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Effect of a Recovery Supplement on Weight Lifting Performance, Muscle Morphology  
and Muscle Protein Accretion

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A dissertation

presented to

the faculty of the Department of Exercise and Sport Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy in Sport Physiology and Performance

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by

Christopher Brian Taber

August 2016

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Keywords: Weightlifting, Protein, Carbohydrate, Supplementation, Periodization

## ABSTRACT

### Effect of a Recovery Supplement on Weight Lifting Performance, Muscle Fiber Morphology and Muscle Protein Accretion

by

Christopher B. Taber

The purposes of this dissertation were to examine the effect of a protein and carbohydrate recovery beverage versus a placebo on weightlifting performance, its effect on muscle morphological changes and specific muscle protein accretion. The following are major findings from the dissertation: 1) Protein and carbohydrate recovery supplementation does not appear to have influence on performance measure in trained weightlifters. This finding may be associated with the short-term nature of this study and the trained population used. 2) Compared with placebo, a protein and carbohydrate beverage provided greater benefits on cross sectional area of type I and type II muscle fibers. Additionally, the block periodization protocol incorporating phase potentiation improved cross sectional area of both groups compared to baseline. 3) Finally, protein and carbohydrate supplementation provided greater benefits on total mTOR and myosin heavy chains 6 & 7. These findings indicate that a protein and carbohydrate beverage provide greater benefits compared with a placebo on cellular signaling, myosin heavy gene expression and muscle fiber increases in trained weightlifters. Improved cross sectional area and increased myosin heavy chains indicate positive adaptations to resistance training combined with supplementation and may indicate improved skeletal muscle qualities necessary for increased power output. The mTOR pathway is the master regulator of cellular growth and increases in total mTOR indicate a greater proclivity for cellular growth and greater activity resulting from resistance training may increase synthesis and accretion of muscle contractile proteins. This

dissertation highlighted several benefits of recovery supplementation, however further longitudinal studies utilizing block periodization and well-trained athletes are necessary to fully elucidate benefits for strength and power athletes.

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## DEDICATION

This dissertation is dedicated my parents and Lucy, without your unwavering love and support this project would not have been possible.

## ACKNOWLEDGEMENTS

Dr. Michael Stone – for building the greatest program in the country and teaching me what excellence truly means.

Dr. Brad DeWeese- for being my chair and providing me with the guidance necessary to finish this project.

Dr. Kimatake Sato- for your unique views on sport science and constant support.

Dr. Charles Stuart- for providing me the opportunity to complete my dissertation. Your knowledge was invaluable for this project.

Mary Howell- for answering all my questions and helping me to complete my project on time.

Garett Bingham- for reading all the permutations of my dissertation and all your help

Stoneage Weightlifting Club- for donating a piece of your leg to science and participating in my study.

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## CHAPTER 1

### INTRODUCTION

Athletes and coaches implement many tactics to improve performance. These tactics can include adjustments in the training regimen such as the manipulation of the volume-load, frequency, or density of sessions within the training process. Further alterations may occur in the athlete's diet and lifestyle to best promote recovery and adaptation from various stressors. Because athletes cannot continue to increase training load without being subjected to overtraining, other methods of recovery must be utilized. Once lifestyle changes such as optimized sleep, implementation of recovery methods and consolidation of outside stressors have been made, the remaining component of successful sport performance is the diet.

#### Introduction to Nutritional Countermeasures in Sports Performance

Using the diet to counteract the catabolic effects of a training session and to exploit the anabolic processes leading to greater adaptation and super compensation has been termed "nutritional countermeasures". These nutritional countermeasures must be implemented in order to dampen the catabolism associated with each training session, as well as replace lost substrates in order to promote anabolism and recovery adaptation. Using diet to bolster performance is not a new topic, sources reaching back to antiquity show that ancient Olympians supplemented with meat in order to perform at their best. Milo of Croton an ancient Olympic wrestler was known to consume up to 20 pounds of meat and bread a day (Grivetti & Applegate, 1997).

The diets of athletes vary widely and should match the demands of each respective sport in order to address all disruptions caused by training and lifestyle (Phillips, 2004, Wilson, & Wilson, 2006). After a needs analysis is completed outlining the demands, of the sport a coach and athlete can begin to work together to build a balanced plan for meeting the nutritional needs

of the athlete in a daily and weekly schedule. Once the diet plan has been established, minor changes may have large outcomes when all other aspects of training have been attuned. One change that can be implemented that may further increase adaptations from training is the logistical and opportune matching of food intake around the training window. This timing of key nutrients mainly carbohydrate and protein around the workout is labeled nutrient timing and has become the source of research and scrutiny.

### Nutrient Timing

In the seminal work by Ivy and Portman appropriately labeled nutrient timing they lay the foundation for the timing of nutrients around training (Ivy & Poortman, 2004). Nutrient timing attempts to exploit the unique internal environment found after a training session and use this time period to increase uptake of nutrients at the cellular level with the end product being improved recovery and adaptation. From this work a multitude of studies have been completed using nutrient timing around the workout window. These studies include a variety of populations (young vs. old) (trained vs. untrained), timing of supplementation (immediate vs. delayed), macronutrient utilized (protein vs carbohydrate), amount of macronutrient, and various resistance training programs. Research on the topic of nutrient timing has been conflicted as to the efficacy of post work nutrition with some authors claiming no effect (Aragon, & Schoenfeld, 2013; Phillips, 2004; Reidy, & Rasmussen, 2016; Schoenfeld, Aragon, & Krieger, 2013) and others presenting evidence for benefits (Beelen, Burke, Gibala, & van Loon, 2010; Cermak, Res, de Groot, Saris et al., 2012; Humli, Lockwood, & Stout, 2010; Ivy, & Poortman, 2004; Ivy & Ferguson 2010; Kerksick et al., 2008; Wilson, & Wilson, 2006). The discrepancy in findings can be attributed to the variations in subjects, supplement administered, timing of supplement administered, training program used and methods used in measuring changes (Aragon, &

Schoenfeld, 2013; Kerksick et al., 2008; Schoenfeld, Aragon, & Krieger, 2013). Because of these inconsistencies it is difficult to compare results across studies and provide suitable nutrition recommendations for coaches and athletes. Within this research there is a paucity of studies using well-trained subjects utilizing sound periodization and programming methods coupled with nutrient timing methods.

Properly timed consumption of the nutrients protein and carbohydrate can influence the internal milieu leading to positive protein synthesis, mitigation of protein breakdown, alternation in hormones and repletion of carbohydrates. The end result of nutrient timing is an increase in anabolic processes leading to tissue synthesis and the extenuation of the catabolic processes found post workout aimed to prevent the breakdown of key proteins and structures. If one can take advantage of these benefits over multiple training sessions the end result can be a hastened recovery following each workout with potential improvements in performance capabilities. Some evidence suggests that the combination of protein and carbohydrate is more effective than either macronutrient alone because they each activate different but cooperative signaling pathways that serve to regulate carbohydrate and protein metabolism (Ivy & Ferguson 2010). The consumption of carbohydrate serves dual functions, first as a promoter of muscle glycogen post workout and secondly as a stimulator of insulin preventing muscle protein breakdown. The consumption of protein provides amino acids, which serve an array of functions in the body from enzymes to structural protein. These proteins, given post workout, can cause a shift from muscle protein degradation found within a catabolic state into muscle protein synthesis that is more reflective of an anabolic state (Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999). The balance between the total protein breakdown and total protein synthesis is termed net protein balance. In order for

benefits to be realized from resistance training the net protein balance needs to be positive and can be achieved through diet and proper nutrient timing post workout.

### Sport Specific Nutritional Countermeasures

The type of training implemented for athletes is a primary determinate of the macronutrient composition of post workout nutrition. Endurance athletes place importance on the replenishment of lost glycogen that occurred during training, whereas strength and power athletes place a premium on the stimulation of maximal protein synthesis and minimizing protein breakdown. Though both groups have different training goals both can take advantage of maximizing protein synthesis and glycogen repletion and preventing muscle protein breakdown. As athletes progress through their careers and competition level increases, prevention of overtraining and proper recovery between workouts becomes increasingly important. Athletes of higher qualification level often train multiple times per day; of importance is muscle glycogen repletion due to the lack of full recovery between sessions.

For sports requiring high maximal strength and high levels of power output, importance should be placed on diet in order to facilitate myofibillar protein synthesis providing increases in muscular cross sectional area (CSA). This specific protein synthesis is important because the CSA of a muscle has been correlated with high force production specifically, the larger the muscle CSA the larger the force which can be produced (Hakkinen, & Keskinen, 1989). In addition this increase in CSA provides the athlete with a base upon which greater strength and power can be developed. Zamparo, Minetti, Prampero (2002) have provided a theoretical model by which following proper training sequencing, athletes can develop maximal muscular power. This model begins with alterations in CSA and altering tissue architecture; this can be

accomplished with higher volumes of resistance training. Following this stage the training focus shifts to the development maximal strength through both central and local factors such as recruitment of specific muscle fiber types and co-contractions of muscle groups. This development of maximal strength is completed by a concurrent decrease in total work volume but increasing intensity. Finally, through the manipulation of volume and through task specific training we can transfer this maximum strength previously created to develop maximum muscular power. Maximum muscular power can be expressed by decreasing fatigue via lower volume load and implementing the highest intensities found throughout the training plan. Muscular power has been cited as a large contributor to performance in strength/power sports such as throwing (Bourdin et al., 2010), sprinting (Cormie, McGuigan, & Newton, 2010b) and weightlifting (Izquierdo, Hakkinen, Gonzalez-Badillo, Ibanez, & Gorostiaga, 2002). Additionally, proper nutritional supplementation may expedite the recovery between training sessions allowing athletes to perform at higher intensities (Beelen, Burke, Gibala, & van Loon, 2010, Nielsen, Farup, Rahbek, de Paoli, & Vissing, 2015). The end result of resistance training is alterations in protein synthesis, providing changes in key structural proteins and enzymes related to high intensity power output. By properly structuring training and implementing a periodized training plan we can positively influence protein synthesis and provide a base on which we can capitalize on these internal changes leading to improved performance.

Due to the complexity of athletic development a multifaceted approach must be incorporated in order to develop all facets of an athlete's life from training to lifestyle. Once, all other aspects of the athletes development have been addressed the final changes that can be implemented are found in the diet through nutritional countermeasures. Many nutritional intervention studies have been completed with varying outcomes utilizing mainly untrained

populations and multiple training methods. Because there are a lack of studies incorporating nutrient timing and well-trained athletes further research is necessary to examine its application for athletes.

### Dissertation Purposes

1. To compare the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo on performance in well trained weightlifters.
2. To compare the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo on muscle morphological changes in the vastus lateralis.
3. To compare the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo on specific muscle protein accretion.

## Operational Definitions

1. Nutrient timing- The systematic timing of macronutrients around the workout window to provide recovery and adaptation.
2. Nutritional countermeasures- Using the diet to counteract the catabolic effects of a training session and to exploit the anabolic processes leading to greater adaptation and super compensation.
3. Periodization is the logical, sequential, phasic method of manipulating training variables in order to increase the potential for achieving specific performance goals while minimizing the potential for overtraining and injury through the incorporation of planned recovery. (DeWeese, Hornsby, Stone, & Stone, 2015)
4. mTOR- Mamalian target of rapamycin is a protein that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy and transcription. (Hay & Sonenberg, 2004).
5. AMPK- 5' AMP-Activated protein kinase is an enzyme that plays a role in cellular energy homeostasis. (Cantó & Auwerx, 2010)
6. Immunohistochemistry- The process of detecting antigens in cells of a tissue section by exploiting the principal of antibodies binding to specific antigens in biological tissues (Ramos-Vara & Miller, 2014)
7. Western blotting – A widely used analytical technique used to detect specific proteins in a sample of tissue homogenate or extract. It uses gel electrophoresis to spate native proteins by 3-D structure or denatured proteins by length of the polypeptide. The proteins are then transferred to a membrane, where they are stained with antibodies

specific to the target protein. (Towbin, Staehelin, & Gordon, 1979) (Renart, Reiser, & Stark, 1979).

8. Rate of force development- Force produced at different times from the initiation of the muscle action.
9. Counter movement jumps- a type of vertical jump involving a pre-jump countermovement using the stretch shortening cycle
10. Static Jumps- a type of vertical jump involving no countermovement at a prescribed squat depth.
11. Isometric Mid-thigh Pull- A test of muscular strength utilizing the mid-thigh pull position. It is characterized by having an immovable bar in the mid-thigh position with the knees at an angle of approximately  $\sim 125^\circ$  and the hips at an angle of  $\sim 145^\circ$ . (Kraska et al., 2009).



## CHAPTER 2

### COMPREHENSIVE REVIEW OF THE LITERATURE

Strength and power sports such as weightlifting and throwing require athletes to produce high levels of force in a specific manner dictated by each sport in order to be successful. In order to enhance performance strength/power athletes use resistance training in order to maximize strength, power and rate of force development (RFD). Once high levels of strength and power are developed athletes can implement sport specific tasks in order to apply strength and power to improved sport performance. To facilitate maximum performance an athletes training must be properly designed and implemented over the course of a training year. Because of the demands of the modern competition calendar, athletes muscle have highly organized and properly periodized training phases in order to drive physiological adaptations and maximize performance during the most important competitions.

Periodization is the logical, sequential, phasic method of manipulating training variables in order to increase the potential for achieving specific performance goals while minimizing the potential for overtraining and injury through the incorporation of planned recovery (DeWeese, Hornsby, Stone, & Stone, 2015). Often, misconstrued are the terms of periodization and programming with the former being a theory or idea and the later implementation of that idea. Periodization can be thought of as the theory behind the training plan that logically divides the training into various fitness phases in order to reach performance goals. Periodization can be presented in the form of an annual plan which subdivides that competition calendar into timelines and phases in order to focus on specific fitness abilities and provide sequencing of training in order to prepare an athlete for competition. Programming is the implementation of the

aforementioned theory into practice and deals with the selection of sets, repetitions, and exercises appropriate to develop various fitness qualities.

Because of the constraints of the modern competition calendar the planning and sequencing of training for athletes must be designed in a way to account for multiple competitions and have planned times for recovery from training. Traditional periodization (TP) was developed to have athletes peak for one major competition during the year and often trained multiple fitness characteristics simultaneously leading to one large peak during the year. TP presents several problems as it relates to the modern competition calendar and includes lack of multiple peaking phases, simultaneous development of fitness qualities and high volumes of work completed in order to train fitness qualities. Due to the constraints of TP, periodization evolved in order to address the downfalls of TP and meet the demands of the modern competition calendar. Block periodization (BP) developed out of the need for peaking for several competitions as well as emphasizing and de-emphasizing different fitness characteristics to allow for proper athletic development. BP utilizes concentrated loads in order to develop fitness characteristics and when these concentrated loads are compiled in sequence allow for superior athletic development. BP that uses phase potentiation allows for training residuals from one training block to manifest themselves in subsequent blocks, which in turn can lead to increased performance later in the training cycle (Stone, Stone, & Sands, 2007).

Strength/power athletes require both high force and high velocity contractions of the muscular system, with the combination of both leading to high power outputs (Slater & Phillips, 2011). In addition to the high power outputs coordination of multiple muscle groups together to create an orchestrated forceful movement is required for success in competition. In order to maximize competition performance we need to organize training to optimize internal physiology

through improved enzyme profiles and altered neural mechanisms as well as cause morphological changes in muscle architecture including increased cross-sectional area (CSA). The combination of internal physiology as well as architectural changes provide the athlete the ability to express high power output during competition providing sequencing is adequate.

In order to achieve these adaptations in the muscle and nervous systems proper training must be coupled with proper recovery and adaptation. The volume of work completed by athletes cannot be increased past a certain point without running the risk of overtraining. Thus a major focus of the training process should be on recovery between sessions in order to improve athletic performance. Athletes undergoing strenuous training have unique needs as it pertains to diet and recovery between training bouts and a large emphasis should be placed on both diet and recovery in order to allow for maximum adaptation. Topics that have been investigated regarding diet within the literature include underlying physiological mechanisms, timing of nutrient delivery, amount of nutrients utilized and effects in multiple populations. By investigating nutrition as it pertains to athletic performance we can begin to develop guidelines for maximizing post training recovery and adaptation. The purposes of the following literature review are to 1) outline the underlying mechanisms behind nutrient timing, 2) provide a brief review of type and timing of macronutrients used in the research, 3) review the subjects, training and methods used for research, and 4) provide a rationale for the utilization of post workout nutrition and block periodization for athletes.

## Underlying Physiological Mechanisms

At its most basic level, training for either endurance or strength/power attempts to elicit positive changes in protein synthesis. If training is designed and implemented properly, athletes can take advantage of this increased protein synthesis leading to improved performance. Of importance for resistance training athletes is the promotion of positive changes in the anabolic cellular signaling pathways known to positively impact protein synthesis leading to accretion of myofibers. The main anabolic pathway is the mammalian target of rapamycin (mTOR), which is considered the master regulator of cellular growth and signaling that allows for the accretion of contractile elements leading to myofibrillar hypertrophy. Downstream targets of mTOR are: p70s6k and 4ebp1, which are positive markers of protein synthesis and are upregulated post exercise (West, Burd, Staples, & Phillips, 2010). The mTOR pathway has many positive upstream inputs that can lead to positive protein synthesis including but not limited to insulin, amino acids, muscular tension, muscular damage and metabolic stress. Through the inclusion of both preferred training methodology and proper nutritional countermeasures one may positively impact most inputs of the anabolic process which may be used to fully stimulate these anabolic pathways with the end product being the synthesis of larger contractile units.

In contrast to the anabolic pathway, strength power athletes should attempt to mitigate the effect of the catabolic pathway AMP-activated protein kinase (AMPK). This catabolic pathway is upregulated when there is a large difference in the AMP/ATP ratio and serves as a cellular energy conservation pathway and can become highly active when substrates become depleted. This pathway provides benefits for endurance training and can cause positive alterations in mitochondrial biogenesis and provide key enzymes leading to greater oxygen utilization. This pathway can cause a downregulation of the mTOR pathways and lead to the breakdown of

substrates for energy and prevent full activation of anabolic processes (Hay & Sonenberg, 2004). Though all training leads to the activation of this pathway to some degree, the type of training implemented and post workout nutrition can serve to diminish the negative effects post workout leading to a greater anabolic response.

