Evaluation of Training, Fueling, and Whey Protein Supplementation in Army Initial Entry Training

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy in Kinesiology

Auburn, Alabama
August 4, 2018

Key Words: performance, muscle, military, body composition, biomarkers, bone

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Abstract

Background: Nutrient intake needs to provide the energy required to support recovery from training, improve performance, and promote health. To date, research on energy expenditure and dietary intake in recruits that participate in Army Initial Entry Training (IET) is sparse. Training in an energy-resticted state may predispose the soldier to musculoskeletal injuries, considered to be the primary medical problem for today's military. Whey protein is a nutritional supplement reported to improve recovery, overall health, body composition, athletic performance and to stimulate bone formation. Specific Aims: 1) Establish the energy and physical training load associated with 14 weeks of IET; 2) Evaluate dietary intake at two times points across 9 weeks of IET; 3) Evaluate the effects of supplement intake on performance, body composition, and musculoskeletal injury. Methods: A double blinded, placebo-controlled intervention study with two IET training companies. The first cohort consumed two servings and the second cohort consumed one serving per day of either whey protein (38.6g protein 19g carbohydrates, and 7.5g fat), or calorie-matched carbohydrate shakes (0.5g protein 63.4g carbohydrates, and 3.9g of fat) across 9 weeks of IET. Outcome measures: Dependent variables were training volume, energy usage, dietary intake, body composition and serum biomarkers. Fitness level was assessed using the Army Physical Fitness Assessment, and musculoskeletal injury data was collected. Most IET soldiers were in a negative net energy balance and thus may not have optimal adaptation when non-supplemented. Overall WP seemed to have a clinically relevant effect on fat free mass, fat mass, and push-up performance.

Note: Chapters 4-7 of this dissertation are inserted as complete manuscripts. Chapters 4 and 5 have been submitted for publication. Thus, there will be duplication of information in introductions and methods sections.

Acknowledgements

To start with I would like to thank God for the opportunity to work towards the completion of this degree, for the strength and encouragement to do so, and for all of the wonderful people He has provided along the way. I would like to thank my wonderful wife for all of her sacrifice, love, and dedications throughout this process. I can say without a doubt that you are the best friend I have ever had and I love you and Josiah very much. I would like to thank my parents, my brother, for loving, caring for me, teaching me how to work hard, and always being there to support me. Dr. Sefton, thank you for all of your time, wisdom, and mentorship. I truly appreciate your patience with my many mistakes and as you say "make better ones next time". Kaitlin, thank you for having my six. You have been a great coworker and friend, and you have always done whatever you were asked to get the job done. I would like to thank my committee. Thank you, Dr. Roberts, for your expertise in protein supplementation and your willingness to take the time to answer my many questions even though I work in a completely different lab. Dr. Lohse, thank you for instilling in me an understanding of the importance of statistical knowledge and a love for "R". In Dr. Sefton's words, "I drank the cool-aid". Dr. Young thank you for all our conversations and for taking the time to talk about stats, ELISA, or Russel Wilson. Thank you Matt, Petey, Cody, Paul, Hayden, Anna, and Leslie, for your friendship and many of you waking up at 2 AM to go to Benning to help us out. Thank you Dr. Witte for taking the time to be my outside reader. I would also like to thank all of our wonderful master's and undergraduate students who have helped so much along the way (Rian,

Sarah, Alex, Krystal, Bryce, Josh, Jessica, Jordan, Angela, JC, and everyone else who has and still is investing in our lab). I would also like to thank all of my friends here in Auburn who have made the journey along the way a very special one. I appreciate your thoughts, prayers, and care. I would like to thank Dr. Rudisill. You have always had our backs and you are a great department/school head. Finally, I would like to offer a BIG thankyou to all of our cadre and military personnel for all of your help in this dissertation and for all that you sacrifice for us. Without you this would not have been possible.

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List of Abbreviations

IET, Initial Entry Training

FFM, Fat Free Mass

WP, Whey Protein

CHO, Carbohydrate

RDA, Recommended Daily Allowance

USG, Urine Specific Gravity

ACSM, American College of Sports Medicine

APFT, Army Physical Fitness Test

1RM, 1 Repetition Maximum

HSL, Hormone Sensitive Lipase

AMP, Adenosine Monophosphate

ADP, Adenosine Diphosphate

ATP, Adenosine Triphosphate

VO_{2max} Maximal Oxygen Uptake

AMPK, Adenosine Monophosphate Kinase

g/kg, Grams per kilogram of body weight per day

REE, Resting Energy Expenditure

Kcal, Calories

PINP, Serum N-terminal propeptide of type 1 collagen

IGF-1, Insulin Growth Factor-1

BMI, Body Mass Index

OPG, osteoprogerin

RANKL, Receptor activator of nuclear factor-κ B ligand

PRT, Physical Readiness Training

MSI, Musculoskeletal Injury

mTOR, Mammalian Target of Rapamycin

PGC-1α, Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 alpha

mRNA, Messenger Ribonucleic Acid

B_3-AR, Beta-3 Adrenergic Receptors

IL-12, Interleukin-12

INF-γ, Interferon-γ

BCAA, Branched Chain Amino Acids

ELISA, Enzyme Linked Immunosorbent Assay

Chapter 1: Introduction

The ability of soldiers to respond and adapt to Initial Entry Training (IET) is an important part of military force readiness. The decline in fitness and increasing obesity rates of the American population over recent decades has resulted from changes in lifestyle factors such as increased television viewing time, reduced outside activities, fewer physically active jobs, and reduced active transportation (e.g. walking, riding bikes) (Brownson, Boehmer, & Luke, 2005). Military recruits mirror the characteristics of the general population, with lower initial fitness levels upon entry to military training and increased failure rates on the IET initial fitness assessment (J. Molloy, Feltwell, Scott, & Niebuhr, 2012a).

Lifetime physical activity and physical fitness prior to beginning IET is important from a physiologic perspective. Physical activity produces structural changes to muscle and bone, which can help prevent micro-damage and improve resiliency to training-induced injuries (Bennell et al., 1997; Liu et al., 2003). Previous physical activity results in larger shaft diameters in long bones and greater cortical thickness in active versus sedentary individuals (Bennell et al., 1997; Liu et al., 2003). Importantly, it has been reported that small increases in cortical thickness (8% increase) can lead to large increases in the amount of axial mechanical loading cycles required to fracture bone (15,000 compared to 1.5 million) (Warden et al., 2005). Skeletal muscles also undergo structural changes that may decrease muscle damage during training (Aagaard et al., 2001; Doering, Reaburn, Phillips, & Jenkins, 2016; Flann, LaStayo, McClain, Hazel, &

Lindstedt, 2011). Additionally, skeletal muscle may adapt to training by more efficiently utilizing protein (Butterfield & Calloway, 1984; Phillips, 2004). Thus, IET soldiers with previous physical activity may better withstand the rigors of IET by reduced levels of exercise-induced muscle damage and by better utilizing dietary protein to support muscle repair.

