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The Relationships Between Energy Balance, Timing and Quantity of Protein Consumption, and Body Composition in Collegiate Football Players

By:

Letal Garber
A Thesis

In Partial Fulfillment of the
Master of Science in Health Sciences
Byrdine F. Lewis School of Nursing and Health Professions
Department of Nutrition
Georgia State University
Atlanta, GA
2016

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ACCEPTANCE

This thesis, The Relationships Between Energy Balance, Timing and Quantity of Protein Consumption, and Body Composition in Collegiate Football Players, by Letal Garber was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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Abstract

Background

Timing and quantity of protein (PRO) consumption are important considerations for muscle protein synthesis (MPS), fat-free mass (FFM) accretion, and body fat % (BF%) reduction. The effect of PRO ingestion on changes in FFM is mediated by many variables. Past studies have focused on specific composition of carbohydrate (CHO) and PRO consumption (CHO vs. PRO + CHO), and have also investigated PRO intake timing at pre-exercise, post-exercise, or both. Other studies have investigated FFM maintenance and growth with increased PRO consumption during catabolic or anabolic phases of energy balance (EB). These mechanisms have been studied in various populations, including healthy untrained individuals, overweight and obese people, and endurance athletes. However, studies have not explored relationships between the amount and timing of PRO ingested, and the state of EB, as it relates to FFM%.

Method/Design

A retrospective analysis design was used to assess relationships between PRO ingestion, timing, and EB on FFM in collegiate football players. Subjects were members of an intercollegiate Division 1 football team, had completed a one-day food and activity record, and had body composition assessed as part of a regular team screening procedure. Data acquisition was supervised by a PhD/Registered Dietitian. Food and activity records were analyzed using NutriTiming®, which predicts RMR via the Harris-Benedict equation, uses a MET-based relative intensity activity scale, and accesses the USDA Nutrient Database for Standard Reference, Release 26 to predict hourly EB and PRO consumption. EB was assessed as ±400 kcal EB (EBR), < 0 kcal EB (NEGEB), and > 0 kcal EB (POSEB). Total useable PRO (TUP) was defined as the sum of PRO consumed in units up to 30g max/meal, a value also assessed relative to EB at the time of ingestion. The goal was to assess the amount and timing of PRO intake with EB as these factors relate to FFM.

Results

Pearson's correlations found that BF% was negatively associated with TUP while in EBR (r=-.253; p=0.049), and FFM% was positively associated TUP in EBR (r=0.279; p=0.030) and in POSEB (r=0.282; p=0.028). NEGEB was positively associated with BF% (r=0.325; p=0.011), and negatively associated with FFM% (r=-0.322; p=0.011).

Conclusions

Results elucidate that players who ingest PRO in a relatively good energy-balanced state had higher FFM% and a lower BF%. Further, those players consuming TUP while in POSEB had an even stronger positive association with FFM% and a stronger inverse association with BF%. These data reject the null hypothesis that football players who consume PRO in POSEB have less FFM% than those who consume PRO in NEGEB.

Abstract Abbreviations: *BF- body fat, CHO – carbohydrate, FFM – fat free mass, Met-metabolic equivalent of task, MPB – muscle PRO breakdown, MPS – muscle protein synthesis, PRO – PRO, RMR-resting metabolic rate*

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List of Abbreviations

AA – Amino Acids

BF – Body Fat

BMD – Bone Mineral Density

BMI – Body Mass Index

CHO – Carbohydrate

CM – Chocolate Milk

DEXA – Dual-energy X-ray absorptiometry

EAA – Essential Amino Acids

EB – Energy Balance

EBR – ±400 kcal EB

ED – Energy Deficit

FFM – Fat-free Mass

FSR – Fractional Synthesis Rate

LEU – Leucine

MPB – Muscle PRO Breakdown

MPS – Muscle PRO Synthesis

NEGEB – Negative Energy Balance

PHE – Phenylalanine

POSEB – Positive Energy Balance

TDR – Time-divided Supplementation Regimen

TFR – Time-focused Supplementation Regimen

TUB – TUPPRO

RDA – Recommended Dietary Allowance

RMR – Resting Metabolic Rate

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Chapter I

Introduction

The intricate metabolic process for stimulating muscle protein synthesis (MPS) has been investigated, with findings that suggest multiple factors are involved, including protein (PRO) ingestion, exercise, and energy balance (EB). PRO consumption has emerged as an important factor for the maintenance or increase in fat-free mass (FFM) when in conjunction with exercise training (Stiegler et al., 2006). Some studies are in agreement that PRO and carbohydrate (CHO) co-ingestion is more effective in increasing MPS than CHO ingestion alone (Willoughby 2007; Murphy and Miller, 2010; Breen et al., 2011; Ferguson-Stegall et al., 2011; Lunn et al., 2012), while other studies have proposed that the composition of energy substrate ingestion does not differentially effect MPS (Atherton et al., 2012; Fujita et al., 2009).

In has become increasingly clear that the timing of PRO intake is an important factor in MPS, and should be a consideration in making recommendations for strategies to increase FFM. Literature relating to the timing of PRO intake has illustrated that ingesting PRO with various timing schemes (pre-exercise, post-exercise, or both) may differentially affect MPS (Aragon et al., 2013; Burk et al., 2009; Areta et al., 2013).

EB is also a variable explored in relation to FFM in individuals. MPB is affected by EB, the difference between energy intake and energy expenditure (Joosen et al., 2006). It has been found that a negative EB up-regulates proteolytic enzymes (such as cortisol) that catabolize muscle PRO and decrease FFM (McIver et al., 2012). Further, muscle PRO breakdown (MPB) associated with exercise may also result in a negative PRO balance (Atherton et al., 2012). Some studies showed greater conservation of FFM in those individuals that ingested a higher PRO intake than their recommended dietary allowance (RDA) despite their participation in energy-deficit trials (Pasiakos et al., 2013, Soenen et al., 2013).

Most findings suggest that, regardless of the training level (novice to elite) of the athletes studied, PRO consumption pre- and post-workout increases muscular anabolism and may also improve physical performance, training session recovery, and strength (Ferguson-Stegall et al., 2011). However, this “anabolic window” of opportunity for PRO accretion is not well studied nor fully understood in football players. Therefore, by performing a cross-sectional analysis of PRO ingestion on a typical practice day, it was hoped to assess the relationship between PRO intake and FFM in collegiate football players. The aim was to improve our understanding of the relationship between timing of PRO consumption, EB, and FFM, which could help to fine-tune current published recommendations offered to these athletes.

Chapter II

Review of Literature

This literature review explores FFM accretion as it relates to energy substrate ingestion, energy substrate timing, and EB.

Background

Energy Metabolism

Energy-yielding macronutrients work synergistically with water and micronutrients to supply the body tissues with the necessary fuel to sustain normal functioning, which is of particularly high importance for athletes because of a high demand for energy (Wolinsky and Driskell, 2008). Further, total energy and macronutrient availability modulates exercise performance and adaptations to training (Wolinsky and Driskell, 2008). Energy-yielding pathways for the energy substrates convert chemical bond energy to adenosine triphosphate (ATP) and creatine phosphate in striated muscle (CP) (Driskell and Wolinsky, 2000). Well-timed and adequate consumption of energy helps to provide the fuel for storing muscle and liver glycogen, and cellular and storage fat, all of which are important energy sources in physical activity. With appropriate consumption of energy substrates, CHO is metabolized to provide fuel to our bodies through both aerobic and anaerobic metabolic processes, fat is utilized for insulation and protection of our vital organs and is also a major source of energy through aerobic metabolisms, and PRO is used to repair and build tissues, but can also provide amino acids (AA) for gluconeogenesis and the manufacture of fats, both of which can be used as a source of energy (Wolinsky and Driskell, 2008). The utilization of PRO for energy is of particular interest in this study, as it is hypothesized that a failure to provide sufficient total energy to satisfy tissue requirements will result in a forced utilization of PRO for energy and a failure of a primary function of PRO: to repair and build tissues.