Consumption of the key nutrients around the workout can facilitate the uptake of nutrients at the cellular level leading to the recovery of lost substrates from isolated training bouts and can lead to activation of cellular pathways stimulating greater protein synthesis and glycogen repletion. Amino acids are one input upstream from mTOR and provide the building blocks for structures and various enzymes and are crucial for rebuilding damaged tissues (Hay & Sonenberg, 2004). Carbohydrate serves dual roles, the first through replenishment of lost carbohydrate during exercise and secondly through the stimulation of insulin post workout providing the uptake of nutrients at the cellular level and the prevention of protein breakdown (Jentjens & Jeukendrup, 2003). Though each macronutrient has benefits when consumed alone, effects can be compounded when consumed simultaneously and may provide greater benefits than either macronutrient consumed alone.

### Carbohydrate Consumption

The consumption of carbohydrates in the post exercise window stimulates the secretion of insulin from the pancreas and has multiple positive effects throughout the body. Insulin is peptide hormone secreted by the beta cells in the pancreas which promotes the storage of carbohydrates and fats. Insulin has a positive effect on glucose uptake at the cellular level by activation of GLUT4 and increased protein synthesis that is insulin mediated. Glucose transport proteins exist in the human body with different isoforms in different tissues. GLUT1 and GLUT4

are found in abundance in skeletal muscle with type 1 being more active at rest and type 4 being activated by exercise and insulin respectively. Jentjens and Jeukendrup, (2003) suggest that two pools of GLUT4 transports exist within the muscle fiber with one pool associated with muscle contraction and the other associated with insulin release. The exact location and action of these separate transport protein pools has not yet been fully elucidated. At rest GLUT4 is synthesized by the ribosomes and stored in GLUT4 storage vesicles (GSV) produced by the Golgi apparatus. When insulin is present it can bind to the insulin receptors at the site of the alpha subunit. This binding leads to auto phosphorylation on tyrosine residues on the beta subunit causing a cascade of events eventually leading to the translocation of GLUT4 from the GSV's to the sarcolemma at the site of the t-tubules. Once stimulated by insulin, the GSV's are transported to the cell membrane by elements in the cytoskeleton including microtubules and actin. Once they arrive at the cell membrane they become tethered to the cell membrane by snare proteins where they can dock and eventually fuse with the lipid bilayers (Bryant, Govers, & James, 2002). Finally, through the process of endocytosis GLUT4 can bring glucose in the cell in order to use it for energy immediately or lead to storage for utilization later (Brooks, Fahey, & Baldwin, 2005; Gropper, & Smith, 2013).

In addition to cellular uptake of nutrients insulin has a large effect on protein synthesis and may have direct control over processes leading to activation of the mTOR pathway. mTOR is an important regulator in growth of mammals in response to the availability of nutrients, and is a key mediator of insulin, insulin-like growth factor 1 and other growth-factor signals for the cell growth machinery (Vander Harr, Lee, Bandhakavi, Griffin, & Kim, 2007). When insulin is present post workout it can bind with the insulin receptor activating insulin receptor substrate 1 (IRS-1). IRS-1 once activated phosphorylates PI(3)K which then phosphorylates protein kinase

B (AKT) this is a crucial step because AKT is directly upstream and activates mTOR eventually leading to activation of P70S6K which is a marker of cell growth and 4E-BP1/2 which is related to mRNA translation and cell proliferation. The PI3k/AKT/MTOR pathway is related to cellular size and cellular growth and the presence of insulin is a potent stimulator of this process and should be exploited by post workout nutrition in order to induce muscular hypertrophy (Vander Harr et al., 2007).

Though carbohydrates can increase GLUT4 translocation through insulin, GLUT4 translocation can also be increased post- workout independent of insulin in a process known as the insulin independent GLUT4 translocation. Some evidence suggests that muscle contractions, nitrous oxide, increased AMPK activity and low muscle glycogen could increase GLUT4 translocation but these pathways have not been fully illuminated (Richter, Derave, & Wojtaaszewski, 2001). The process of GLUT4 translocation independent of insulin is called the contraction-mediated glucose uptake and states that increase in cytosolic calcium with each wave of sarcolemma depolarization lead to translocation of GLUT4 to the cell membrane is key to this process but has not been fully clarified (Wright, 2007). The calcium/calmodulin-dependent pathways may lead to further increases in GLUT4 content allowing insulin to have increased effects after training (Wright, 2007). The combination of insulin mediated GLUT4 translocation and insulin independent GLUT4 translocation provide the cells with the ability to transport glucose into the cell and aid in the repletion of muscle glycogen post training.

Glucose can be used by the cells for energy or stored for later utilization in the form of glycogen. Both enzymatic activity during the workout and energy status intra- and post-training dictate the pathway in which glucose can be utilized. If energy is needed, glucose can be broken down through glycolysis. Glycolysis is a metabolic pathway that converts glucose into pyruvate

and during this 10-step process, liberates ATP and nicotinamide adenine dinucleotide (NADH) which can be used by the cells providing energy during exercise (Richter, E.A., Hargreaves, M. 2013). Glucose storage is necessary during the post-training window and glucose that travels to the cell can be stored as muscle glycogen. The presence of insulin post-training, as well as depletion of glycogen and possibly the up regulation of AMPK, provides the stimulus for the repletion of lost muscle glycogen during exercise by blocking the effects of glucagon and catecholamine's leading to storage of glycogen. Post exercise, one focus is the repletion of glycogen and is completed through several steps initiated by the facilitated diffusion of glucose through the cell membrane by GLUT4. Upon entry into the cell, glucose is phosphorylated by hexokinase to form glucose-6-phosphate, which transfers a phosphate during the reaction catalyzed by the enzyme phosphoglucomutase to form glucose-1-phosphate (G-1-P). The combination of G-1-P with uridine triphosphate (UTP) leads to the formation of uridine diphosphate-glucose and uridine diphosphate (UDP). Following this step, the enzyme glycogenin catalyzes the attachment of the initial 6-8 glucose molecules by combining with another glycogenin protein at the tyrosine anchor point. The next step is controlled by glycogen synthase, which causes the synthesis of glycogen from glucose by utilizing UDP-glucose and lengthening the glycogen chain. Finally, glycogen branching enzyme transfers glucose molecules 6-7 residuals in length to the interior of a glycogen molecule. The end result of many additions of these glycogen branches initially forms proglycogen which occurs rapidly and later takes the form of macro glycogen which can be affected by diet and takes additional time to form (Gropper & Smith, 2013; Jentjens & Jeukendrup, 2003). These two windows of glycogen repletion provide the need for immediate carbohydrate consumption following training as well as sustained adequate consumption following training extending into the recovery period.



## Amino Acid Consumption

Protein follows a similar process as glucose where it is broken down in digestion and transported to cells via the blood but is found in the form of free amino acids. Once arriving at the cell specialized proteins known as amino acid transporters which are specialized membrane transport proteins facilitate the diffusion of amino acids from the blood across the cell membrane into the myocyte where the final step is utilization by the ribosomes for protein synthesis. These transport proteins work through facilitated diffusion and carrier proteins dependent on type of amino acids present in the blood. Human L-type amino acid transporter 1 (LAT1) a transport protein is found in abundance in many tissues including skeletal muscle and transports large branched chain amino acids (leucine, isoleucine, valine) inside the cell (Wagner, Lang, Broer, 2001). Once inside the cell these amino acids are transported to the ribosome in order to be used for protein synthesis and creation of specific proteins through the process of transcription and translation of mRNA which has been mentioned previously from the activation of the mTOR pathway. Amino acids have a positive effect on the mTOR pathway by co-operating with Rheb-GTP to switch on mTORC1 leading to the activation of the initiation and elongation steps of mRNA translation and perhaps promoting other anabolic processes. This process has not been fully elucidated but is thought to occur from amino acids effects on TSC1/2 by inhibiting its action and stimulation TCTP which converts Rheb GDP, it's inactive form, to Rheb GTP. Rheb GTP completes the event through activation of mTOR (Proud, 2007). Another possibility for amino acids positively affecting the mTOR pathway is the presence of branch chained amino acids, especially leucine, that may inhibit the activation of amp-activated protein kinase (AMPK) which is an enzyme related to cellular homeostasis and is upregulated when there is a lack of nutrients present and is involved in cellular survival and catabolism. Evidence has been

presented that leucine may be converted to acetyl CoA and used in the mitochondria inhibiting AMPK (Tokunaga, Yoshino, & Yonezawa, 2003).

Proteins serve a wide array of functions and are the building blocks of enzymes, tissues and cell membranes to provide a few examples. Post exercise the function of protein is to switch from a catabolic state to an anabolic state by preventing the breakdown of proteins and providing the building blocks for anabolism and protein synthesis. Once inside the cell these amino acids are used by the ribosomes to form numerous proteins (Alberts et al., 2003). For coaches and athletes the protein synthesis most important is the formation of contractile proteins eventually leading to increased hypertrophy and the creation of key enzymes to be used in bioenergetics to provide the energy necessary to complete sporting tasks.

#### Dual consumption of Amino Acids and Carbohydrates

The post exercise internal environment in the absence of insulin is a catabolic state where the stress hormones cortisol, glucagon, epinephrine and norepinephrine are highly active and serve to liberate substrates. In order to quell these catabolic effects the stimulation of insulin post workout has an antagonistic effect on these stress hormones and serves to limit protein breakdown. Without the consumption of amino acids and carbohydrate following the workout degradation typically outpaces protein synthesis leading to a negative net protein balance. Because net protein balance is determined by protein synthesis and degradation, insulin serves to limit the degradation while amino acids serve to stimulate protein synthesis. These catabolic hormones play a vital role on substrate mobilization during exercise but these processes need to be reversed in the recovery process and this can be facilitated by proper macronutrient consumption and timing.

In conclusion, due to the complex nature of the post-training internal environment, careful consideration should be placed on the consumption of macronutrients around the training window in order to maximize anabolic pathways and negate the catabolic pathways. The following sections will investigate the use of proper amounts and timing of macronutrients as well as the training plans that may provide the most benefit in concert with nutrient timing. Finally, in order to assess the changes in athletes using nutrient timing focus should be placed on sensitive measurement techniques in order to capture small changes within athletes.

### Macronutrient Breakdown of Post Workout Recovery

Immediately following a training session a unique internal environment is present where through proper supplementation we can take advantage of the cellular and molecular pathways in order to increase protein synthesis. Through the combination of protein and carbohydrate consumed following training we can take advantage of this internal environment by maximizing protein synthesis with amino acids and prevent protein degradation with carbohydrate via insulin. Of importance following exercise is the timing and amount of macronutrients consumed in order to maximally stimulate anabolism and mitigate the negative effects of catabolism. Work by Ivy (2004), Phillips (2006), Kerksick (2008), and Nackerio and Larumbe-Zabala (2015) have provided evidence for the positive effects of post workout nutrition, while work by Schoenfeld (2013), Aragon (2013), Phillips (2004) and Reidy and Ramussen (2016) have failed to support these hypotheses.

The outcomes from these previously mentioned studies are mixed with authors claiming no benefits to small benefits in favor of post workout nutrition. To date the author has found no

study stating any negative performance effects from post workout supplementation in athletes or healthy populations. Because of purported effects are small researchers may dismiss these effects due to lack of statistical significance but they may be increasingly important for athletes as they develop to elite status. The difference between first and last place in modern elite competition is very small and minor adjustments to training and diet may provide an effect large enough to alter placing in competition. Because these effects manifest themselves as very minor changes, often hard to detect within a short time frame further investigations are warranted in nutrient timing for elite and sub-elite athletes.

Work in nutrient timing has shown that the combination of protein and carbohydrate may be more beneficial than consuming either nutrient alone (Ivy & Poortman, 2004). The exact amounts of carbohydrates and protein as well type have yet to be fully illuminated though several recommendations have been found in the literature to provide maximal benefits. Beelen et al. (2010) propose the ingestion of 0.8g - 1.2g•hr of carbohydrate and a protein ingestion of ~9g of essential amino acids or 20 grams of intact whey protein. Of particular importance is the inclusion of the branched chain amino acid leucine, which has a stimulatory effect on the mTOR pathway and is needed for maximal protein synthesis. Additionally, the ratio of 4:1 carbohydrates to protein can provide benefits to athletes post workout (Kerksick et al., 2008). Many recommendations have been suggested following exhaustive exercise leading to depletion of intramuscular glycogen stores (Beelen et al., 2010; Jentjens & Jeukendrup, 2003). Resistance training can cause a significant drop in intramuscular glycogen stores following intense exercise (Haff et al., 2000) and this must be replenished before the next training session. Unlike typical endurance training, resistance training with a heavy eccentric component can alter glycogen repletion in the muscle in the days succeeding exercise altering recovery (Nielsen et al., 2015).

In order to mitigate this disruption, carbohydrates should be consumed post workout to replenish lost muscle glycogen before the next training session occurs.

Immediately following a resistance training session the first 30 minutes to an hour is known as the anabolic window (Aragon & Schoenfeld, 2013). Taking supplementation during this this anabolic window purportedly provides benefits greater than delayed feedings of the same macronutrients though not all studies agree. In a review of the literature by Aragon & Schoenfeld (2013) they challenge the anabolic window theory stating that total protein consumption throughout the day is more indicative of muscular hypertrophy than the benefits of the anabolic window. However, this review article contained a total of 7 studies and only contained one well trained subject population and may not be representative of athletic populations. The research is decidedly mixed on the outcome of nutrients consumed around the anabolic window and can be attributed to the populations utilized and the amount and timing of supplement administered.

#### Immediate vs delayed feedings

Early studies have investigated the effect of nutrients either before or after exercise with Tipton et al. (2001) showing that protein supplementation before exercise providing greater benefits than the same amount of nutrient after. The authors suggest the positive increase in pre feeding is the increased delivery of nutrients to the muscles utilized during exercise. Later studies have examined the effect of immediate or delayed feeding of nutrients around the workout window. Esmark et al. (2001) showed that in older adult's immediate feedings vs delayed feedings improved cross sectional area better than delayed feedings of two hours. Wycherly et al. (2010) used 34 untrained older males and found no difference between feedings

before exercise and 2 hours post exercise in 16 weeks of progressive resistance training. In contrast Hoffman (2007) used 33 well trained athletes and examined protein supplementation before and after exercise vs morning and evening feedings and found no difference in body composition and both groups improved strength from baseline. Finally, in a study by Cribb and Hayes (2006), 33 recreationally trained body builders consumed protein either pre and post exercise vs morning and evening and reported increases in cross-sectional area and increased 1 repetition maximum (1RM) in the immediate vs delayed protein group. The results of these studies provide a contrast in well trained vs untrained subjects as well as younger vs older subjects and it important to note that none of these studies used the same amount of supplement or matched resistance training protocols.

#### Subject Characteristics and How They Relate To Nutrition

In addition to amount and timing of supplements varying subject populations have been used in nutritional research with both young and old being investigated as well as trained and untrained subjects. The outcomes from these studies need to be carefully examined when attempting to provide guidelines for athletes engaging in strenuous training. Training status may have a large impact on utilization of nutrients with Phillips (2004) ascertaining that untrained subjects have differing protein requirements than trained subjects. This difference can be attributed to increased utilization of amino acids post workout with trained subjects requiring less amino acids following and isolated bout of training. Additionally, the time course for increased protein synthesis may be longer for untrained subjects compared to well-trained athletes due to positive adaptations in recovery from training stimuli. This information is of importance when examining the literature because many studies incorporate untrained subjects

as well as subjects in older populations, which may provide different outcomes than well-trained subjects using the same protocol.

### Young vs Old

A majority of nutritional supplementation studies have been conducted using young subjects incorporating both protein and EAA supplementation as well as immediate vs delayed feedings. A summary of these studies is presented in table 2.1. Additional studies have been completed utilizing older subject utilizing untrained individuals response to protein supplementation, EAA acid ingestion and high and low protein diets. A summary of these findings is presented in table 2.2.