IET soldiers with lower physical fitness likely train at higher relative intensities in comparison to higher fit soldiers. For instance, lower fit individuals require greater muscle recruitment to complete the same exercise/activities relative to their more fit counterparts (Ploutz, Tesch, Biro, & Dudley, 1994; Turner, 2016). A contextual example for IET soldiers is ruck marching. An IET soldier with a one-repetition squat max (1RM) of 50 kg carrying a 25 kg ruck sack is ambulating with a load at 50% of his 1RM; whereas an IET soldier with a 1RM of 100 kg is carrying a load that is only 25% of his 1RM. Thus, IET soldiers with lower fitness levels are likely consistently training at higher relative intensities, which could more easily lead to overtraining. The result of these factors is that IET soldiers with lower previous physical activity levels and initial physical fitness are at a higher risk for musculoskeletal injuries (MSI). MSI result in lost training time and are the primary reason for medical discharge and attrition (Jones et al., 1993; Jones, Thacker, Gilchrist, Kimsey Jr., & Sosin, 2002; Lisman, O'Connor, Deuster, & Knapik, 2013; J. Molloy et al., 2012a; Plavina, 2004; Shaffer, Brodine, Almeida, Williams, & Ronaghy, 1999).

High rates of medical discharge and attrition are detrimental to military force readiness. Currently only 25% of individuals between the ages 17 and 24 are eligible for military service and few of the eligible 25% enlist for military service (Teyhen, 2014). A reduced recruiting population combined with high medical discharge and attrition rates make it difficult for the United States military services to maintain the required force levels needed for national security.

Medical discharge and attrition also result in a high financial burden. It has been estimated that the cost of replacing one soldier who withdraws from IET is approximately $31,000-$57,000; resulting in an estimated cost of $384 million per year in IET and $683 million per year for soldiers with 1-2 years of service (J. Molloy et al., 2012a; Teyhen, 2014). MSI during IET is an important contributor to attrition and readiness of soldiers with 1-2 years of service. Previous MSI is an important risk factor for future injury in active duty personnel, an important factor for discharge from military service (O. Hill, Bulathsinhala, Scofield, Haley, & Bernasek, 2013). Furthermore, MSI typically results in missed training, often requiring the IET soldier to repeat or restart training. Thus, injuries experienced during IET can have acute effects on force readiness and attrition, and chronic effects by increasing the likelihood of sustaining future MSI. Strategies to improve the ability of IET soldiers to physically adapt and overcome the stressors of training are needed in order to maintain military readiness and lower costs. In 2015 Army Medical Command rolled out the Army Performance Triad program, an initiative that focuses on sleep, nutrition, and exercise to improve soldier performance, health and readiness (Army Medicine, 2014). A recent joint position statement from the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine (ACSM) (D. Thomas, Erdman, & Burke, 2016a) also addressed the importance of nutrition for performance in athletes. These authors suggest that in order to promote optimal performance, nutritional intake should be considered in the context of overall energy expenditure requirements (energy expenditure/availability) as well as training specificity (intensity, duration, anaerobic versus aerobic modalities) (D. Thomas et al., 2016a). Currently there is limited knowledge on the energy expenditure, training intensity, or nutritional needs in IET environments. Conversely, many studies have been conducted in athletic populations suggesting that modulation of dietary

intake of protein, fat, and carbohydrate can have significant effects on performance and adaptation to training (Balsom, Gaitanos, Söderlund, & Ekblom, 1999; Doering et al., 2016; Horvath, Eagen, Fisher, Leddy, & Pendergast, 2000; H Kato, Suzuki, Bannai, & Moore, 2016a; McGlory, Devries, & Phillips, 2016; Simonsen et al., 1991). It has been suggested that nutritional intake from diet alone may be inadequate to support training adaptations during high volume training, and that nutritional supplementation may be necessary to meet nutritional needs (Brouns et al., 1989; Westerterp, 2001). In this regard, whey protein is a promising nutritional supplement for improving performance, body composition, and health (Ha & Zemel, 2003; Hulmi, Lockwood, & Stout, 2010; Moore & Soeters, 2015).

Whey protein is a nutritional supplement often used by active, healthy, and diseased populations for its ergogenic and health benefits (Ha & Zemel, 2003). Much of the whey protein sold in the United States as a supplement comes from bovine milk. The whey protein fraction of milk is separated from the curds during processing and is contained in a liquid form before it is filtered into a dry whey protein concentrate (Marshall, 2004). Whey protein concentrate can be broken down into smaller peptides by breaking the peptide bonds between the concentrated proteins to form whey protein hydrolysate (Marshall, 2004). Whey protein hydrolysate contains the same amino acid content, but the peptides are pre-digested to lower molecular weight peptides (smaller) to promote rapid absorption and to reduce allergic reactions (J Farup et al., 2016; Morifuji et al., 2010; Restani, Ballabio, Di Lorenzo, Tripodi, & Fiocchi, 2009). Historically, whey protein was used to treat stomach ailments, sepsis, and wounds (Smithers, 2008). Over time, the therapeutic properties of whey protein were less appreciated, and until the late 20th century, whey protein was viewed as a waste product of cheese manufacturing and disposed (Smithers, 2008). Over the past few decades, the utility of whey protein has generated

interest due to reports of its high nutritional quality (includes all essential Amino acids), rapid digestion rates, and bioactive properties (Almeida, Alvares, Costa, & Conte-Junior, 2016; Ha & Zemel, 2003; Jeewanthi, Lee, & Paik, 2015; Patel, 2015).

Whey protein is characterized as having a high biological value. One reason is the composition of amino acids in whey (Moore & Soeters, 2015). There are two broad categories of amino acids: dispensable or non-essential and indispensable or essential amino acids (Bos, Gaudichon, & Tomé, 2000). The body can synthesize non-essential amino acids (arginine, glutamine, glutamic acid, tyrosine, cysteine, alanine, asparagine, proline, serine, glycine and aspartic acid), whereas essential amino acids (lysine, threonine, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan and histidine) must be obtained from dietary sources. Complete proteins contain sufficient amounts of all of the essential amino acids. Whey protein is a complete protein source made up primarily of α-lactalbumin and β-lactoglobulin, which are polypeptides that are rich in essential amino acid (Almeida et al., 2016). Whey protein peptides are broken down into amino acids in the lumen and are rapidly absorbed in the blood (Bos et al., 2000) where they circulate to the tissues acting as powerful stimulators of protein synthesis (Bos et al., 2000).

Research on whey protein supplementation has primarily been conducted in strength athletes; whereas Army IET soldiers are a hybrid functional athlete that requires strength and endurance to complete mission tasks. These tactical athletes may require unique approaches to training, nutrition, and supplementation, as opposed to the typical strength or endurance athlete that has been the primary subject of research to-date. Thus, the primary aims of the current research are:

1. Specific Aim 1: Establish the energy and physical training load associated with completion of 14 weeks of IET.
2. Specific Aim 2: Evaluate dietary intake during 9 weeks of IET training.
3. Specific Aim 3: Evaluate the effects of supplementation with whey protein versus a carbohydrate placebo on fitness performance, body composition, physiological biomarkers, health, and MSI.

