6 ± 23.4 kg; BF: 23.3 ± 11.2 %) or a 27 g/d carbohydrate control group (CG) (n=5; two girls, three boys, age: 15.2 ± 1.6 y; Tanner: 4.6 ± 0.9 ; Height: 1.7 ± 0.1 m; Mass: 69.8 ± 15.4 kg; BF: 20.5 ± 10.1 %) (Table 1).

Supplementation Protocol

The WP group consumed 24 g of whey protein (Optimum Nutrition Gold Standard 100% Whey, Double Rich Chocolate, Optimum Nutrition, Inc., Aurora, IL) daily for four weeks. The whey protein consisted of 120 calories, 1.5 g fat, 3 g carbohydrate, and 24 g protein (blend of whey protein concentrate, whey protein isolates, and whey peptides). The CG group consumed a 27 g carbohydrate placebo (Carnation Breakfast Essentials Powder Drink Mix, Rich Milk Chocolate, Nestle HealthCare Nutrition Inc., Florham Park, NJ) daily for four weeks. The placebo consisted of 130 calories, 0.5 g fat, 27 g carbohydrate (19 g maltodextrin, 8 g dietary fiber), and 5 g protein. The whey protein dosage was based on manufacturers recommendation and the most common dosage taken by adults. This dosage is also most representative of what teenage athletes have reported taking and is therefore

more reflective of real-world situations, unlike prescribing dosage by body weight, for example.

Supplements were consumed immediately after training or at a similar time of day on non-training days. Supplements were of similar consistencies, amount, and flavor. Supplements were in powder form and distributed in individual generic packages on a weekly basis. Participants were instructed to mix their given supplement with 10 oz of water. Compliance was monitored by having participants consume their given supplement in the presence of study personnel on training days. Additionally, participants recorded the date and time when the supplement was consumed on log sheets. Empty packets were returned and counted at the end of each one-week period.

Urinalysis

Urine was collected as a noninvasive way of assessing kidney function at baseline and post. Protein (mg/dL), glucose (mg/dL), ketone (mg/dL), blood, pH, nitrite, and leukocyte content was visually assessed via color-block reagent strips (Siemens Multistix® 10 SG Reagent Strips, Siemens Medical Solutions, Malvern, PA).

Proteinuria is the excessive elimination of protein in the urine and is a characteristic of glomerular dysfunction (43). Proteinuria is present by definition when the protein concentration in urine is at least 30 mg/dL (43). The amount of protein in the urine was assessed via color-block reagent strips as either negative, 1+ (30 mg per dL), 2+ (100 mg per dL), 3+ (300 mg per dL) or 4+ (1,000 mg per dL) (27). Values

below 30 mg/dL are therefore presented as negative, or 0 mg/dL, which was used for analysis.

Analysis for glucose excretion was defined in a similar fashion, with a negative result being anything >100 mg/dL, and thus 0 mg/dL was used for analysis. Negative ketone excretion was defined as being > 15 mg/dL, and therefore treated as 0 mg/dL for analysis. Nitrite, leukocyte, and blood excretion was defined as either positive or negative, therefore when a negative result occurred, it was reported as 0 for analysis.

Athletic Performance Testing

Measures of athletic performance were assessed at baseline and post. Performance testing was completed during a single session and supervised by National Strength and Conditioning Association Certified Strength and Conditioning Specialists. All participants had a previously established history of sport participation and resistance training experience (≥ 6 months), therefore no familiarization for the athletic performance tests was required as participants were already familiar. Muscular strength was assessed via bench press and squat 1RM following similar guidelines done by Faigenbaum et al. (14). Agility was assessed via 5-10-5 pro-agility run measured via automatic timing (Brower Timing Systems, Draper, UT) and power was assessed via vertical jump measurement (VERTEC, Power Systems, Knoxville, TN). Both tests were performed three times, and the peak of the three trials was used for analysis. Tests were completed in the following order: vertical jump, 5-10-5 pro-agility run, squat 1RM, bench press 1RM. All participants completed the same standardized full body warm up prior to all testing. Full rest was given between every

trial and every test. Pre and post testing was also performed at the same time of day ($\pm$ 0.5 hr).

Anthropometrics

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 216, Seca North America, Chino, CA). Participants wore light clothes and no shoes. Measurements were taken from the floor to the top of the head, with feet together on the floor and as close to the wall without touching.

Body Composition Testing

Body composition, including body mass (BM), percent body fat (PBF), and lean body mass (LBM), was assessed at baseline and post via bio-electrical impedance analysis (BIA; InBody 770, InBody, Cerritos, CA) according to manufacturer's guidelines. Each participant was instructed to attend both pre and post testing sessions euhydrated, which was confirmed as a urine specific gravity ≤ 1.020 (21) in order to increase measurement accuracy.

Heel ultrasonography (GE Achilles EXP II ; General Electric Co, USA) was used to measure BMD at baseline and post according to manufacturer's guidelines. Heel BMD was reported as stiffness index. Stiffness index value, as stated by the manufacturer, is the basic measurement of bone density given by the device. The device utilizes quantitative ultrasound (QUS). QUS is based on the principle that bone, as a porous material, will absorb, scatter and transmit sound wave dependent on stiffness, density and volume of the material (17). Both sound attenuation and the sound velocity are combined to form the stiffness index value.

Dietary Recall

All participants completed a one-day diet log at baseline and post. Participants recorded all food and drink (except water), including brand if possible, method of preparation, and amount. Diet logs were analyzed using Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool (National Institutes of Health, Bethesda, MD) for mean calorie, fat, carbohydrate, and protein intake.

Statistical Analysis

Baseline dependent variable differences between groups were compared using independent samples t-tests. Differences in athletic performance measures, body composition, and urinalysis measures were analyzed by a two-way mixed factorial analysis of variance (ANOVA). Statistical analysis was performed using SPSS (Version 25, Chicago, Ill). Significance was set at $p \leq 0.05$. Values are presented as mean $\pm$ SD.

RESULTS

Baseline Differences Between Groups

There were no baseline differences ($p \geq 0.05$) in age, body mass, height, tanner stage, vertical jump, 5-10-5 pro agility run, 1RM squat, 1RM bench press, PBF, LBM, BMD. Additionally, no baseline differences ($p \geq 0.05$) were observed for mean calorie, carbohydrate, protein, or fat intake (Table 1).

Urinalysis

The results indicated that there were no significant ($p \geq 0.05$) changes for leukocyte, nitrite, pH, protein, blood, ketone, and glucose content in urine over time.

There were also no main effects revealed for time X group interaction for any urinalysis measure (Table 5).

Athletic Performance Tests

The results indicated that there was no group X time interaction ($p \geq 0.05$) for any measure of athletic performance. However, there was a significant ($p = 0.003$) increase in bench press 1RM over time for both groups (Table 2).

Body Composition

The results indicated that there were no significant ($p \geq 0.05$) changes over time for BM, PBF, LBM, or BMD for the WP or CG. There was no main effect revealed for time and no time X group interaction was observed for any body composition variable (Table 3).

Dietary Recall

The results indicated that there were no significant differences ($p \geq 0.05$) at baseline, post, over time, or between groups for any nutritional intake variable (Table 4). However, it is important to note that the post-intervention diet logs were recorded one-day post consumption of the final day of supplement intervention period. Therefore, the macronutrient data represented at post does not feature the presence of either supplement in both groups.

DISCUSSION

Kidney Functioning

In the present study, there were no significant changes in biomarkers of kidney functioning for the WP or CG. Thus, whey protein should be considered a safe dietary

supplement in teenage athletes given the lack of changes from baseline to post-testing in biomarkers of kidney functioning. This is in agreement with previous research done by Lothian et al. (28). Eleven children (age 12.6 ± 3.6 y) with mild to moderate asthma supplemented with whey protein (18.2 g/d) and researchers monitored changes in serum urea and creatinine. After one month of daily supplementation, there were no significant changes in serum urea or creatinine. While a lower dose than the one given in the present study, the results of this study suggest that an increase in daily protein intake in children has no adverse effect on kidney functioning.

Athletic Performance

In the present study, there were no significant changes observed between groups for vertical jump, 5-10-5 pro agility run, squat 1RM, or bench 1RM. Additionally, there were no differences observed over time for both groups for vertical jump, 5-10-5 pro agility run, or squat 1RM. However, a significant increase in bench press 1RM was revealed over time for both groups. Thus, the addition of whey protein supplementation to the diet of teenage athletes, administered over a short period of time, resulted in no considerable improvements in common tests of athletic performance.

Previous studies investigating any form of protein supplementation in young athletes with the goal of improving performance and/or muscle mass are scarce. Laskowski and Antosiewicz (25) randomly assigned twelve young judoists to a 0.5g/kg body mass/day soy protein (mean age 16.8 ± 0.4 y) or an isocaloric control group (mean age 15.6 ± 1.3 y). Participants continued their normal training and after four weeks of supplementation, it was reported that while both groups had significant

increases in VO₂max and Wingate test performance (peak power output and total work output), increases were significantly higher in the soy protein group than the control group. The dosage and type of protein in this study differs from that of the present study, however. While not done in an exclusively teenage population, Wilborn et al. (49) reported that in combination with eight weeks of structured resistance training, a 24 g whey protein supplement improved vertical jump, broad jump, 5-10-5 pro agility run, and 1 RM bench and leg press testing in a young female population (mean age 20 ± 1.9 y).

Similar results were reported by McAdam et al. (29). Participants (mean age 19 ± 1 y) were randomly assigned to either a whey protein group (77g/d) or an isocaloric carbohydrate control group. After eight weeks of military basic training, the whey protein group performed significantly more push-ups than the carbohydrate control group. An important note to make is that the whey protein dose utilized in this study was significantly higher than that of the present study, but did feature a teenage post-pubertal, although not high school age, population. Antonio et al. (2), however, reported contrasting results. After assigning untrained women (mean age 26.9 ± 1 y) to either an essential amino acid group (average dose of 18.3 g of essential amino acids) or a placebo group and engaging in six weeks of combined aerobic and resistance training, it was reported that while no significant changes in muscular strength were seen, treadmill time to exhaustion increased significantly in the amino acid group. The results of these studies suggest that a longer intervention period (i.e. at least 8 weeks) may be needed to elicit positive changes in athletic performance as a result of whey protein supplementation.

Body Composition

In the present study, there were no significant changes observed between groups for body mass, lean body mass, or percent body fat. Additionally, there were no differences observed over time for both groups for any of the aforementioned variables. Therefore, these results would suggest that supplementing with whey protein over a short term period has no influence, positive or negative, on typical measures of body composition. A longer intervention period is likely needed to observe any kind of change.