PRO Metabolism

PRO provides the structural framework for cells and organs, catalyzes biochemical reactions, and regulates intra- and intercellular communication (Welle, 2012). PRO are broken down through proteolysis to their constituent AA and for PRO mass maintenance, new PRO must be synthesized. The term “turnover” normally refers to the simultaneous process of degradation and synthesis of body PRO. Synthesis of new PRO is mandatory for cell replication and growth, and accounts for only a portion of the human PRO metabolism, while a smaller amount is made up of regulatory PRO such as hormones and rate-limiting enzymes. Oxidation of AA, glycation, and other cellular damage makes the turnover process of PRO essential for the maintenance and integrity of body PRO. While CHO provide the main fuel source for our bodies, the turnover of body PRO produces a dynamic amino acid pool for structural functions (Wolinsky and Driskell, 2008).

Energy Substrate Ingestion and Fat-Free Mass

Some studies isolated the type of energy substrate as their independent variable, leaving the timing of consumption the same in both groups. One of these studies featured 20 untrained males with the average age of 19 years who were matched by age, total body mass, and leg press strength after baseline testing (Willoughby 2007). The purpose of this study was to investigate the effects of supplemental PRO and AA on muscle performance and anabolism. First, percutaneous muscle biopsies (50–70 mg) were obtained prior to supplement ingestion before the exercise session at week one and 24 hours following the last exercise session at the end of week 10. Next, participants were randomly assigned to supplement groups either containing 20 g PRO (14 g whey + casein + 6 g AA) or 20 g dextrose placebo (PLA). The solution was ingested one-hour before and one-hour after exercise. The subjects exercised four times/week for 10 weeks using three sets of 6-8 repetitions at 85-90% of 1 repetition maximum (RM). The significant finding was that the PRO supplement resulted in greater increases in FFM ($p<0.05$) than the dextrose supplement. Results showed respective increases in total body mass, FFM, and thigh mass of 4.35 ± 2.88, 2.70 ± 1.31, and 0.41 ± 0.03 kg for PLA and increases of 7.00 ± 2.32, 5.62 ± 0.98, and 0.73 ± 0.08 kg for PRO. In addition, the data showed 0.20 ± 0.08 and 0.61 ± 0.03 kg increases in relative bench press and leg press strength, for PLC; however, PRO underwent increases of 0.48 ± 0.02 and 1.13 ± 0.12 kg in relative bench press and leg press strength. These researchers concluded that PRO is more effective than CHO as a nutrient that upregulates markers of MPS and ultimately improve performance.

Resistance training requires planning of PRO ingestion around the exercise sessions for effective muscle PRO recovery. Energy substrates and FFM have also been studied in normal, non-athlete subjects involved in aerobic exercise. One study in particular found that PRO+CHO supplementation increased MPS more than a CHO supplement in 12 healthy older adults of an average age of 59 years (Murphy et al., 2010). Immediately after exercise, subjects ingested a 60 g CHO drink or an isocaloric PRO beverage (40 g CHO + 20 g whey). The researchers focused on LEU uptake via direct measurement of whole-body PRO with a LEU infusion, sampling blood, and expired air. Their results showed that non-oxidative LEU was higher (p=0.001) in the PRO group than in the CHO group, suggesting that consumption of a PRO beverage increases whole body PRO more than a CHO beverage.

Similar findings were found in a single-blind, cross-over study (Breen et al., 2011). This study focused on ten well-trained male cyclists with an average age of 29 who were recruited from local cycling clubs. Well-trained cyclists were those participants who undertook two or more training sessions per week of 1–5 hours in duration. Cyclists also averaged 7 ½ years of cycling experience. They completed two trials in randomized order to reveal MPS after ingestion of a CHO or CHO + PRO beverage immediately and 30 minutes post-exercise. The CHO beverage was 25.2 g of CHO and the CHO + PRO beverage was 25.4 g of CHO plus 10.2 g of whey PRO isolate dissolved in 250 mL of cold water. A second identical beverage was consumed 30 minutes after the first beverage was finished. These doses provided a total of 50.8 g CHO and 20.4 g PRO for the CHO + PRO solution and a total CHO intake of 50.4 g for the CHO solution. All tests were completed within a 4-week period with both treatment trials separated by 14–21 days. The researchers controlled for discrepancies in energy substrates by standardizing the participants' diet two days prior to each treatment. Analysis of muscle biopsies showed a significant increase ($p = 0.025$) of MPS in cyclists ingesting the CHO+PRO beverage versus participants drinking the CHO beverage alone. Specifically, the MPS rates were ~35% higher for CHO+PRO compared with CHO. In addition, serum insulin increased to a greater extent for CHO+PRO (285 ± 32%) than for CHO (60 ± 8%; $p < 0.001$). Further, researchers found that mTOR phosphorylation (an important pathway in MPS) was increased at post-exercise for the CHO+PRO group ($p = 0.1$), and decreased for post-exercise ingestion of CHO ($p = 0.08$).

Investigators assessed chocolate milk’s (CM) efficacy on post-exercise muscle recovery using three beverages (CM-CHO+PRO, isocaloric CHO drink, and PLA drink) without otherwise changing the participant’s diet and training regimen (Ferguson-Stegall et al., 2011). Thirty-two healthy, recreationally active yet untrained (16 males and 16 females) subjects between 18 and 35 years old completed the study. Specifically, subjects could not have exercised regularly more than three hours a week over the last two years, and had VO_2 max values of <40 mL/kg/min for females and <45 mL/kg/min for males in order to fit the classification of recreationally active yet untrained. The beverages were ingested one-hour after each exercise session (60 min/d of cycling, 5 d/week for 4.5 weeks at 75-80% of maximal VO_2). These beverages were CM, CHO, or PLA. The CM (organic low-fat CM) and CHO drink (grape-flavored Kool-Aid) were matched in calories and fat, while the PLA drink was an artificially-sweetened grape-flavored zero calorie beverage. Dual energy X-ray absorptiometry (DEXA) was used to measure whole body and regional changes in fat mass, and lean mass, as well as bone mineral density (BMD). The results showed greater improvements ($p<0.05$) in FFM in subjects consuming the CM than in those that drank the CHO supplement. In particular, whole body FFM had increased by a 1.4 kg accretion in relation to 0.6 kg in the CHO group and 0.8 kg in the PLA group. The researchers concluded that nutritional supplementation of these energy substrates may increase the magnitude of training adaptations compared to the exercise stimulus alone.

Other researchers examined the effects of fat-free chocolate milk on PRO turnover during recovery from endurance exercise and presented similar results as the aforementioned study (Lunn et al., 2012). Fourteen male runners with the average age of 24 years were recruited from similarly matched groups of recreational and club-level runners to participate in two trials separated by one week. There was a set of protocols that consisted of a 45-min controlled exercise bout followed by a 3-hour recovery period during which subjects consumed one of two experimental beverages. After completing baseline testing, subjects consumed a diet with the same amount of calories throughout the 14 days of the study. PRO intake was prescribed at 1.5 g/kg per day, fat intake was ≤30% of total daily energy intake, and CHO intake was 6 g/kg per day. After the 45-min run at 65% of VO_2 max, subjects randomly consumed a single bolus (480 mL) of either the CHO + PRO drink (fat-free CM) or a CHO drink containing 74.0 g of sweetened grape-flavored drink mix prepared in bottled water. Both beverages contained 296 kcal, but the CHO + PRO drink consisted of 16 g PRO (64 kcal), whereas the CHO drink was non nitrogenous. The CHO content of the CHO beverage (74 g, 296 kcal) was greater than that of the CHO + PRO beverage (58 g, 232 kcal) and neither drinks contained fat.