Our hypotheses are:

1. EIT soldiers will be in a negative energy balance due to large volumes of training and inadequate caloric intake
2. IET soldiers will consume below the recommendations for macronutrient intakes for athletic populations
3. Whey protein will:
    a. Increase fat free mass gains, increase fat mass loss, improve push-up and sit-up performance during IET.
    b. Result in higher testosterone levels, reduced cortisol, reduced interleukin 6, increased bone formation and reduced bone resorption, and increased IGF-1 levels during IET
    c. Result in reductions in MSI rate, total medical visits, and profile days during IET.
4. Carbohydrate placebo will have improved run time performance during IET

The following literature review will discuss the basics of energy metabolism and will segue into considerations regarding caloric intake for soldiers engaged in IET. The importance of

dietary protein will be discussed, and this is followed by a discussion on the physiological (i.e., skeletal muscle, bone and immune system) benefits of whey protein supplementation. Finally, a gap in the scientific literature and purpose statement of this research will be posited.

Chapter 2: Literature Review

Background and Basics of Exercise Metabolism

Diet and nutrition are crucial to the body's ability to adapt to exercise training (D. Thomas et al., 2016a). Nutritional intake must match the specific nutrient requirements of the athlete in order to promote proper physiological adaptation to training (D. Thomas et al., 2016a) and maintain an appropriate energy balance (Westerterp, 2001). Nutritional intake that exceeds energy expenditure can lead to adverse changes in body composition that can reduce exercise performance; whereas negative energy balance can lead to insufficient adaptation to training (A. Loucks, 2004a). Current nutritional recommendations for military personnel are determined by U.S. Army Regulation 40-25 (Medical Services, 2017) and are based on the joint statement of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine (D. Thomas et al., 2016a). The updated regulation 40-25 suggests 0.8-1.6 g/kg/d of protein intake, 4-8 g/kg/d of carbohydrate, and less than 30% of total calorie intake from fat. These recommendations are drawn from studies in athletic populations which are typically specialized for a specific type of athlete (aerobic, strength, or power). Tactical athletes, such as military personnel, first responders, and firefighters must complete a variety of functional tasks that require endurance, strength, power, and agility. No known work has been completed to date that details the specific dietary needs of this population.

Training Demands of Military IET

Few studies have objectively measured training intensity or estimated energy expenditure during IET. Soldiers engaged in IET are exposed to high volumes of physical activity covering a wide range of low, moderate and high intensity activities (JJ Knapik, Hauret, Canada, Marin, & Jones, 2011). Research to date has found that IET soldiers covered on average six to seven miles of ambulatory activity per day during nine weeks of basic combat training (JJ Knapik et al., 2011) covering over 620 miles throughout a full training cycle (Sefton, unpublished data). One study using accelerometers to examine physical activity reported that IET soldiers participated in an average of 311 (light = 174 minutes, moderate = 102, vigorous = 34 minutes per day) minutes per day of physical activity, and approximately 445 minutes in sedentary activity (Redmond, Cohen, Simpson, Spiering, & Sharp, 2013; K Simpson et al., 2013a). It is important to note that of the 445 minutes in sedentary activity levels, only 225 of those minutes were spent seated. Cumulatively across the basic training cycle IET soldiers spent approximately 6,000 more minutes standing than sitting (19,000 standing and 13,000 minutes sitting) (Redmond et al., 2013; K Simpson et al., 2013a). Studies show that standing energy expenditure is on average 9% higher compared to a seated position (Levine, Schleusner, & Jensen, 2000). Thus, when IET soldiers are sedentary the energy usage is elevated compared to other populations due to the long periods of standing.

High intensity exercise is also required during IET; examples include morning physical training, running an obstacle course, or buddy team live fire drills where IET soldiers must rapidly move from point to point and advance down range to eliminate targets. IET soldiers were reported to spend approximately 131 minutes per day in moderately high and approximately 37 minutes per day in high intensity physical activity (Redmond et al., 2013; K Simpson et al.,

2013a). Some of these higher intensity requirements involve strength such as battlefield casualty evacuation or marching carrying loads that can range from under 4.5 to 34 kg depending on the training activity or mission (Mala et al., 2015; K Simpson et al., 2013a).

In summary, IET soldiers complete physically demanding training that includes on average 144 minutes of low intensity, over 100 minutes of moderate, and 37 minutes per day of vigorous intensity exercise (Redmond et al., 2013; K Simpson et al., 2013a). Current recommendations by the ACSM suggest that individuals participate in 150-300 minutes per week of moderate intensity exercise, or 75-150 minutes per week of vigorous intensity exercise (Powers, 2014). It is evident that soldiers engaged in IET are superseding these recommendations, possibly putting them at risk for overtraining/under-recovery situations. Furthermore, it is likely that the estimates above of time IET soldiers spent in varying physical activity classifications are underestimated. In the aforementioned study by Redmond et al., accelerometers were collected at approximately 4:00 PM each day, missing any evening physical activity that may have occurred (Redmond et al., 2013). Thus, IET soldiers well exceed the ACSM recommendations for moderate and high intensity exercise making proper nutritional intake even more important for optimal physiological adaptation to physical training. It is essential to understand the nutritional substrates used during different physical activity intensities in order to provide informed recommendations for nutritional intake to promote optimal performance and recovery IET soldiers.

Basics of Skeletal Muscle Energy Metabolism

Type I muscle fibers are primarily recruited to provide the necessary force production for lower intensity exercises such as standing, walking, or jogging. Energy to fuel type I fibers

comes from re-synthesis of ATP via coordinated actions of the Krebs cycle and electron transport chain during aerobic metabolism. The Krebs can resynthesize ATP using substrates from glycolysis or via β-oxidation of free fatty acids from stored (adipose tissue or intramuscular) or exogenous (diet) sources. The aerobic energy system (oxidative phosphorylation) can use both carbohydrates and fat for ATP resynthesis, with fat serving as the primary substrate at low to moderate intensity physical activity (L. van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001). Free fatty acid availability and utilization during exercise increases due to the release of epinephrine, norepinephrine, cortisol, and growth hormone as physical activity duration increases (Horowitz, 2003). Catecholamines (epinephrine and norepinephrine) bind to specific adrenergic receptor subtypes. At low intensity exercise they bind to β-adrenergic receptors, stimulating lipolysis by increasing the activity of hormone sensitive lipase (HSL) via cyclic-AMP (Adenosine Monophosphate) (Langfort et al., 1999). Increased release of cortisol and growth hormone also plays an important role in the stimulation of HSL and release of free fatty acids for aerobic metabolism, which may have an additive role when acting in concert (Djurhuus et al., 2004; Horowitz, 2003; Vijayakumar, Novosyadlyy, Wu, Yakar, & LeRoith, 2010). Epinephrine, cortisol, and growth hormone, also antagonize the insulin stimulated uptake of glucose by peripheral tissues which further promotes the utilization of free fatty acids as duration of exercise increases (Baron, Wallace, & Brechtel, 1987; Baron, Wallace, & Olefsky, 1987; Kilgour, Baldwin, & Flint, 1995; Kim & Park, 2017; Vijayakumar et al., 2010).