Table 2.1 *Studies that Incorporated Young Subjects*

Author	N (Training Status)	Intervention	Results
Andersen et al. (2005)	22 Untrained Men	25g Whey or 25g Maltodextrin	↑ Type I and II Fibers in Protein Group Only
Antonio et al. (2000)	19 Untrained Women	18.3 g EAA or 18.3g Cellulose	No significant changes
Ballard et al. (2006)	52 Untrained Men & Women	42g Protein + 21g CHO or CHO Placebo	No significance between groups
Bird et al. (2006)	32 Untrained Men	6g EAA or 6g EAA+CHO	↑EAA+ CHO showed greater FFM gains
Candow(2006)	27 Untrained Men & Women	Whey + sucrose or Maltodextrin + sucrose	↑ in lean mass for protein group
Coburn(2006)	33 Untrained Men	20g whey + 6.2g Leucine Or 26.2g Maltodextrin	No significant changes between groups
Cribb & Hayes(2006)	23 Moderately trained	40g Whey, 43 glucose	↑ In CSA type II fibers

Table 2.1 (continued)

	Males	7g creatine immediately or morning/evening	↑ Lean body mass Immediate group
Erskine et al.(2012)	33 untrained Men	20g Protein or placebo	No significant changes
Hartman et al.(2007)	56 Untrained Men	17.5g milk, soy, or CHO Placebo	↑ In type II fibers and FFM for protein groups
Hoffmann et al (2007)	21 Well-trained Men	42g Protein or CHO Placebo	No significant changes
Hoffmann et al. (2009)	33 Well-trained Men	42g Protein immediately or morning/evening	No significant changes
Humli et al. (2009)	31 Untrained Men	15g whey or placebo	↑ in CSA of vastus in protein group
Josse et al. (2010)	20 Untrained Women	18g protein vs maltodextrin Placebo	↑in lean mass for protein group and↓ in fat mass for protein
Kerksick et al. (2006)	36 Untrained Men		
Miekle et al. (2009)	39 Untrained Men	20g whey or 20g maltodextrin	No significant changes
Rankin et al. (2004)	19 Untrained Men	0.21G/kg of Milk or CHO Beverage post exercise	No significant changes
Rozenek et al. (2002)	73 Untrained Men	106g Pro +356g CHO, Isocaloric CHO or Control group	↑ in body mass and lean body mass for supplement groups
Shebani et al. (2010)	32 Well-trained Men	42g Protein +18g CHO Or 63g CHO	↑ 1RM squat for Supplement group
Viellevoye et al.(2010)	29 Untrained Men	15g EAA+ 15 CHO or 30g of CHO	No statistical difference between groups
Walker et al. (2010)	30 Moderatly Trained Men & Women	19.7 g Whey or CHO Placebo	↑ FFM for protein group
Weisgarber et al. (2012)	17 Untrained	Protein 0.3/kg or CHO	No difference between



Table 2.1 (continued)

	Men & Women	Placebo	groups
Wiloughby (2007)	19 Untrained Men	20g Protein or placebo	No significant change

Table 2.2 *Studies that Incorporated Older Subjects*

Author	N	Intervention	Results
Bembem et al. (2010)	42 Untrained Men	35g pro+creatine, 35g protein, 5g creatine, or placebo	No significant difference between groups
Campbell (1995)	12 Untrained Men & Women	High Pro 1.6g/kg or Low Pro 0.8/kg	No difference between groups
Candow(2006)	29 Untrained Men	Protein 0.3/kg or CHO Placebo	No significant changes between groups
Eliot et al.(2008)	42 Untrained Men	35g Whey+CHO or CHO Placebo	No significant changes
Esmark et al.(2001)	13 Untrained Men	10g Milk/ Soy	↑ CSA for immediate group Immediate or delayed.
Goddard et al. (2002)	17 Untrained Men	12g AA and72Fructose	No significant changes between groups
Holm et al. (2008)	44 Untrained Women	10g protein + 31 CHO or 6 grams of CHO	↑in lean body mass and strength in protein group
Iglay et al.(2009)	36 Untrained	Higher protein 1.2g/kg Or low protein 0.9g/kg	No difference between groups
Kukulijan(2009)	91 Untrained Males	13.2 g protein vs control	No difference between groups

Table 2.2 (continued)

Verdijk(2009)	28 Untrained	10g Casein or Placebo	No significant changes
Wycherly et al. (2010)	34 Untrained Men & Women	21 g protein + 29g CHO immediate or delayed	No significant difference between groups

Note: CHO=carbohydrate, EAA=essential amino acids, CSA=cross sectional area, PRO=protein

When investigating these same studies for outcomes' relating to strength, evidence provides insight into the benefits of post workout supplementation for improving strength in young and older subjects. Of all the aforementioned studies only the studies by Antonio et al., (2000), Erskine et al. (2012), Veillevoye et al. (2010), Weisgarber et al. (2012), Bembem et al. (2010), Kukuljan et al. (2009), Rankin et al. (2004) White et al. (2009), failed to show any difference between groups or both groups showed improvement. Due to many studies utilizing untrained subjects it may be difficult to differentiate the effects of the resistance training protocols benefits from those of the supplementation alone. Evidence on strength gains in well trained athletes has been unequivocal and can be attributed to differences in methodologies, a small number of studies completed and low numbers of subjects in these studies.

### Trained vs Untrained

In an investigation of 41 studies regarding protein supplementation around the workout window few studies used trained or well-trained athletes. Cribb and Hayes, (2006), Hoffman et al. (2007), Hoffman et al. (2009), Walker et al. (2010), Sheibani, Refahiat, & Noura, (2010). Cribb and Hayes, (2006) using recreationally trained bodybuilders investigated the effect of protein and carbohydrate before and after training vs morning and evening for 10 weeks. The

authors found the group using before and after training supplementation gained greater cross-sectional area and 1RM strength compared with morning and evening supplementation. Walker in (2010) used moderately trained men and women and investigated the changes from body weight exercises and running for 8 weeks and noted changes in bench press strength for the protein group and improvements in fat-free mass, lean body mass and total mass for the protein group only. In opposition a study by Hoffman (2007) observing protein vs carbohydrate placebo in a 12 week periodized study found increases in bench press strength for the protein group only but no significant changes for squat strength. Moreover, they found no statistical changes in body composition for either group. Shaibani and colleagues (2010) completed a 10-week study with 32 experienced strength/power athletes investigating supplementation with protein vs a placebo on measures of strength, power, and body composition as well as blood measures of testosterone, cortisol, growth hormone and IGF-1. The authors reported increases in lower body strength in the protein group but failed to show any statistical difference in any further measures between protein and placebo groups. Finally, in a study by Hoffman (2009) using well trained young men the authors monitored changes over 10 weeks using protein before and after exercise or morning and evening and found no changes in body composition and similar increases in 1RM and 5RM strength with no differences between groups.

Because of the lack of studies using well-trained athletes, it may be difficult to draw conclusions based on the previous studies as they relate to athletes. Only the studies by Hoffman (2007; 2009) and Shaibani (2010) used well-trained athletes and only one implemented a periodized training plan. Because the effects of training and supplementation may be altered in well-trained subjects, further research is necessary to provide best practice implementation of nutritional countermeasures around the workout to provide benefits and bolster performance.

### Training Plans Implemented in Studies

Another important facet of nutritional literature is the type of training plan implemented in the research. The most implemented protocols for training are progressive resistance training studies lasting ~12 weeks and utilized 3 or more days a week of resistance training incorporating major muscle groups. Due to the complex nature of the modern training schedule most if not all athletes use some form of modern periodization in preparation for important competitions. Because of these concepts researchers may be interested in the effects of periodization vs typical progressive resistance training in combination with nutritional countermeasures.

To date the author can confirm no nutritional studies incorporating true block periodization with appropriate programming using well-trained athletes. A number of studies erroneously claimed the prescription of periodized plans, however most relied on progressive resistance training prescribed to a pool of untrained subjects with varying types of supplementation. The general progression is linear in nature and uses set with 10-12 repetitions followed by sets of 8-12 and final ending with sets of 6-8. Though this scheme works well for controlled training studies its application to real world training is limited and thus provides difficulties in drawing conclusions for well-trained athletes using periodized programs. According to Plisk and Stone (2003), the very nature of periodization removes linearity as a major emphasis of the concept is the staging and cycling of workloads in order to increase the potential for achieving specific performance goals.

The aforementioned linear loading of subjects in previous nutritional countermeasure investigations may provide adequate stimulus to induce stress, but may fail to provide the necessary recovery in order to achieve optimal adaptation from the applied stimulus. Because of

a paucity of well-designed periodized training plans more research should be completed in order to provide best practice nutritional countermeasures for athletes.

In a study by Antonio (2000) researchers investigated the effects of an essential amino acid supplement vs a placebo containing cellulose on measures of body composition and exercise in untrained women. The protocol involved 6 weeks of a modified periodization scheme using repetitions ranging from 6-12 and 20 minutes of aerobic exercise. The authors found no changes in body composition or strength measures but did report statistical difference in time to exhaustion for a treadmill test favoring the essential amino acid group. Furthermore, Hoffman in 2007 utilized progressive periodized resistance training in collegiate football players noting only changes in lower body strength development for supplementation group with all other measure showing no changes or improving to a similar degree. Finally, in a study with recreationally trained males Viellevoye et al.(2010) compared EAA vs a placebo over the course of 12 weeks implementing a linear progressive program. Authors found increases in both groups in terms of body mass changes and found that EAA supplementation caused greater increases in gastrocnemius architecture. Conclusions for this study were EAA supplementation may be more beneficial for subjects with lower nitrogen balance and lower initial strength levels.

Though evidence has been presented showing little to no effects using progressive resistance training not all studies have provided the same conclusions. Candow et al. in 2006 completed a 6 week training study with 27 young adults supplementing with whey protein, soy protein or a sucrose placebo. The training involved 4 days a week of resistance training of all major muscle groups and the completed 4-5 sets of 6-12 repetitions at 60-90% of 1RM. The authors concluded that whey and soy may provide minimal benefits compared with resistance training or placebo consumed post resistance training. Later in a study completed by Humli and

colleagues in 2009 they investigated the acute and long term effects of protein ingestion on muscle hypertrophy and gene expression. Thirty one untrained males completed 21 weeks of progressive resistance training and they were divided into protein, placebo or control groups. At the conclusion of the study researchers found that effects from resistance training were enhanced by protein supplementation following exercise and may provide positive effects on gene expression relating to hypertrophy. Finally, in a study in 2010, Josse et al. examined the effects of fat free milk in untrained women over a 12 week linear progressive resistance training protocol. Authors found positive effects due to the consumption of milk in the post exercise period on measures of strength, fat loss, muscle mass and possibly bone turnover.

As with earlier studies the outcomes of progressive resistance training studies are unequivocal on the outcomes of protein supplementation following exercise. Because the aforementioned studies did not contain protocols that followed the same progression model of resistance training methods it is difficult to ascertain benefits across subjects or find benefits of specific amounts or timing of macronutrients. Because progressive loading for resistance training is commonly used for non-athlete population's additional studies should be completed employing sound periodization and similar amounts of macronutrients using athletes in order to derive evidence regarding its efficacy.

#### Measurement Techniques used in Research

Of importance for researchers is how to monitor the changes that can occur with supplementation and resistance training. Due to the time course of hypertrophy being a relatively lengthy process, longitudinal studies must be employed in order to find meaningful changes in subjects. Additionally, changes in muscle hypertrophy over time may be minimal and

measurement tools may fail to detect these small changes within the muscle. Various methods of measurement have been used in order to track changes in body composition and muscle fibers. These methods include dual-energy X-ray absorptiometry (DXA), magnetic resonance imaging (MRI), hydrostatic weighing, skinfold calipers, muscle sonography and muscle biopsies. Because changes in muscle fibers may be miniscule the type of measurement used may have large outcomes at the completion of the study. Methods that can capture small changes in muscle fiber composition and architecture would be preferred compared with methods that provide a more global measure of muscular changes such as segment girths and skinfold calipers. The most sensitive measurement tools currently employed are: hydrostatic weighing, muscle biopsies, MRI, sonography and DXA. Skinfold measurements and muscle girth measurements may provide insights in body composition changes and are relatively simple to implement but may fail to capture the minor changes within the muscle.

The majority of studies investigating nutritional interventions with resistance training are concerned with body composition changes in the subjects. Secondary outcomes from these studies are generally strength or performance related characteristics such as strength, power or endurance. Measurements are performed pre intervention, post intervention and frequently measurements are performed at various time points within the training study in order to assess changes. Measurements frequently utilized are total body mass, fat mass (FM) and fat free mass (FFM). Additionally, measurements may involve cross sectional area changes, skin fold thickness and muscle circumference. Because muscle hypertrophy is a long term remodeling process and occurs first at the cellular level, methods implemented should be sensitive enough to capture small changes. Specific measurement tools can be utilized in order to provide a clearer picture of changes within the muscle, DEXA can provide insights on bone mass, MRI and

sonography can access cross-sectional area changes and muscle biopsies can provide information regarding cellular adaptations and protein synthesis.

### Dual-Energy X-ray Absorptiometry

Currently, the most common measurement tool used is the DEXA scan this may be due in part to equipment availability, cost effectiveness and the procedure being noninvasive in nature. Because the DEXA uses computer imaging and assessment software the analysis and data reporting can be completed easily researchers will use this to compute body composition changes. The DEXA uses a three-component chemical model and can quantify fat, soft lean tissue and bone mineral (Eston and Reilly 2009). Studies using DEXA have provided mixed results with studies reporting no changes (Antonio et al., 2000; Bembem et al., 2010; Eliot et al., 2008; Hoffmann et al., 2007; Hoffmann et al., 2009; Rankin et al., 2004; Verdijk et al., 2009; Weisgarber, Candow, & Vogt, 2012; Wycherly et al., 2010) and others noting statistically significant changes (Ballard, Specker, Binkley, & Vukovich, 2006; Bird, Tarpinning, & Mariano, 2006; Candow et al., 2006; Cribb and Hayes, 2006; Hartman et al., 2007; Holm et al., 2008; Josse et al., 2010; Kerksick et al., 2006; and Walker et al., 2010). Because the DEXA provides a three component model it is good for long term changes in body composition but may be limited in scope in providing details at the cellular level or detecting small changes within given muscular segments.

### Magnetic Resonance Imaging

In order to quantify small changes that occur during the course of research studies advanced techniques must be implemented. One such technique requires the use of an MRI. MRI has been used to examine changes in muscle architecture, cross sectional area and body



composition changes. Studies using MRI are uncommon in nutritional intervention studies due to the cost and availability to researchers. Four notable studies have examined body composition changes using an MRI device, Coburn et al. (2006) and Erskine (2012) both found no significant changes with training and supplementation whereas Esmark et al. (2001) and Humli et al. (2009) found statistical alterations with supplementation. The MRI may provide excellent data for researchers regarding changes in muscle over the course of a training study but due to the cost and lack of availability this measurement tool may be unsuitable for all researchers to use in nutritional intervention studies.

### Muscle Biopsies

Muscle biopsies provide the researcher with detailed information relating to muscle fiber composition and cross sectional area at the cellular level. During this procedure researchers remove a small sample of muscle tissue from a given muscle generally the vastus lateralis using a hollow barrel needle and then process the biopsy for utilization in muscle fiber staining procedures or protein immunoblots. Because muscle biopsies extract muscle tissue this procedure provides investigators with a clear view of the changes within the muscle and allow for detailed quantification of cellular changes. Because this procedure is invasive it may not be applicable for all populations and requires a medical doctor to complete the procedure. Additionally, due to the localized nature of the biopsy conclusion of total body changes in muscle fiber composition or body composition changes cannot be established.

Studies involving muscle biopsies have demonstrated more positive benefits in regards to nutrient timing then previously mentioned studies not using biopsies. One study utilizing muscle biopsies completed by Mielke et al. (2009) failed to demonstrate any significant changes

between a whey and leucine mixture versus maltodextrin consumed before and immediately following training in 39 untrained males. Studies completed by Andersen et al. (2004), Bird, Tarpinning, and Mariano (2006), Cribb and Hayes (2006), Esmark et al. (2001), Hartman et al. (2007), Humli et al. (2009), Wilouby, Stout, and Wilborn (2007), and Holm et al. (2008) all presented evidence that nutrient timing provided greater benefits than the placebo or control groups. Because the muscle biopsy can detect small changes in the muscle fibers and due to the short nature of the training studies this technique may provide evidence that may be undetected by other techniques.

### Sonography

An emerging technique being used for assessment of muscular adaptations is sonography better known as ultrasound. Ultrasound incorporates sound waves at high frequencies and can provide a noninvasive view of the internal environment of the human body and is most commonly used as a diagnostic tool for medical purposes. This technique can be utilized by researchers as a measurement tool of skeletal muscle providing an image which can be analyzed by a computer and provide data on pennation angle, fascicle length, muscle thickness and total muscle cross sectional area. Studies by Candow et al. (2006), Viellevoye et al. (2010), Weisgarber et al. (2012), used ultrasound in combination with other methods in order to assess changes in muscle cross-sectional area. All three of these studies found no statistical difference between groups regarding nutrient timing.

### Miscellaneous Techniques

In addition to the techniques mentioned above several other miscellaneous techniques can be found in the literature that examines the efficacy of nutrient timing. Research by Mickle et al.

(2009), Wilouby et al. (2007), Rozenek, Ward, Long, and Garhammer (2002), and White et al. (2009) all utilized the technique of hydrostatic weighing in which subjects are submerged in a known quantity of water and based on Archimedes principal and known standards, the practitioner can calculate the body composition of the subject. Outcomes from these studies were mixed with some finding benefits Wilouby et al. (2007) and others finding no statistically significant changes Miekle et al. (2009), Rozenek et al. (2002), White et al., (2009). Finally, in a study by Goddard, Williamson, and Trappe, (2002), the researchers utilized computerized tomography in order to compare the effects of EAA supplementation vs fructose and failed to demonstrate and significant changes in cross-sectional area.

Because a multitude of methods exist for the measurement of changes within the muscular system researchers need to consider which methods will be most advantageous in assessing changes. In general MRI, DEXA, and muscle biopsies may provide the best benefit in determining small changes in the muscle but due to cost effectiveness and availability these methods may not always be an option. Other methods can provide strong evidence of changes over time and include hydrostatic weighing and ultrasound and are often found in the research in combination with other measurement techniques. Lastly, the use of skinfolds and girths and circumferences may provide the practitioner with quick easily trackable data but may fail to provide quantitative data in order to make an informed conclusion. These methods should be combined with more sensitive measurement techniques in order to provide supporting evidence.

### Summary

Coaches and athletes alike are continually searching for methods that provide benefits from training and recovery in order to bolster and improve performance. One important method

for increasing performance is nutritional alterations, specifically utilization of macronutrients to expedite recovery from training sessions. Therefore the purpose of this dissertation is to examine the effects of a recovery protein and carbohydrate supplement versus a calorie free placebo on performance, body composition and protein synthesis. From the literature review we can conclude the following: 1) immediate post exercise the internal environment is in a state of catabolism and properly timed nutrients can counteract the negative effects of training and provide vital nutrients leading to anabolism and adaptation, 2) many studies have investigated nutrient supplementation around the workout window using multiple populations and various amounts of nutrients and timing of these nutrients, 3) an due to the conflicting outcomes from these studies further investigations are warranted using best practice training methods and sound nutritional countermeasures in order to assess changes in athletes.