Research investigating the effects of whey protein supplementation in youth are limited. Leidy et al. (26) reported that daily consumption of a high protein breakfast (35g/d) over the course of 12 weeks in young adults (mean age 19 ± 1 y) reduced gains in fat mass compared to a control. Additionally, overweight adolescents (age 12-15 y) who consumed 35g/d of skimmed milk, casein, or whey protein for 12 weeks had similar increases in lean mass index, but significantly greater increases in fat mass index and body weight than those who consumed water, despite a similar total caloric intake (3,24).

In a young adult athletic population, results have been more consistently positive. Taylor et al. (41) indicated that after eight weeks of whey protein supplementation (24 g) in female college basketball players (mean age 21 ± 3 y), lean mass significantly increased and fat mass significantly decreased compared to a placebo. Similarly, Candow et al. (7) reported that combined with resistance training, six weeks of whey protein supplementation (1.2 g/kg body mass) resulted in significantly greater gains in lean tissue mass than a placebo in young adults (mean

age 24.0 ± 6 y). These results suggest that whey protein supplementation in young adult populations is effective for improving body composition.

No changes were seen in regard to markers of BMD. This is to be expected, as the four week intervention of the present study was too short of a time period to see any changes in BMD, positive or negative, regardless of the supplementation or resistance training. Research in adult populations, however, have reported no negative effects of a prolonged (≥ 1 y) high protein diet or whey protein supplementation on bone health (1,20).

Additionally, no changes in urine pH were seen over time or between groups (Table 5). Urine pH is a measure of the amount of acid in the urine. A normal urine pH value can range from 4.6 to 8.0, with a lower pH being considered more acidic and a higher pH being considered more alkaline (40). A low urine pH could potentially indicate an increased acid load of the body, and to compensate for this, the body may take calcium out the bones in an attempt to neutralize the increase acid (47). Therefore, a low urine pH could be a sign of reduced BMD. However, no changes in urine pH were seen over time or between groups and urine pH was within the aforementioned normal range at both time points for both groups. This further supports the hypothesis that whey protein supplementation should be considered safe for this population.

Protein Supplementation and Dietary Protein Intake

In the present study, there were no significant changes observed over time or between groups for mean calorie, fat, carbohydrate, and protein intake. Analysis of total dietary protein intake and body weight at pre and post for both groups revealed

that at baseline, the WP group was consuming 1.5 g/kg/bw of protein, while the CG was consuming 1.7 g/kg/bw dietary protein. At post, without their respective supplements, the WP was still consuming 1.5 g/kg/bw of protein, while the CG increased slightly to 1.8 g/kg/bw. With the addition of their respective supplements (Table , the WP group was revealed to be consuming 1.7 g/kg/bw at post while the CG was revealed to still be consuming 1.8 g/kg/bw of protein.

Current protein recommended dietary allowance (RDA) for healthy adults over the age of 19 is 0.8 g/kg/day (42), while the Acceptable Macronutrient Distribution Range for protein in children (ages 4-18) is 10-30% of total energy intake (20). However, there appears to no general consensus regarding recommended protein intake for active children and adolescents. Values ranging from 0.6 – 2.9 g/kg/day have been proposed (6,37,45).

A recent review by Moore suggests that protein requirements in active children and adolescents are similar to those of athletic adult populations, not only because of the anabolic nature of physical activity, but also because of the need to support the rapid increases in lean body mass that occur in this population (31). Therefore, Moore proposes a daily intake of ~1.6 g/kg/day in this population in order to meet the metabolic demand necessary to sustain a neutral or even positive whole body net protein balance (31). Based on this information, it would appear that the participants in the CG were meeting the proposed necessary protein needs throughout the study (1.7 g/kg/bw pre and 1.8 g/kg/bw post), while those in the WP group were not (1.5 g/kg/bw pre and post). Only with the addition of the supplement (1.7 g/kg/bw) did the WP group meet the proposed protein needs. Therefore, both groups should not be

considered high protein, as they were only meeting the proposed protein needs (in the WP group this was only due to the addition of the supplement). Future studies should look to investigate the safety of high protein intake ($> 2\text{g/kg/day}$) in this population.

Conclusion

In summary, daily whey protein supplementation had no adverse effects on kidney functioning or bone health in this population. Furthermore, the results of the present study indicate that whey protein supplementation, at least over a short term period, has no effect, positive or negative, on body composition and typical measures of athletic performance. Further research is needed to investigate if positive changes in body composition and athletic performance can occur over a longer intervention period. To the best of our knowledge, this study is the first of its kind investigating the effects of whey protein on health and performance in teenage athletes.

Despite the fact that the aim of the present study was to examine the effects of a daily addition of whey protein to the diet of teenage athletes while they continued their normal sport specific and resistance training regimen, this could be considered a limitation, as the training of the participants was not controlled. However, participants were matched according to sport, season, and strength and therefore received similar training, mitigating this issue. Future studies could still look to control the training of participants. A strength of the present study was the collection of diet logs at baseline and post.

PRACTICAL APPLICATIONS

In the present study, daily whey protein supplementation over a one month period did not improve body composition or tests of athletic performance in teenage athletes, compared to a carbohydrate placebo. However, no adverse effects on kidney health were revealed as a result of the whey protein supplementation. This data suggests that for an athletic, high school age population, whey protein supplementation is safe over a short period of time. Therefore, future researchers should feel comfortable conducting long term intervention studies, with the main focus investigating the effectiveness of whey protein on performance in this population.

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TABLES

Table 1: Anthropometrics and baseline differences between groups (mean $\pm$ SD).

Variable	WP (n=5)	CG (n=5)	p value
Age (years)	16 $\pm$ 1	15.2 $\pm$ 1.6	0.141
Height (cm)	170.8 $\pm$ 12.3	167.7 $\pm$ 10.8	0.737
Body mass (kg)	78.7 $\pm$ 23.4	69.7 $\pm$ 15.4	0.807
Tanner stage	4.2 $\pm$ 0.8	4.6 $\pm$ 0.9	1.000
Vertical jump (inches)	15.3 $\pm$ 5.6	14.9 $\pm$ 5.2	0.636
5-10-5 pro-agility run (seconds)	5.3 $\pm$ 0.7	5.4 $\pm$ 0.6	0.501
1RM squat (kg)	94.5 $\pm$ 45.5	96.8 $\pm$ 43.2	0.848
1RM bench press (kg)	56.8 $\pm$ 26.4	56.4 $\pm$ 25.6	0.911
Percent body fat	23.3 $\pm$ 11.2	20.5 $\pm$ 10.1	0.951
Lean body mass (kg)	59.2 $\pm$ 17.8	55.3 $\pm$ 13.5	0.446
BMD (Stiffness Index)	123.8 $\pm$ 12.8	107.5 $\pm$ 29.5	0.157
Calories (kcal)	2240.6 $\pm$ 437.1	2196.1 $\pm$ 591.8	0.062
Carbohydrates (g)	230.7 $\pm$ 90.0	283.3 $\pm$ 74.4	0.237
Protein (g)	121.0 $\pm$ 18.1	121.2 $\pm$ 19.7	0.057
Fat (g)	74.3 $\pm$ 28.5	65.5 $\pm$ 33.9	0.515

WP = whey protein group, CG = carbohydrate placebo control group, 1RM = one repetition max.

Table 2: Differences in measures of athletic performance between groups over time (mean $\pm$ SD). * denotes significant difference from pre values in corresponding group.

Measure	Group	Pre	Post	Time		Time X Group	
				p value	Partial eta sq.	p value	Partial eta sq.
Vertical jump (inches)	WP	15.3 $\pm$ 5.6	14.2 $\pm$ 4.8	0.838	.006	0.067	0.358
	CG	14.9 $\pm$ 5.2	15.8 $\pm$ 4.0				
5-10-5 pro-agility run (seconds)	WP	5.3 $\pm$ 0.7	5.5 $\pm$ 0.6	0.235	0.171	.100	0.301
	CG	5.4 $\pm$ 0.6	5.3 $\pm$ 0.5				
Squat 1RM (kg)	WP	94.5 $\pm$ 45.5	98.2 $\pm$ 50.0	0.078	0.338	0.643	0.028
	CG	96.8 $\pm$ 43.2	102.7 $\pm$ 44.8				
Bench 1RM (kg)	WP	56.8 $\pm$ 26.4	59.7 $\pm$ 25.7*	0.003*	0.748	0.194	0.228
	CG	56.4 $\pm$ 25.6	61.8 $\pm$ 28.1*				

WP = whey protein group, CG = carbohydrate placebo control group, 1RM = one repetition max.

Table 3: Differences in body composition between groups over time (mean $\pm$ SD).

Measure	Group	Pre	Post	Time		Time X Group	
				p value	Partial eta sq.	p value	Partial eta sq.
Body mass (kg)	WP	78.7 $\pm$ 23.4	79.2 $\pm$ 23.7	0.071	0.350	0.580	0.040
	CG	69.7 $\pm$ 15.4	70.7 $\pm$ 15.3				
Percent body fat	WP	23.3 $\pm$ 11.2	23.3 $\pm$ 10.4	0.671	0.038	0.617	0.038
	CG	20.5 $\pm$ 10.1	20.2 $\pm$ 10.2				
Lean body mass (kg)	WP	59.2 $\pm$ 17.8	59.5 $\pm$ 18.8	0.180	0.212	0.514	.055
	CG	55.3 $\pm$ 13.5	56.3 $\pm$ 13.8				
BMD (Stiffness Index)	WP	123.8 $\pm$ 12.8	118.5 $\pm$ 25.2	0.428	0.107	0.984	.000
	CG	107.5 $\pm$ 29.5	102.5 $\pm$ 17.7				

There were no significant differences ($p \geq 0.05$) over time or between groups for any measure of body composition. WP = whey protein group, CG = carbohydrate placebo control group.

Table 4: Differences in macronutrient intake between groups over time without their respective supplement (mean $\pm$ SD).

Measure	Group	Pre	Post	Time		Time X Group	
				p value	Partial eta sq.	p value	Partial eta sq.
Calories (kcal)	WP	2240.6 $\pm$ 437.1	2077.4 $\pm$ 859.9	0.312	0.145	0.225	0.202
	CG	2196.0 $\pm$ 991.8	2243.0 $\pm$ 1291.6				
CHO (g)	WP	230.7 $\pm$ 90.0	221.5 $\pm$ 82.7	0.612	0.039	0.829	0.007
	CG	283.3 $\pm$ 74.4	260.4 $\pm$ 124.4				
PRO (g)	WP	121.0 $\pm$ 18.1	120.9 $\pm$ 16.8	0.405	0.142	0.332	0.188
	CG	121.2 $\pm$ 19.7	126.8 $\pm$ 19.8				
Fat (g)	WP	74.3 $\pm$ 28.5	65.5 $\pm$ 20.9	0.482	0.073	0.094	0.350
	CG	65.5 $\pm$ 33.9	78.3 $\pm$ 51.3				

There were no significant differences ($p \geq 0.05$) over time or between groups for any nutritional intake variable. WP = whey protein group, CG = carbohydrate placebo control group, CHO = total dietary carbohydrate intake, PRO = total protein intake.