The body adapts to physical training by improving utilization of free fatty acids as a fuel for exercise (Klein, Coyle, & Wolfe, 1994; Nordby, Saltin, & Helge, 2006). This is likely due to an increase in fat transporters (fatty acid translocase, fatty acid binding protein), mitochondrial

content, intramuscular stores of lipids, and enhanced ability to utilize fat in ATP production in response to endurance training (Bonen, Dyck, Ibrahimi, & Abumrad, 1999; Kiens, Kristiansen, Jensen, Richter, & Turcotte, 1997; Talanian et al., 2010; MA Tarnopolsky et al., 2007). Thus, after IET soldiers progress through the first few weeks of training their bodies are able to better utilize fat as a substrate for energy production.

Fat is required for survival and is an important macronutrient for fueling physical activity (Horvath et al., 2000; D. Thomas et al., 2016a). Inadequate fat intake can hamperperformance (Horvath et al., 2000). Fat intake has been discouraged in the general public and research literature due to its high energy yield per gram (9 kcal/gram as opposed to 4 kcal/g of protein and carbohydrate) and its reported negative impact on health (J. Hill, Melanson, & Wyatt, 2000; Hooper et al., 2001). These recommendations could result in IET soldiers avoiding fat intake with a potentially negative impact on training performance. Recent evidence suggests that many of the negative side effects reported with higher consumption of dietary fat are offset or negated by physical activity (Molteni et al., 2004; Venkatraman, Feng, & Pendergast, 2001). The high energy density of fat provides a larger pool of energy per gram of dietary intake compared to protein or carbohydrates, helping soldiers meet energy requirements during high training volumes (Horvath et al., 2000; A. Loucks, 2007). Repeated bouts of exercise result in preferential increases in fat oxidation and lipolysis, resulting in increased reliance on fat as a substrate (Stich et al., 2000). Some investigations into low fat diets have revealed negative effects on performance. One study found low fat diets reduce intramuscular triglyceride stores and reduce oxidation of fat in comparison to a moderate (30%) intake of healthy fat (Coyle, Jeukendrup, Oseto, Hodgkinson, & Zderic, 2001). Another study reported that runners who were fed low fat (16%) versus moderate fat (31%) diets not only had reduced intramuscular

triglyceride stores, but also a reduction in endurance performance in a time to exhaustion run at 80% VO_2max (Horvath et al., 2000). A study evaluating the effects of varying dietary fat intake and endurance performance found that higher fat diets (40%) increased time to exhaustion in comparison to lower fat diets (18%) in trained male runners by approximately 20%. (Hoppeler, Billeter, Horvath, Leddy, & Pendergast, 1999). Another study examined the effects of varying dosages of dietary fat intake on performance (Venkatraman et al., 2001). They found that fat intakes of 30% resulted in 19% and 24% increases in endurance time to exhaustion at 80% VO_{2max} compared to 15% dietary fat intake in female and male runners. They also evaluated increases in fat intake up to 40% of daily calories, but found no additional benefit in comparison to 30% fat intake (Venkatraman et al., 2001).

Type II muscle fibers rely predominately on glycolysis for ATP synthesis. There is an increased reliance on the glycolytic pathway for energy production and an increase in recruitment of type II fibers to meet the force production demands as exercise intensity increases (Gollnick, Piehl, & Saltin, 1974). Increases in exercise intensity also results in systemic changes, such as shunting of blood from internal organs and adipose tissue to the working muscle, increased production of lactate and release of hydrogen ions, and increases in adenosine monophosphate kinase (AMPK) activity, all of which act to inhibit lipolysis (Daval, Foufelle, & Ferré, 2006; Farrell, Joyner, & Caiozzo, 2012; Powers, 2014). Peak oxidation of fat as a substrate occurs around 60-65% VO_2max and decreases to negligible levels at approximately 85-87% VO_2max (Achten & Jeukendrup, 2003; Peric, Meucci, & Nikolovski, 2016). As intensity increases glycogen becomes the primary substrate utilized for energy production (Achten & Jeukendrup, 2003; Peric et al., 2016; L. J. van Loon et al., 2005).

Glycolysis relies on carbohydrates in the form of glucose-6 phosphate from blood glucose and/or glycogen stores in the liver and skeletal muscle for synthesis of ATP. Working muscles preferentially use carbohydrate stored in the muscle as glycogen during higher intensity exercise, as opposed to blood glucose as a source of glucose-6 phosphate for glycolysis (Gollnick et al., 1974; Powers, 2014). Inorganic phosphate, ADP, and AMP accumulate in the muscle as intensity increases, which stimulates glycogen phosphorylase. Glycogen phosphorylase is the enzyme responsible for hydrolysis of glucose polymer linkage bonds and is a key step in the production of glucose-6 phosphate for glycolysis. Increases in exercise intensity, epinephrine stimulation of skeletal muscle, and glycolytic flux leads to a linear increase in glycogen depletion, beginning with type I fibers and progressing to type II fibers as intensity increases from 30-150% VO_2max (Gollnick et al., 1974). In the late 1960's Bergstrom et al (Bergström, Hermansen, Hultman, & Saltin, 1967) was one of the first to investigate the importance of muscle glycogen on performance in response to dietary manipulation. These authors reported that the acute consumption of carbohydrate-rich diets significantly increased muscle glycogen content in comparison to high fat or high protein intake. The carbohydrate rich diet was correlated to significantly improved endurance performance while cycling at 75% intensity (Bergström et al., 1967). Subsequent studies have confirmed these findings (Rauch, Gibson, Lambert, & Noakes, 2005; Simonsen et al., 1991).

Muscle glycogen content is highly dependent on acute dietary intake of carbohydrates to replenish stores (Galgani & Ravussin, 2008). The maximal capacity of the body to store carbohydrates as glycogen has been suggested to be approximately 15 grams of carbohydrate per kg of body weight in males (Acheson et al., 1988). It has been shown that consuming a carbohydrate-loaded (10g/kg body weight) diet for three days resulted in higher power outputs

during a two hour session of cycling that was interspersed with sprints (Rauch et al., 2005). However, excess intake of carbohydrate when muscle glycogen stores are saturated can lead to increased de novo lipogenesis from carbohydrates and storage as triglycerides in adipose tissue (Acheson et al., 1988; Ameer, Scandiuzzi, Hasnain, Kalbacher, & Zaidi, 2014; Dich et al., 2000). Thus, many investigations have sought to determine optimal dietary intakes of carbohydrate to maximize glycogen stores, minimize fat deposition, and optimize exercise performance (Acheson et al., 1988; Dich et al., 2000; Simonsen et al., 1991; MA Tarnopolsky et al., 2001). One study (Simonsen et al., 1991) examined the effects of 10g/kg body weight intake of carbohydrate vs. 5g/kg body weight over four weeks in rowing athletes. These authors reported that muscle glycogen levels were much higher and average power output was increased during high volumes of training in the high carbohydrate group compared to a lower carbohydrate intake group who consumed 5 g/kg of body weight per day (Simonsen et al., 1991). Another study (MA Tarnopolsky et al., 2001) examined the effects acute dietary carbohydrate intakes of 6.1, 7.9, and 10.5 g/kg of body weight per day in males. These authors reported that the total glycogen was increased, but neither proglycogen nor macroglycogen (the subtype of muscle glycogen that is more responsive to carbohydrate intake) content increased in the in the 7.9g/kg group. In contrast, macroglycogen and total glycogen were both increased in those who consumed 10g/kg group (MA Tarnopolsky et al., 2001). These studies suggest: 1) muscle glycogen is important to exercise performance; and 2) adequate intake of carbohydrate in the diet is important in maintaining muscle glycogen stores for higher intensity exercise performance in carbohydrate-adapted athletes.