## CHAPTER 3

### EFFECTS OF A PROTEIN AND CARBOHYDRATE RECOVERY BEVERAGE ON PERFORMANCE IN WELL TRAINED WEIGHTLIFTERS

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Prepared for submission to *International Journal of Sports Physiology and Performance*

## **ABSTRACT**

The purpose of this study was to examine the effects of a recovery supplement compared with a placebo on performance measures in trained weightlifters. 10 trained weightlifters (Age =  $30.8 \pm 5.1$  years, Height =  $177.4 \pm 4.0$  cm, body mass =  $94.3 \pm 12.4$  kg, training age =  $5.3 \pm 2.9$  years) completed a 12 week training program utilizing block periodization. A double blind placebo protocol was utilized to compare effects between treatment and placebo groups. Jump height, scaled peak power, peak force and rate of force development were compared between groups using a series of 2x4 (group x time) mixed measures ANOVA's. All athletes improved on performance measures however, no statistical difference was found between treatment and placebo groups on measures of jump height ( $p=.186-.736$ ), peak power ( $p=.446-.969$ ) with weighted and unweighted jumps and no statistical difference was found for peak force ( $p=.238$ ) or rate of force development ( $p=.250$ ) with isometric mid-thigh pulls. These findings indicate that recovery supplement utilized provided no additional performance benefits compared with a placebo in a 12 week block periodization protocol in trained weightlifters.

**Keywords:** *protein, supplementation, weightlifting, block periodization*

## INTRODUCTION

The sport of weightlifting consists of the snatch and the clean and jerk with the greatest weight lifted in each discipline giving an athlete a competition total. This total is compared with all other athletes in their respective weight class to determine a winner. Coaches and athletes are continually looking for ways to bolster weightlifting performance through training methods, recovery methods and lifestyle changes. For weight class athletes such as weightlifters, body mass and composition have a large impact on performance.<sup>1</sup> It is advantageous for athletes to maximize the amount of lean body mass in relation to fat mass in order to lift the most weight in their respective class. One potential method to optimize sport performance and body composition is the use of nutritional countermeasures in the diet to offset the negative aspects of training and provide both enhanced recovery and adaption from the applied training stimulus.<sup>2</sup>

Nutrient timing deals with the consumption of macronutrients around the workout window to facilitate recovery from training in the form of protein synthesis and glycogen repletion while mitigating the negative effects of catabolism that accompany training.<sup>3</sup> Several investigations on nutrient timing suggest that nutrient availability can serve as a potent modulator of many of the acute responses and chronic adaptations to both endurance and resistance-based training.<sup>4</sup> The major macronutrients manipulated around the workout window are carbohydrates and protein, which in appropriate doses can lead to the attenuation of protein degradation, the increase in protein synthesis and the repletion of muscle glycogen post training.<sup>5</sup> By combining protein and carbohydrates together following training athletes can attempt to recover from isolated training bouts more efficiently due to the additive effects of both macronutrients consumed simultaneously compared with either alone.<sup>3,5,6</sup> These additive effects can be attributed to insulin stimulation, down regulation of catabolic hormones and activation of anabolic hormones.<sup>3</sup>

The consumption of amino acids following exercise stimulates protein synthesis and decreases protein breakdown.<sup>7</sup> Maximal stimulation of muscle protein synthesis has been shown to occur with consumption of ~6 grams of essential amino acids or ~20 grams of whole proteins.<sup>5</sup> Following training there can be a shift towards a state of catabolism consisting of an imbalance in the rates of protein degradation and accretion leading to an altered nitrogen balance.<sup>8</sup> Consuming adequate protein can stimulate protein synthesis and cause a positive shift in total nitrogen balance. Immediately following a resistance training session a unique internal environment is present where there is an increase in anabolic cellular signaling pathways leading to creation of contractile proteins; by consuming amino acids during this time provides the necessary nutrients to facilitate this process. The increased activation of the mammalian target of rapamycin (mTOR) pathway serves as an upstream marker of anabolic growth and stimulates the initiation growth factors 4-EBP1 and p70S6k which are main activators of mRNA transcription and translation which directly control contractile protein synthesis.<sup>9</sup> There are many “inputs” to stimulate the mTOR pathway which include but are not limited to amino acids, growth factors, mechanical stress and energy availability.<sup>10</sup> By incorporating both protein and carbohydrate supplementation in conjunction with well-designed training, the stimulation of all inputs of the anabolic pathways to facilitate training adaptations may be achieved.

The ingestion of carbohydrates serves dual roles by stimulating the release of insulin and causes increases in total protein accretion by diminishing protein breakdown. The stimulation of insulin by carbohydrates facilitates the uptake of nutrients at the cellular level and can lead to glycogen repletion by increased absorption of glucose by GLUT4.<sup>11</sup> Additionally, muscle contractions, nitrous oxide, increased 5' AMP-activated protein kinase (AMPK) and calcium influx also serve as mechanisms leading to insulin independent glucose uptake at the cellular level.<sup>11</sup> Following



exercise a more catabolic state occurs with the catabolic hormones glucagon and cortisol as well as the catecholamine's epinephrine and norepinephrine upregulated to liberate energy needed to match training demands. The increase in insulin, insulin-like growth factor 1, growth hormone and testosterone following training leads to the diminishment of these catabolic hormones and the stimulation of anabolic pathways leading to glucose uptake and repletion as well as downregulation of protein degradation.<sup>3</sup> Additionally, a potent stimulator of the AMPK is low energy status which serves to regulate cellular homeostasis by inhibiting the previously mentioned anabolic pathways.<sup>4</sup> Immediately following exercise the co-ingestion of carbohydrates as well as amino acids provide the necessary growth material to mitigate the inhibitory effects of the AMPK pathway.

The literature investigating supplementation has been inconsistent in its outcomes regarding the benefits of protein. The discrepancies found can be attributed to subjects, protein amounts, methodology and various measurement techniques. Reviews of protein supplementation have shown both positive effects on body composition and strength<sup>12</sup> whereas Reidy & Rasmussen<sup>13</sup> have failed to demonstrate differences in adaptation resulting from supplementation. It is important to note the authors have found no studies showing any negative adaptations with protein consumption in conjunction with training. Carbohydrate consumption following exercise has resulted in more consistent outcomes regarding performance, with post exercise consumption of carbohydrates being the most important factor in glycogen synthesis.<sup>5</sup> Combining protein and carbohydrates in conjunction with resistance training has been shown to stimulate improvements in strength and body composition when compared with control or placebo groups.<sup>6</sup> The conflicting nature of nutrient timing research can be attributed to: 1) subjects used; 2) timing of supplements; 3) amount of macronutrients consumed; 4) training protocol implemented and 5)

measurement techniques employed. Because of these various conflicting variables more research is necessary to clarify the effects of nutrient timing for athletes.

In order to measure performance changes in weightlifting appropriate field tests must be employed to capture the changes that accompany training. Two such methods commonly found in the literature are the use of vertical jumps and isometric mid-thigh pulls. Studies have investigated a relationship between vertical jump testing and isometric mid-thigh pulls to weightlifting performance. Studies by Beckham et al.<sup>14</sup> and Haff et al.<sup>15</sup> have shown moderate to strong relationships correlating isometric mid-thigh pulls with performance in the snatch, clean and jerk and total. Haff et al.,<sup>16</sup> completed a study investigating isometric and dynamic muscle actions and have shown that isometric testing may provide information about dynamic movements and be predictive of the ability to generate force quickly. In addition the testing of vertical jumps consisting of static and counter movements jumps have shown predictive abilities in weightlifters when examining jump height (JH) and peak power (PP).<sup>17,18</sup>

Previous nutrient timing research has provided information about untrained populations with relatively few incorporating well trained athletes<sup>19,20</sup> and to the authors knowledge no studies have been conducted employing block periodization. Block periodization and appropriate programming has been shown to be efficacious and efficient compared to other programs, particularly among athletic populations.<sup>21-23</sup> One might expect more efficacious programs coupled with supplementation to produce superior results. Therefore, the primary purpose of this study was to examine the effects of a recovery beverage compared with a calorie free placebo on vertical jumps and isometric mid-thigh pull performance in trained weightlifters in a 12 week study utilizing block periodization.

## METHODS

### *Subjects*

Ten trained weightlifters participated in this study and were randomly assigned to a treatment or a placebo group. Athletes descriptive can be found in table 3.1. Inclusion criteria required that each subject had been training regularly for weightlifting competition for a minimum of one year and free of injury for the past 6 months. Each subject read and signed a written informed consent. This study was approved by the East Tennessee State University Institutional Review Board.

Table 3.1 Subject Demographic Information

	Treatment		Placebo		<i>p</i>
N	5		5		
Age (y)	28.4	±5.4	33.7	±3.2	.131
Height (cm)	179.6	±4.5	175.2	±2.8	.103
BM (kg)	95.3	±12.3	93.5	±15.3	.839
Training Age(y)	5.2	±3.2	4.9	±3.4	.890
EST1RM (kg)	170.6	±31.8	155.0	±38.9	.507
EST 1RM STR/BW	1.8	±0.3	1.7	±0.3	.517

Note: values are means± standard deviations, EST 1RM STR/BW= ratio of back squat to body mass.

### *Experimental Design*

A double blind placebo study using a repeated measures design was used to test out hypotheses and determine the relationship between changes in static jumps, countermovement jumps and isometric mid-thigh pulls in treatment and control groups respectively. Before the study subjects

were randomly assigned to either the treatment group or a placebo group. The treatment group received a protein and carbohydrate beverage immediately following each workout and the placebo group consumed a calorie free beverage immediately post workout. The protein carbohydrate beverage contained 230 calories consisting of 16g of hydrolyzed whey protein and 41 grams of carbohydrates consisting of sucrose and dextrose. The placebo group received calorie free fruit punch drink containing no calories from either protein or carbohydrate. The supplement and placebo beverages were placed in opaque shaker bottles with subject numbers placed on the lid of the bottle to ensure anonymity. Subjects were instructed to consume no additional supplements during the study and to refrain from eating for 30 minutes following the consumption of post workout beverage. Each subject completed 4 jump testing sessions and 4 isometric mid-thigh pull testing sessions conducted the week following the conclusion of a training block.

### *Training plan*

A twelve week periodized training plan was completed for this study. A block periodization protocol was utilized for the duration of this study. Each subject completed four training sessions per week consisting of general strength exercises, weightlifting movements and their weightlifting derivatives. Training sessions followed a Monday, Wednesday, Thursday, Saturday schedule for the duration of the study. A detail of the training plan implemented is contained in table 3.2 and exercises implemented can be found in table 3.3

Table 3.2: 12 Week Training Plan

Week	Sets x Reps	Daily Intensities (Mon, Wed, Thur, Sat)
1	3x10	M, M, L, L
2	3x10	MH, MH, ML, ML
3	3x10	H, H, L, VL
4	3x5(1x5)	ML, ML, L, VL

5	5x5	M, M, ML, ML
6	3x3(1x5)	MH, MH, VL, L
7	3x2(1x5)	ML, M, ML, L
8	5x5	H, MH, ML, L
9	3x3(1x5)	MH, M, L, L
10	3x2(1x5)	ML, L, VL, Meet
11	3x5	M, M, ML
12	3x5	L, L, VL

Note: VL=very light, L= light, ML=medium light, M=medium, MH=medium heavy, H=heavy, Meet=competition day.

Table 3.3: Exercise Selection

Weeks	Exercises: Monday & Thursday	Wednesday	Saturday
1-3	Back Squat	Snatch	SGSS
	Strict Press	CGSS	Snatch
	Dumbbell Press	CG Pull from PP	SGSLDL
		CGSLDL	DB Row
4-7	Back Squat	Snatch	SGSS
	Push Press	CGSS	Snatch
	BN Press	CG Pull from BKN	Clean and Jerk
	DB Press	CGSLDL	SGSLDL
		CG Row	SG Row
8-10	Back Squat	Snatch	SGSS
	Jerk	CGSS	Snatch
	DB Press	SG pull from FLR	Clean and Jerk
		CGSLDL	SGSLDL
		DB Row	DB Row
11-12	Back Squat	Power Snatch	-
	DB Press	CGSS	-
	Fr Raise	CG Pull from PP	-
		SLDL	
		DB Row	

Note: BN Press=behind the neck press, DB Press= dumbbell press, Fr Raise= front raise, CGSS=clean grip shoulder shrug, SGSS=snatch grip shoulder shrug, DB Row= dumbbell row, CG Pull from PP=clean grip pull from power position, CG pull from BKN= clean grip pull from below the knee, SG Pull from FLR= Snatch grip pull from the floor, CGSLDL= clean grip stiff leg deadlift, SGSLDL= Snatch grip stiff leg deadlift, CG Row= clean grip row, SG Row= snatch grip row

### *Jump Testing Session*

Prior to jump testing sessions each athlete performed a standardized warm up of 25 jumping jacks, 10 body weight squats followed by a standardized back squat warm up consisting of 5 repetitions with 40kg, 5 repetitions with 60kg and 5 repetitions of 80kg respectively. Following this warm-up 2 minutes of rest was provided before jump testing was initiated. Immediately following this rest period the athlete completed a series of 10 jumps under 5 different weighted conditions with two trials at each weight and one minute of rest between each trial. The first two conditions tested were unweighted static and countermovement jumps using a near weightless PVC pipe held on the upper back in the back squat position. The remaining 3 conditions consisted of static jumps with 40kg, 60kg and 80kg respectively.

All jumps were performed on a dual force plate setup (2 separate 45.5 x91 cm force plates; RoughDeck HP, Rice Lake, WI) sampling at 1,000 Hz which subjects squatting down to a knee angle of 90°, received a countdown and jumped as high as possible. Variables collected were (JH) and (PP) scaled to body mass for each weight: 0kg countermovement, 0 static, 40kg static, 60kg static and 80kg static respectively.

#### *Isometric Mid-Thigh Pull Testing Session*

Prior to each Isometric mid-thigh pull testing session subjects performed a standardized warm up of 25 jumping jack, 5 repetitions of mid-thigh pulls with 20kg and 3 sets of 5 repetitions of mid-thigh pulls with 60Kg. Following the warm up, 2 minutes of rest were provided before initiation of the testing session. During this rest period, subject bar height was measured in the rack to assure a consistent knee angle of 125° and hip angle of 145°. Subjects then performed two warm up trials with 50% and 75% of maximum effort with one minute of rest between each trial. After

the second rest period athletes performed two maximum effort trials at 100% of maximum effort and were instructed to pull “as fast and as hard as you can” to ensure a maximum effort.<sup>14</sup>

Testing for the isometric mid-thigh pull were performed on a dual force plate setup (2 separate 45.5 x91 cm force plates; RoughDeck HP, Rice Lake, WI) in a custom power-rack that allowed for adjustment of bar height and fixation at various heights. Only 100% trials were considered for analysis. Variables collected were peak force (PF) and rate of force development at 250ms(RFD@250).

#### *Data and Statistical Analysis*

The static jump, countermovement jumps and isometric mid-thigh pull data were collected and analyzed using a customized LabVIEW program (2012 Version, National Instruments Co., Austin TX, USA). Voltage data obtained from the force plates was filtered using a digital low-pass Butterworth filter with a cutoff frequency of 10Hz in order to remove and noise from the signal. Peak values of force and power were extracted from the force-time and power-time data, respectively from each individual force plate.

Intraclass correlation coefficients (ICC) were used to determine the test-retest reliability of JH and scaled PP for jumps and PF and RFD@250 for isometric pulls. A series of 2x4 (group x time) a mixed-design ANOVA's were used to compare treatment and placebo groups for each weighted jump condition and mid-thigh pulls. All statistical analyses were performed with SPSS 22 (IBM, New York, NY) and statistical significance for all analyses was set at  $p \leq 0.05$ .

## RESULTS

JH, scaled PP, PF and RFD@250ms displayed high test-retest reliability with ICC values of 0.99, 0.98, 0.95 and 0.76 respectively. Descriptive data for Jumps and pulls are displayed in table 3.1 and 3.2. No statistically significant values were obtained for jumps for group by time. Statistical outputs for jumps are details in table 3.3. No statistically significant values were obtained for isometric mid-thigh pulls for PF ( $F_{3,24}=1.508, p=.238$ ) or RFD@250 ( $F_{3,24}=1.460, p=.250$ ).

Table 3.4 Descriptive data for Jumps

Condition	Group	Week1	Week4	Week8	Week12
PPCM0 (W•bm <sup>2/3</sup> )	Treatment	243.8±48.5	232.0±52.8	244.5±63.8	233.0±48.6
	Placebo	230.1±5.9	224.5±18.8	232.5±12.5	233.2±15.9
	Combined	236.9±33.4	228.3±37.6	238.5±43.8	233.2±34.1
PPSJ0 (W•bm <sup>2/3</sup> )	Treatment	252.9±36.8	240.5±42.5	252.9±51.7	242.7±39.3
	Placebo	221.1±19.1	215.9±26.9	223.8±24.2	223.1±20.9
	Combined	237.0±32.3	228.2±35.9	238.3±41.0	232.9±31.4
PPSJ40 (W•bm <sup>2/3</sup> )	Treatment	243.1±33.8	232.9±34.6	246.7±44.6	241.6±38.8
	Placebo	210.5±21.1	204.0±21.7	213.5±18.6	208.9±17.4
	Combined	226.8±31.7	218.5±31.2	230.1±36.7	225.3±33.1
PPSJ60 (W•bm <sup>2/3</sup> )	Treatment	233.1±32.3	227.1±36.3	232.7±44.1	227.9±37.6
	Placebo	201.6±24.2	195.3±18.4	203.1±14.8	199.1±18.2
	Combined	217.4±31.6	211.2±31.9	217.9±34.7	213.5±31.8
PPSJ80 (W•bm <sup>2/3</sup> )	Treatment	228.0±32.8	219.5±38.9	223.4±43.4	218.7±44.5
	Placebo	193.8±23.3	191.3±19.4	198.1±12.3	191.6±18.1
	Combined	210.9±32.3	205.4±32.6	210.8±32.9	205.1±35.0
JHCM0(cm)	Treatment	0.38±0.08	0.36±0.08	0.37±0.09	0.37±0.07
	Placebo	0.33±0.04	0.33±0.05	0.34±0.06	0.34±0.05
	Combined	0.36±0.06	0.34±0.07	0.36±0.07	0.36±0.07
JHSJ0(cm)	Treatment	0.33±0.07	0.31±0.07	0.33±0.07	0.32±0.06
	Placebo	0.28±0.04	0.28±0.05	0.29±0.06	0.29±0.05
	Combined	0.31±0.07	0.30±0.07	0.31±0.07	0.31±0.06
JHSJ40(cm)	Treatment	0.21±0.04	0.19±0.04	0.20±0.05	0.21±0.05
	Placebo	0.17±0.03	0.16±0.03	0.17±0.04	0.16±0.02
	Combined	0.19±0.04	0.18±0.04	0.19±0.04	0.19±0.04
JHSJ60(cm)	Treatment	0.16±0.04	0.15±0.04	0.15±0.05	0.15±0.04
	Placebo	0.13±0.02	0.12±0.02	0.13±0.02	0.12±0.02
	Combined	0.15±0.03	0.14±0.04	0.14±0.04	0.14±0.03
JHSJ80(cm)	Treatment	0.12±0.03	0.11±0.04	0.11±0.04	0.11±0.04



Placebo	0.09±0.02	0.09±0.02	0.10±0.01	0.09±0.01
Combined	0.11±0.03	0.10±0.03	0.11±0.03	0.10±0.03

Note: values are means± standard deviations, PP=scaled to body mass, JH=jump height, CM=countermovement, SJ=static jump.