The significant findings showed a higher mixed muscle fractional synthesis rate (FSR) and lower whole body proteolysis in runners ingesting CM versus those that drank the CHO beverage ($p<0.05$).

Although the above studies generally agree that ingestion of energy substrates increase MPS more than fasting, there is evidence that competes with this finding. One such study measured FSR in participants either ingesting a solution or fasting prior to a high-intensity resistance exercise (Fujita et al., 2009). The foundation of this study was the understanding that acute bouts of resistance exercise stimulate MPS within just one hour of exercise, and may remain elevated for one to two days post-exercise. The researchers were curious whether or not energy substrate ingestion in the form of a PRO + CHO beverage would induce a more significant increase in FSR than the control (fasting) after acute bouts of resistance exercise. The reason for looking at FSR is because it is a marker of MPS, which may help researchers assess energy substrates and their relationships on stimulating muscle fiber accretion. The participants were 22 young (average 26 years old), healthy subjects (13 male, 9 female). These subjects were physically active yet not engaged in a resistance or endurance exercise training program during the length of the trial. They were allocated into two groups (fasting control group, and essential amino acid (EAA) + CHO group). The experimental group ingested a solution of EAA + CHO one-hour before beginning an exercise bout and two hours post-exercise. Their FSR was measured through stable isotopic methods and muscle biopsies. Muscle FSR decreased in the fasting group but increased at one-hour post-exercise ($p<0.05$), while the supplement group experienced a muscle FSR increase immediately after ingestion then a return to basal values and remain unchanged one-hour post exercise. The authors concluded that EAA + CHO ingestion before resistance exercise does not enhance post-exercise MPS compared with exercise with no energy substrate intake.

Timing of Energy Substrate Consumption and Fat-Free Mass

Pre-Exercise

Type of energy substrate is frequently investigated, yet some researchers also focus on the timing scheme of ingesting energy substrates (pre-exercise, post-exercise, and both) and their attributed effect on MPS in healthy participants. One study suggested that ingestion of a PRO + CHO solution pre-exercise contributes to greater MPS than intake of this solution post-exercise (Tipton et. al., 2001). This investigation included six healthy human subjects (3 males, 3 females). Subjects with a mean age of 30.2 years were considered recreationally active and were instructed to refrain from physical exercise for ≥24 h before being studied. They were also told to maintain a consistent dietary intake pattern throughout the duration of the study. At least one week before the initial infusion study, each subject was familiarized with the leg press and leg extension machine, and their one-repetition maximum was determined on each. Immediately after the first muscle biopsy, subjects performed an intense leg resistance exercise bout. Before initiation of the resistance exercise routine, subjects consumed either a 500-mL bolus of the EAC solution (PRE) or a PLA solution (POST). The investigators measured phenylalanine (PHE) concentrations across the leg by priming a continuous infusion of L-[ring-(2) H (5)] PHE. The exercise bout consisted of 10 sets of eight repetitions of leg press at 80% of 1RM followed by eight sets of eight repetitions of leg extension at 80%

of 1RM (session would last 45-50 minutes). The results showed blood and muscle PHE concentrations increase by 130% after consumption in both trials (pre- and post-exercise). However, the delivery of AA an hour into exercise following pre-exercise consumption was significantly greater ($p<0.05$) than when the solution was ingested post-exercise. This led to the authors' conclusion that the response of net MPS is greater when the solution is ingested immediately before resistance exercise due to an increased delivery of AA to the leg during that period (although these findings have been debated based on a flawed methodology). Specifically, the researchers admitted that the results may be biased toward the pre-exercise trial because they followed the participants the entire three hours following the consumption of EAC, whereas during post-exercise, they only followed the participants for two hours (Tipton et al., 2001).

Post-Exercise

Other researchers tested a different approach to timing of PRO ingestion throughout a 12-hour period in order to determine how the quantity and timing of PRO ingestion after a single bout of resistance exercise influence the muscle anabolic response throughout the entire day (Areta et al., 2013). Twenty-four healthy trained males with at least two years of high-intensity resistance training experience (≥2 times per week) were assigned to three groups (n=8 in each group). They were each provided with individualized pre-packed meals for the three days prior to an experimental trial that were standardized in calories and provided 1.5 g PRO/kg per day. No exercise was allowed during the 48-hour period prior to a trial. The participants ingested 80 g of whey PRO after resistance exercise in three varying timing schemes; 10 g every 1 ½ hours, 20 g every three hours, and 40 g every six hours. Muscle biopsies taken at rest showed that MPS increased more in those that ingested PRO every three hours than the other timing schemes ($p<0.02$).

Another study that deserves mention was one that investigated the timing of PRO intake and muscle hypertrophy and strength in elderly people (Esmarck et al., 2001). Thirteen men with an average age of 74 years participated in a 12-week resistance training program (three times/week) while receiving oral liquid PRO (10 g PRO, 7 g CHO, 3 g FAT) either immediately (P0 group) after or two hours post-exercise (P2 group). These subjects had not participated in resistance training within the last five years, an inclusion criterion for the study. The exercise began with a cycling bout on a cycle ergometer (5-10 minute warm-up), followed by resistance training consisting of three different concentric strength exercises: leg press, lat pulldown, and knee extension (8-12 repetitions, 3-5 sets). Muscle hypertrophy was evaluated by MRI and muscle biopsies, muscle strength by dynamic and isokinetic strength measurements, and body composition was determined by use of DEXA. Food records were obtained over four days and plasma insulin response was also considered by the researchers. At the completion of the 12-week program, the cross-sectional area of the quadriceps and the mean fiber area increased in the P0 group, whereas no significant increase was observed in P2. Further, both dynamic and isokinetic strength increased in P0, by 46% and 15%, respectively ($p< 0.05$), whereas P2 only improved in dynamic strength by 36 % ($p<0.05$). The researchers concluded that the immediate ingestion of energy substrate rather than delaying energy substrate intake post-exercise is more beneficial for muscle hypertrophy during resistance training.

Many previously mentioned studies focused on how PRO ingestion around exercise (resistance or aerobic) is associated with FFM and MPS. However, there is also evidence of the synergistic effects of resistance training and PRO ingestion on MPS (Atherton et al., 2012, Burd et al., 2011). Burd and his colleagues explained this in their study that examined the effect of PRO ingestion in two states: unexercised-rested muscle and 24 hours post-exercise on MPS. The researchers recruited fifteen healthy (deemed healthy by routine medical questionnaire) recreationally active men (mean age: 21 years). The participants reported engaging in lower body exercise, either resistance or resistance and aerobic exercise more than 3x/week for the past six months. The subjects received a primed, constant infusion of PHE to measure MPS after PRO feeding at rest (FED; 15 g whey PRO) and 24 h after resistance exercise (EX-FED). Participants performed four sets of maximal strength to failure leg exercises at varying intensity. The findings showed that PRO ingestion stimulated rates of MPS above fasting rates by 0.016 ± 0.002%/hour and the response was enhanced 24 hours after resistance exercise ($P = 0.003$). Thus, the authors concluded that MPS continues to occur even four hours post-exercise, which is an important consideration when administering PRO after physical activity. They explained further that PRO ingestion the following day after exercise sensitizes the muscle to PRO feeding for at least 24 hours, ultimately expanding the window of opportunity for PRO ingestion.

Although difficult to determine whether certain military personal are novice, recreationally active, or trained athletes, there are investigations of post-exercise PRO supplementation on muscle soreness during basic military training (Flakoll et al., 2003).