While primarily used by cells for protein synthesis, amino acids are also used by the body for energy metabolism. Protein consumed in the diet is digested by proteolytic enzymes in the

gut and absorbed into the blood stream as amino acids. The amine groups must first be removed through a process called deamination (Rui, 2014). This liberates the carbon backbone of the amino acid and allows it to be converted into Krebs cycle intermediates (Bender, 2012; Rui, 2014). The carbon skeletons can then be exported to the cytoplasm to produce glucose (gluconeogenesis) or be oxidized and provide hydrogen to the electron transport chain (direct oxidation and ATP production) (Bender, 2012; Rui, 2014). The fate of the carbon skeletons depends on many factors such as intensity and duration of exercise and if a person is in the fed or fasted state (Rui, 2014).

Gluconeogenesis is the process of producing glucose from a non-carbohydrate source and occurs primarily in the liver. Production of glucose is important because it is the substrate used in glycolysis to produce ATP, lactate, or pyruvate-acetyl coenzyme A (Acetyl coA) which enters the Krebs cycle for oxidation and further ATP production (Rui, 2014). In short, gluconeogenesis reverses the direction of glycolysis to produce glucose that can either be stored as liver glycogen or exported to the blood stream to circulate to other tissues (Houston, Tupling, & Tidus, 2001). Many of the reactions in glycolysis are near equilibrium and can be reversed by the addition/removal of substrates or products (mass action). However, there are three steps in the glycolytic pathway that are irreversible. The enzymes involved are: pyruvate kinase, phosphofructokinase, and hexokinase/glucokinase (J. Hill et al., 2000; Rui, 2014). In order for gluconeogenesis to occur these three steps must be bypassed.

Pyruvate kinase reacts with phosphoenolpyruvate and produces pyruvate in the late stages of glycolysis (Powers, 2014). Pyruvate can then be converted to acetyl CoA and enter the Krebs cycle (Talanian et al., 2010). To bypass the pyruvate kinase reaction in gluconeogenesis the intermediates in the Krebs cycle must be converted to oxaloacetate (Bender, 2012; Rui,

2014). Next, phosphoenolpyruvate carboxykinase (PEPCK-C) interacts with oxaloacetate to reform phosphoenolpyruvate to bypass the pyruvate kinase step of glycolysis. PEPCK-C is a key step in gluconeogenesis from amino acids (Bender, 2012; Houston et al., 2001; Rui, 2014). Deletion of PEPCK-C in animal knockout models prevents production of glucose from amino acids (Hakimi et al., 2005; Rui, 2014). Once phosphoenolpyruvate is formed glycolysis can reverse and flux via mass action until the phosphofructokinase (PFK) reaction (Houston et al., 2001). During glycolysis PFK adds a phosphate to fructose-6-phosphate, to form fructose-1, 6 bisphosphate. During gluconeogenesis the enzyme fructose-1,6-bisphosphatase performs the opposite action and removes the phosphate to form fructose-6-phosphate, thus bypassing the PFK reaction (Bender, 2012; Houston et al., 2001; Rui, 2014). Next, the reversal of glycolysis continues via mass action until glucose-6-phosphate is formed at the last irreversible step, the glucokinase/hexokinase reaction. During glycolysis, glucokinase in the liver and hexokinase in skeletal muscle, react with glucose-1-phosphate to form glucose-6-phosphate (Houston et al., 2001). The fate of glucose-6-phosphate can differ depending on the location and the metabolic status of the body. In the liver, glucose-6-phosphate can be converted into glucose-1-phosphate by phosphoglucomutase (Sutherland, Cohn, Posternak, & Cory, 1949) and subsequently liver glycogen, or it can react with glucose-6-phosphatase and produce free glucose (Rui, 2014; Van Schaftingen & Gerin, 2002). Under conditions that challenge blood glucose homeostasis, such as prolonged exercise or fasting, glucose-6-phosphate is converted to free glucose, translocated to the cell membrane, and released into the blood to help maintain blood glucose (Rui, 2014). Conversely, skeletal muscle does not contain the enzyme glucose-6-phosphatase and therefore can only form glycogen from glucose-6-phosphate (Pelley, 2007).

Direct oxidation of amino acid carbon skeletons in the Krebs cycle can occur in the liver as well as skeletal muscle (Owen, Felig, Morgan, Wahren, & Cahill, 1969; Suryawan et al., 1998). Amino acids can be de-aminated and enter the Krebs cycle as intermediates such as either, pyruvate, alpha-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate to name a few (Rui, 2014). In the Krebs cycle they serve as a substrate for oxidation by nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) (Bender, 2012). This reaction yields NADH and FADH, which translocate to the inner mitochondrial membrane and enters the electron transport chain (Powers, 2014). The electron transport chain uses the hydrogen donated from the amino acid carbon skeletons to create a gradient of hydrogen across the inter-mitochondrial membrane, which will be used to provide energy for ATP synthesis at the fourth complex of the electron transport chain (Powers, 2014). The result of amino acid oxidation ranges from a net loss of 2.5 to a gain of 37.5 ATP depending on the amino acid metabolized (Bender, 2012). Only glycine results in a net loss of ATP, and most result in at least a net gain of 12 ATP (Bender, 2012).

Early investigations into the role of protein as a substrate have revealed that protein contributes minimally to energy production during exercise. Individuals performing cycling exercise at 45% VO_2 max expended approximately 567 ± 83 total kcals, the energy yield from oxidation of amino acids averaged 3.7 % or 21 ± 8 kcals (Calles-Escandon et al., 1984). The relative role of protein in energy production seems to increase as exercise duration increases and muscle glycogen levels begin to fall (Wagenmakers et al., 1991). One study revealed that cycling exercise under glycogen depleting conditions resulted in an inverse relationship between glycogen levels and stimulation of amino acid oxidation enzymes (Wagenmakers et al., 1991). Another study found that cycling at 75% VO_2 max to exhaustion results in increases in plasma

and muscle ammonia concentration (MacLean, Spriet, Hultman, & Graham, 1991). This suggests that amino acid oxidation increased, because ammonia is a byproduct of amino acid metabolism and was found to increase in serum and the muscle (MacLean et al., 1991). Additionally, plasma essential amino acids and muscle essential amino acids were elevated post exercise, whereas branched chain amino acids were not (MacLean et al., 1991). The authors interpreted these findings as amino acid oxidation increasing across an exercise bout, whereas branched chain amino acids may be preferentially utilized for metabolism (MacLean et al., 1991). Together this suggests that muscle initially metabolizes muscle glycogen early in an endurance bout, with amino acids becoming more important during later stages of exercise (MacLean et al., 1991).