Table 3.5 Descriptive Data for Isometric Pulls

Condition	Group	Week1	Week4	Week 8	Week12
PF(N)	Treatment	5341.9±809.1	5256.7±1048.3	5131.7±662.7	5221.8±650.5
	Placebo	4559.5±1214.1	4772.0±1343.9	4887.9±949.9	4742.6±977.2
	Combined	4950.7±1056.5	4014.4±1164.6	5009.8±782.8	4982.1±822.4
RFD@250(Ns)	Treatment	9390.7±999.5	7300.6±1260.4	7808.9±974.1	6846.9±2320.9
	Placebo	8874.0±3295.1	9059.9±4178.6	9039.4±3519.7	8190.4±3200.2
	Combined	9132.4±2311.7	8180.3±3053.9	8424.2±2519.5	7518.7±2728.9

Note: values are means± standard deviations

Table 3.6 ANOVA Results for Jump height & Peak Power

Condition		df	error	F	<i>p</i>
Peak power:	CM0kg	3	24	.920	.446
	SJ0kg	3	24	.806	.503
	SJ40kg	3	24	.160	.922
	SJ60kg	3	24	.083	.969
	SJ80kg	3	24	.528	.667
Jump Height:	CM0kg	3	24	.777	.518
	SJ0kg	3	24	.895	.458
	SJ40kg	3	24	.692	.566
	SJ60kg	3	24	.427	.736
	SJ80kg	3	24	1.740	.186

Note: CM=countermovement jump, SJ=static jump

## DISCUSSION

This study examined the effects of a recovery supplement versus a placebo in trained weightlifters in a 12 week block periodization training protocol. The primary findings of this

study are as follows. No statistical significant group by time interactions occurred for JH or PP at any unweighted or weighted conditions and no group by time interactions occurred for PF or RFD@250 for isometric mid-thigh pulls.

These findings are consistent with Reidy & Rasmussen<sup>13</sup> who found a diminished protein supplementation effect occurring over a prolonged exposure stimulus after exercise training. The subjects in this study had a high training age and were currently trained before the study, which may explain the negated effect of the protein supplement on performance measures. These current findings are inconsistent with the work of Cermak et al.<sup>12</sup> who have demonstrated strength effects from protein supplementation in both younger and older subjects. The subjects in this study had a high relative strength to body mass ratio before the training protocol which may explain the equivocal strength changes found in both groups from pre to post training. In two studies completed by Hoffman et al.<sup>19,20</sup> the investigators demonstrated changes in 1rm strength for upper and lower body in trained athletes consuming a protein supplement compared with a placebo. These effects could not be replicated during this training protocol which employed a smaller sample size but better trained athletes than the previously mentioned studies.

There are a number of variables the authors believed would illuminate the use of recovery supplementation for athletes: 1) this training protocol used block periodization which incorporated heavy and light days in order to facilitate recovery and adaptation which is in contrast with previous studies that used progressive loading; 2) this study utilized trained subjects which would negate the learning effect of training which is often observed in untrained or minimally trained individuals; 3) this study implemented consistent monitoring techniques following each training block in an attempt to capture the adaptations that occurred within the

training protocol; 4) this study incorporated stronger subjects in both treatment and placebo groups who may be closer to their genetic ceiling.

There are several limitations to this study that should be considered when examining the data for conclusions: 1) the average ages of the subjects contained in this study are older than typically found in collegiate athletes training and competing; 2) these athletes were trained at the inception of this study and thus the stimulus of training and subsequent adaptation from training may be diminished due to previous training history. Over time athletes who train consistently find it progressively harder to adapt as an athlete approaches their genetic potential and thus the effect of the recovery supplement may be less effective due to the nature of diminishing returns of training protocols; 3) this study incorporated a training period of 12 weeks which is a short time span in order to investigate effects of training or supplementation which may have considerable residual training effects that may not manifest themselves at a later time point. Block periodization using phase potentiation capitalizes on the effects of previous training blocks to bolster performance at later time. The effects of this study may be magnified at later training blocks and due the short nature of this study did not measure any long term training effects; 4) the subject pool for this study was small which can increase the potential of finding no statistical changes due to the nature of small sample sizes. Because of the paucity of trained individuals, it is difficult to recruit and place trained athletes on the same training protocol.

## **PRACTICAL APPLICATIONS**

In conclusion, the evidence does not appear to support the hypothesis that protein supplementation would provide greater performance benefits than a placebo in trained

weightlifters. Because training is an investment with current training manifesting itself as increased performance at a later date further studies should be implemented utilizing nutrient timing and block periodization over the course of a macrocycle in order to access performance outcomes. Due to the limitations stated above subsequent studies should be conducted utilizing larger samples sizes of trained athletes as well as extending the duration of these studies to investigate long term performance changes in order to access the efficacy for nutrient timing and supplementation for athletes.

## **ACKNOWLEDGEMENTS**

The authors would like to thank the members of the Stoneage Weightlifting Club who participated in this study. There are no conflicts of interest. There are no professional relationships with companies or manufacturers who will benefit from the results of the present study for each author.

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## CHAPTER 4

### EFFECTS OF A PROTEIN AND CARBOHYDRATE RECOVERY BEVERAGE ON MUSCLE MORPHOLOGY IN TRAINED WEIGHTLIFTERS

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Prepared for submission to *European Journal of Sport Science*



## **ABSTRACT**

The purpose of this study was to examine the effects of a recovery supplement compared with a placebo on muscle morphology in trained weightlifters. 10 trained weightlifters (Age =  $30.8 \pm 5.1$  years, Height =  $177.4 \pm 4.0$  cm, body mass =  $94.3 \pm 12.4$  kg, training age =  $5.3 \pm 2.9$  years) completed a 12 week training protocol implementing block periodization. A double blind placebo protocol was utilized to compare effects between treatment and placebo groups. Vastus lateralis and muscle fiber cross sectional area was compared between groups using a series of 2x2 (group x time) repeated measures ANOVA's. All athletes improved cross-sectional area of the vastus lateralis, type I and type II muscle fibers. Greater changes in type I and type II muscle fibers were observed for the treatment group but not for vastus lateralis cross sectional area. These findings indicate that recovery supplement utilized provided greater changes at the cellular level but not the muscular level compared with a placebo in a 12 week block periodization protocol in trained weightlifters.

**Keywords:** Carbohydrate, Protein, Supplementation, Weightlifting

## INTRODUCTION

Weightlifting is a weight class sport with competitors attempting to lift the most weight in two separate disciplines, the snatch and the clean and jerk.(Chiu & Schilling, 2005) In order to maximize performance a combination of the muscular and nervous systems must synergistically coordinate to impart the high force and velocity necessary to lift the barbell successfully overhead. Initial improvements in strength training occur via neural mechanisms with muscular adaptations occurring at a slower rate.(Häkkinen, 1989; Narici, Roi, Landoni, Minetti, & Cerretelli, 1989) As training progresses, adaptations in strength are more difficult to achieve and morphological adaptations in the muscle become more important.(Cormie, McGuigan, & Newton, 2010) Morphological changes within the muscle can be altered by resistance training and can include cross-sectional area (CSA), muscle thickness, pennation angle, fascicle length and fiber type composition.(Cormie, McGuigan, & Newton, 2011) These specific morphological changes within the muscle may also be altered by nutritional countermeasures which can assist in tissue remodeling and cellular growth of contractile proteins.(Dowling, Topisirovic, Fonseca, & Sonenberg, 2010; Dunlop & Tee, 2009) By combining resistance training and nutritional countermeasures coaches and athletes can attempt to alter muscle architecture positively leading to increased performance.

Previous research has demonstrated that maximal strength is of importance for success in weightlifting and other strength sports.(Stone, Moir, Glaister, & Sanders, 2002; Stone et al., 2005) In order to increase maximal strength, athletes can utilize resistance training to alter the nervous system and muscle architecture leading to more forceful contractions of the motor units or increased size of contractile units.(Haff, Whitley, & Potteiger, 2001) The maximal isometric force generating capacity of a human muscle fiber is directly proportional to its CSA regardless

of fiber type.(Cormie et al., 2011) Therefore as a strength-power athlete progresses through their competitive career importance should be placed on increasing CSA to continue to improve performance. Of importance for strength power athletes is the contribution of the muscle fiber type within the muscle for force generating capabilities. Type II muscle fibers have a greater capacity to generate power per unit of CSA compared with type I fibers.(Cormie et al., 2011) Strength power athletes contain a similar proportion of type I to type II fiber compared with untrained populations but fiber size of type II fibers is greater in weightlifter which is largely affected by genetics and further altered by systematic resistance training protocols.(FRY et al., 2003) In order to maximize performance for weightlifters an increase in type II muscle fiber CSA is beneficial for force generating capabilities related to weightlifting performance.

Further morphological alterations in pennation angle (PA) and fascicle length (FL) can alter contractile properties of skeletal muscle leading to increases in force and velocity respectively. PA of the muscle affects the force generating capabilities of contractile units by adding more sarcomeres in parallel leading to increased CSA and subsequent increases in maximal strength.(Stone, Stone, & Sands, 2007) FL alters the contractile units by adding more sarcomeres in series and is related to the length of the muscle fiber and is directly proportional to its velocity capabilities.(Cormie et al., 2011) FL may be altered by specific resistance training that incorporates high velocity contractions and ballistic movements but this has not been fully elucidated. The shortening velocity of human muscle is ultimately limited by enzymes kinetics and the rate of cross bridge dissociation by ATP, therefore there is a limit on how fast human muscle can contract.(Nyitrai et al., 2006; Sargeant, 2007) Faster cross bridge cycling rates occur in type II compared to type I muscle fibers therefore designing training plans which attempt to

generate larger type II CSA may lead to greater performance benefits for strength and power athletes.

Both protein supplementation(Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999) and exercise(Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997) cause increases in protein synthesis following exercise. Evidence has been presented that protein intake can have direct effects on cellular signaling pathways that lead to accretion of the contractile proteins actin and myosin when combined with resistance exercise.(Hulmi et al., 2009; Reitelseder et al., 2011)

Furthermore, the inclusion of carbohydrates with protein following exercise may provide further benefits by activating upstream inputs of the anabolic signaling pathways(Dunlop & Tee, 2009) and causing decreases in protein degradation that accompanies resistance training.(Kerksick et al., 2008) Protein consumed with resistance training has been shown to augment adaptations that follow each training session and has been shown to cause increases CSA when combined with chronic training as well as expedite recovery from training.(Hulmi, Lockwood, & Stout, 2010) These increases in CSA can be attributed to increases in the anabolic signaling pathways related to the mammalian target of rapamycin (mTOR) pathway that is dually activated by both resistance training and amino acid ingestion following exercise.(Reidy & Rasmussen, 2016) By consuming adequate amounts of protein and carbohydrate following training athletes can attempt to improve recovery as well as facilitate the accretion of contractile proteins leading to increased muscle CSA.

By combining resistance training with nutritional countermeasures athletes endeavor to alter muscle morphology through changes in muscle architecture consisting of changes in total CSA and muscle fiber type characteristics. These adaptations in muscle architecture may lead to performance benefits that accompany resistance training. Therefore, the purpose of this paper is

to investigate the effects of a protein and carbohydrate recovery beverage vs a calorie free placebo on muscle morphological changes in trained weightlifters.

## METHODS

### *Materials*

Vector red alkaline phosphatase substrate kits (SK-5105) and Vector SG peroxidase kits (SK-4705) were purchased from Vector Laboratories (Burlingame, CA). Monoclonal anti-myosin (skeletal-fast) alkaline phosphatase (A4335) was purchased from Sigma (St. Louis, MO). Monoclonal mouse anti-slow muscle myosin (MAB1628) was purchased from Millipore (Temecula, CA)

### *Subjects*

Ten trained weightlifters participated in this study (Age =  $30.8 \pm 5.1$  years, Height =  $177.4 \pm 4.0$  cm, body mass =  $94.3 \pm 12.4$  kg, training age =  $5.3 \pm 2.9$  years). Descriptive data for the treatment and placebo group can be found in table 4.1. Inclusion criteria required that each subject had been training regularly for Olympic weightlifting for a minimum of one year and free of injury for the past 6 months. Each subject read and signed a written informed consent form. This study was approved by the East Tennessee State University Institutional Review Board.

Table 4.1 Subject Demographic Information

	Treatment		Placebo		<i>p</i>
N	5		5		
Age (y)	28.4	$\pm 5.4$	33.7	$\pm 3.2$	.131
Height (cm)	179.6	$\pm 4.5$	175.2	$\pm 2.8$	.103
BM (kg)	95.3	$\pm 12.3$	93.5	$\pm 15.3$	.839
Training Age(y)	5.2	$\pm 3.2$	4.9	$\pm 3.4$	.890

EST1RM (kg)	170.6 ±31.8	155.0 ±38.9	.507
EST 1RM STR/BW	1.8 ±0.3	1.7 ±0.3	.517

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Note: values are means± standard deviations, EST 1RM STR/BW= ratio of back squat to body mass.

### *Experimental Design*

A double blind placebo study design was used to test hypotheses and determine the relationship between changes in muscle morphology in treatment and control groups, respectively. Before the study, subjects were randomly assigned to either the treatment group or a placebo group. The treatment group received a protein and carbohydrate beverage immediately following each workout and the placebo group consumed a calorie free beverage immediately post workout. The protein carbohydrate beverage contained 230 calories consisting of 16g of hydrolyzed whey protein and 41 grams of carbohydrates consisting of sucrose and dextrose. The placebo group received calorie free fruit punch drink containing no calories from either protein or carbohydrate. Subjects were instructed to consume no additional supplements during the study and to refrain from eating for 30 minutes following the consumption of post workout beverage. Muscle biopsies were collected from each subject before and after the training study and completed ultrasound measurements immediately following each muscle biopsy collection.

### *Training plan*

A twelve week training plan using phase potentiation and block periodization was completed for this study. Each subject completed four training sessions per week consisting of general strength exercises, weightlifting movements and their weightlifting derivatives. Training session followed a Monday, Wednesday, Thursday, Saturday schedule for the duration of the study.

Table 4.2: 12 Week Training plan

Week	Sets x Reps	Daily Intensities (M, W, T, S)
1	3x10	M, M, L, L
2	3x10	MH, MH, ML, ML
3	3x10	H, H, L, VL
4	3x5(1x5)	ML, ML, L, VL
5	5x5	M, M, ML, ML
6	3x3(1x5)	MH, MH, VL, L
7	3x2(1x5)	ML, M, ML, L
8	5x5	H, MH, ML, L
9	3x3(1x5)	MH, M, L, L
10	3x2(1x5)	ML, L, VL, Meet
11	3x5	M, M, ML
12	3x5	L, L, VL

Note: VL=very light, L= light, ML=medium light, M=medium, MH=medium heavy, H=heavy, Meet=competition day, Intensities were based on sets and repetitions(Stone et al., 2007)

Table 4.3: Exercise Selection

Weeks	Exercises: Monday & Thursday	Wednesday	Saturday
1-3	Back Squat	Snatch	SGSS
	Strict Press	CGSS	Snatch
	Dumbbell Press	CG Pull from PP	SGSLDL
		CGSLDL	DB Row
		DB Row	
4-7	Back Squat	Snatch	SGSS
	Push Press	CGSS	Snatch
	BN Press	CG Pull from BKN	Clean and Jerk
	DB Press	CGSLDL	SGSLDL
		CG Row	SG Row
8-10	Back Squat	Snatch	SGSS
	Jerk	CGSS	Snatch
	DB Press	SG pull from FLR	Clean and Jerk
		CGSLDL	SGSLDL
		DB Row	DB Row
11-12	Back Squat	Power Snatch	-
	DB Press	CGSS	-
	Fr Raise	CG Pull from PP	-
		SLDL	
		DB Row	

Note: BN Press=behind the neck press, DB Press= dumbbell press, Fr Raise= front raise, CGSS=clean grip shoulder shrug, SGSS=snatch grip shoulder shrug, DB Row= dumbbell row, CG Pull from PP=clean grip pull from power position, CG pull from BKN= clean grip pull from below the knee, SG Pull from FLR= Snatch grip pull from the floor, CGSLDL= clean grip stiff

leg deadlift, SGSLDL= Snatch grip stiff leg deadlift, CG Row= clean grip row, SG Row= snatch grip row

### *Muscle Biopsies*

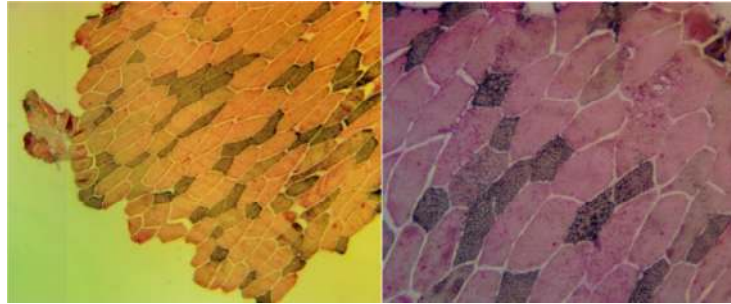
Percutaneous needle biopsies of vastus lateralis were performed using a 5-mm Bergstrom-Stille needle under suction after an overnight fast as previously described.(Stuart et al., 2006) A 50- to 100-mg specimen was quickly blotted, and a portion was mounted on cork for sectioning. The remainder of the sample was frozen in an isopentane slurry cooled over liquid nitrogen. All the samples were then placed in liquid nitrogen and stored at -80°C for later analysis.