Researchers selected 387 US Marine recruits (with a minimum age of 18 years) from six platoons for a double-blind randomized-control study. The participants were randomly allocated to three different treatment groups. They were instructed to continue with the same daily activities including dietary intake, training activities, and exercise. The exception was a total of 27 training sessions of conditioning hikes (3, 5, and 10-miles) and 24 days of running (1-3 miles), sit-ups, push-ups and pull-ups. Group one (n=128) received a nonnutritive PLA tablet after completion of their exercise. The second group (n=129) did not receive PRO but had 8 g CHO and 3 g FAT in the solution post-exercise (Con), while the third group (n = 130) were administered 10 g PRO, 8 g CHO, and 3 g FAT (PRO) after their physical activity. Muscle soreness immediately post-exercise was reduced by PRO supplementation vs. PLA and control groups on both days 34 and 54. Specifically, on the 54th day of training, muscle soreness immediately after the final physical function test was improved significantly ($p<0.05$) more with Pro (26%) than with PLA (9%) and Con (13%). Researchers concluded that post-exercise PRO supplementation may not only enhance MPS but also has the potential to positively impact health, muscle soreness, and hydration during prolonged intense exercise training. This can easily be applied to an individual at any level of athleticism (Flakoll et al., 2003).

With the knowledge that post-exercise PRO ingestion is beneficial for muscle anabolism, researchers still question whether there is indeed an anabolic window of opportunity for PRO ingestion post-exercise (Rasmussen et al., 2000). Specifically, they experimented with a mixture of EAA and CHO delivered after physical activity to see if it enhanced

MPS by timing the energy substrate delivery one or three hours post-exercise. Six participants (three men, three women) with a mean age of 34 years were recruited for a randomized control trial. With the exception of one volunteer, subjects were recreationally active but were not involved in a consistent resistance or endurance exercise program. Subjects were randomly assigned to receive either the EAA (6 g EAA, 35 g sucrose) then a PLA drink or the PLA then EAA drink at one or three hours post-exercise. The three-compartment model for measuring MPS was an infusion of PHE, femoral arterial and venous blood sampling, and muscle biopsies. PHE net balance and MPS were significantly increased ($p<0.05$) when the drink was taken one or three hours after exercise but not when the PLA was ingested at one or three hours. Further, the EAA-CHO drink produced similar anabolic responses at one and three hours. The authors concluded that combining EAA with CHO stimulates muscle anabolism by increasing MPS at one or three hours post-exercise.

Researchers that agree post-exercise ingestion of PRO enhances MPS and muscle recovery do not allude to the concentration needed before oxidation occurs. Therefore, researchers implemented a study on the direct relationship of a dose-response ingestion of whole PRO to MPS after resistance exercise (Moore et al., 2015). The study was done on six healthy active males (mean age: 22 years) who had four or more months of previous recreational weight-lifting experience (range was 4 months to 8 years). Participants went to the laboratory on five different occasions separated by at least one week. They each performed an acute bout of leg resistance exercise and then drank a beverage containing 0, 5, 10, 20, or 40 g of egg PRO in a randomized order. The researchers measured whole-body LEU oxidation and albumin PRO synthesis by a primed constant infusion of [1-^{13}C] LEU (an EAA metabolized in the lean tissues of the body). Body mass did not change over the course of the controlled diet (EER via Harris Benedict equation and 1.6 activity factor) given to the participants, suggesting they were in EB. The results showed that dietary PRO ingestion maximally stimulated MPS in a dose-response manner at 20 grams ($p<0.01$). Oxidation of LEU was maximal at 20 and 40 grams of ingested PRO ($p<0.01$). Thus, dietary PRO consumed post-exercise that is in excess of the rate at which AA can be incorporated into tissues may lead to irreversible oxidation.

Pre-Exercise and Post-Exercise

It is possible that splitting a PRO-CHO dose, provided pre- and post-exercise is more effective in increasing FFM than a similar amount provided prior to training. One study hypothesized that applying this method in prolonged resistance training may be better than ingestion of the supplement immediately before exercise (Burk et al., 2009). The subjects were 13 untrained men aged 18-19 years and evaluated during two eight-week training and supplementation periods. In the first period (time-focused supplementation regimen-TFR), the subjects consumed the supplement (60 g of PRO) in the morning and afternoon, right before the training session. In the second period (time-divided supplementation regimen-TDR), the men ingested the PRO supplement in the morning and the second five hours after training. They measured body composition using DEXA and found that the FFM increased with TDR (from 62.4±1.2 to 63.5±1.3 kg, $p=0.046$), with no evident changes with TFR. Further, there was also an increase in 1RM strength in the squat exercise in the TDR regimen ($r=0.569$; $p=.041$) that the authors attributed to the increased FFM. Therefore, these findings of a TDR approach to training may have practical implications in enhancing FFM.

Distribution of PRO ingestion and MPS have also been investigated in healthy individuals not performing physical activity, besides the 30 minutes of light aerobic activity the first and last day of testing (Mamerow et al., 2014). The researchers used a 7-day crossover feeding design with a 30-day washout period and measured changes in MPS in response to isocaloric and isonitrogenous diets with PRO at breakfast, lunch, and dinner distributed evenly (EVEN) or skewed (SKEW) (more PRO given at dinner). Eight healthy male (n=5) and female (n=3) volunteers between the ages of 25 and 55 years old participated in this study. The participants were physically active but not athletically trained and they could not participate in a diet or exercise program concurrently with the study. Muscle biopsies and blood samples were also taken during primed constant infusion of PHE. The 24-hour FSR was 25% higher in the EVEN group than the SKEW group (p=0.003), indicating that consuming moderate amounts of PRO at each meal is more effective at stimulating MPS than bolus PRO consumption at one meal.

Despite the suggestion that consuming moderate amounts of PRO is more effective in stimulating MPS than bolus ingestion, a recent study challenged this finding by inquiring whether delivery of PRO that was divided would differentially affect MPS than a bolus administration of the same concentration of EAA (Mitchell et al., 2015). Sixteen healthy young men with an average age of 20 years were recruited for a study on EAA ingestion and regulation of the “muscle-full” effect. They self-reported to be recreationally active and were instructed not to commit to heavy exercise 48 hours prior to the study. The participants were either assigned to the same dose of 15 g mixed-EAA in a single does (bolus group n=8) or in four fractions of 45-minute intervals (spread group n=8). The researchers assayed plasma insulin and EAA concentrations and took muscle biopsies to quantify PHE and determine MPS. Although researchers found a gradual aminoacidemia in spread administration over bolus PRO delivery ($p<0.01$), identical anabolic responses were observed, and both methods resulted in MPS returning to fasting rates within two hours, regardless of the differences in circulating EAA. Therefore, the researchers concluded that EAA delivery is not an essential determinant of anabolism at young men at rest.

A similar discovery was made when researchers chose to investigate the quantity of PRO ingested rather than the mode of delivery of PRO (Kim et al., 2015). Twenty-four healthy older (range 52-55 years old) subjects recruited via local newspaper advertisements participated in a randomized control trial, yet only 20 were included in the final analysis. Subjects were randomly allocated into the following four PRO RDA groups: 0.8 g (1RDA), 1.5 g/kg/day (~2RDA). Each RDA group had a subgroup of uneven (U: 15/20/65%) or even distribution (E: 33/33/33%) patterns of intake for breakfast, lunch, and dinner over the day. Subjects were studied with primed continuous infusions of l-[$^2H_5$] PHE and l-[$^2H_2$] tyrosine on day four following three days of diet habituation. Positive nitrogen balance (NB) was achieved at both PRO levels, but NB was greater in 2RDA vs. 1RDA ($p=0.0001$), without effects of distribution on NB. The greater NB was due to the higher MPS with 2RDA vs. 1RDA ($p=0.0018$). Thus, the authors concluded that MPS was greater with 2RDA vs. 1RDA, regardless of distribution patterns. Whole body net PRO balance was greater with PRO intake above recommended dietary allowance (0.8 g PRO g/kg/day) in mixed meals.