The role of protein in energy metabolism can also be affected by fueling. Consuming high (1.8 g/kg body weight) versus lower protein intakes (0.7 g/kg body weight) resulted in 40 and 46% increase in leucine oxidation at rest and during exercise (Bowtell et al., 1998). Other studies have shown higher protein intake in conjunction with lower carbohydrate diets lead to increased protein metabolism; whereas higher carbohydrate intake has an amino acid sparing effect. Nitrogen excretion (indicator of protein metabolism) was found to linearly increase from 7% to over 31% at 4 hours post-exercise when participants consumed a carbohydrate depleted diet (Lemon & Mullin, 1980). Compare this to the 6.5% during exercise and 10.9% at 4 hours of recovery in the same subjects consuming a 3-day carbohydrate rich diet (Lemon & Mullin, 1980). Furthermore, the authors estimated protein metabolism could have accounted for 10.4% of total calorie expenditure during exercise, but only 4.4% when carbohydrate intake is higher (Lemon & Mullin, 1980). Another study revealed that individuals consuming a high protein diet and given a glucose supplement during exercise demonstrated a 20% decrease in leucine oxidation during exercise in individuals who consumed a 7-day high protein diet (Bowtell et al.,

2000). Interestingly, the reduction in leucine oxidation did not occur in those who consumed a 7-day low protein diet (Bowtell et al., 2000). Therefore, overall diet can influence the source and amount of a macronutrient during exercise. Additionally, the effect of supplementation on exercise is dependent on the composition of the overall diet.

Collectively, protein can contribute to energy metabolism either directly via amino acid metabolism or indirectly via gluconeogenesis. The extent to which protein is utilized in energy metabolism is relatively low and depends upon the type of exercise being performed as well as the amount of protein and carbohydrate in the diet. The relationship between use of different macronutrients in energy metabolism can differ based on supplementation.

Dietary Protein

Dietary protein intake is crucial for performance and health. Current recommended dietary allowance (RDA) by the Institute of Medicine suggests a dietary intake of 0.8 g/kg body weight per day for the average individual (Institute of Medicine, 2002). It has been reported that protein intake at this level is inadequate to support training adaptation in active individuals (Campbell et al., 2007).

Load-bearing physical activity induces structural and volumetric changes in skeletal muscle. These structural changes consist of increased numbers sarcomeres in series (increased length of myofibril) and parallel (increased width of a myofibril), changes in pennation angle of the muscle, and other myofibrillar structural changes (Aagaard et al., 2001; Blazevich, Cannavan, Coleman, & Horne, 2007; Seynnes, de Boer, & Narici, 2007). Dietary protein intake is a key component of this adaptation as it provides amino acids that serve as building blocks for synthesis of the key structural and functional muscle proteins. Rodent data have demonstrated

that rats consuming low amounts of protein (5% of kcal intake) experience structural disturbances and damage, such as breakdown of the z-lines of sarcomeres in skeletal muscle, compared to rats consuming adequate protein amounts (20% kcal) (Oumi, Miyoshi, & Yamamoto, 2001). Many studies have been conducted to determine the protein needs in resistance trained athletes and active individuals (Cermak, de Groot, Saris, & van Loon, 2012; Lemon, Tarnopolsky, MacDougall, & Atkinson, 1992a; MA Tarnopolsky et al., 1992). One study (Lemon et al., 1992a) found that 1.4-1.5 g dietary protein per kilogram of body weight is needed in strength training individuals to maintain nitrogen balance (thought to be reflective of the protein requirement). The authors suggests that to maintain positive nitrogen balance (and support muscle protein synthesis) protein intake should be closer to of 1.7 g/kg for individuals participating in strength training (MA Tarnopolsky et al., 1992). However, others have reported that increasing protein intake to 2.6 g/kg of body weight yielded no additional benefit for hypertrophy or performance (Lemon et al., 1992a). Protein requirements are also influenced by overall caloric intake (E. Helms, Zinn, Rowlands, & Brown, 2014a). A systematic review found that protein intake of 2-3 g/kg of fat-free mass may be necessary to support optimization of body mass and muscular performance for resistance-trained individuals training during energy restriction (E. Helms et al., 2014a). Thus, protein needs must be considered in the context of training intensity and type as well as overall caloric intake. These factors add another level of complexity to determining optimal dietary protein to promote strength and higher intensity performance.

Protein intake is also important for recovery and adaptation to endurance training. Dietary protein is thought to be important for repair and adaptation of muscle proteins, mitochondrial adaptation, and recovery in response to endurance training (Doering et al., 2016;

M Hansen, Bangsbo, Jensen, Bibby, & Madsen, 2015a; Moore, Camera, Areta, & Hawley, 2014). The base protein requirement has been shown to be at minimum 0.94 g/kg body weight in endurance-trained individuals (M Tarnopolsky, 2004). Additionally, comparisons of protein intake in endurance training males to the daily recommendations (0.8 g/kg) revealed that 1.2 g/kg of body weight are needed to establish positive nitrogen balance, and that 0.87 g/kg was inadequate for endurance-trained athletes (Gaine et al., 2006). Several studies (H Kato et al., 2016a; M Tarnopolsky, 2004) have supported the notion that the current RDI's are inadequate for endurance training individuals. One study used the amino acid oxidation method to evaluate the protein needs of endurance training individuals (training 3 days). These authors reported that the average protein intake needed for people involved in endurance training was 1.6 g/kg of body weight (H Kato et al., 2016a). This finding is in contrast to the nitrogen balance studies that recommend intakes of 1.2 g/kg/d. A comparison of methods is beyond the scope of this review, but some of the difference could be explained by intensity of training and the possibility that the nitrogen balance method underestimates protein requirements (Elango, Humayun, Ball, & Pencharz, 2010; Humayun, Elango, Ball, & Pencharz, 2007; H Kato et al., 2016a).

Nutritional Considerations for IET Soldiers

The literature is dominated by studies assessing the protein requirements of endurance or strength trained athletes/individuals (Phillips, 2006). However, soldiers engaged in IET have a wide range of strength, endurance, and functional demands placed on them on a daily basis. Successful, non-injured completion of IET sets the soldier up for a long and successful career. Research needs to be conducted specifically to determine optimal nutritional intake for soldiers engaged in IET in order to optimize performance. Recommendations for intakes for a specific

macronutrient should not be isolated to that macronutrient (protein, carbohydrate, or fat), but should be considered in the context of the overall caloric intake and other macronutrient requirements. This must be considered to ensure: 1) macronutrients requirements are not compromised by increased intake of one macronutrient; and 2) overall caloric intake is not excessive which could lead to adverse changes in body composition.

Determination of caloric intake and energy expenditure is needed to understand energy lost from basal metabolism as well as the energy used during physical activity. Typically, methodologies for measuring metabolism are not practical for use with large populations. The Harris-Benedict equation was developed to estimate individual resting energy expenditure and correction factors have been developed to take into account increased energy needs of active populations to get a non-invasive estimate of energy expenditure (Roza & Shizgal, 1984). The Harris-Benedict equation is listed below along with the correction factors for highly active individuals.