Table 5: Differences in macronutrient intake between groups over time with the addition of their respective supplements (mean $\pm$ SD).

Measure	Group	Pre	Post	Time		Time X Group	
				p value	Partial eta sq.	p value	Partial eta sq.
Calories (kcal)	WP	2240.6 $\pm$ 437.1	2197.4 $\pm$ 859.9	0.664	0.029	0.218	0.208
	CG	2196.0 $\pm$ 991.8	2373.0 $\pm$ 1291.6				
CHO (g)	WP	230.7 $\pm$ 90.0	239.8 $\pm$ 79.4	0.973	0.000	0.868	0.004
	CG	283.3 $\pm$ 74.4	287.4 $\pm$ 124.4				
PRO (g)	WP	121.0 $\pm$ 18.1	134.9 $\pm$ 16.8	0.652	0.044	0.464	0.112
	CG	121.2 $\pm$ 19.7	131.8 $\pm$ 19.8				
Fat (g)	WP	74.3 $\pm$ 28.5	67.0 $\pm$ 20.9	0.537	0.057	0.100	0.339
	CG	65.5 $\pm$ 33.9	78.8 $\pm$ 51.3				

There were no significant differences ($p \geq 0.05$) over time or between groups for any nutritional intake variable with the addition of their respective supplement. WP = whey protein group, CG = carbohydrate placebo control group, CHO = total dietary carbohydrate intake, PRO = total protein intake.

Table 6: Differences in urinalysis measures between groups over time (mean $\pm$ SD).

Measure	Group	Pre	Post	Time		Time X Group	
				p value	Partial eta sq.	p value	Partial eta sq.
Leukocytes (total cell count)	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				
Nitrite content	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				
Protein (mg/dL)	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				
pH	WP	6.5 $\pm$ 0.9	6.1 $\pm$ 0.7	0.237	0.170	0.860	.004
	CG	7.2 $\pm$ 0.4	6.9 $\pm$ 0.9				
Blood (total cell count)	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				
Ketone (mg/dL)	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				
Glucose (mg/dL)	WP	0 $\pm$ 0	0 $\pm$ 0	1.000	-	1.000	-
	CG	0 $\pm$ 0	0 $\pm$ 0				

There were no significant differences ($p \geq 0.05$) over time or between groups for any urinalysis measure. WP = whey protein group, CG = carbohydrate placebo control group.

APPENDICES

Appendix A: Review of the Literature

Abstract

The pursuit of excellence in athletic performance is not solely an adult interest. Long-term athletic development models start as early as age five in some sports (Lloyd et al., 2016). In addition to the physical aspects of training, young athletes are also mirroring the nutritional and supplementation practices of adult athletes (Naylor & Cherubini, 2008). Major supplement companies are already ahead of the curve in terms of marketing, having nutritional supplementation products specifically marketed towards young athletes with claims of improved performance. While research on the use of supplements among adult athletes is well known (Erdman et al., 2006; S.-H. S. Huang et al., 2006), few studies on supplement use by teenage athletes have been done. Research has been reported that between 22.3% to 71% of high school athletes were using some form of supplementation (McDowall, 2007). Whey protein is a common and popular supplement taken by adults and young athletes. However, knowledge regarding its safety and efficacy in youth athletes is currently insufficient. Common reasons cited for supplement use in youth athletes is similar to that in adults: improvement of athletic performance and recovery from fatigue (Wiens, Erdman, Stadnyk, & Parnell, 2014). While the benefits (Devries & Phillips, 2015; Maughan et al., 2018) and safety (Campbell et al., 2007) of whey protein supplementation in adults is undisputed, the safety nor the performance enhancing benefits of whey protein supplementation in youth athletes has been investigated. This study will seek to better

understand the effects of this supplement on the health and athletic performance of young athletes ages 13-18.

Introduction

Whey protein is one of several primary proteins found in cow's milk. Whey protein is typically defined as a mixture of proteins isolated from whey, which is the liquid remaining after cow's milk has been curdled and strained. Whey is a by-product of the cheese and curdle manufacturing process and was previously considered a waste product. However, whey was found to have several health applications, and was no longer considered a by-product of the cheese manufacturing process. Instead, it became viewed as a co-product (Marshall, 2004), with importance placed on its production. The commercial success of whey protein has led to the development of high quality whey protein supplements by manufacturers. Whey protein contains all of the essential amino acids the body requires (Walzem, Dillard, & German, 2002). Whey protein, in its powdered form, is an incredibly popular dietary supplement and is purported to have immunoenhancing effects as well as positive benefits on exercise performance and body composition.

Whey protein is typically taken with intent to improve athletic performance and to recover from fatigue in adults (D. P. J. Cribb, 2005). Common reasons cited for supplement use in youth athletes is similar to that in adults: improvement of athletic performance and recovery from fatigue (Wiens et al., 2014). Teenage athletes are being increasingly exposed to an expanding market of nutritional supplements and tend to be heavily influenced by professional athletes, as well as their own peers, when regarding supplementation use. Many young athletes decide on their supplementation

use without advice from others, occasionally seeking help from a parent, trainer, or coach, or in rare circumstances, doctors and dieticians (Diehl et al., 2012; Petróczi et al., 2007). Researchers have reported that young athletes are taking supplements with the goals of staying healthy, increasing energy, and improving immune function (Wiens et al., 2014).

Few studies on supplement use by teenage athletes have been done. Several different studies have reported that between 22.3% to 71% of high school athletes were using some form of supplementation (McDowall, 2007). Among a high school cohort of 270 athletes, 58% had used some form of supplementation (Kayton, Cullen, Memken, & Rutter, 2002). A study of young elite Japanese athletes participating in the 2010 Youth Olympic Games reported the most popular supplement used was protein powder, with 21.3% of athletes reported using it (Sato et al., 2012). A survey of elite young UK athletes reported that athletes were taking whey protein with the goal of maintaining strength and increasing their ability to train longer (Petróczi et al., 2008). Another survey study, this time in young, non-elite Canadian athletes, reported that protein supplements were the most popular supplement choice, despite an already high dietary intake of protein (Parnell, Wiens, & Erdman, 2016). This study helps to further establish the popularity of protein supplementation in youth athletes, in this case in a non-elite population.

Prior research has suggested that resistance training resulted in a downregulation in protein metabolism in healthy children (Rodriguez, 2005). This highlights a potential need for greater protein intake in pre-pubescent children participating in a resistance training program to avoid that downregulation in protein

metabolism. However, after puberty, there is research suggesting that the growth spurt observed during puberty is associated with a marked increase in the efficiency of dietary protein utilization for growth (Beckett et al., 1997). The results of this study suggest that post-pubertal children are more efficient at utilizing protein and therefore whey protein can be a potentially safe ergogenic aid for teenage athletes.

No research has been done investigating the effects of whey protein on kidney function in an under-18 population. Research on adults has been extensive, however. Contrary to popular belief, it has been well documented that a high protein diet (considered to be protein intake greater than the recommended daily amount) does not have any adverse effects on kidney function in healthy adults (Martin, Armstrong, & Rodriguez, 2005).

While there has been no direct research investigating the effect of whey protein supplementation on body composition and athletic performance in an under-18 population, the positive effects on an adult population have been well documented. Compared to a control, whey protein supplementation combined with resistance training, can help preserve fat free mass, increase lean body mass, and decrease body weight and body fat (Miller, Alexander, & Perez, 2014b). Similarly, it has been well established that whey protein supplementation can improve athletic performance and muscle strength compared to a control (Hayes & Cribb, 2008; Pasiakos, McLellan, & Lieberman, 2015).

A combination of a proper diet and resistance training has been suggested to increase mineral density in youth (Faigenbaum et al., 2009). Research investigating the effect of whey protein on bone health in humans in has been scarce, with the

majority of research focusing on rats, and none being done in an under-18 population. This research on rats has been positive however, showing that whey protein had the ability to increase femoral bone strength (Kato et al., 2000; Kim, Kim, Kim, Imm, & Whang, 2015; Takada et al., 1997). Similar research that has been done in humans has focused on using milk basic protein (MBP), a component of whey protein, to improve bone health. MBP has been suggested to stimulate proliferation and differentiation of osteoblastic cells as well as suppress bone resorption (Takada, Aoe, & Kumegawa, 1996). Studies utilizing daily MBP supplementation for six months have revealed a positive effect on bone mineral density in comparison to a placebo (Aoe et al., 2001; Yamamura et al., 2002).

While the benefits of whey protein supplementation in adults is well documented (Morton et al., 2018), the safety nor the performance enhancing benefits of whey protein supplementation in youth athletes has been investigated. Based on the aforementioned research, it was deemed pertinent that the safety and efficacy of whey protein in teenage athletes be investigated. Thus, the purpose of this study was to investigate the effects of whey protein supplementation on athletic performance, body composition, and biomarkers of bone and kidney health in teenage athletes.

Supplement Use Amongst Teenage Athletes and Reasons For

Supplement use by adults has been well documented. However there have been limited studies done investigating supplement use by teenage athletes. Therefore, it is first important to establish supplement use among teen athletes and their motivations behind taking them. A review article of supplement use by young athletes done by Jill A. McDowall reported that between 22.3% and 71% of child and adolescent athletes

took at least one supplement regularly (McDowall, 2007). While health and illness prevention were cited as the main reasons for taking supplements, enhanced athletic performance was also reported as a strong motivating factor among young athletes. The review also suggests that females use supplements more frequently than males and their reasons were for improved health and recovery, as well as replacing an inadequate diet.

Bell et al. investigated nutritional supplement use in adolescent athletes between the ages of 13 and 19 (Bell, Dorsch, McCreary, & Hovey, 2004). Data was collected from 333 adolescent athletes (190 male, 139 female, 4 no respondents) via survey. The most popular nutritional supplement was multivitamins, with 42.4% of males and 43.1% of females reported currently taking a multivitamin. Protein supplements were the next most prevalent, with 16.7% of male and 9.5% of females reported currently taking some form of protein supplementation. Furthermore, 47.3% of males and 35.3% of females believed protein supplements had a performance enhancing quality. This study establishes that not only are teenage athletes taking nutritional supplements, but they are taking them with the belief that they will enhance performance.

In a similar vein, Scofield and Unruh also investigated dietary supplement use among adolescent athletes (Scofield & Unruh, 2006). One-hundred thirty-nine high school athletes across a wide range of sports and activities (99 males, 34 females, mean age = 15.8 ± 1.19 years) volunteered to participate in this study. 22.3% of the participants reported that they were currently taking a dietary supplement. The most popular reported dietary supplement was protein, with 23.7% reported taking it. This

study helps to continue to establish that protein supplements are prevalent among teenage athletes. This study was limited however due its small size.