Verijk et al., (2009) contested the idea that pre-exercise and post-exercise PRO supplementation further augments MPS after resistance training. The objective of their study was to assess the benefits of timed PRO supplementation on the increase in muscle mass and strength during prolonged resistance-type exercise training in healthy elderly men who habitually consume adequate amounts of dietary PRO. Timed PRO supplementation has also been considered in an older population (mean age 72 years) who normally have adequate intakes of PRO. The researchers recruited 26 participants to a progressive, 12-week resistance-type exercise program with two experimental groups (PRO or PLA) immediately before and after each exercise session. All subjects were living independently and had no history of participation in any structured exercise training program in the past five years. There were three sessions per week and during the 5-minute warm-up and cooling-down procedures, the subjects received 250 mL of a beverage with either only water (PLA) or 10 g PRO as casein hydrolysate. Thus, the PRO group received 20 grams of PRO each session. One-repetition maximum tests were regularly performed during the intervention. Muscle anabolism was measured at the whole-body level via DEXA and CT scans. The 1 RM strength increased in both groups ($p<0.001$) and the DEXA and CT scans showed similar adaptations in leg muscle mass in both groups ($p<0.001$). The researchers proposed that timed PRO supplementation immediately pre- and post-exercise does not further enhance MPS and strength after prolonged resistance-type exercise in those individuals who habitually ingest their RDA of dietary PRO.

Energy Balance and Fat-Free Mass

The rate of MPB is increased in the fasted state. Exercise increases both MPS and MPB when the participant is in the fasted state and when the net PRO balance is negative. Despite the rise of MPS post-exercise, net PRO balance may still remain negative because the rate of MPB exceeds that of MPS. Therefore, in order to achieve a positive net muscle PRO balance, amino acid availability, and thus MPS must increase even more. This can be achieved by enhancing amino acid availability and allowing the rate of MPS to surpass the rate of MPB, which itself may become suppressed (Kumar et. al 2009). This general idea is the backbone of many studies analyzing MPS and FFM in subjects who exercise in an anabolic state versus exercising in an energy deficit (ED).

Studies have assessed FFM when the daily PRO amount was increased in some subjects and kept stable in others (Flakoll et al., 2003, Kim et al., 2015). Decreases in FFM mainly occur from inadequate per-meal intakes of PRO, leading to a negative PRO balance. Similarly, this decrease in FFM has been shown to be the result of a reduced rate of post-meal PRO availability related to the type/quality of PRO ingested (McIver et al., 2012). For example, body composition was assessed before and after the 3-month dietary intervention, which consisted of PRO-rich supplements in the PRO group (n=12) and an isoenergetic (equally active) combination of CHO and fat supplements in the control group (n=12). Daily PRO intake was calculated from 24h urinary nitrogen. Body composition was measured by DEXA, and subjects were weight stable and did not change their habitual physical activity. Daily PRO intake increased in the PRO group during the intervention compared to baseline versus the control group that did not change their PRO intake. FFM of the PRO group had increased ($p<0.01$), and fat mass had decreased ($p<0.05$), while the control group had not changed. These findings suggested that individuals in a positive PRO balance may see greater increases in FFM than those in a negative PRO balance.

There is literature that suggests increasing PRO ingestion above the RDA while in an ED conserves FFM (Pasiakos et al., 2013). Researchers recruited military personnel and civilians from various research settings. Volunteers were required to be physically active (physical activity 3–4 d/wk) and recreationally fit as indicated by baseline-study screening (VO_2 max = 40–60 mL kg/min). There were 32 men (11 military, 21 civilians) and seven women (7 civilians) that completed the randomized controlled study. This study was designed to assess the effects of dietary PRO intake on body composition and MPS responses to short-term ED. Subjects were either allocated to a RDA dose of PRO (0.8 g/kg), 2x RDA (1.6 g/kg), or 3x RDA (2.4 g/kg) while on an ED diet (30% decrease in calories) for 31 days. The first phase was a 10-day weight maintenance period in which the participants consumed a diet consistent with their recommended energy requirements. Volunteers lost weight regardless of dietary PRO. However, the proportion of weight loss due to reductions in FFM were lower in those that consumed PRO 2x RDA and 3x RDA than those that consumed RDA PRO ($p<0.05$). These results suggested conservation of FFM when PRO ingestion is increased during times of ED, such as in exercise.

A similar study (Soenen et al., 2013) with a longer trial period (6 months of energy restriction) investigated a similar intervention on 72 overweight participants (24 women and 48 men). Originally, the researchers recruited 80 participants via advertisements in local newspapers, and eight of the subjects dropped out during the first two weeks. These participants were overweight or obese (BMI>25 kg/m^2) and aged between 18 and 80 years. They were allocated into two groups: normal PRO diet (n=36) and high PRO diet (n=36). PRO intake was consistent in the normal PRO diet (0.8 g/kg) and high PRO diet (1.2 g/kg) groups throughout the study ($p<0.001$). BMI and body fat (BF) mass similarly decreased in both groups ($p<0.01$), however, FFM changed favorably (increased) with the high PRO diet compared with the normal PRO diet group ($p<0.05$). These studies proposed that subjects in an ED and greater PRO intakes conserve their FFM more efficiently than those participants with lower PRO intakes.

One study added resistance exercise as another variable to possibly potentiate MPS (Areta et al., 2014). The researchers employed multiple experimental interventions to determine the best formula for preservation of muscle mass. Sixteen young (average age of 27 years for men, 28 years for women), healthy, resistance-trained subjects (7 females, 8 males) completed the study. Body composition was measured a one to two weeks before the first experimental trial using DEXA. The four experimental interventions were EB at rest, ED at rest, and then ED with resistance exercise both with and without PRO feeding (15 g and 30 g). MPS was measured by infusing PHE enrichments and the resting post-absorptive MPS after ED was lower compared with EB (0.019 vs 0.026%/h) ($p<0.001$). However, resistance exercise in ED returned MPS to values comparable with resting EB in the acute post-exercise recovery period. Resistance exercise followed by 15- and 30-g PRO ingestion increased post-exercise MPS ~16 and ~34% above resting EB (0.030 and 0.038%/h, $p<0.02$). Taken collectively, the results demonstrated that a combination of resistance exercise with increased PRO availability post-exercise can enhance rates of MPS during short-term ED, which could in the long term preserve muscle mass.

Other researchers investigated the effects of endurance training and concurrent ED on body composition. In particular, one study focused on endurance racing while ingesting varying macronutrients and associations with EB and body composition (Paulin et al., 2015). Thirteen relatively lean (BF% = 21.9) participants (12 male, 1 female) with an average age of 40 years participated in a 500 mile race aiming to reach the South Pole finishing line located at the American South Pole Research Station. The researchers hypothesized that due to the nature of the duration and intensity of the race, food and drink choices become less appealing or tolerable, causing the athlete to under fuel and end up in an ED. Dietary analysis for caloric, macronutrient and micronutrient intake was performed using a suitable software package (Training Peaks dietary software, Peaksware LLC, CO, USA). The average loss of lean mass throughout the race was 2.0 ± 4.1 kg ($n = 13$). There was a significant inverse correlation between change in lean mass, PRO ($p = 0.03$) and energy intake ($p = 0.03$). The rate of muscle breakdown is accelerated when muscle PRO oxidation exceeds synthesis, which usually occurs in proportion to intensity and duration of the sporting activity. These findings suggested that a diet in

PRO was better suited to athletes competing over long distances at lower intensities, in order to better maintain FFM.