Modified Harris-Benedict Equation for Resting Energy Expenditure (REE)(Roza & Shizgal, 1984):

REE = 88.362 + (13.397 x body weight (kg)) + (4.799 x Height (cm)) - (5.677 x age (yrs.))

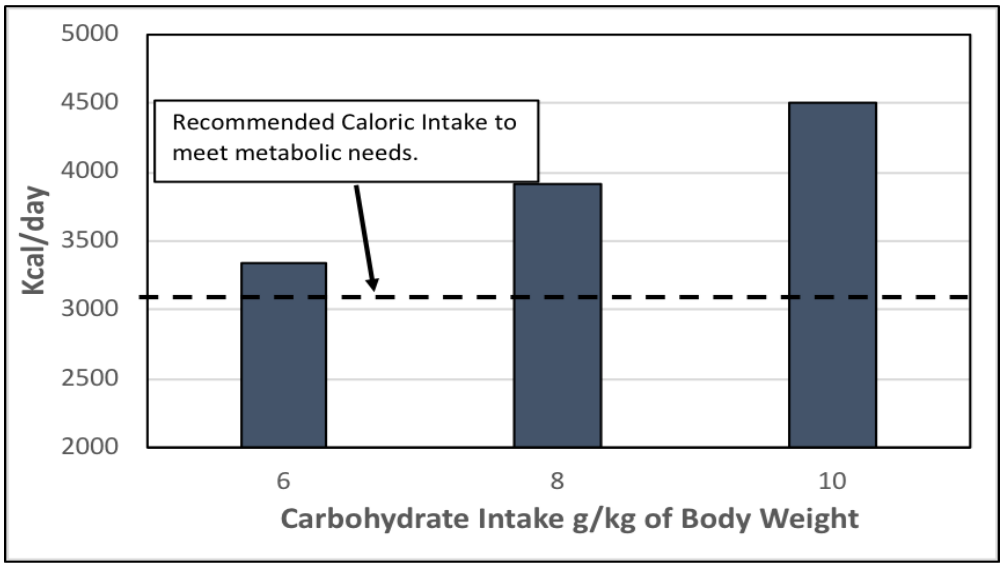
Table 1. Correction factors for Harris-Benedict equation

Activity zone	Description of Activity Levels	TDEE calculation
1	Little to no exercise	REE x 1.2
2	Light exercise (1–3 days per week)	REE x 1.375
3	Moderate exercise (3–5 days per week)	REE x 1.55
4	Heavy exercise (6–7 days per week)	REE x 1.725
5	Very heavy exercise (twice per day, extra heavy)	REE x 1.90

Dietary fat intake is important to athletic performance and energy balance due to its relatively high-energy yield per gram (9 kcal/g as opposed to 4 kcal/g for protein and carbohydrate). Recommended dietary fat intake is approximately 30-35% of total caloric intake based on research in endurance athletes. This is based on the studies suggesting negative benefits of lower fat diets on endurance performance Hoppeler et al., 1999; Horvath et al., 2000; Venkatraman et al., 2001 and research that indicates endurance athletes who restrict fat intake generally have inadequate overall caloric intake (Horvath et al., 2000; A. Loucks, 2007).

Recommended carbohydrate intake is approximately 6-10 g/kg of body weight, even though higher levels of carbohydrate consumption (8-10 g/kg of body weight) have been shown to improve muscle glycogen stores (MA Tarnopolsky et al., 2001; D. Thomas et al., 2016a). Few differences were found in muscle glycogen between carbohydrate intakes of 6 and 7.9 g/kg of body weight (MA Tarnopolsky et al., 2001). Thus, if glycogen can be maintained with a lower carbohydrate intake this could be beneficial for maintenance of other macronutrients while still maintaining an acceptable overall caloric intake. Figure 1 represents estimated macronutrient consumption for a 72 kg IET soldier at carbohydrate intakes of 6, 8, or 10 g/kg of body weight. The intake recommendations listed in Figure 2 for IET soldiers are based on the Harris-Benedict equation and the correction factor for the highest level of physical activity(Roza & Shizgal, 1984).

Figure 1. Calorie intake at each recommended carbohydrate intake

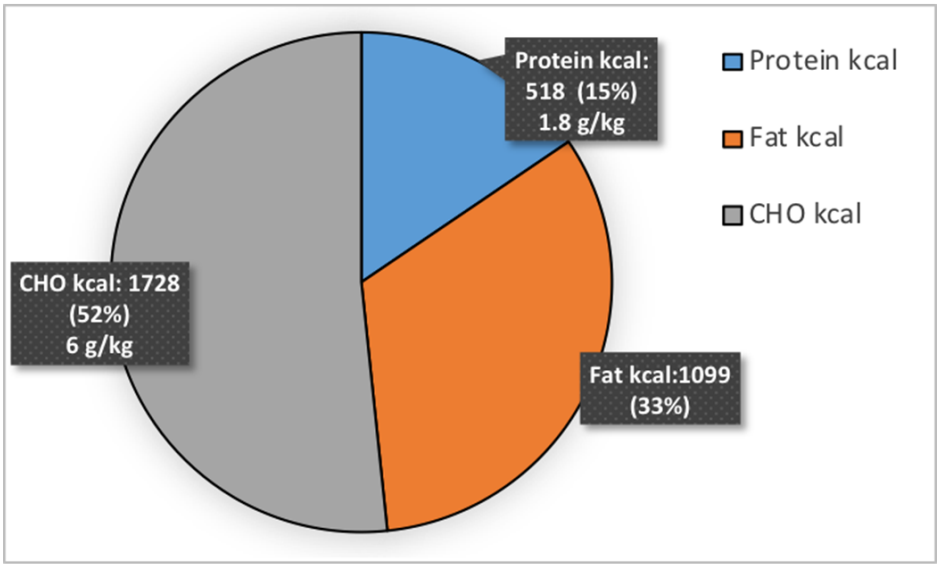


Legend: This figure illustrates overall calorie intake based on 6, 8, or 10 g/kg per day of carbohydrate intake for a 72 kg male IET solder. Also plotted is the estimated calorie needs for the IET soldier as estimated by the Harris Benedict equation using the highest physical activity correction factor. Abbreviations: kcal, calories; g/kg, grams per kilogram of body weight per day;

If intake of other macronutrients is kept at the necessary levels, increasing carbohydrate intake to 8 or 10 g/kg increases overall caloric intake above the estimated needs. This excess of caloric intake can lead to increases in adiposity, likely negatively impacting physical performance. It is also important to note, that although the higher levels of carbohydrate intake resulted in higher power output during training sessions, ultimately there was no significant improvement in performance on endurance tests after 3 weeks of training on a higher carbohydrate diet in comparison to moderate intakes (Hulston et al., 2010). Thus, making a tradeoff of restricting other macronutrient intake for the sake of increasing carbohydrate intake to maintain caloric balance may not be beneficial for performance.