Parnell et al. sought to quantify dietary intakes in young Canadian athletes from a wide variety of sports, with a secondary aim of evaluating dietary supplement use in the context of nutrient needs, health, and performance (Parnell et al., 2016). One-hundred and eighty-seven (84 male, 103 female) athletes were recruited from sporting communities and local public schools. Ages ranged from 11 to 18 years. Analysis by age groups of 11–13 years and 14–18 years reported an increased use of protein powder in the older age group with regular use at 18%, occasional at 45%, and never at 37% vs. 10% regular, 23% occasional, and 68% never in 11–13 years ($p = 0.001$). Protein supplements were most popular in this cohort; despite an already high dietary intake of protein. This study helps to further establish the popularity of protein supplementation in youth athletes, in this case in a non-elite population. This also establishes supplement use in a younger, potentially prepubescent, population (i.e. 11-13 years).

Research investigating the nutritional supplementation practices in elite UK junior national track and field athletes has also been performed (Nieper, 2005). Survey data was taken from 32 athletes (20 males, 12 females, mean age = 18 y) to assess the prevalence and type of supplement used, the reasons for use, knowledge of supplements, and sources of information. Results reported that 62% of athletes took at least one supplement regularly. Additionally, supplement use was higher in females (75%), than males (55%), although the relationship was not significant ($p = 0.45$). Reasons cited for using supplements were for health (45%), to enhance the immune

system (40%), and to improve performance (25%). This helps to further establish the reasons behind supplement use in teenage athletes. This study was limited however by its small sample size and lack of identification of specific supplements.

Teenage athletes in the UK were again examined, this time by Petróczi et al. The purpose of this survey study was to investigate nutritional supplement use by elite young UK athletes, and to establish whether a rationale versus practice incongruence exists, and to investigate the sources of information (Petróczi et al., 2007). The authors surveyed 403 athletes (64.5% males, 33.0% females, mean age 17.66 ± 1.99 y). Statistically significant reasons for taking whey protein were reported to be to maintain strength and increase ability to train longer. The researchers observed no agreement between athletes' rationale and behavior in relation to nutritional supplements, except for creatine. Health professionals, nutritionists and physiotherapists, followed by coaches, were the most frequently advised by young athletes when regarding what supplements to take. Young athletes in the present sample appear to be less health conscious and more performance focused than their adult counterparts. Since this appears to be the case, providing youth athletes with a safe supplement that can potentially enhance performance (i.e. whey protein) would be beneficial for this population and hopefully reduce the likelihood that they would turn to illegal and/or dangerous performance enhancing drugs/supplements.

Another survey of elite young athletes, this time in a Japanese population, was done by Sato et al. This study collected data from 75 athletes from the Japanese national team competing in the 2010 Youth Olympic Games in Singapore (Sato et al., 2012). Data was collected from 75 athletes (26 male, 49 female, mean age 16 ± 1.1 y).

Supplements were divided into two categories: dietary supplements and sport foods, and ergogenic aids. A total of 47 (62.7%) athletes used one or more supplement regularly. Under the dietary supplements and sports foods category, the most popular supplement product was protein, used by 16 athletes (21.3%). Under the ergogenic aid category, amino acids were the most frequently consumed supplements, used by 33 athletes (44.0%). Recovery from fatigue was the most frequently cited purpose for supplement use, followed by improvement of athletic performance and supplementation of diet. This study helps to further validate the belief that young athletes are taking supplements, with protein being particularly popular.

Similarly, Braun et al. sought to investigate dietary supplement use, this time in elite young German athletes (Braun et al., 2009). One-hundred sixty-four elite young athletes across various sports completed a survey that assessed supplement use during the previous four weeks. These surveys showed that 80% of athletes reported using at least one supplement, with no significant differences between types of sports ($p = 0.53$). Vitamins (76%) and minerals (87%) were the most popular, followed by sport beverages (69%), carbohydrate supplements (64%), and protein/amino acid products (30%). The use of protein products was greater in males than in females (42% v. 20%), however the relationship was not significant ($p = 0.07$). Among athletes who used or had used supplements, 63% stated that they did so for “maintenance of health”, while 44% stated they did so for “improvement of immune functions.” Athletes who cited performance-related reasons had been using significantly more protein ($p < 0.001$) and carbohydrate ($p < 0.05$) products. This

study indicates that while protein supplementation may not be the most common nutritional supplement taken, it is for athletes believing it will improve performance.

Duellman et al. sought to determine whether male high school football players, who choose to take protein supplements, have more misconceptions regarding their effectiveness than those who did not (Duellman, Lukaszuk, Prawitz, & Brandenburg, 2008). Sixty-one male high school football players were recruited for the study. Thirty-nine identified as users of protein supplements. Thirty-two of those users identified gaining muscle as the main reason for taking protein supplements. The results also indicated that protein supplement users had greater levels of misconceptions about protein supplementation than the non-protein users. The results of this study further indicate the need for continued research into this topic with a primary goal being able to provide youth athletes proper education about correct supplement usage.

Muscle Strength and Athletic Performance

Protein supplements are frequently consumed by athletes and recreationally active adults of all ages with the goal of improving muscle strength and mass and therefore subsequent performance. Research has suggested that both pre and post exercise ingestion of whey protein can increase muscle protein synthesis, resulting in a positive net protein balance, which is important for muscle hypertrophy (Hulmi, Lockwood, & Stout, 2010). The use of protein supplements have also been documented as a potential treatment for sarcopenia (Nabuco et al., 2018), which is the loss of skeletal muscle mass as one ages. However, the breadth of this section of the review will focus on the effects of protein supplementation, specifically whey protein

when possible, on measures of athletic performance and muscle strength in healthy young-to-middle-aged adults.

In order to assess its effectiveness on muscle strength, whey protein is often combined with resistance training, or given to resistance trained individuals (Naclerio & Larumbe-Zabala, 2016). A primary benefit of resistance training is increased muscle strength, and whey protein is often added to resistance training protocols in an effort augment these effects.

Investigating this idea, Burke et al. conducted one of the first studies examining the effects of whey protein supplementation combined with resistance training on muscle strength (Burke et al., 2001). Twenty-seven participants (18 female, 9 male) who were not participating in resistance type training participated in the study. Participants were randomly and equally placed into either a whey protein, soy protein, or a placebo group. Knee extension and flexion peak torque, and bench press and squat strength were used to assess muscle strength. Whey protein dosage was 1.2 g/kg body mass/day while the placebo was 1.2 g/kg body mass/day of maltodextrin. All participants followed the same resistance training protocol for 12 weeks. Only knee extension peak torque was reported to have increased significantly in the whey protein group compared to the placebo group. There were no differences revealed for knee flexion peak torque, squat and bench press strength between the groups. Participants only supplemented for the first six weeks of the study, and not the final six weeks. This almost certainly affected any potential gains in strength made as a result of the whey protein. This study is noteworthy however in that provides some rationale for whey protein supplementation and allows for continued investigation.

Following Burke's lead, Coburn et al. utilized a randomized double-blind design to compare whey protein and a placebo, combined with resistance training, on muscle strength (Coburn et al., 2006). Thirty-three men (mean age 22.4 ± 2.4 y) were divided into three groups: a supplement group ($n = 11$), a carbohydrate placebo group ($n = 12$), and a no supplement control group ($n = 10$). The supplement contained 20 g of whey protein and 6.2 g of leucine. Participants participated in a unilateral dynamic constant resistance leg extension exercise protocol three times per week for eight weeks. Supplements were consumed 30 minutes prior to and immediately after each exercise session. One repetition max testing on a unilateral leg extension machine was performed at weeks 0 and 8. The whey protein group's 1RM strength increased significantly ($p \leq 0.05$) at the end of the eight weeks compared to both the placebo and the control group. This study shows that in combination with resistance training, whey protein supplementation can increase strength gains compared to a carbohydrate placebo. However, this study did not assess dietary protein intake at any point. This leads to the possibility that the whey protein supplement may have been distributed to a group of people that were protein deficient compared to the placebo group.

Willoughby and colleagues also investigated the effects of whey protein supplementation on muscle strength over the course of 10 weeks (Willoughby, Stout, & Wilborn, 2007). Nineteen untrained males were randomly assigned to either a 40 g/d whey protein group ($n = 9$), or a 40 g/d dextrose group ($n = 10$). All participants engaged in the same resistance training protocol four times per week. Strength was assessed via bench press and leg press 1RM. At the end of 10 weeks, the whey protein group had significantly higher ($p \leq 0.05$) increases in bench press and leg press 1RM

than the dextrose group. A strength of this study was that macronutrient intake within each group and between the two groups did not change significantly over the course of the 10 weeks, and that both groups engaged in the same resistance training protocol, the observed improvements in muscle strength in the protein group are most likely due to the ingestion of the protein supplement. This study aids in establishing whey protein as an effective ergogenic aid.

Walker et al. also reported similar results, this time without a structured resistance training intervention (Walker et al., 2010). Thirty moderately trained male participants (mean age 26.9 y) were separated into either a combined 19.7 g whey protein and 6.2 g leucine group (n = 18) or an isocaloric placebo group (n = 12). Participants engaged in at least three days of total body training per week. After eight weeks of daily supplementation, participants in the whey protein group had significant increases in 1RM bench press and one-minute push-up test compared to week one, while the placebo group did not see any significant changes. Neither the supplement nor the placebo saw any improvements in other non-traditional measures of athletic performance and muscle strength, such as a three mile run or one-minute abdominal crunch test. This study suggests that whey protein has more advantages for increasing upper body strength. A limitation of this study was that the training of the participants was not controlled, but only matched for similarities between participants.

McAdam et al. investigated the effects of whey protein supplementation on the battery of tests featured in Army Physical Fitness Test (McAdam et al., 2018). The Army Physical Fitness Test consists of two minute push-up test, two minute sit-up test, and a two mile run. Sixty-nine fit male US Army soldiers (mean age 19 ± 1 y)

were separated into two groups: a 77 g/d whey protein group (n = 34) and an isocaloric carbohydrate group (n = 35). Participants consumed their given supplement twice daily for eight weeks and underwent the Army Physical Fitness Test at weeks two and eight. Compared to the carbohydrate group, the whey protein group performed significantly more push-ups ($p \leq 0.05$). No differences were seen in sit-up and two mile run test. This study again shows the effectiveness of whey protein supplementation in improving upper body strength.

Whey protein is often compared to other protein supplements in an attempt to see if one is more beneficial than the other. Cribb et al. compared the effects of whey protein and casein protein on muscle strength (P. J. Cribb, Williams, Carey, & Hayes, 2006). Thirteen male recreational bodybuilders were separated into either a whey or casein group and all participated in a 10 week resistance training program. Participants consumed 1.5 g/kg of body weight of their given supplement daily. Strength was assessed via 1RM bench press, squat, and cable pulldown. The whey protein group showed significant increases ($p \leq 0.05$) in all three strength tests compared to the casein group. While it showed positive effects, this study was limited by its small sample size. This study does show that whey protein is perhaps better than other protein sources at improving muscular strength.