Summary

Football training is intense and may occur twice a day or even more. These training periods may lead to acute muscle damage characterized by either acute muscle soreness or delayed muscle soreness (24-48 hours post-exercise) making recovery an essential component of training (Kersick et al., 2008). Researchers have suggested that achievement of a positive EB could aid FFM accumulation (Weinheimer et al., 2010). Investigators have concluded that PRO ingestion is more effective in increasing FFM than CHO alone (Ferguson-Stegall 2011; Murphy and Miller, 2010). Further, studies also found that subjects who were on higher PRO versus lower PRO intakes had a greater increase in FFM at the end of the trial (Soenen et al., 2013; Pasiakos et al., 2013). Researchers tend to spotlight post-exercise PRO ingestion (typically 1-hour post-exercise) as the most effective method of muscle PRO accretion and recovery (Aragon et al., 2013; Shoenfeld et al., 2013). The review of literature mainly focused on cyclists and healthy individuals, while studies on football players remain scarce. Past studies showcased significant results ($p<0.05$) when using retrospective analysis to analyze PRO intake and changes to muscle mass (Bopp et al., 2008; Moore et al., 2015). Retrospective studies allow the researcher to analyze specific exploratory questions using pre-existing data in a relatively inexpensive way. In addition, this method grants access to large data sets that may be analyzed for longer observation periods, and is a convenient approach to answering time-sensitive questions (Motheral et al., 2003). Therefore, the purpose of this study is to assess the relationship of PRO ingestion, timing, and EB on FFM in football athletes. Specifically, the aim of the proposed retrospective analysis is to isolate energy substrate timing as the independent variable and investigate any significant effect it may have induced on FFM (dependent variable).

Hypothesis

Hypothesis: Football players who consume PRO in an anabolic state (POSEB) have higher FFM% than those who consume PRO in a catabolic state (NEGEB).

> Null Hypothesis: Football players who consume PRO in an anabolic state (POSEB) have lower FFM% than those who consume PRO in a catabolic state (NEGEB).

Chapter III

Methods

Subjects

The study used a retrospective analysis design, and was submitted for IRB approval. Since the study was noninvasive and utilized a data set that has already been obtained, it was an expedited review and subsequently approved. Subjects came from a sample population of collegiate level male football players on a NCAA Division 1 football team. The study excluded those players with any recent injuries. Previous studies utilized relatively small sample sizes (e.g., 10 subjects) that resulted in lower statistical power (Park et al., 2009). We had data from approximately 70 football players of varying positions, making this a robust review. All subjects were assigned a unique ID number unrelated to any personally identifying information. Due to inconsistent data on nine of the football players, only 61 final subjects were used for the statistical analysis.

Study Protocol

The independent variable was timing of daily PRO intake (over 24 hours) assessed on an hourly basis and the dependent variable was FFM. The players completed a diet entry form (see Appendix) where they were able to specifically describe what they ate and drank in 24 hours for three days. An example was provided for the players to reference when filling out the form. The NutriTiming® Activity Factor Scale Descriptions helped the subjects figure out the best factor that matches their daily activities. Since this sample was extracted from the target population of collegiate football players, the "exercise" mentioned was their daily training regimen during the football season (September-December). The researcher had access to body composition data including BMI, FFM, height, and weight of each player, which was measured by multi-current bioelectrical impedance analyzer (BIA). The researcher matched the day of the BIA reading with the analysis provided by NutriTiming® for an accurate representation of the variables.

Data Analysis

NutriTiming® provided both 24-hour and hourly EB values, and also provided an assessment of total and hourly macronutrient intake, including PRO (measured in grams), which can be simultaneously analyzed with state of EB at the time of consumption. This allowed assessment of the player's PRO intake by amount and time at any point of the day. It was also possible to investigate differences in FFM in players that consumed most of their PRO in an anabolic state versus those players that took in most of their PRO in a catabolic state. This was done by extracting total useable protein (TUP) from their overall caloric intake throughout a 24-hour span. More specifically, TUP was measured by noting where athletes consumed $\leq$ 30 grams of PRO throughout the day and calculating the sum. TUP consumed in an energy balance (EBR) was the amount of PRO utilized by the muscles in a relatively balanced caloric range (±400 kcal). TUP ingested while in a positive EB (POSEB) was the amount of PRO ingested in an EB over 0 kcal, the player's anabolic state. TUP consumed in a negative energy balance (NEGEB) was the amount of PRO ingested in an EB below 0 kcal, the player's catabolic state.

Statistical Analysis

SPSS was utilized for creation of the data set. Linear regression analysis allowed the researcher to see if any of the PRO variables (consumption, timing, and balance) had a relationship with FFM. Descriptive statistics helped the researcher measure central tendency and spread among the aforementioned variables. Correlation statistics helped group multiple variables against each other to see how they are related (e.g., BMI and amount of PRO consumed prior to football exercise). When looking at the mean of PRO ingested while in <0 EB, an independent t-test compared players that fell above or below the mean to determine whether there was statistical evidence of different associations with body composition in the two groups.

Chapter IV

Results

Descriptive Statistics. Data were included on a total of 61 subjects between the ages of 18 to 23 with the mean age of 20.4 years (**Table 1**). The height range of the football players was from 5'1" to 6'4" and averaged 6', while the weight range was 153 lb. to 308 lb., with an average of 227 lb.

Table 1: Subject Characteristics (N=61)	
Variables	**Mean ± SD**
Age	20.4 ± 1.3
Height (in.)	72.4 ± 3.6
Weight (lb.)	227.9 ± 42.6

The BF% range was 6.7 to 29.4% and the mean BF% was 18.3%, a relatively lean subject group (**Table 2)**. Fat mass was a range from 13 to 87 lb. (average of 43.8 lb.) and FFM mass % was a minimum of 70% and 93.3% with an average of 81.7%. FFM ranged from 132.8 lb. to 227.6 lb. with an average of 184 lb. Total body water ranged between 97.2 lb. to 166.6 lb. and averaged 184 lb.

Table 2: Body Composition (N=61)	
Variables	**Mean ± SD**
Fat Mass (%)	18.3 ± 5.8
Fat Mass (lb.)	43.8 ± 21.5
Fat-Free Mass (%)	81.7 ± 5.8
Fat-Free Mass (lb.)	184 ± 23.2
Total Body Water (lb.)	134.7 ± 17

The nutrient data were representative of energy intake in a 24-hour time span (**Table 3**). Total kcal intake in collegiate football players ranged from 1223 kcal to 8445 kcal and averaged at 3715 kcal. PRO intake (gm) ranged from 34.4 g to 446.7 gm and averaged 166.8 gm. The PRO intake measured in g/kg average was a minimum of 0.34 g/kg and maximum of 4.38 g/kg with an average of 1.7 g/kg. PRO intake as a measurement of % kcal was a low of 11% and a high of 35% with an average of 20%. TUP measured in gm ranged from 34.4 gm and 334 gm and averaged 105.2 gm/day. Further, TUP consumed in EBR ranged from 0 to 334 gm with an average of 77.8 gm. TUP consumed in POSEB had the same range and an average of 61.9 gm. TUP ingested in NEGEB was an average of 43.2 gm with a range from 0 to 160.37 gm.