### *Quantification of Muscle Fiber Type Composition and Fiber Size*

Fiber composition was determined using methods described by Behan et al.(Behan, Cossar, Madden, & McKay, 2002) Muscle sections were stained for bright-field light microscopy in a two-step method using commercial monoclonal antibodies for fast and slow isoforms of myosin heavy chain. After acetone fixation and incubation with 1% normal rabbit serum, the slow myosin antibody was applied, followed by a peroxidase-conjugated rabbit anti-mouse IgG antibody. The fast myosin antibody was then applied. Slides were alcohol dehydrated, cleared with xylene, and preserved in synthetic medium. This technique allows discrimination of type I, type IIa, and type IIx (Figure 1a). All sections were coded and then quantified independently by two observers who were unaware of which subject or treatment the image represented. Fiber diameter was measured using ImageJ version 1.49 at 10x magnification for all slides.(Figure 2b)



Figure 4.1a: Muscle fiber for quantification at 4x magnification. 2b: Measurement at 10x

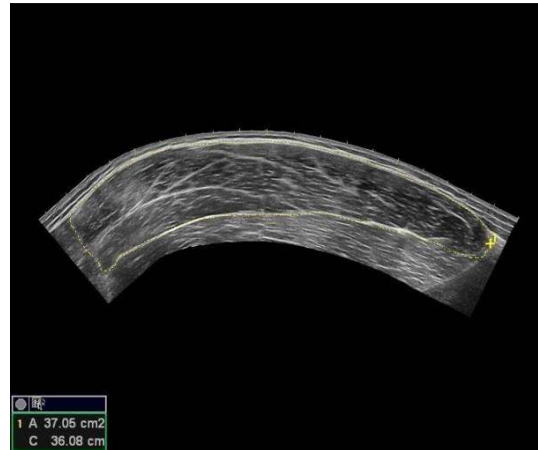


### *Muscle Architecture*

A GE logiq P6 ultrasound (General Electric, Fairfield, CT, USA) was used to examine cross-sectional area (CSA), PA and FL of the vastus lateralis for the duration of the study. The initial muscle biopsy incision site served as a landmark for ultrasound measurements and was used at all time points. A ML6-15, 7.5MHz ultrasound probe was used for all measurements to capture images of the vastus lateralis on the corresponding leg used for the muscle biopsy. Measurements were taken with the athletes rested on their side and hips perpendicular to the examination table in the axial plane with a knee angle of  $120\pm 5^\circ$  as measured by a goniometer.(Wells et al., 2014) During each measurement session the location was marked with a permanent marker and the probe was oriented perpendicular to the muscle for each sample. CSA was measured by placing the probe on the muscle and moving it in the transverse plane to collect a cross-sectional image using the LogicView Function of the ultra sound device. (Figure 2) The reliability of this method has been determined previously(da Matta & de Oliveira, 2012), CSA was measured by tracing the inter-muscular interface in the cross sectional images. (e Lima, da Matta, & de Oliveira, 2012; Howe & Oldham, 1996; Seymour et al., 2009) The examiner took 3 cross-sectional images from each sonogram. Images were collected pre intervention and

following the cessation of the training protocol coinciding with the week of the muscle biopsies. The means of CSA were assessed from the images and used for further analysis.

Figure 4.2: Vastus lateralis cross sectional area



### *Statistical Analysis*

Intraclass correlation coefficients (ICC) were used to determine the test-test reliability of the ultrasound CSA. A 2x2 (group x time) mixed-design analysis of variance was used to compare treatment and placebo groups for changes in CSA. A 2x2 (group x time) repeated measures analysis of variance was used to compare muscle fiber size changes from immunohistochemistry samples. Effect sizes were generated and interpreted as trivial, small, moderate, large, very large, and nearly perfect when Cohen's  $d$  was 0.0, 0.2, 0.6, 1.2, 2.0 and 4.0 based on the scale by Hopkins (Hopkins, 2014). All statistical analyses were performed with SPSS 22 (IBM, New York, NY) and statistical significance for all analyses was set at  $p \leq 0.05$ .

## **RESULTS**

The intraclass correlation for CSA was 0.96. No interaction (group x time) or group effect was present for the vastus lateralis CSA data but a statistically significant time effect was observed,

$F(1, 8) = 9.558, p=0.015, d=0.6$ . Fischer's least significance difference revealed statistically significant effect on CSA for the placebo group from pre to post ( $p= 0.026$ ) with no significance for the treatment group ( $p>0.05$ ). No statistical significance was found for the interaction (group x time), time effect or group effect was observed for type I fibers. No interaction (group x time) effect was observed for type II fibers but a statistically significant time effect was observed  $F(1, 8) = 5.476, p=0.047, d= 1.0$ . A statistically significant group effect was found for type II fibers  $F(1, 8)= 9.117, p=0.017, d=1.18$ . Fischer's least significant difference revealed no statistical difference for group ( $p>0.05$ ) and approached significance for time ( $p=0.076$ ) for the treatment group. Descriptive data for vastus lateralis CSA, type I fibers and type II fibers can be found in table 4.3.

Table 4.4 Descriptive Data for Muscle Architecture

Condition	Group	Pre	Post
CSA (cm <sup>2</sup> )	Treatment	37.8±1.4	39.1±2.2
	Placebo	39.9±3.2	42.1±4.0
	Total	38.8±2.5	40.6±3.4
Type I (µm <sup>2</sup> )	Treatment	2642±66.8	3369±113.1
	Placebo	2932±41.8	3038±10.1
	Total	2780±50.2	3206±56.7
Type II (µm <sup>2</sup> )	Treatment	4105±34.0	5102±55.4
	Placebo	3557±49.1	4128±19.6
	Total	3826±18.1	4608±47.7

Note: All data are means± standard deviations

## DISCUSSION

This study compared the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo on muscle morphological changes in weightlifters. The primary outcome of this

study was that a protein and carbohydrate recovery beverage provided greater benefits than a placebo on type II muscle fiber CSA but no additional benefits on vastus lateralis CSA or type I muscle fiber CSA. A secondary finding was the periodized training protocol using phase-potentiated blocks implemented for the duration for the study had a positive effect on vastus lateralis CSA and type I and type II muscle fiber CSA for both groups compared to baseline.

Compared to baseline both groups improved CSA at the muscular and cellular level with greater changes at the cellular level for the treatment group and statistically significant changes in type II muscle fibers for the treatment group compared to the placebo. These findings are in contrast to Andersen et al.(Andersen et al., 2005) who found increases in both type I and type II muscle fibers for the protein group only. The current study found statistically significant changes in type II fibers which may be related to the nature of the resistance training combined with recovery supplementation. This study aligns with previous research by Hartman et al.(Hartman et al., 2007) and Cribb & Hayes(Cribb & Hayes, 2006) who found increases in type II fibers for the protein supplementation groups compared with carbohydrate equated placebo. The previously mentioned studies used untrained and moderately trained individuals which may display differing responses to resistance training and supplementation. Previous investigations by Hoffman(Hoffman, Ratamess, Kang, Falvo, & Faigenbaum, 2007; Hoffman et al., 2009) failed to show significant differences between treatment and control groups in well trained males The current study contained trained subjects who had been currently resistance training for more than one year and displayed greater changes in fiber CSA from pre to post and greater changes for the treatment group compared with the placebo.

The vastus lateralis CSA revealed a greater response from the placebo group from pre to post which may be related to individual responses to the training protocol. From a practical perspective both groups means improved to a similar degree from pre to post with a larger standard deviation being found in the placebo group compared to the treatment group. Though the strength levels of the treatment and placebo group were not statistically different, the treatment group was stronger at the initiation of the study, potentially placing them closer to their genetic ceiling which may have led to a difference in response to the resistance training protocol.

Strength and power sports, particularly weightlifting require high power outputs in competition which are underpinned by high force and relatively high velocity contractions. Due to fiber type limitations in shortening velocity in human muscle high force contractions must be periodically utilized in order to continue developing higher power outputs.(Nyitrai et al., 2006) The recovery supplementation utilized in this study caused an increase in type I and type II fiber CSA for both groups with a larger increase in type II fibers being found in the treatment group. Practically, this may lead to greater improvements in performance by increasing muscle CSA and allowing for greater force production when those muscular CSA changes are caused by increases in type II fibers. Additionally, with the larger increases in type II fibers compared with type I fibers training may alter the type I/II fiber size ratio allowing for more forceful contractions of skeletal muscle.(Häkkinen, Komi, & Tesch, 1981)

Though this study was to an extent limited by subject size and therefore lacked statistical power from which to draw strong conclusions regarding the efficacy of protein supplementation for weightlifters it contained a homogenous group of individuals who had a similar training

background. From a practical perspective the inclusion of a protein and carbohydrate recovery beverage conferred greater benefits at the cellular level compared with a placebo. Additionally the supplementation had a larger effect on type II muscle fibers which are advantageous for strength and power development. Secondly, this study was limited to 12 weeks in length where adaptations caused by training and supplementation may not come to fruition until cellular proteins have developed in sufficient amounts resulting in larger CSA. Future investigations should attempt to look at long term supplementation over the course of an annual plan and combine supplementation with trained athletes in order to assess effectiveness for sporting performance.

## **CONCLUSION**

The protein and carbohydrate recovery supplement used for this study increased type II muscle fiber CSA but not type I CSA or vastus lateralis CSA compared with a placebo in weightlifters. Both groups improved from pre training to post training with greater CSA found at the cellular level but not the muscular level for the treatment group. The greater CSA in type II fibers may be advantageous for strength and power athletes who utilize both high force and high velocity contractions for performance. From a practical standpoint the inclusion of a recovery supplement utilizing carbohydrates and proteins may facilitate greater adaptations to training protocols that employ resistance training during training.

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## CHAPTER 5

### EFFECTS OF A PROTEIN AND CARBOHYDRATE RECOVERY BEVERAGE ON MUSCLE PROTEIN ACCRETION IN WEIGHTLIFTERS

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Prepared for submission to *Clinical Journal of Endocrinology and Metabolism*

## **ABSTRACT**

The purpose of this study was to examine the effects of a recovery supplement containing protein and carbohydrate compared with a placebo given immediately after each training session on muscle protein accretion in trained weightlifters. 10 trained weightlifters (Age =  $30.8 \pm 5.1$  years, Height =  $177.4 \pm 4.0$  cm, body mass =  $94.3 \pm 12.4$  kg, training age =  $5.3 \pm 2.9$  years) completed a 12 week training protocol implementing block periodization. A double blind placebo protocol was utilized to compare effects between treatment and placebo groups. Total and phosphorylated mTOR and AMPK were compared from baseline to the completion of the intervention for treatment and placebo groups. Gene expression of myosin heavy chain 1, 6 and 7 were compared between treatment and placebo groups from baseline to completion of the intervention. Independent samples t-test revealed increases in total mTOR for the treatment group ( $p=0.044$ ) but not AMPK ( $p=0.159$ ), phosphorylated mTOR ( $p=0.385$ ) or phosphorylated AMPK ( $p=0.430$ ). No statistical difference was found between treatment and placebo groups on myosin heavy chain gene expression for myosin heavy chain 1 ( $p=0.08$ ), 6 ( $p=0.08$ ) or 7 ( $p=0.37$ ). These findings indicate that protein supplementation has a positive effect on total mTOR expression and may have a positive effect on myosin heavy chains 1 and 6 in trained weightlifters.

**Keywords:** Carbohydrate, Protein, Supplementation, Weightlifting

## INTRODUCTION

Human skeletal muscle expresses significant plasticity of phenotype in response to an applied training stimulus. Skeletal muscle in response to resistance training stimulus undergoes a transformative process where cellular signaling pathways lead to increase in contractile proteins that over time express themselves in muscular hypertrophy. Diametrically opposed to these anabolic cellular signaling pathways are the cellular survival and homeostatic regulatory pathways that are integrated with energy availability and homeostatic balance. Attempting to shift the balance between the anabolic pathways and catabolic pathways is the basis for adaptation-recovery and can be altered via training, recovery methods including nutritional interventions.

The giant protein kinase, mammalian target of rapamycin (mTOR), is the major regulator of cell growth and proliferation via protein synthesis for mammals.<sup>1</sup> Two distinct forms of mTOR exist with the first mTOR complex 1 which contains raptor and the second mTOR complex 2 which contains rictor.<sup>2</sup> Complex 1 is controlled by growth factors, energy levels, cellular stress, insulin and amino acids and regulates cell growth, proliferation and protein synthesis.<sup>3</sup> Complex two is controlled by amino acids and insulin and activates protein kinase B (Akt) which is an upstream activator of complex 1 and regulates actin cytoskeleton reorganization, cell survival and ribosome biogenesis.<sup>2,4,5</sup> mTOR complex 1 has downstream targets relating to cell growth (P70S6k), proliferation (4eBP1) and mRNA transcription and translation.(eIF4G) Following downstream activation of mTOR targets is the creation of the cellular contractile elements actin and myosin. Of importance for resistance training adaptations is the creation of myosin heavy chains within the sarcomere for muscle contraction. Myosin heavy chains are the primary determinant of ATPase activity and are responsible of the speed of skeletal muscle shortening.<sup>6</sup>

By manipulating the inputs into the human body via training and nutrition we can alter the upstream and downstream pathways related to the mTOR complexes 1 and 2 leading to specific cellular signaling and protein synthesis related to performance.<sup>7,8</sup>

Adenosine monophosphate-activated protein kinase (AMPK) is an enzyme that regulates cellular energy homeostasis and controls the balance between catabolic and anabolic processes.<sup>9</sup> AMPK is an energy sensing pathway associated with changes in the ATP/AMP-ADP ratios. Upstream inputs leading to activation of AMPK are exercise, nutrients, hormones, hypoxia and ischemia.<sup>9</sup><sup>12</sup> Downstream effects of activation of AMPK leads to autophagy and fatty acid oxidation as well as inhibition of gluconeogenesis, lipid and protein synthesis.<sup>12</sup> Because AMPK can directly inhibit the anabolic processes related to mTOR it is imperative to structure training and nutritional countermeasures to mitigate the catabolic effects of the AMPK pathway.

To strike a positive imbalance between the anabolic pathways of mTOR and the catabolic pathways of AMPK there must be a integration of training and nutrition which has the potential to facilitate recovery and adaptation. Because both pathways are tightly regulated and integrate information from the cellular environment and nutritional status, training and nutrition can be combined in such a way as to upregulate protein synthesis leading to accretion of contractile proteins and inhibit the negative aspects of the catabolic pathways potentially amplifying cellular protein synthesis and growth. Currently the most efficacious way to merge training and nutrition is by utilizing block periodization and implementing sound nutritional countermeasures.<sup>13-15</sup>

Periodization can be defined as a logical and phasic method of manipulating training variables in order to increase the potential for achieving sport specific performance.<sup>16</sup> Previous investigations have shown the efficacy of periodization and proper sequencing of training variables for

strength/ power athletes.<sup>13,14,17-19</sup> By sequencing the training plan into blocks, practitioners can utilize concentrated training stimuli to drive specific protein synthesis at key times of training year and prevent maladaptation and overtraining. Additionally, through manipulations in training intensity and volume, dissipation of training fatigue can be achieved allowing athletes to express the highest levels of fitness in competition.<sup>20</sup> In order to ensure optimal performance a training plan must be created before the implementation of training which incorporates all aspects of the athlete's lifestyle to account for training and lifestyle stressors that may impact recovery. Once competition dates are scheduled the annual plan can be designed incorporating training, travel, recovery, and nutritional countermeasures to enable optimal performance

Nutritional countermeasures deal with the ingestion of specific macronutrients to offset the negative aspects of training thus facilitating recovery and adaptation from training sessions. These countermeasures serve as an immediate and delayed effector of recovery by altering the acute responses to training and the delayed effects related to recovery from training. Acutely nutrition serves to fuel training by providing the energy necessary to complete training tasks and delay fatigue that accompanies training. Chronically, nutrition serves to prepare for following subsequent training sessions, recover from previous training stresses and provide nutrients for growth. The main macronutrients manipulated around the training window are carbohydrates and protein. By altering the timing and amounts of carbohydrates and protein we can attempt bolster training, recover from training and prepare for subsequent training sessions.

Muscle glycogen is the primary fuel source during moderate to high intensity exercise<sup>21</sup> and the reduction in muscle glycogen levels has been associated with fatigue.<sup>22</sup> Low muscle glycogen levels is one upstream activators of the AMPK pathway<sup>9</sup> and may inhibit activation of the mTOR pathway.<sup>1</sup> One method to offset the negative effects of low muscle glycogen is the consumption

of adequate carbohydrates following training to promote muscle glycogen formation and storage. In a review on glycogen repletion following exercise has provided evidence that ingestion of 1.2 g •kg/hr being beneficial in the recovery from exercise though many of these studies reviewed were completed on endurance type exercise.<sup>23</sup> There may be an rapid and delayed time course for muscle glycogen repletion with the former occurring the first hour after exercise and the latter occurring after this immediate phase concludes.<sup>21</sup> Due to these glycogen repletion windows it may benefit the athlete to ingest adequate carbohydrate immediately following exercise to exploit this immediate phase then utilize the diet in order to utilize the delayed phase. Additionally, eccentric exercise that is inherent to resistance training may alter glycogen re-synthesis in the days following training placing importance on adequate carbohydrate consumption in conjunction with training.<sup>24</sup>

Training and amino acids both have a positive effect on protein synthesis, however training has an accompanying protein degradation that unaltered can lead to a negative net protein balance.<sup>25</sup> In order to negate this protein degradation the consumption of amino acids promotes protein synthesis and can blunt the effects of protein breakdown if consumed in adequate amounts. Studies on stimulation of protein synthesis have demonstrated that consumption of either 20g of intact whey protein or ~9g of essential amino acids is necessary to maximally stimulate protein synthesis post exercise.<sup>23</sup> Of the essential amino acids, the content of the branched chain amino acid leucine is necessary for maximal protein synthesis. Consumption of adequate levels of leucine (1-1.7g) which is found in sufficient levels in whey and casein proteins is necessary for maximal muscle protein accretion.<sup>26</sup>

Consumption of carbohydrates and amino acids independently have positive effects on post exercise recovery but when consumed together may provide synergistically greater benefits than

either macronutrient consumed alone.<sup>15,23,27</sup> These additive effects may be attributed to increased release of insulin, increased glycogen re-synthesis, increased protein synthesis and a reduction in protein breakdown.<sup>27</sup>

Due to the strict regulation of the cellular signaling pathways and the ability of training and nutrition to cause positive or negative stimulation, it warrants investigation into the optimal methods to promote adaptation. By consuming protein and carbohydrates combined with training athletes can activate the anabolic pathways and attempt to inhibit the negative aspects of the catabolic pathways, leading to recovery and subsequent adaptation. Therefore, the purpose of this paper is to investigate the effects of a protein and carbohydrate recovery beverage on the specific muscle protein accretion and cellular signaling in weightlifters.