Joy et al. compared whey protein supplementation versus rice protein supplementation (Joy et al., 2013). Twenty-four resistance trained males (mean age 21.3 ± 1.9 y) were randomly divided into either a 48 g whey group (n = 12) or a 48 g rice group (n = 12). All participants engaged in the same resistance training protocol three times per week for eight weeks and consumed their given supplement after each

training session. Bench press and leg press 1RM were used to assess strength. Unlike prior research, whey protein supplementation did not present an advantage over its comparison. While both groups saw increases in strength at the end of the eight weeks, there was no difference between groups. These results suggest that differences in protein composition appear to be less relevant when consumed in high doses.

While most research typically focuses on a male population, Wilborn et al. chose to examine the effects of whey versus casein protein in female athletes (Wilborn et al., 2013b). Sixteen female basketball players (mean age 20 ± 1.9 y) were randomly divided into a 24 g whey ($n = 8$) or 24 g casein group ($n = 8$). All participants participated in the same supervised four-day per week resistance training program for eight weeks. Supplements were consumed 30 minutes prior to and immediately after training. At weeks zero and eight, participants underwent vertical jump, broad jump, 5-10-5 agility, and 1RM bench press and leg press testing. After eight weeks, there were no differences seen between groups for any of the aforementioned measures. All measures did increase, however. While this study shows no performance advantage between whey and casein, it does show positive benefits for protein supplementation in a female population.

Positive effects of whey protein supplementation on muscle strength have been seen in some, but not all cases. In some untrained populations, research has documented no differences in measures of muscle strength between whey and a placebo after engaging in a structured resistance training protocol for eight weeks (Herda et al., 2013; Mielke et al., 2009; Weisgarber, Candow, & Vogt, 2012). Furthermore, it has also been reported that a combination supplement of whey protein,

creatine, and carbohydrates has no advantages over a carbohydrate-only supplementation when consumed post-exercise in relation to muscle strength, endurance, and anaerobic cycling performance (Chromiak et al., 2004). This suggests that whey protein supplementation has more efficacy in a trained population.

While not as established in the literature as research on the effects of whey protein in resistance trained population and/or on strength measures typically associated with resistance training, there is a growing body of research investigating the effects of whey protein on aerobic and endurance sport performance. This research, however, has been mixed at best. Acute whey protein supplementation has been suggested in some instances to have a negative effect on cycling time trial performance (Macdermid & Stannard, 2006; Schroer, Saunders, Baur, Womack, & Luden, 2014), while in others it has reported no negative effect (Oosthuysen, Carstens, & Millen, 2015). Time to exhaustion treadmill runs have been reported to significantly improve after 6 weeks of amino acid supplementation in an untrained population (Antonio, Ellerbroek, & Carson, 2018). Additionally, in elite endurance athletes, whey protein has been reported to improve performance and markers of recovery (Hansen, Bangsbo, Jensen, Bibby, & Madsen, 2015; W.-C. Huang et al., 2017).

Body Composition

In addition to being taken for its positive effects on muscle strength, whey protein is often taken as a weight loss/maintenance aid. Similar to its effects on muscle strength and athletic performance, the use of whey protein, combined with or without resistance training, has been documented to improve several parameters of body composition, including lean body mass and body fat (Miller et al., 2014). Research has

suggested that diets higher in protein and lower in carbohydrate are most advantageous for promoting weight loss and preserving lean body mass (Hession et al., 2009; Wycherley et al., 2012). Whey protein has also been documented to increase satiety and suppress appetite more than other proteins (Anderson, Tecimer, Shah, & Zafar, 2004; Hall, Millward, Long, & Morgan, 2003). In addition, whey protein has been suggested to be superior to other protein sources for increasing and supporting muscle protein synthesis (Devries & Phillips, 2015; Phillips, Tang, & Moore, 2009).

A notable study done Volek et al. aimed to determine whether whey protein promotes greater increases in muscle mass than soy protein or a carbohydrate control (Volek et al., 2013). Sixty-three healthy participants (37 male, 26 female) were randomly divided into three groups: 1.4 g/kg/body weight whey protein (n = 19, mean age 22.8 ± 3.7 y, mean body mass 74.1 ± 15.7 kg), 1.4 g/kg/body weight soy protein (n = 22, mean age 24.0 ± 2.9 y, mean body mass 72.0 ± 8.4 kg), and 1.1 g/kg/body weight carbohydrate (n = 22, mean age 22.3 ± 3.1 y, mean body mass 72.4 ± 14.9 kg). All participants engaged in the same nine month whole body resistance training program. Body composition, measured via DXA, was assessed at three, six, and nine months. While body mass and lean body mass increased significantly at three months and remained higher at six and nine months in all groups, lean body mass was significantly greater at all time points in the whey protein group compared to the other two groups. While body mass and body fat percentage decreased significantly across all time points, no differences were seen between groups. These results suggest that daily whey protein supplementation, in particular over a long period of time, can promote significant increases in lean body mass.

Similar results were also reported by Cribb and colleagues (P. J. Cribb et al., 2006). Comparing 90g/d of whey protein to 90g/d of casein over the course of 10 weeks, it was revealed that the group supplementing with whey protein had significantly ($p < 0.01$) greater gains in lean mass (5.0 ± 0.3 kg) and significantly ($p < 0.05$) greater losses in fat mass (-1.5 ± 0.5 kg) compared to the casein group. All participants engaged in the same resistance training protocol. This suggests that the amount of whey protein consumed is important, with higher amounts being perhaps more beneficial for reducing fat mass and increasing lean mass.

Similar to testing its effects on muscle strength, whey protein is often combined other supplements in attempts to augment its effectiveness. In this vein, Hulmi et al. compared the effects of whey protein with or without added carbohydrates on body composition (Hulmi et al., 2015). Eighty-six active male participants were divided into three groups: a 30 g whey protein group ($n = 22$), a 34.5 g isocaloric maltodextrin group ($n=21$), and a combination 30 g whey protein and 34.5 g of maltodextrin group ($n = 25$). Participants also engaged in a structured resistance training protocol two to three times per week for 12 weeks and consumed their given supplement post training and on training days only. Body composition was assessed via DXA. While all groups showed increases ($p \leq 0.05$) in fat-free mass, the whey protein group had a larger relative increase (per kg of bodyweight) in fat-free mass than both the carbohydrate only group and the combined group. Total fat mass also saw a significant decrease after 12 weeks in both the whey protein only and combination groups, but not the carbohydrate only group. This study suggests that if

one is aiming to reduce fat mass, consuming whey protein after exercise provides an advantage over carbohydrate supplementation.

While most research focuses on a male resistance trained population, Taylor et al. examined whey protein supplementation in female basketball players (Taylor, Wilborn, Roberts, White, & Dugan, 2016). Fourteen healthy Division III female basketball were randomly assigned to 24g whey protein group ($n = 8$, mean age 20 ± 2 y) or a 24 g placebo group ($n = 6$, mean age 21 ± 3 y) for eight weeks. Body composition was assessed via DXA at zero and eight weeks and participants consumed their given supplement prior to and immediately after their training sessions. The study took place during the preseason period of the season, where players engaged in basketball specific speed and agility training three days per week, and an undulating resistance training program four days per week. Over the course of the eight weeks, the whey protein group gained more lean mass ($+1.4$ kg, $p = 0.003$) than the placebo group ($+0.4$ kg, $p = 0.095$). The whey protein group also lost more fat mass (-1.0 kg, $p = 0.003$) than the placebo group (-0.5 kg, $p = 0.41$). While limited by its small sample size, this study presents efficacy for consumption of whey protein in a female population engaged in a concurrent training program.

While most studies combine whey protein supplementation with resistance training, Frestedt and colleagues chose to simply add whey protein to the diet of their participants, with no resistance training intervention (Frestedt, Zenk, Kuskowski, Ward, & Bastian, 2008). One-hundred and six participants were randomly assigned to a twice daily 10g whey protein group or an isocaloric maltodextrin control group. Participants consumed their given supplement 20 minutes before breakfast and 20

minutes before dinner each day for 12 weeks. Body composition, assessed via DXA, was measured at 0, 4, 8, and 12 weeks. At 12 weeks, it was revealed that the whey protein group lost significantly more body fat (2.81 vs. 1.62 kg, $p = 0.03$) and lost less lean mass (1.07 vs. 2.41 kg, $p = 0.02$) than the control group. While all participants were assigned a diet plan that allowed for similar protein, carbohydrate, fat, and kilocalorie intake between groups, this study was limited by the fact the physical activity was not controlled. What this study does show, however, is that whey protein can be an effective weight management supplement without the addition of a structured training program.

A recent systematic review and meta-analysis examining the effects of whey protein on body composition in females also reported similar results (Bergia, Hudson, & Campbell, 2018). An analysis of 13 studies analyzing a total of 488 female participants was conducted comparing the effect of whey protein to a control on body composition. Intervention periods ranged from six weeks to 12 months and whey protein dosage ranged 6 g/d to 48 g/d. Relative to a control, it was revealed that whey protein increased lean mass (+0.37 kg, 95% CI: 0.06 to 0.67) more than a control (-0.20 kg, 95% CI: -0.67 to 0.27), without changing fat mass (-0.20 kg, 95% CI: -0.67 to 0.27). When combined with a decreased caloric intake, the benefits of whey protein on lean mass improved (+0.90 kg, 95% CI: 0.31 to 1.49). However, this benefit on lean mass was lost when whey protein was combined with resistance training (+0.23 kg, 95% CI: -0.17 to 0.63). This reviews again highlights the positive changes that whey protein supplementation can have on lean body mass without the need to engage in resistance training.

In some experienced resistance trained populations however, the effects of whey protein are more varied. Hoffman et al. examined changes in body composition over the course of 12 weeks in 21 experienced (≥ 2 years of prior resistance training) strength and power athletes (Hoffman, Ratamess, Kang, Falvo, & Faigenbaum, 2007). Participants were divided into a 42 g protein group ($n = 11$, mean age 20.3 ± 1.6 y) and an isocaloric placebo group ($n = 10$, mean age 21.0 ± 1.2 y) and engaged in a four-day per week, split resistance training routine. Participants consumed their given supplement twice daily: once in the morning and again post training. Body composition was assessed via DXA at 0, 6, and 12 weeks. While no significant changes in body mass, lean body mass or percent body fat were observed in either group from 0 to 12 weeks, lean body mass did increase by 1.4 kg in the protein group compared to only 0.1 kg increase in the placebo group. This was only a trend, and no significance was seen. This may be explained by the relatively low caloric intake seen by the participants as consuming insufficient calories may compromise the body's ability to increase its lean mass.