Table 3: Nutrient Intake (N=61)

Variables	**Mean ± SD**	**Range**
Kcal Intake	3715 ± 1431.6	1223-8445
PRO Intake (gm)	166.8 ± 78.3	34.42-446.27
PRO Intake (gm/kg)	1.7 ± .8	0.34-4.38
PRO Intake (% Kcal)	20 ± 5	10.78-35.28
Total PRO (up to 30 gm/meal)	105.2 ± 46.4	34.41-334.02
TUP (EBR)	77.8 ± 62.5	0.00-334.02
TUP (>0 EB)	61.9 ± 68.1	0.00-334.02
TUP (<0 EB)	43.2 ± 40.8	0.00-160.37

Key: EB is ± 400 kcal, >0 EB is anabolic state, <0 EB is catabolic state

Associations (**Table 4**). BF% was negatively associated to TUP in EBR (r=-0.283; p=0.027). BF% was also negatively associated with TUP in POSEB (r=-0.286; p=0.025). FFM% was positively associated with TUP in EBR (r=.279; p=0.030), and with TUP in POSEB (r=0.282; p= 0.028). The TUP in NEGEB showed significant positive associations with fat mass% (r=0.325; p=0.011), fat mass (lb.) (r=0.358; p=0.005), and FFM (lb.) (r=0.386; p=0.002). FFM% was negatively associated with TUP in NEGEB (r=-0.322; p=0.011).

Table 4: Associations between Body Composition and PRO Intake (N=61)

Variables		**Useable Prot. (Up to 30 gm)**	**Useable Prot. (± 400 kcal)**	**Useable Prot. (>0 kcal EB)**	**Useable Prot. (<0 kcal EB)**
Fat Mass %	R	-0.140	-0.283*	-0.286*	0.325*
	P	0.282	0.027*	0.025*	0.011*
Fat Mass (lb.)	R	0.014	-0.134	-0.296*	0.358*
	P	0.914	0.305	0.021*	0.005*
Fat-Free Mass %	R	0.136	0.279*	0.282*	-0.322*
	P	0.297	0.030*	0.028*	0.011*
Fat-Free Mass (lb.)	R	0.142	-0.049	-0.225	0.386*
	P	0.275	0.707	0.081	0.002*
*Values with * are statistically significant (p≤0.05)*					

Table 5: PRO Intake (g/kg) Associations with Body Composition (N=61)

Variable		**Weight (lb.)**	**Body Fat %**	**Fat Mass (lb.)**	**Fat-Free Mass (lb.)**	**Fat-Free Mass %**
PRO (g/kg)	Correlation	-.347	-.361	-.372	-.296	.352
	Sig (2-tailed)	.006	.004	.003	.021	.005

Table 5 shows that PRO intake, measured in grams, was negatively associated with weight (r=-0.347; p=0.006), BF% (r=-0.361; p=0.004), fat mass (r=-0.372; p=0.003), and FFM (r=-0.296; p=0.021). PRO intake was also positively associated with FFM% (r=.352; p=0.005). The independent group t-test showed that eating PRO while in NEGEB was associated with more BF% (p=0.031) and fat mass (p=0.014) and a lower FFM% (p=0.033).

Results Summary

The athletes were relatively lean with an average BF% of 18.3. The PRO intake average (measured in g/kg) had a minimum of 0.34 g/kg and maximum of 4.38 g/kg with an average of 1.7 g/kg. BF% was negatively associated to TUP in EBR (r=-0.283; p=0.027) as well as TUP consumed in POSEB (r=-0.286; p=0.025). FFM% was positively associated with TUP in EBR (r=.279; p=0.030), and with TUP in POSEB (r=.282; p= 0.028). The TUP ingested in NEGEB was significantly and positively associated with fat mass % (r=0.325; p=0.011), fat mass (lb.) (r=0.358; p=0.005), and FFM (lb.) (r=0.386; p=0.002). FFM% was negatively associated with TUP in NEGEB (r=-0.322; p=0.011). PRO intake (g/24 hr) was negatively associated with weight (r=-0.347; p=0.006), BF% (r=-0.361; p=0.004), fat mass (r=-0.372; p=0.003), and FFM (r=-0.296; p=0.021). PRO intake (g/24 hr) was also positively associated with FFM% (r=0.352; p=0.005).

Chapter V

Discussion and Conclusions

The purpose of this research was to investigate how PRO ingestion, timing and distribution effects FFM in collegiate level football athletes. The significant findings indicated that players who ingest PRO for MPS in a relatively balanced energy state had more FFM% and a lower BF%. Further, those players who consumed their TUP in an anabolic state had an even stronger positive association with FFM% and an inverse association with BF%. Subjects who ingested their PRO in a negative EB, or a catabolic state, had higher BF% and fat mass and lower FFM% than those players who consumed their PRO in an anabolic state. FFM is everything in the body that is not fat; muscle, water, bone, connective tissue, etc. FFM% was a better indicator of changes in muscle because it measured the % of total FFM over the total body mass. This explains the discrepancies in the associations between ingested protein and FFM measured in lb. versus FFM%.

The discoveries in this study support the hypothesis that football players who consume PRO in POSEB have more FFM than those who consume PRO in a NEGEB. These results also suggest that TUP alone is not a significant determination of FFM accretion, and EBR should also be considered. This finding can be applied to the current nutrition recommendations given to college-level football players. Educating athletes on how much PRO they must ingest based on their body composition and energy expenditure is one component of the nutrition recommendation. Further, these results on FFM accrual in EB could be a tool to help players maintain an EBR state throughout their practice season. Their ingested PRO may more effectively increase MPS, and possibly improve body composition and performance.

The findings suggested that those football players who consumed more PRO in an energy-balanced state had a lower BF% and higher FFM%, regardless of what form of PRO they ingested (PRO-CHO or PRO). Although there are multiple studies that claim PRO or PRO + CHO combinations are more effective in stimulating MPS than a CHO energy substrate, these variables were not analyzed in this particular study (Breen et al., 2011; Ferguson-Stegall. 2011; Lunn et al., 2012; Murphy et al., 2010; Willouhby, 2007). Prospective studies can investigate which type of energy substrate is most effective with increasing FFM in football players, while maintaining them in an energy balanced state throughout their practice day. These data agree with previous discoveries that a negative EB up-regulates proteolytic enzymes that catabolize muscle PRO and decreases FFM (McIver et al., 2012). In addition, the general consensus obtained from previous literature is that PRO ingested in an EB or anabolic state induces MPS and FFM accretion, which is parallel to the significant results in the current study (Flakoll et al., 2003; McIver et al., 2012; Pasiakos et al., 2013; Soenen et al., 2013).

Timing of PRO ingestion in football athletes varied in this particular study, where other studies kept timing the same while adjusting for type of PRO consumed (Willoughby 2007). What is understood is that the timing scheme that allowed for a balanced intake of

PRO throughout the day without letting the athlete go into an ED was associated with a larger accretion of FFM in the athlete. Past studies showed an increase in FFM with a TDR approach to PRO ingestion (pre-exercise ingestion and post-exercise ingestion) (Burk et al., 2009; Mamerow et al., 2014). In this study, athletes maintaining EB throughout the practice day had a higher FFM than those in an ED, which is aligned with this aforementioned finding of a TDR approach to ingestion of PRO. Further, maximizing FFM accretion by attaining a positive EB is also supported by some past research (Weinheimer et al., 2010). Some authors opposed this concept when their results showed similar muscular adaptations in their subjects whether they consumed their PRO spread out through the day or in one sitting (Kim et al., 2015; Mitchel et al., 2015; Verjick et al., 2009).

Those players who consume most of their PRO while in EBR or POSEB have more FFM than those players who consume most of their PRO in an ED. This is a noteworthy finding because it can be used as a general recommendation for football athletes looking to improve their performance while maintaining their muscle strength. Currently the recommendation for athletes is to never go longer than three to four hours without PRO. Researchers support this recommendation after finding that MPS is greater in athletes that consume their PRO every three hours than any other timing scheme (Areta et al., 2013). Existing guidelines suggest 1.2 to 1.7 g/kg as the amount of PRO needed to improve aerobic capacity through mitochondria synthesis and build muscle mass and strength (Rosenbloom, 2009). The findings are at the high end of this recommendation, with consumption of PRO in the football players an average of 1.7 g/kg. PRO intake was positively associated with FFM accretion, suggesting that the more PRO the players ingested, the more FFM they had. Although consuming more PRO may have brought the athletes in EBR or POSEB state, allowing this optimal MPS phenomenon.