## **METHODS**

### *Materials*

mTOR, AMPK, phospho-mTOR and phospho-AMPK antibodies (2972S, 2532S, 2971S, 2531S, rabbit anti-human) were purchased from Cell Signaling (Danvers, MA). Extended duration chemiluminescence substrate (QK226059) was purchased from Thermo Scientific (Rockford, IL). NuPAGE 3-8% Tris-Acetate gels was purchased from Life Technologies (Carlsbad, CA). Antibodies against myosin heavy chain 1 and 7 (SAB2104768, MABS755) were purchased from Sigma Aldrich (St. Louis, MO). Antibodies for myosin heavy chain 6 (PAB20052) were purchased from ABNOVA (Neihu, Taiwan) Wes Master kit 66-440 (PS-MK40) was purchased from Protein Simple (San Jose, CA).



## *Subjects*

Ten well trained weightlifters participated in this study (Age = $30.8 \pm 5.1$  years, Height =  $177.4 \pm 4.0$  cm, body mass =  $94.3 \pm 12.4$  kg, training age =  $5.3 \pm 2.9$  years). Descriptive data for the treatment and placebo group can be found in table 5.1. Inclusion criteria required that each subject had been training regularly for weightlifting competitions for a minimum of one year and free of injury for the past 6 months. Each subject read and signed a written informed consent form. This study was approved by the East Tennessee State University Institutional Review Board.

Table 5.1 Subject Demographic Information

	Treatment		Placebo		<i>p</i>
N	5		5		
Age (y)	28.4	$\pm 5.4$	33.7	$\pm 3.2$	.131
Height (cm)	179.6	$\pm 4.5$	175.2	$\pm 2.8$	.103
BM (kg)	95.3	$\pm 12.3$	93.5	$\pm 15.3$	.839
Training Age(y)	5.2	$\pm 3.2$	4.9	$\pm 3.4$	.890
EST1RM (kg)	170.6	$\pm 31.8$	155.0	$\pm 38.9$	.507
EST 1RM STR/BW	1.8	$\pm 0.3$	1.7	$\pm 0.3$	.517

Note: values are means $\pm$  standard deviations, EST 1RM STR/BW= ratio of back squat to body mass.

## *Experimental Design*

A double blind placebo study using a pre-post design was used to test out hypotheses and determine the relationship between expression of mTOR, AMPK, phospho-mTOR, phosphor-AMPK, myosin heavy chains 1, 6 and 7 in treatment and control groups respectively. Before the study subjects were randomly assigned to either the treatment group or a placebo group. The treatment group received a protein and carbohydrate beverage immediately following each

workout and the placebo group consumed a calorie free beverage immediately post workout. The protein carbohydrate beverage contained 230 calories consisting of 16g of hydrolyzed whey protein and 41 grams of carbohydrates made up of sucrose and dextrose. The placebo group received calorie free fruit punch drink containing no calories from either protein or carbohydrate. The supplement and placebo beverages were placed in opaque shaker bottles with subject numbers placed on the lid of the bottle to ensure anonymity. Athletes were instructed to consume no additional supplements during the study and to refrain from eating for 30 minutes following the consumption of post workout beverage. Athletes were regularly reminded and questioned not to deviate from the agreed supplementation routine.

### *Training plan*

A twelve week block periodization training plan using phase potentiation was completed for this study.<sup>13,14</sup> Each subject completed four training sessions per week consisting of general strength exercises, weightlifting movements and their respective derivatives. Training sessions followed a Monday, Wednesday, Thursday, Saturday schedule for the duration of the study. A detail of the training plan implemented is contained in table 5.2 and exercises implemented can be found in table 5.3.

Table 5.2: 12 Week Training plan

Week	Sets x Reps	Daily Intensities (Mon, Wed, Thurs, Sat)
1	3x10	M, M, L, L
2	3x10	MH, MH, ML, ML
3	3x10	H, H, L, VL
4	3x5(1x5)	ML, ML, L, VL
5	5x5	M, M, ML, ML
6	3x3(1x5)	MH, MH, VL, L
7	3x2(1x5)	ML, M, ML, L
8	5x5	H, MH, ML, L
9	3x3(1x5)	MH, M, L, L
10	3x2(1x5)	ML, L, VL, Meet

11	3x5	M, M, ML
12	3x5	L, L, VL

Note: VL=very light, L= light, ML=medium light, M=medium, MH=medium heavy, H=heavy, Meet=competition day.

Table 5.3: Exercise Selection

Weeks	Exercises: Monday & Thursday	Wednesday	Saturday
1-3	Back Squat Strict Press Dumbbell Press	Snatch CGSS CG Pull from PP CGSLDL DB Row	SGSS Snatch SGSLDL DB Row
4-7	Back Squat Push Press BN Press DB Press	Snatch CGSS CG Pull from BKN CGSLDL CG Row	SGSS Snatch Clean and Jerk SGSLDL SG Row
8-10	Back Squat Jerk DB Press	Snatch CGSS SG pull from FLR CGSLDL DB Row	SGSS Snatch Clean and Jerk SGSLDL DB Row
11-12	Back Squat DB Press Fr Raise	Power Snatch CGSS CG Pull from PP SLDL DB Row	- - -

Note: BN Press=behind the neck press, DB Press= dumbbell press, Fr Raise= front raise,

CGSS=clean grip shoulder shrug, SGSS=snatch grip shoulder shrug, DB Row= dumbbell row, CG Pull from PP=clean grip pull from power position, CG pull from BKN= clean grip pull from below the knee, SG Pull from FLR= Snatch grip pull from the floor, CGSLDL= clean grip stiff leg deadlift, SGSLDL= Snatch grip stiff leg deadlift, CG Row= clean grip row, SG Row= snatch grip row

### *Muscle Biopsies*

Muscle biopsies were performed the week preceding the study and during the final week of the training protocol. Percutaneous needle biopsies of vastus lateralis were performed using a 5-mm Bergstrom-Stille needle under suction after an overnight fast as previously described.<sup>28</sup> A 50- to 100-mg specimen was quickly blotted, and a portion was mounted on cork for sectioning. This sample was frozen in an isopentane slurry cooled over liquid nitrogen. All the remainder of the samples were frozen in nitrogen and stored at -80°C for later analysis. A muscle specimen was used as a reference sample for comparison was obtained from a post-mortem.

### *Immunoblots*

30µg protein from muscle homogenate or fractions was separated on a 3-8% polyacrylamide gel using the Laemmli system,<sup>29</sup> transferred to a nitrocellulose membrane, subjected to blocking with 2.5% bovine serum albumin (BSA) in PBS, incubated with either AMPK, mTOR, pmTOR, or pAMPK antibodies in 1.25% BSA, and developed with the enhanced chemiluminescence reagent and digital images were obtained. Image analysis was performed on digital files using Quantity One version 4.6.3 software from Bio-Rad (Hercules, CA).

### *Wes Kit*

1µg protein from muscle homogenate was combined with a 5x master mix. Samples were subjected to blocking with Wes antibody diluent (proprietary blend), incubated with either antibodies to myosin heavy chain 1, 6, or 7 and developed using a luminol-peroxidase mix and images were captured and analyzed using the Compass software version 2.7.1 from Protein Simple (San Jose, CA).

## Statistical Analysis

Independent samples t-test was used to compare %fold change in means of treatment and placebo groups from baseline to conclusion of the study. Effect sizes were generated and interpreted as trivial, small, moderate, large, very large, and nearly perfect when Cohen's *d* was 0.0, 0.2, 0.6, 1.2, 2.0 and 4.0 based on the scale by Hopkins<sup>30</sup>. All statistical analyses were performed with SPSS 22 (IBM, New York, NY) and statistical significance for all analyses was set at  $p \leq 0.05$ .

## RESULTS

Data for immunoblotting can be found in table 5.4. No statistical significance was found for total AMPK ( $p=0.159$ ), pAMPK ( $p=0.430$ ) and pmTOR ( $p=0.385$ ). Total mTOR reached statistical significance ( $p=0.04$ ) from baseline to post intervention. Myosin heavy chain data can be found in table 5.5. No statistical significance was found for MHC1 ( $p=0.37$ ). MHC1 and MHC6 approached significance with ( $p=0.08$ ) for each group respectively. % change from baseline data for immunoblotting and myosin heavy chains can be found in figure 5.1 and 5.2.

Table 5.4: Immunoblotting Data

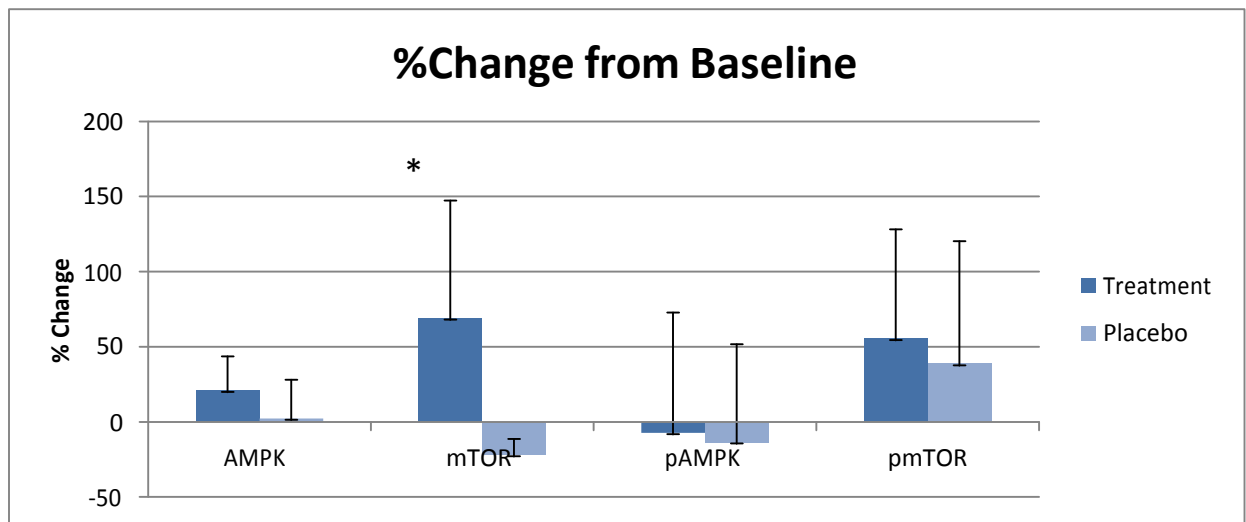
Condition	Group	Mean	SD	<i>p</i>	<i>d</i>
AMPK	Treatment	120.9	25.9	0.159	0.75
	Placebo	102.3	29.3		
mTOR	Treatment	169.0	88.9	0.044*	1.60
	Placebo	78.0	11.9		
pAMPK	Treatment	92.9	91.0	0.430	0.08
	Placebo	86.5	74.2		
pmTOR	Treatment	155.4	83.0	0.385	0.19
	Placebo	138.5	93.0		

Note: \*= $p < 0.05$

Table 5.5: Myosin Heavy Chain Data

Condition	Group	Mean	SD	<i>p</i>	<i>d</i>
MHC1	Treatment	124.7	44.7	0.08	0.95
	Placebo	94.1	6.6		
MHC6	Treatment	232.6	158.5	0.08	0.92
	Placebo	119.2	67.9		
MHC7	Treatment	93.1	18.5	0.37	0.20
	Placebo	97.1	21.3		

Figure 5.1: % Change from Baseline for Immunoblotting



Note: \*= $p < 0.05$

Figure 5.2: % Change from Baseline for Myosin Heavy Chains

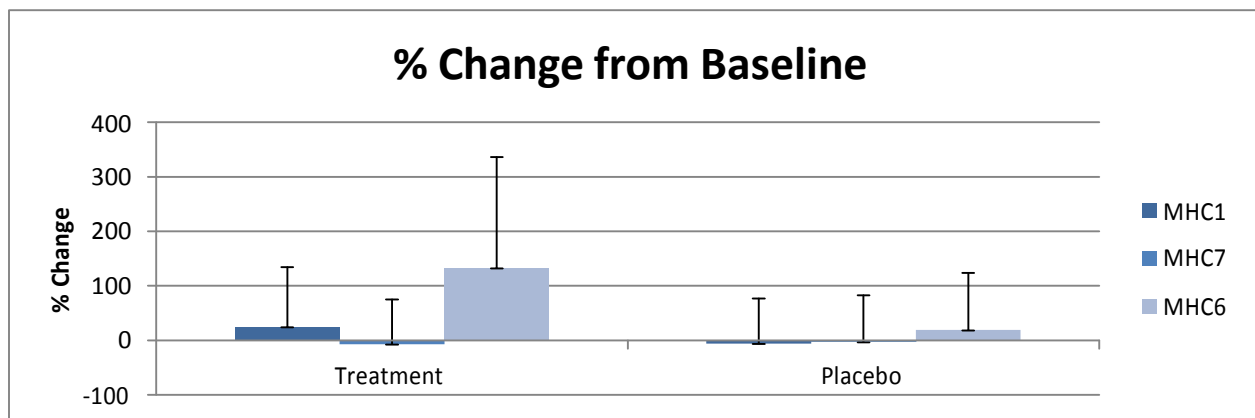
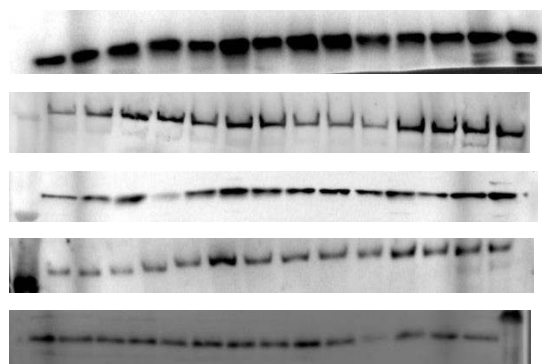
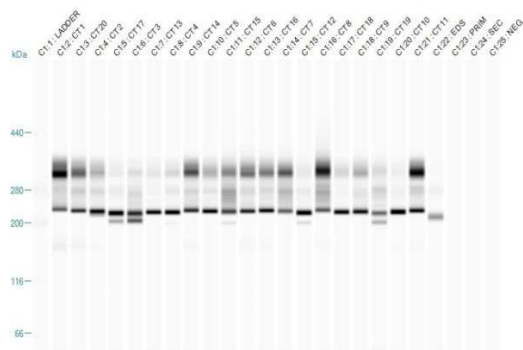


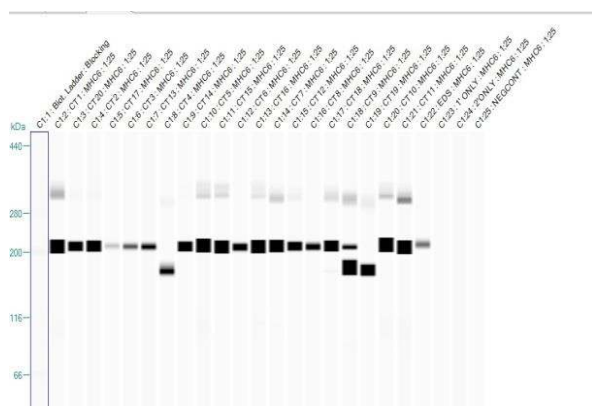
Figure 5.3: Wes Kit and Immunoblotting Data



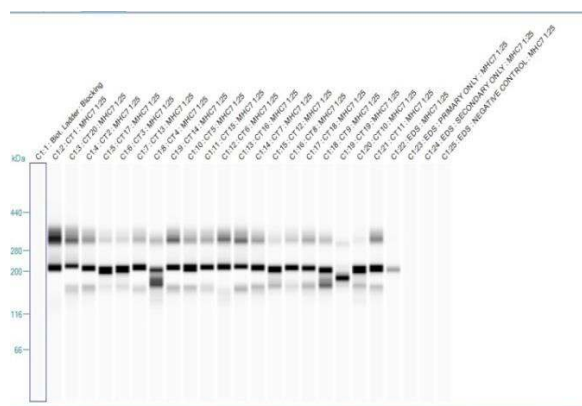
A



B



C



D

Note: Figure A=AMPK, MTOR, pAMPK, pmTOR, Actin control; B=MHC1; C=MHC6; D=MHC7.

## DISCUSSION

This study compared the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo on muscle protein accretion in weightlifters. The primary outcome of this study was that a protein and carbohydrate recovery beverage provided greater protein accretion of total mTOR and favorably shifted gene expression of MHC 1 & 6 compared to a calorie free placebo.

No statistically significant changes in protein accretion were observed for total AMPK, phosphorylated AMPK, phosphorylated mTOR or gene expression of MHC 7.