Protein Intake and Kidney Function

Diets high in protein have been previously rumored to have a negative effects on kidney functioning. This idea has received particular attention recently, with the increase in popularity of diets that feature high protein intakes, such as the paleo, ketogenic, and Atkins diet. The concern being that the primary job of the kidneys is to filter waste from the bloodstream, and that excess protein increases the rate at which this occurs. This increased rate, also known as glomerular hyperfiltration, may cause damage to the kidneys, and thus cause waste products to build up in the body

(Kalantar-Zadeh & Fouque, 2017). There is experimental data in animal models showing that long-term dietary protein intake exceeding 1.5g/kg of body weight per day may cause glomerular hyperfiltration (Hostetter, Meyer, Rennke, & Brenner, 1986). Most kidney issues caused by increased protein intake in humans have occurred in individuals either at risk for chronic kidney disease or already have known kidney disease, and similar claims in humans free of kidney issues have gone unsupported (Friedman, 2004).

In support of this claim, Elswyk et al. conducted a systematic review investigating renal health in healthy individuals with high protein intakes (Van Elswyk, Weatherford, & McNeill, 2018). The authors examined both randomized controlled trials (RCTs) and observational epidemiologic studies (OBS). High protein intake was defined as being above the United States recommended daily allowance ($> 0.8\text{g/kg}$ or 10-15% of total energy intake), or protein intake greater than or equal to 20% of total energy intake but less than 35%. Twenty six studies (18 RCTs, 8 OBS) of healthy adults free of any kidney disease were examined. While eight RCTs reported an increase in glomerular filtration rate (GFR) with increased protein intake, all rates were consistent with normal kidney function. Similarly, all eight of the OBS reported no associations between protein intake and GFR. All studies in this review had an intervention/observational period of ≤ 6 months. Average participant number in the RCTs was 45.6, although the studies ranged from five to 378. Average participant number in the OBS was 4,402, and studies ranged from 42 to 15,792. Based on the evidence from this review, high protein intake, at least over a short term period, is consistent with normal kidney functioning in healthy adults. It should be noted

however, that this review was unable to determine if increased protein intake from a particular source, like plants or animals, affected kidney health differently.

A similar systematic review and meta-analysis also investigating high protein intake on kidney function was conducted by Devries et al. (Devries et al., 2018). However, they sought to compare high protein intakes with normal or low protein intakes. A high protein intake was defined as either ≥ 1.5 g/kg body weight, $\geq 20\%$ of total energy intake, or ≥ 100 g/day, while normal/low protein intake was defined as $\geq 5\%$ less total energy intake from protein per day as compared to the high protein intake. A total of 28 studies were analyzed, and all of the studies were RCTs. Total participant count was 1,358, with studies ranging from six to 307 participants. Changes in GFR were again utilized to assess kidney function, and analyses were conducted using postintervention GFR and the change in GFR from pre to post. Changes in GFR pre/post intervention period did not differ ($p = 0.16$) between protein intakes. However, a small increase in GFR was observed after in high protein intakes ($p = 0.002$), and a linear relationship was observed between protein intake and GFR in the post-only comparison ($r = 0.332$, $p = 0.03$), but not between protein intake and the change in GFR ($r = 0.184$, $p = 0.33$). The results of this research continue to support the notion that high protein intakes do not adversely affect kidney function in healthy adults. Additionally, it suggests that low protein intake does not necessarily protect against increases in GFR and kidney health.

Protein and Bone Health

Similar to the misconceptions about protein and kidney health, it has also been speculated that a high protein intake can reduce calcium in bones, potentially causing

osteoporosis. This theory is based on the idea that a high protein intake increases the acid load of the body, and to compensate for this, the body takes calcium out of bones to neutralize this increased acid (Barzel & Massey, 1998). However, in order to maintain bone structure, particularly as one ages, there is a certain amino acid requirement from dietary protein needed (Wallace & Frankenfeld, 2017), creating something of a paradox. Therefore, it is important to investigate these claims to determine the true relationship between protein and bone health.

Darling et al. conducted the first systematic review and meta-analysis regarding this topic in 2009 (Darling, Millward, Torgerson, Hewitt, & Lanham-New, 2009). Thirty-one cross-sectional surveys examining bone mineral density (BMD), bone mineral content (BMC), and bone markers were included in the systematic review. BMD was assessed at various sites, including the lumbar spine, calcaneus, femoral neck, femur, hip, and radius. Little evidence was suggested of a negative relationship between protein intake and BMD. Fifteen cross sectional surveys reported a significant positive relationship between protein intake and BMD at least one site. However, 18 studies reported no significant correlation between protein intake and at least one BMD site. No studies showed a significant increase in BMD loss with increased protein intake, and it was revealed that protein intake was not a significant predictor of BMD. Similar results were revealed for bone markers, as the cross-sectional surveys showed little evidence that protein intake had any influence. Results were more mixed for BMC, as four cross sectional surveys reported a positive correlation between protein intake and BMC for at least one site, while two reported no significant correlation, and only one survey reported a significant negative

correlation between protein intake and BMC. Overall, the results of this systematic review and meta-analysis show that while there may be a small benefit of protein intake on bone health, there is at least no detrimental effect.

A more recent systematic review and meta-analysis in 2017 was done by Shams-White et al. in conjunction with the National Osteoporosis Foundation (Shams-White et al., 2017). Sixteen RCTs and 20 prospective cohort studies were analyzed for the relationship between dietary protein intake with or without vitamin D on BMD. There was evidence from five RCTs that higher protein intake may actually have a protective effect on lumbar spine BMD compared with lower protein intake (95% CI: 0.06%, 0.97%). However, no effect was reported for total hip, femoral neck, or total body BMD. Overall, there was no evidence of adverse effects of higher protein intake on BMD.

In an attempt to examine the relationship between protein intake and bone health for an extended period, Antonio et al. investigated the effect of a high-protein diet on BMD in exercise trained women over a one year period (Antonio et al., 2018). A high protein diet was defined as > 2.2 g/kg/day. BMD was assessed via dual-energy DXA. Twenty seven female participants (mean age = 37 y) participated in the study. Participants engaged in a minimum of resistance and/or aerobic training three times per week. No changes ($p > 0.05$) were reported over the course of the study for BMC, BMD, and lumbar spine BMC and BMD. These results suggest that a high protein diet has no adverse effects on BMD over a long term period.

While all of the aforementioned research has simply investigated a high-protein intake, it is important to investigate the specific effects that whey protein

supplementation has on bone health. Kerstetter et al. attempted to do this by examining the effects of whey protein supplementation in older Caucasian adults (Kerstetter et al., 2015). A randomized, double-blind, placebo-controlled study design was utilized. Two-hundred and eight men and women were separated into two groups: a 45 g/d whey protein group (mean age = 69.9 ± 6.1 y) and an isocaloric carbohydrate control group (mean age = 70.5 ± 6.4 y). Participants consumed their given supplement daily for 18 months and BMD and body composition was assessed via DXA at baseline, nine and 18 months. No significant differences ($p \geq 0.05$) were reported between groups for lumbar spine, total femur, and femoral neck BMD. Total lean mass ($p = 0.011$) and truncal lean mass ($p = 0.003$) were significantly lower in the carbohydrate group compared to baseline, while lean mass in the protein group was unchanged from baseline. While not significant, total lean mass ($p = 0.069$) was higher in the protein group at 18 months. Lean trunk mass was significantly ($p = 0.048$) higher in the protein group at 18 months. Total fat mass also significantly ($p = 0.018$) increased over the 18 months in the carbohydrate group. A similar change was not seen in the protein group. The results of this study suggest that whey protein supplementation can promote positive changes in body composition without adversely affecting skeletal health. While whey protein supplementation did not negatively impact bone health, it did not positively impact it either.

The results of the previous study were also in agreement with those reported by Zhu et al. several years prior. They also utilized randomized, double-blind, placebo controlled study (Zhu et al., 2011). Two-hundred and ninety healthy postmenopausal women were separated into two groups: a 30 g/d whey protein group (mean age = 74.2

± 2.8 y) and a 2.1 g/d protein placebo group (mean age = 74.3 ± 2.6 y) and tracked for two years. BMD was assessed via DXA at baseline and at one and two years. While there was a significant decrease in hip DXA over the two years, there was no difference between groups. These results further demonstrate that while whey protein supplementation has no adverse effect on bone, it was also not beneficial for bone health.

Similarly, Wright et al. also reported no benefits of whey protein supplementation on bone health (Wright, McMorrow, Weinheimer-Haus, & Campbell, 2017). Unlike the previous studies that investigated healthy older populations, this study looked at middle age overweight and obese adults. Again, a double blind, randomized, placebo-controlled study design was used. Participants ($n = 186$, 108 females, 78 males, mean age = 49 ± 8 y, BMI: 30.1 ± 2.8 kg/m²) consumed either a 0, 20, 40, or 60 g/d whey protein supplement daily for 36 weeks. Participants also took part in a resistance training program twice per week and an aerobic training program one day per week. BMD and BMC were assessed via DXA. There were no differences seen for any whey protein dose and BMD and BMC after the 36 weeks. These results further support the notion that whey protein supplementation has no positive or negative impact on bone health, in this case in an overweight and obese middle age population.

Conclusion

While mixed at times, a significant body of evidence has readily exhibited a number of positive benefits that whey protein supplementation can have on muscle strength, athletic performance, and body composition. These benefits extend from

young healthy adult populations to elderly populations with sarcopenia and have been documented to occur in as little as 8 weeks. Furthermore, whey protein has been reported to be more effective than other protein sources at improving these measures and has been reported to be effective with or without the addition of a structured training program.

Additionally, considering the lack of side effects that high protein intakes have on bone and kidney health, concerns for supplementing with whey protein in an under-18 population should be lessened. Based on the current literature, whey protein presents itself as a popular supplement in a teenage athlete population and appears to be growing in popularity. Therefore, based on the knowledge that whey protein is popular amongst teenage athletes and has been well documented to be an effective ergogenic aid in young adult populations, it would appear beneficial to investigate the effectiveness of whey protein supplementation in an under-18 population. Since little to no research of this nature has been done, future research should investigate these ideas across a wide variety of populations, both pre and post pubertal, and for intervention periods of at least eight weeks in length.

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APPENDIX B: Parental Permission Form for Research

THE
UNIVERSITY
OF RHODE ISLAND

Disa Hatfield, PhD

Department of Kinesiology

Effect of whey protein supplementation on athletic performance and immune function in teenage athletes

Page 1 of 3

Parental / Legally Authorized Representative Permission Document for Research

BACKGROUND

Your child has been invited to take part in a research project described below. Our names are Disa Hatfield and Andrew Stranieri and we are asking for permission to include your child in this study because we are hoping to make important discoveries about the interactions between whey protein supplementation and health in teenage athletes through this research, and we cannot do it without your help.

This research study is called the "effect of whey protein supplementation on athletic performance and immune function in teenage athletes." The primary purpose of the study is to investigate the effects of whey protein supplementation on athletic performance in teenage athletes, while a secondary purpose is to simultaneously investigate the effect of whey protein supplementation on the athletes' immune, bone, and kidney health. There will be many safeguards throughout this study to reduce and prevent risk or discomfort to your child. If at any point in this study your child feels uncomfortable or does not want to participate anymore please do not hesitate to tell one of us.