One possible limitation was that the retrospective analysis was done on one day out of the football player's practice season. This one day may not have been representative of the athlete's usual PRO intake, limiting generalizability of the results. Another limitation is that this study was done via secondary analysis of one-day body composition and nutrient analysis. Thus, no further follow-up was possible with these athletes. Another confounding factor not considered was individual response to insulin, a PRO that muscle is extremely sensitive to immediately post-exercise. Multiple studies reviewed by Koopman et al., (2005) have suggested that elevated insulin concentrations can stimulate the uptake of selected AA, MPS rate and effectively inhibit proteolysis. The combination of CHO + PRO tends to induce a great anabolic response, and since we were solely looking at PRO consumption, it was difficult to determine whether or not some players had greater FFM accretion due to this combination. Specifically, several studies have given credit to CM as an effective source of combined CHO + PRO that induces an anabolic response (Ferguson-Stegall et al., 2011; Lunn et al., 2012). Due to the fact that this study was a retrospective analysis done on male collegiate football players, this may limit application of the data to other sports. Future studies could investigate the relationship of PRO consumption, EB, and FFM in other sports such as wrestling, basketball, soccer, and others.

This research serves a practical purpose for the college level football player. If the athlete applies the recommendation of not going longer than three to four hours without PRO and also is equipped with the knowledge that the body only absorbs about 30 grams of PRO at a time, they avoid consuming an excess of PRO to make up for their losses (Areta et al., 2013, Moore et al., 2015). Consuming excess PRO above the RDA may not be superior to consuming modest amounts of PRO throughout the day while maintaining EBR. The breakdown of PRO requires more water than metabolism of CHO and FAT, due to the excretion of nitrogen (Berning et al., 2006). Therefore, as PRO metabolism increases, so does the risk of dehydration. It is important to consume more fluids to make up for the water losses. Athletes are at increased risk for dehydration due to their water losses through sweat and expiration, so excess PRO may be an additive factor to dehydration. Another possible detrimental effect of higher PRO intakes is increased risk in osteoporosis or bone fractures (Heaney and Layman, 2008). High PRO intakes have been shown to affect calcium homeostasis, resulting in increased calcium excretion, and decreased BMD (Heaney and Layman, 2008). This is an important consideration for athletes because bone and muscle strength both impact performance. The absorbable capacity of PRO is evident in the results; if TUP is an average of 105.2 grams and the average ingested without considering EB is 166.8 grams, there is PRO that is not being utilized. If these subjects consume their PRO a few times a day while maintaining an EB, they may see an increase in FFM, effective recovery and perhaps an improvement in athletic performance.

Prospective studies could expand on this finding of players with positive associations of TUP and FFM accretion. The researchers could do a longitudinal study that follows the players throughout their football season. They could then assess what specific type of PRO combination (i.e., PRO, PRO + CHO, and LEU) increases FFM in players while controlling for EB. Investigation on differences in PRO ingestion and FFM among the various football positions may allude to specific recommendations for every type of football athlete. Researchers can use the available data to further investigate whether various player positions (e.g., defensive lineman, running backs, and quarterbacks) differentially impact FFM. Due to the limitations of this study, we did not include PRO timing as an independent variable on FFM accretion. Building upon the findings of EB and FFM adaptations, later studies can incorporate PRO ingestion timing: pre-exercise, during exercise, post-exercise to their data analysis.

Conclusions

The findings of this study suggested that collegiate level football players who ingested PRO in EBR had more FFM% and a lower BF%. Further, players who consumed their TUP in POSEB had an even stronger positive association with FFM% and an inverse association with BF%. These data reject the null hypothesis that football players who consume PRO in POSEB have less FFM% than those who consume PRO in NEGEB. The results of this study are in general agreement with past studies regarding FFM accretion in anabolic states and through post-exercise PRO ingestion (Flakoll et al., 2003; McIver et al., 2012; Pasiakos et al., 2013; Soenen et al., 2013). Football players are involved in anaerobic activity that requires PRO for recovery after short bursts of energy.

If they stay within EBR (± 400 kcal) or in a POSEB throughout the majority of their day, they can optimize AA delivery to their muscles with as little as a 20 g PRO per meal (Areta et al., 2013; Moore et al., 2015). This could mean less wasted PRO, less cost of PRO supplements, and more efficient use of ingested energy substrates. New research can further elucidate whether player positions may differentially impact FFM. Prospective studies can also look into energy substrate timing and FFM of a similar population of collegiate level football players.

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Appendix

NutriTiming® Data Entry Form

Instructions: Completing this form will help us understand whether the amount of energy (calories) you consume comes close to matching the energy (calories) you expend. This form provides a way of entering your energy expended by using an 'Activity Factor', and your energy consumed by using a description of the foods and drinks you ate. The information is entered by hourly units, so you don't have to remember precisely the time you had an activity or ate some food. Rather, you are asked to enter when you had an activity, its intensity by using the activity factor scale, and how long you did it (example: I had a slow jog between 10 and 11 in the morning that lasted for 30 minutes). Use the NutriTiming Activity Factor Scale Descriptions to help you figure out the best factor to enter when describing an activity. When entering food, describe the food and the way it was prepared fully (example: chicken breast with no skin that was baked; or fried, battered chicken breast, etc), and the amount you consumed (example: 1 apple; 1 ½ cups; 15 red grapes; 1 large banana, etc.). A factor of 1.5 is considered normal daytime activity, and we will assume a factor of 1.5 unless you indicate otherwise. A factor of 1 is equal to sleep, and a factor greater than 1.5 suggests you are doing something more vigorous than normal daytime activity. Please enter a full 24 hours of all your activities and all the foods/drinks you consume. Use the example below to help you understand how to enter the information.

NutriTiming Activity Factor Scale

Factor	Description
1	**Resting, Reclining:** Sleeping, reclining, relaxing
1.5	**Rest +:** Normal, average sitting, standing daytime activity
2.0	**Very Light:** More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth
2.5	**Very Light +:** Working harder than 2.0
3.0	**Light:** Movement with upper and lower body. Equivalent to household chores
3.5	**Light +:** Working harder than 3.0; Heart rate faster, but can do this all day without difficulty
4.0	**Moderate:** Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable
4.5	**Moderate +:** Working harder than 4.0. Heart rate noticeably faster, breathing faster
5.0	**Vigorous:** Breathing faster and deeper, heart rate faster, must take occasional deep breath during sentence for conversation
5.5	**Vigorous +:** Working harder than 5.0. Breathing faster and deeper, and must breath deeply more often to carry on conversation
6.0	**Heavy:** You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high
6.5	**Heavy +:** Working harder than 6.0. You can barely talk but would prefer not to. This is as hard as you can go, but not for long
7.0	**Exhaustive:** Can't continue this intensity long, as you are on the verge of collapse and are gasping for air. Heart rate is pounding

Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
****Begin Example****					
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (Frozen-Kellogg)	3
				Maple Syrup	2 Tablespoons
				1 % Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1 % Milk for Coffee	2 Tablespoons
10am	11am	5.0	Jog 30 minutes	Gatorade	16 Ounces
12noon	1pm	1.5	Nothing Special	Medium size beef sandwich with white	1 Sandwich
				bread, mayonnaise, lettuce, and tomato.	
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
5pm	6pm	4.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium Size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped; no butter)	100 Calorie Pack