Compared to baseline the treatment group expressed greater percent fold increases in total mTOR compared with the placebo group. Within this greater increase in total amount of mTOR, phosphorylated mTOR increased to a greater extent in the treatment compared to the placebo group possibly indicating greater activation of anabolic signaling. Activation of mTOR can lead to downstream signaling of 4E-BP1 and p70S6K which are indicators of translation initiation and related to increased muscle protein synthesis rates following resistance exercise.<sup>31</sup> By combining resistance exercise with carbohydrates and protein athletes can attempt to increase muscle protein synthesis above baseline and mitigate the effects of protein breakdown leading to a net positive nitrogen balance. Theoretically if this is done over time the cumulative effects will manifest themselves as increased contractile proteins and lead to greater hypertrophy.<sup>32</sup> Increases in AMPK signaling inhibit anabolic processes and can directly inhibit the mTOR pathway.<sup>33</sup> Changes in total AMPK and phosphorylated AMPK from baseline to the completion of the study were largely unaffected by protein and carbohydrate supplementation.

MHC's contain ATPase and are necessary for human muscle contraction and various isoforms have been identified in human muscle.<sup>34</sup> Human skeletal muscle shortening velocity is limited by enzyme kinetics and rate of cross bridge dissociation with greater velocities being observed in type IIx and IIa compared to type I muscle fibers.<sup>35</sup> Therefore, in an attempt provide greater velocity of shortening training which emphasizes stimulation of type II fibers and especially type IIx can be beneficial for strength and power athletes. MHC 1, 6 and 7 are expressed in greater amounts in type IIx, IIa and I muscle fibers respectively.<sup>34</sup> Gene expression of MHC 1 and MHC 6 were greater compared to baseline for the treatment group compared to the placebo



during the intervention. This increased gene expression of MHC 1 and 6 may indicate a positive relationship between protein and carbohydrate supplementation combined with resistance training leading to greater stimulation of type II muscle fibers.

This study is in agreement with previous research that has demonstrated increase in mTOR following ingestion of protein and a combination of protein and carbohydrate. Hulmi et al.<sup>36</sup> demonstrated increases in mTOR signaling with administration of whey protein following exercise in untrained males. Dreyer et al.<sup>37</sup> provided a carbohydrate and essential amino acids immediately following exercise with untrained males and provided evidence that combined supplementation also increased mTOR signaling. Less is known about the responsiveness of cellular signaling over time and some evidence has been presented that protein supplementation combined with resistance training may have diminishing benefits over time.<sup>38</sup> However the present study presents evidence that even in trained subjects a protein and carbohydrate supplementation provided greater benefits for anabolism compared to a placebo.

There are several limitations to this study that should be considered when examining the data for conclusions: 1) this study was short term in nature and thus presents challenges when attempting to draw conclusions for chronic adaptations; 2) this study contained a small sample size (n=10) which was comprised of a homogenous group of trained weightlifters; 3) the placebo group contained no calories compared to the treatment group which introduced a confounding variable of increased caloric content of the treatment beverage; 4) the subject pool for this study was small which can increase the potential of finding no statistical changes due to the nature of small sample sizes. Because of the paucity of well-trained individuals, it is difficult to recruit and place trained athletes on the same training protocol. Future investigations should attempt to utilize trained subjects of different training backgrounds with larger sample sizes and for longer

duration studies in order to illuminate the effects of protein and carbohydrate supplementation for strength and power athletes.

## **CONCLUSION**

The protein and carbohydrate supplement used for this study had a positive effect on total mTOR but little effect on AMPK, phosphorylated AMPK or phosphorylated mTOR. Compared to a placebo the treatment group had greater gene expression of MHC 1 and 6 which are correlated with type II muscle fibers. Greater increases in size and proportion of type II fibers are advantageous adaptations for strength and power athletes. These positive adaptations can occur due to resistance training and may further be enhanced with proper supplementation containing both protein and carbohydrates following training. Further investigations are necessary to fully elucidate the effects of protein and carbohydrate supplementation in combination with resistance training for strength and power athletes.

## **ACKNOWLEDGEMENTS**

The authors would like to thank the members of the Stoneage Weightlifting Club who participated in this study. There are no conflicts of interest. There are no professional relationships with companies or manufacturers who will benefit from the results of the present study for each author.

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## CHAPTER 6

## SUMMARY AND FUTURE INVESTIGATIONS

The purpose of this dissertation was to compare the effects of a protein and carbohydrate recovery beverage versus a calorie free placebo in trained weightlifters. In order to examine these effects this dissertation was divided in to separate but related research questions: 1) the effects of the recovery beverage on performance in weightlifters compared with a placebo, 2) the effects of the recovery beverage on muscle morphological changes compared with a placebo, 3) the effects of the recovery beverage on muscle protein accretion and gene expression compared with a placebo.

The first investigation failed to demonstrate any performance improvements due to protein and carbohydrate supplementation compared with a placebo beverage. Measurements of static and countermovement jumps as well as a maximal isometric were used to look for differences between treatment and placebo groups. The lack of performance increases may be related to the time course of muscle hypertrophy and delayed realization of fitness qualities that may come to fruition in later phases of training (Stone, Stone & Sands 2007). Additionally, final measurements for the study occurred after a major competition which may have confounded results due the high levels of emotional and physical stress that was placed on the athletes prior the final testing sessions.

The second investigation provided evidence that the training plan used for this study improved muscle morphology for both groups by showing improvement in cross sectional area for the vastus lateralis, type I and type II. Within these improvements the recovery beverage provided greater benefits for type I and type II muscle fibers compared with the placebo group. Additional benefits were not observed at the level of the vastus lateralis with slightly greater improvements

being observed in the placebo group though the magnitude of change in means was similar between groups. This evidence suggests a positive change at the cellular level related to supplementation and resistance training in weightlifters. Increased cross sectional area is related to force generating capabilities of skeletal muscle and supplementation may provide the material needed for positive structural adaptations and thus increased force production ability.

The final investigation was aimed at the specific muscle protein accretion and gene expression related to cellular growth and positive adaptations to resistance training. This study showed positive adaptations in total mTOR and phosphorylated mTOR for treatment group and small but not statistically significant decreases in mTOR activation for the placebo group. Additionally, positive adaptations were observed in myosin heavy chains 1 and 6 gene expression, which are correlated with type IIX and type IIA muscle fibers. Increases in these myosin heavy chains are beneficial adaptations to resistance training and are related to shortening velocity of human muscle. This in combination with the increases in muscle fiber size seen in the second investigation indicates positive adaptations leading to increase power output of skeletal muscle in weightlifters.

This dissertation attempted to encapsulate the changes in performance, muscle morphology and protein accretion related the ingestion of a recovery protein and carbohydrate beverage following exercise. Though this dissertation illuminated several benefits of recovery supplementation following exercise further research is necessary to fully elucidate benefits of supplementation for strength and power athletes. Future investigations should continue to use trained populations of athletes and block periodization protocols combined with recovery supplementation.

Additionally, future studies should attempt to use long term studies over the course several months to look for performance benefits due to delayed training effects and the length time

course related to hypertrophic muscle improvements. Finally, larger sample sizes should be incorporated in studies with control and placebo groups to provide additional insights into benefits of supplementation with athletes.

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APPENDICES

APPENDIX A: ETSU Institutional Review Board Approval



Office for the Protection of Human Research Subjects  
• Box 70565 • Johnson City, Tennessee 37614-1707  
Phone: (423) 439-6053 Fax: (423) 439-6060

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**IRB APPROVAL – Initial Full Review**

September 11, 2015

Christopher Taber

**Re:** Effect of a Recovery supplement on Weightlifting Performance and Muscle Fiber Composition **IRB#:** 0815.7f  
**ORSPA #:**

The following items were reviewed and approved by the convened IRB.

- NPS xform, PI CV, \*ICD version 07/29/2015

The item(s) with an asterisk(\*) above noted changes requested by the convened board.

On **September 1, 2015**, a final approval was granted for a period not to exceed 12 months and will expire on **August 31, 2016**. The expedited approval of the requested changes will be reported to the convened board on the next agenda.

The following **enclosed stamped, approved Informed Consent Documents** have been stamped with the approval and expiration date and these documents must be copied and provided to each participant prior to participant enrollment:

- Informed Consent Document (ICD version 9.10.2015 stamped 9.01.2015)

Federal regulations require that the original copy of the participant's consent be maintained in the principal investigator's files and that a copy is given to the subject at the time of consent.

**Projects involving Mountain States Health Alliance must also be**

**approved by MSHA following IRB approval prior to initiating the study.**

Unanticipated Problems Involving Risks to Subjects or Others must be reported to the IRB (and VA R&D if applicable) within 10 working days.

Proposed changes in approved research cannot be initiated without IRB review and approval. The only exception to this rule is that a change can be made prior to IRB approval when necessary to eliminate apparent immediate hazards to the research subjects [21 CFR 56.108 (a)(4)]. In such a case, the IRB must be promptly informed of the change following its implementation (within 10 working days) on Form 109 ([www.etsu.edu/irb](http://www.etsu.edu/irb)). The IRB will review the change to determine that it is consistent with ensuring the subject's continued welfare.

Sincerely,  
George Youngberg, M.D., Chair  
ETSU/VA Medical IRB

cc:



*Accredited Since December 2005*



APPENDIX B: Informed Consent Document  
INFORMED CONSENT DOCUMENT

~~This Informed Consent will explain about being a participant in a research study. It is important that you read this material carefully and then decide if you wish to be a volunteer.~~

Principal Investigator:

Christopher B. Taber, M.S.

Co-Investigators:

Charles A. Stuart, M.D., Dr. Michael H. Stone, Ph.D., Dr. Brad DeWeese, Ph.D., Dr. Kimatake Sato, Ph.D.

Title of Project:

Effect of a Recovery supplement on Weightlifting Performance and Muscle Fiber Composition

**PURPOSE:** The purpose of this research study is to see if a protein and carbohydrate supplement has an effect on muscle fibers. In this protocol, we will carefully measure the changes in several muscle proteins and their fiber cross-sectional area caused by twelve weeks of resistance training combined with recovery supplementation. During this study participants will receive either a recovery protein and carbohydrate beverage or a calorie free placebo drink immediately following each resistance training session. Since this is a randomized, placebo controlled, double-blinded study, neither the subject nor the investigators will know which subjects receive the protein and carbohydrate supplement until the study is completed. Four training sessions will occur weekly. All subjects must be willing and able to undergo strength training before they start the program. All subjects must be injury free for the past six months preceding this study. All subjects must be over 18 years of age at the time of the initiation of the study.

To measure changes in your muscle induced by the resistance training program, we will take two very small pieces of muscle from your thigh muscle (vastus lateralis) using a needle under local anesthesia. One biopsy will be done before and the other at the end of twelve weeks. During the protocol, you will also have strength testing, jump performance testing and an estimate of the proportion of body fat and muscle. You will be one of 10 men who will participate in this study.

~~**DURATION:** Your participation will last about 12 weeks and the entire project may last about a year. Before scheduling the study, you will be asked a few questions about your health, training status and injury history. After signing this consent form, whether or not you participate in the recovery drink group or calorie free placebo group will be determined by a randomization procedure. In the week before your first muscle biopsy, your strength will be tested; you will have your body fat content measured using skinfold calipers and you will have a test measuring jumping performance. The time needed for strength and jump testing will be 30minutes and the time needed for the muscle biopsy will be 1 hour.~~

The next week you will begin with the resistance training program. Every week you will report to the laboratory to complete your four resistance training sessions and once a week you will test your jumping performance. Approximately every four weeks you will report to the laboratory in order to test your strength. During the last week of this protocol, a second muscle biopsy will be performed and a final strength test and jump performance measurement will

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by the ETSU-VA IRB

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occur. Normal training sessions last about 1 hour in addition to your normal training you will need to add 10 additional minutes on Mondays workout to adjust for additional vertical jump monitoring and 20 minutes every 4 weeks on Wednesday to investigate the effects of your training on isometric mid-thigh pulls.

PROCEDURES: In the week before your first muscle biopsy, body fat will be measured using the skinfold caliper. The body fat will be measured in the Exercise and Sports Science lab. You will also have a strength test that measures how much force you can produce during a brief bout of intense exercise and a jumping performance test. Biopsy of thigh muscle will be done in the ETSU Physicians and Associates clinic building. The night before your first muscle biopsy, you should not eat or drink anything except water after 10:00 PM. At about 10:00 AM, the muscle biopsy will be done. The muscle biopsy is done with you lying on an exam table. The skin and muscle surface will be numbed with lidocaine. Any adverse reactions to lidocaine or other anesthetic agents should be reported to the investigator before beginning this study. A small cut (7-10 mm) in the skin will be made and a 5 mm diameter muscle biopsy needle will be introduced. A small snip of muscle (about 100 mg - the size of an apple seed) will be taken and quickly frozen in special containers. Usually one stitch is placed in the skin and an elastic bandage is applied. Normal physical activity may be resumed when you leave, but strenuous exercise should be delayed until the next day. Once the cut has healed the stitch will be removed at the same medical office where the biopsy occurred and this should require only a few minutes for removal.

On days when more than one subject has these procedures, the start time may be offset to later in the morning. Failure to adhere to the resistance or monitoring program is sufficient reason to terminate your participation. After consuming the recovery beverage you will be asked not to consume any other foods or beverages in the first 30 minutes after your workout.

ALTERNATIVE PROCEDURES/TREATMENTS : This is a voluntary research project to assess recovery supplementation and resistance training effectiveness in improving weightlifting performance and muscle fiber composition. Subjects who choose not to participate may withdraw from our study and may pursue other diet and exercise programs on their own.

POSSIBLE RISKS/DISCOMFORTS: Each muscle biopsy is done using sterile techniques. Pain can occur, but the use of lidocaine minimizes the discomfort and the elastic bandage minimizes bruising. Reaction to lidocaine can rarely occur. There may be a slight chance of infection or bruising at the biopsy site as well as a possibility of bleeding at puncture site. The skin should be kept clean and dry for about a week after the biopsy. There may be some soreness at the muscle biopsy site for a day or two. Possible risks associated with training are similar to any resistance training program. Possible discomfort may arise due to the increase in the exercise regimen. Though weightlifting is inherently safe injuries are possible. Possibly injures could be muscle strains, muscle sprains, muscle tears and damage to ligaments and tissues. Protein consumption is well tolerated and beverage is commercially available to all consumers. A possible risk may be gastrointestinal discomfort.

POSSIBLE BENEFITS: Volunteers who participate in this study may learn best practice resistance training and new forms of beneficial exercise. They may have success in jumping

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performance, improve their strength and their general feeling of well-being. Additionally, volunteers will receive expert coaching and monitoring of their progress for twelve weeks.

**COMPENSATION FOR MEDICAL TREATMENT:** East Tennessee State University (ETSU) will pay the cost of emergency first aid for any injury that may happen as a result of your being in this study. ETSU makes no commitment to pay for any other medical treatment. Claims against ETSU or any of its agents or employees may be submitted to the Tennessee Claims Commission. These claims will be settled to the extent allowable as provided under TCA Section 9-8-307. For more information about claims call the Chairman of the Institutional Review Board of ETSU at 423-439-6055.

**FINANCIAL COSTS:** There is no cost to you for your participation in this study.

**COMPENSATION IN THE FORM OF PAYMENTS TO RESEARCH PARTICIPANTS:** There is no compensation for this study.

**VOLUNTARY PARTICIPATION:** Participation in this research experiment is voluntary. You may refuse to participate. You can quit at any time. If you quit or refuse to participate, the benefits or treatment to which you are otherwise entitled will not be affected. You may quit by calling Dr. Stuart, whose phone number is 423-439-6282 or Christopher Taber, whose phone number is 240-310-5116. You will be told immediately if any of the results of the study should reasonably be expected to make you change your mind about staying in the study. Sports science protocols require 24 hours to review the informed consent before making a decision. No instructors who have any influence over grade, etc. may obtain consent.

**CONTACT FOR QUESTIONS:** If you have any questions, problems or medical research-related problems at any time, you may call Dr. Stuart at 423-439-6282 or Christopher Taber at 240-310-5116. You may call the Chairman of the Institutional Review Board at 423/439-6054 for any questions you may have about your rights as a research subject. If you have any questions or concerns about the research and want to talk to someone independent of the research team or you can't reach the study staff, you may call an IRB Coordinator at 423/439-6055 or 423/439-6002.

**CONFIDENTIALITY:** Every attempt will be made to see that your study results are kept confidential. A copy of the records from this study will be stored in Quillen College of Medicine Department of Internal Medicine for at least 5 years after the end of this research. The results of this study may be published and/or presented at meetings without naming you as a subject. Although your rights and privacy will be maintained, the Secretary of the Department of Health and Human Services, the FDA, the ETSUNA IRS, and personnel particular to this research (Ors. Stuart, Stone, and Mr. Taber) have access to the study records. Your (medical) records will be kept completely confidential according to current legal requirements. They will not be revealed unless required by law, or as noted above.

**I am the subject or am authorized to act on behalf of the subject. I have read this information, and I will re** **3fP.BY** **of this form after it is signed.**

By the ETSU IRB  
SEP 01 2015  
By [Signature]  
Chair IRB Coordinator

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By signing below, you confirm that you have read or had this document read to you. You will be given a signed copy of this informed consent document. You have been given the chance to ask questions and to discuss your participation with the investigator. You freely and voluntarily choose to be in this research project.

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SIGNATURE OF PARTICIPANT

DATE

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PRINTED NAME OF PARTICIPANT

DATE

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SIGNATURE OF INVESTIGATOR

DATE

SIGNATURE OF WITNESS (if applicable)

DATE

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By the ETSU IRB  
SEP 01 2015

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12/10/2016

by  
IRB

ETSU IRB

## VITA

### CHRISTOPHER BRIAN TABER

- Education: Ph.D. Sports Physiology and Performance, East Tennessee State University, Johnson City, TN, 2016
- M.S. Exercise Science, California University of Pennsylvania, California, PA, 2011
- B.S. Kinesiology, Pennsylvania State University, State College, PA, 2010
- Waynesboro Area Senior High School, Waynesboro, PA, 2005
- Professional Experience: Sports Scientist/ Strength and Conditioning Coach and Doctoral Fellow, East Tennessee State University Weightlifting Team. 2013-2015
- Publications: Taber, C., Bellon, C., Abbott, H., & Bingham, G. E. (2016). Roles of Maximal Strength and Rate of Force Development in Maximizing Muscular Power. *Strength & Conditioning Journal*, 38(1), 71-78.