STUDY PROCEDURE

No changes will be made to your child's training. They will still follow the same training schedule/program that their coach assigns to them. In addition to the normal, sport specific training your child will participate in, your child will also be randomly assigned to one of two groups: a whey protein supplement group or a placebo supplementation (i.e. carnation instant breakfast) control group. Your child will consume their randomly selected supplement on training days for the duration of the study.

Various outcome measures will also be assessed throughout the study as well. Baseline measures will be assessed one week prior to the start of the study, mid-test measures will be assessed at multiple points throughout the study (pre, two to five times equally spaced out throughout the duration of the study, and post), and post-test measures will be assessed at the end of the study.

Measures of athletic performance will be assessed twice: pre and post study. The types of athletic performance tests will depend on the fitness and approval of your child and may include such tests as a 40 yard dash, vertical jump, and bench press and squat one repetitions maximum tests.

Measures of immune, bone, and kidney health will be measured pre, mid (2-5 times), and post. Immune and kidney health will be assessed via urine and saliva. Bone health will be measured via heel ultrasonography. Ultrasound scans of the heel are a quick, portable, radiation-free, and overall easy screening tools. Therefore, heel ultrasonography is an appropriate and feasible method for measuring bone density and tendon health in a teenage population.

Whey protein dosage will be 120 calories, 1.5 grams fat, 3 grams carbohydrate, 24-25 grams protein. This is based on manufacturers recommendation's and the most common dosage taken by adults. This dosage is also most representative of what teenage athletes have reported taking and is therefore more reflective of real-world situations, unlike prescribing dosage by body weight, for example. Control supplement dosage (carnation instant breakfast) will be 130 calories, 0.5 grams fat, 27 grams carbohydrate, 5 grams protein.

RISKS

The possible risks and discomforts of this study are minimal. There is the possibility of some stomach discomfort from the supplement or even an allergic reaction to one or several of the ingredients. While rare, any potential stomach discomfort should subside quickly. You may check with your pediatrician prior to making a decision if you wish.

BENEFITS

There are numerous possible benefits of your child's participation in this study. Your child will learn about their body composition and their bone density. In addition, the general health of their immune system will also be assessed.

TIME COMMITMENT

On two days (once at the start of the study and once at the end of the study) of the study, the child will perform athletic performance tests. These will only take about one hour each, or two hours in total for the duration of the study. At two to four points throughout the study, saliva, urine, and anthropometric measurements will be taken. These should only take about 15 minutes each time, or thirty to sixty minutes in total for the duration of the study. Throughout the duration of the study, on training days, the child will consume their given supplement and record their training in a log. This should only take about five minutes each time. If the child is training three times a week, this would mean 15 minutes a week spent performing these tasks, or one to two hours in total during study. In total, over the course of the study the total time commitment of the child should be about three to five hours.

YOUR RESPONSIBILITIES

Your responsibilities are minimal. All measures are non-invasive and will take place at the child's training location. The only two responsibilities of yours are to help your child complete a one-day dietary recall prior to and after the intervention period, as well as reviewing the ingredient list of the supplement to ensure that your child is not allergic to anything.

CONFIDENTIALITY

The results of the research study may be published, but your child's name will not be used in any way. All results and data will be kept confidential. Every child's information will be assigned a code number so that individual identities will be known only to the researcher and any coded information stored on a computer will be password protected. The two researchers will be the only people with access to these records. All identifying data will be destroyed at the end of the study. All information will be stored securely in a locked file cabinet in Dr. Disa Hatfield's office in Independence Square II for three years after the completion of the study and then destroyed.

PERSON TO CONTACT

If you are not satisfied with the way this study is performed, you may discuss your complaints with Disa Hatfield, anonymously, if you choose. In addition, if you have questions about your child's rights as a research participant, you may contact the office of the Vice President for Research, 70 Lower College Road, Suite 2, University of Rhode Island, Kingston, Rhode Island, telephone: (401) 874-4328. If you have any questions about your child's rights as a research participant, you may contact me at dhathfield@uri.edu or (401) 874-5183.

Institutional Review Board: Contact the Institutional Review Board (IRB) if you have questions regarding your rights as a research participant. Also, contact the IRB if you have questions, complaints or concerns which you do not feel you can discuss with the investigator. The University of Rhode Island IRB may be reached by phone at (401) 874-4328 or by e-mail at researchintegrity@etal.uri.edu.

Vice President for Research and Economic Development: You may also contact the Vice President for Research and Economic Development by phone at (401) 874-4576.

VOLUNTARY PARTICIPATION

Your child's participation in this study is voluntary. If you choose not to allow your child to participate, or if your child does not choose to participate, that is perfectly okay. You or your child may withdraw from participation at any time.

CONSENT

By signing this consent form, I confirm I have read the information in this parental permission document and have had the opportunity to ask questions. I will be given a signed copy of this parental permission document. I voluntarily agree to allow my child to take part in this study.

Printed Name of Child

Printed Name of Parent/Guardian

Signature of Parent/Guardian

Date

Relationship to Child

Printed Name of Person Obtaining Consent

Signature of Person Obtaining Consent

Date

APPENDIX C: Child Assent Form for Research

Disa Hatfield, PhD
Department of Kinesiology

Effect of whey protein supplementation on athletic performance and immune function in teenage athletes

Page 1 of 3

Child Assent Form for Research

WHO ARE WE AND WHAT ARE WE DOING?

Our names are Disa Hatfield and Andrew Stranieri. We are inviting you to take part in a research study because we are trying to learn more about how taking whey protein might affect your athletic performance. You should feel free to ask questions. If you have more questions about this study later, please call Disa Hatfield, Ph.D. or Andrew Stranieri, the persons responsible for this study, at (401)-874-5183.

WHY ARE WE ASKING YOU TO BE IN THIS RESEARCH STUDY?

The primary purpose of the study is to investigate the effects of whey protein supplementation on athletic performance in teenage athletes, while a secondary purpose is to simultaneously investigate the effect of whey protein supplementation on the athletes' immune, bone, and kidney health.

WHAT HAPPENS IN THIS RESEARCH STUDY?

No changes will be made to your training. You will still follow the same training schedule/program your coach assigns to you. You will also be randomly assigned to one of two groups: a whey protein supplement group or a placebo supplementation control group. You will be given your randomly chosen supplement to consume on training days for the duration of the study. You will not know if you are taking the supplement or the placebo, as both should look and taste the same. Be assured that both are safe to consume and will not negatively impact your training in any way.

Prior to, during, and after the study, various different measures of your physical health will be assessed. Before and after the study, your strength, power, and agility will be measured through the use of various athletic performance tests detailed below. Please check the box next to the tests you are comfortable doing.

Athletic Performance tests:

☐ 40 yard dash (run fast)	☐ Vertical Jump (jump high)
☐ Bench Press 1 RM	☐ Squat 1 RM
☐ T-Test cone drill for agility	☐ 5-10-5 shuttle drill for agility

Also, at multiple points during the study, your immune and bone health will be also be assessed. These tests are done by collecting urine and saliva and are very simple. You will urinate into a cup and drool into a tube. Lastly, your bone health will be measured via heel ultrasonography. Ultrasound scans of the heel are a quick, portable, and radiation-free. All you have to do is place your heel in the machine.

WILL ANY PART OF THE RESEARCH STUDY HURT YOU?

The possible risks and discomforts of this study are minimal. There is the possibility of some stomach discomfort from the supplement or even an allergic reaction to one or several of the ingredients. While rare, any potential stomach discomfort should subside quickly.

WILL THE RESEARCH STUDY HELP YOU OR ANYONE ELSE?

There are numerous possible benefits of your participation in this study. You will learn about your body composition and bone density. In addition, the general health of your immune system will also be assessed.

WHO WILL SEE THE INFORMATION ABOUT YOU?

Your participation in this study is confidential. None of the information will identify you by name and your confidentiality will be maintained always through the assignment of specific codes. All records will be stored in a locked room in a locked file cabinet or password protected computer located in room 205 of the Independence Square building and will be kept for 5 years after the completion of the study per government regulations. Access to your results will be restricted to the investigators and the data will be presented in pooled form. In any written reports or publications, you will not be identified in any way.

WHAT IF YOU HAVE QUESTIONS ABOUT THE RESEARCH STUDY?

If you are not satisfied with the way this study is performed, you may discuss your complaints with Andrew Stranieri or Dr. Disa Hatfield (401) 874-5183, anonymously, if you choose. In addition, if you have questions about your rights as a research participant, you may contact the office of the Vice President for Research and Economic Development, 70 Lower College Road, Suite 2, University of Rhode Island, Kingston, Rhode Island, telephone: (401) 874-4328.

DO YOU HAVE TO BE IN THE RESEARCH STUDY?

The decision to take part in this study is up to you. You do not have to participate. If you decide to take part in the study, you may quit at any time. If you wish to quit, simply inform either Andrew Stranieri or Dr. Disa Hatfield of your decision.

AGREEING TO BE IN THE STUDY

I was able to ask questions about this study. Signing my name at the bottom means that I agree to be in this study and my questions have been answered. My parent/guardian and I will be given a copy of this form after I have signed it.

Printed Name

Sign your name on this line

Date

Printed Name of Person Obtaining Consent

Signature of Person Obtaining Consent

Date

The following should be completed by the study member conducting the assent process if the participant agrees to be in the study. Initial the appropriate selection:

_____ The participant is capable of reading the assent form and has signed above as documentation of assent to take part in this study.

_____ The participant is not capable of reading the assent form, but the information was verbally explained to him/her. The participant signed above as documentation of assent to take part in this study.

APPENDIX D: Baseline Data Collection Sheet

Week 0 Pre-Screening/Pre-Test Measurements

Date:

Subject #:

Group:

Pre-Test Data Collection Checklist (week 0)

PA Questionnaire	_____
Nutrition Questionnaire	_____
HHQ	_____
Diet Logs	_____
Parent Consent Form	_____
Child Assent Form	_____
Anthropometrics	_____
Height: _____	
Weight: _____	
Age: _____	
Gender: _____	
Tanner Stage: _____	

Saliva Collected	_____
Urine Collected	_____
Bone Density (heel ultrasonography)	_____
Body Composition (BIA)	_____

Pre-Test Performance Measures (week 0)

Vertical Jump

Trial 1 _____

Trial 2 _____

Trial 3 _____

5-10-5 Pro-Agility Test

Trial 1 _____

Trial 2 _____

Trial 3 _____

APPENDIX E: Post-Test Data Collection Sheet

Week 4 Post-Test Measurements

Date:

Subject #:

Group:

Post-Test Data Collection Checklist (week 4)

Diet Logs

Anthropometrics

Height: _____

Weight: _____

Age: _____

Saliva Collected

Urine Collected

Bone Density (heel ultrasonography)

Body Composition (BIA)

Post-Test Performance Measures (week 4)

Vertical Jump

Trial 1 _____

Trial 2 _____

Trial 3 _____

5-10-5 Pro-Agility Test

Trial 1 _____

Trial 2 _____

Trial 3 _____

Squat 1 RM Test	
Warm-ups:	_____
Lift 1:	_____
Lift 2:	_____
Lift 3:	_____
Lift 4:	_____
Lift 5:	_____
Lift 6:	_____

Lift 1: _____

Lift 3: _____

Lift 5: _____

Bench Press 1 RM Test

Warm-ups: _____

Lift 1: _____

Lift 2: _____

Lift 3: _____

Lift 4: _____

Lift 5: _____

Lift 6: _____

Lift 1: _____

Lift 3: _____

Lift 5: _____

This image shows a single sheet of white paper with horizontal blue ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

APPENDIX F: Urinalysis Data Collection Sheet

Pre	Post
Date:	
Subject #:	
Group:	
<hr/>	
Leukocytes:	Neg Trace Small Mod Large
Nitrite:	Neg Pos
Protein (mg/dL):	Neg Trace 30 100 300 2000+
pH:	5.0 6.0 6.5 7.0 7.5 8.0 8.5
Blood:	Neg Trace Mod Trace Small Mod Large
USG:	1.000 1.005 1.010 1.015 1.020 1.025 1.030
Ketone (mg/dL):	Neg 5 15 40 80 160
Glucose (mg/dL):	Neg 100 250 500 1000 2000+

APPENDIX G: Health History Questionnaire

HEALTH HISTORY QUESTIONNAIRE

Study _____ Subject # _____
 Name _____ Sex _____ Age _____ DOB _____
 Street _____
 City _____ State _____ Zip _____ Phone _____
 Email _____

PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS AND PROVIDE DETAILS FOR ALL "YES" ANSWERS IN THE SPACES AT THE BOTTOM OF THE FORM

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2. Has your doctor ever denied or restricted your participation in sports or exercise for any reason?
☐	☐	3. Do you ever feel discomfort, pressure, or pain in your chest when you do physical activity?
☐	☐	4. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	5. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	6. Does your heart race or skip beats during exercise?
☐	☐	7. Has a doctor ever ordered a test for your heart? (i.e. EKG, echocardiogram)
☐	☐	8. Has anyone in your family died for no apparent reason or died from heart problems or sudden death before the age of 50?
☐	☐	9. Have you ever had to spend the night in a hospital?
☐	☐	10. Have you ever had surgery?
11. Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated.		
☐	☐	High blood pressure
☐	☐	Elevated cholesterol
☐	☐	Diabetes
☐	☐	Asthma
☐	☐	Epilepsy (seizures)
☐	☐	Kidney problems
☐	☐	Anemia
☐	☐	Heart problems
☐	☐	Lung problems
☐	☐	Chronic headaches
☐	☐	Coronary artery disease
☐	☐	12. Have you ever gotten sick because of exercising in the heat? (i.e. cramps, heat exhaustion, heat stroke)
☐	☐	13. Have you had any other significant illnesses not listed above?
☐	☐	14. Do you currently have any illness?
☐	☐	15. Do you know of <u>any other reason</u> why you should not do physical activity?
16. Please list all medications you are currently taking. Make sure to include over-the-counter medications and birth control pills.		
Drugs/Supplements/Vitamins	Dose	Frequency (i.e. daily, 2x/day, etc.)

DETAILS:

IF YOU SELECTED YES TO ANY OF THE QUESTION ABOVE PLEASE PROVIDE DETAILS (WHAT HAPPENED AND WHEN)

17. Please list all allergies you have.

Substance

Reaction

_____	_____
_____	_____
_____	_____

YES	NO
☐	☐

18. Have you smoked? If yes, #/day _____ Age Started _____ If you've quit, what age? _____
Cigarettes/Cigars/Pipes _____

19. Do you drink alcoholic beverages? If yes, how much? _____ How often? _____

20. Tanner stage (see picture) ☐ I ☐ II ☐ III ☐ IV ☐ V

21. Do you have a family history of any of the following problems? If yes, note who in the space provided.

☐ High blood pressure	_____	☐ Heart disease	_____
☐ High cholesterol	_____	☐ Kidney disease	_____
☐ Diabetes	_____	☐ Thyroid disease	_____

22. Please check the box next to any of the following body parts you have injured in the past and provide details.

☐ Head	_____	☐ Hip	_____	☐ Calf/shin	_____
☐ Neck	_____	☐ Thigh	_____	☐ Shoulder	_____
☐ Upper back	_____	☐ Knee	_____	☐ Upper arm	_____
☐ Lower back	_____	☐ Ankle	_____	☐ Elbow	_____
☐ Chest	_____	☐ Foot	_____	☐ Hand/fingers	_____

YES	NO
☐	☐
☐	☐
☐	☐

23. Have you ever had a stress fracture?

24. Have you ever had a disc injury in your back?

25. Has a doctor ever restricted your exercise because of an injury?

26. Do you currently have any injuries that are bothering you?

27. When was the date of your last menstrual cycle?

ADDITIONAL IF YOU SELECTED YES TO ANY OF THE QUESTION ABOVE PLEASE PROVIDES DETAILS (WHAT HAPPENED AND WHEN)

DETAILS:

APPENDIX H: Physical Activity Questionnaire

Physical Activity Questionnaire

Cardiovascular/Aerobic Exercise

Do you currently engage in cardiovascular exercise on a regular basis?

_____ Yes _____ No

If yes, what mode(s) of exercise do you perform? _____

How many times per week do you perform this/these exercise(s)? _____

What is the duration and intensity of exercise (ex: 30 minutes at 3.2 [mph](#),
3.0% grade on the treadmill)? _____

For how many years have you been performing this type of exercise? _____

Have you participated in any other physical activity (other than resistance exercise) on a
regular basis in the last 5 years?

_____ Yes _____ No

If yes, what type? _____

Resistance Exercise (Weight Training)

Do you currently engage in cardiovascular exercise on a regular basis?

_____ Yes _____ No

If yes, how many days per week? _____

List some examples of common exercises you perform. _____

APPENDIX I: Nutritional History Questionnaire

Nutritional History Questionnaire

1. Do you use alcohol? _____ Yes _____ No
If yes, how many times per week. _____
What is the total amount? _____
2. Do you drink caffeinated coffee or colas? _____ Yes _____ No
If yes, how many per week? _____
3. Are you now or have you ever been on a diet? _____ Yes _____ No
If yes, please explain _____

4. Do you consider yourself overweight? _____ Yes _____ No
Do you consider yourself underweight? _____ Yes _____ No
5. Number of meals you usually eat per day: _____
6. Do you usually eat breakfast? _____ Yes _____ No
7. Number of times per week you usually eat the following:
_____ Beef _____ Fish _____ Pork _____ Chicken
_____ Desserts _____ Fried Foods _____ Fast Food
8. Do you regularly use any of the following? (please circle)
Butter Sugar Sweeteners Salt Whole Milk
9. How would you describe your nutrition habits? (please circle)
good fair poor
10. Please describe your knowledge of nutrition. (please circle)
very knowledgeable knowledgeable no knowledge
11. Do you regularly use any vitamin or mineral supplementation?
_____ Yes _____ No
If yes, please list: _____

12. Do you use any other form of supplementation (i.e. herbal remedies, food supplements, performance enhancing drugs, etc?)
_____ Yes _____ No
If yes, please list: _____

13. Have you ever taken protein, casein protein, soy protein, sports nutrition bars, protein shakes or smoothies?

_____ Yes _____ No

If yes, are you currently using these products now on a regular (weekly) basis?

_____ Yes _____ No

Please list the manufacture and name of any of these products you have ever had: _____

If you are currently using any of these products, how many times per week do you eat/drink each product?: _____

If you aren't currently taking any of these product, but have in the past, how often did you use them?: _____

When was the last time you used them and which type did you have?: _____

APPENDIX J: One Day Food Record Sheet

SUBJECT #: _____

Pre

Post

Group: A B

One Day Food Record

Write down everything you ate yesterday. Include all meals and snacks and the amount eaten.

Meal & Time	Food or Beverage Item	Amount	Method of Preparation	Do not write in this space
Breakfast				
Snack				
Lunch				
Snack				
Dinner				
Evening				

Source: Adapted with permission from Rickheim P, Flader J, Carstensen KM. Type 2 Diabetes BASICS.
Minneapolis MN: IDC Publishing; 2000.

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SUBJECT #: _____

Pre

Post

Group: A B

5-Step Multiple-Pass Method

Detail Cycle	1 Quick List	<ul style="list-style-type: none">• Simple list of all foods eaten• No order to list• Specify a 24-hour period• One food/drink per line
	2 Forgotten Foods	<ul style="list-style-type: none">• Read Foods from Forgotten Foods list• Read one line at a time• Give participants time to think and respond
	3 Time & Occasion (Activities)	<ul style="list-style-type: none">• Suggest common activities associated with eating/drinking• Ask participants to think about their activities• Ask participants to circle what time the food was consumed
	4 Describe the food	<ul style="list-style-type: none">• Brand/Restaurant name of food/drink• Describe the food/drink• How it was prepared• How it was served<ul style="list-style-type: none">◦ What, if anything, did you add or eat with the food/drink
	4 Amounts	<ul style="list-style-type: none">• Use visuals in recall kit<ul style="list-style-type: none">◦ How much was consumed◦ What size was food or drink
	5 Final Probe	<ul style="list-style-type: none">• Final review of recall for forgotten foods/drinks• Review forms for completeness

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24-Hour Food Recall Forgotten Foods

There are some foods that people tend to forget they ate.

- Did you have any **crackers, breads, rolls, or tortillas** that you may have forgotten about?
- How about any **hot or cold cereals**?
- **Cheese** added as topping on vegetables or on a sandwich?
- Did you have any **chips, candy, nuts, or seeds**?
- **Fruit** eaten with meals or as a snack?
- What about **coffee, tea, soft drinks, or juices**?
- Any **beer, wine, cocktails, brandies**, or any other drinks made with liquor that you may have forgotten?

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APPENDIX K: Weekly Supplement Log Sheet

Please fill out and return, along with Tupperware

Did you take your supplement today?

Monday___ Tuesday___ Wednesday___ Thursday___ Friday___

Saturday___ Sunday___

Take the supplement with water, and after training, if possible

How many days this week did you go to AMP? _____

How many days this week did you have practice? _____

Any competitions? _____

IF YOU MISS A DAY, THAT IS FINE, JUST LEAVE IT.

DO NOT TAKE DOUBLE IF YOU MISS.

REMINDER! PLEASE DO NOT CHANGE YOUR DIET! THANK YOU!

Along with any questions, if you have any side effects, please stop taking the supplement and let us know

-Call 401-874-5183

-email: astranieri@uri.edu or doch@uri.edu