Dietary intake of protein is recommended at approximately 1.7-1.9 g/kg of body weight per day of protein based on the literature for strength and endurance athletes. This research suggests endurance and strength training individuals require increased dietary protein in order to promote recovery and adaptation to training (H Kato et al., 2016a; Lemon et al., 1992a; MA Tarnopolsky et al., 1992). Nitrogen balance studies suggest lower protein intake could achieve nitrogen balance (1.0-1.5 g/kg protein) (Gaine et al., 2006; Lemon et al., 1992a; M Tarnopolsky, 2004), while others suggest that nitrogen balance studies underestimate protein needs (Elango et al., 2010; Humayun et al., 2007; H Kato et al., 2016a). Therefore, to ensure adequate intake of dietary protein in active individuals it is likely that higher intakes (i.e., 1.7-1.9 g/kg body weight) are needed (Figure 2).

Figure 2. Macronutrient recommendations for military athlete



Legend: This figure illustrates the nutritional recommendations for a 72 kg male IET soldier based on the research literature for athletes and the Harris-Benedict equation for the highest physical activity correction factor. Abbreviations: kcal, Calories; g/kg, grams per kilogram of body weight, CHO, carbohydrate

Whey Protein Supplementation

Effects of Whey Protein Consumption on Bone Metabolism

Bone is a highly metabolic tissue that is constantly adapting and remodeling. Bone adaptation is important for resistance to fracture, and remodeling bone to meet lifestyle demands (Siddiqui & Partridge, 2016). Disruptions in remodeling or adaptation can lead to disease states such as osteoporosis or result in bone injuries such as stress fractures or stress reactions. Bone remodeling consists of local and systemic regulation of three types of bone cells: osteoblasts, osteoclasts, and osteocytes (Siddiqui & Partridge, 2016). Osteoblasts are bone-forming cells that originate from mesenchymal stem cells. Osteoblasts secrete type 1 collagen and proteoglycans, which make up the majority of the bone matrix (osteoid tissue). Osteoblasts also secrete proteins such as osteocalcin, which are mineralized in the matrix to give bone its rigid properties (Crockett, Rogers, Coxon, Hocking, & Helfrich, 2011; Siddiqui & Partridge, 2016). Osteocytes are mature bone cells that have differentiated from the osteoblast cell lineage. Osteocytes are the most prevalent bone cell and are located in the bone matrix. Osteocytes function to monitor the stressors placed on the bone matrix and help stimulate the adaptive response to these stimuli (Crockett et al., 2011).

Mechanical loading is one of the primary stimuli for bone adaptation and remodeling (Hughes, Popp, Yanovich, Bouxsein, & Matheny, 2016). Mechanical loading from resistance training and/or endurance training have been shown to promote beneficial structural adaptations that promote bone health in humans. One study (Fujimura et al., 1997) in young males revealed that resistance training increased bone formation without increases in bone resorption. This finding is in contrast to the traditional understanding of bone remodeling in which requisite resorption of bone must occur by osteoclasts before osteoblasts can be recruited to the surface for

bone formation (Hughes et al., 2016; Siddiqui & Partridge, 2016). Another study implemented a combined program of resistance, cardiovascular, and physical activity-based training. They found increases in bone mineral density and thickness of the cortical/periosteal areas of the femur and femoral neck (Eleftheriou et al., 2012). Cross-sectional studies of athletes in comparison to age-matched sedentary individuals revealed athletes had increased cortical thickness and bone width (Liu et al., 2003). These structural changes lead to increased resistance to bone fatigue from repetitive loading and improved the overall health and strength of bone. Endurance exercise alone has also been shown to stimulate bone formation (Langberg, Skovgaard, Asp, & Kjær, 2000). One marker of bone formation (carboxyterminal propeptide of type I procollagen) decreased immediately post-race in response to marathon running, but then increased and peaked 72 hours post-exercise before returning to baseline on day five. In the same study, a bone resorption marker (immunoactive carboboxyterminal cross-linked telopeptide) increased immediately post marathon but returned to baseline the next day (Langberg et al., 2000). A longitudinal study (Bennell et al., 1997) of power and endurance athletes also supports benefits of physical training on bone health, revealing increases in bone mineral density in the femur of both power and endurance athletes. These authors also reported that power athletes exhibited increased lumbar spine bone mineral density, whereas endurance athletes exhibited increased lower body bone mineral density (Bennell et al., 1997). These findings suggest that there could be a beneficial effect of power (or resistance) and endurance (or cardiovascular) training for systemic bone health.

Soldiers engaged in IET frequently experience deleterious impacts of physical training on bone health. Studies assessing risk factors for musculoskeletal injuries during military training have revealed that low physical activity levels or fitness levels at the onset of training increase

the risk of MSI (Bedno, Cowan, Urban, & Niebuhr, 2013; Heir & Eide, 1996; C Milgrom et al., 1998; C. Milgrom, Simkin, Eldad, Nyska, & Finestone, 2000; Shaffer et al., 1999; Shwayhat, Linenger, Hofherr, Slymen, & Johnson, 1994; Wyss, Von Vigier, Frey, & Mader, 2012). These risk factors would result in decreased thickness and density of bone, thereby reducing the amount of loading cycles required to cause bone micro fractures (Beck et al., 2000; Hughes et al., 2016; Jepsen et al., 2013). Thus, IET soldiers who are less active before attending IET would likely decreased cortical thickness in long bones and be more likely to sustain stress fractures or stress reactions due to the repetitive loading resulting from high volumes of ambulatory activity and load carriage during military training.

It is also noteworthy to mention the high-energy expenditure, lower energy intake, and sleep deprivation may occur during IET. These factors have been reported to decrease bone formation and increase bone resorption (Hughes et al., 2014). This is critical as stress fracture injuries are a serious and expensive problem in the IET environment. It is the leading cause of hospitalization and results in over 68,000 bed days per year (Jones, Canham-Chervak, Canada, Mitchener, & Moore, 2010). Energy restriction or low energy availability has a negative impact on bone metabolism. For instance, research in previously sedentary women indicates low energy availability due to restricted energy intake in conjunction with exercise resulted in increased biomarkers of bone resorption and suppression of markers of bone formation (Ihle & Loucks, 2004). The same effect of energy restriction on bone turnover has also been reported in male endurance athletes who consumed a 50% energy restricted diet or a non-energy restricted diet during two separate periods of 3-days of 60-minute treadmill running. Serum N-terminal propeptide of type 1 collagen (PINP), a biomarker of collagen synthesis, was reduced in the energy-restricted conditions in conjunction with a decrease in serum insulin growth factor-1

(IGF-1, although there was no decrease in osteocalcin which is a marker of mineralization of bone) (Zanker & Swaine, 2000). Together these results suggest that energy restriction produces a reduction in collagen synthesis in the bone matrix, which may be reflective of inadequate bone formation in response to training.

Animal studies have also reported a negative effect of caloric restriction on bone health and resistance to stress fracture in response to training. A study conducted in young and old female rats revealed that chronic energy restriction resulted decreased bone formation; whereas restricted calcium intake did not have an impact on bone formation (Talbott, Rothkopf, & Shapses, 1998). Another study in young male mice subjected to energy restriction found significant reductions in number and thickness of trabeculae, cortical bone thickness, bone mineral density and content, and osteoblasts, and increases in osteoclasts (Hamrick, Ding, Ponnala, Ferrari, & Isales, 2008). There were significant reductions in the stiffness and area moment of inertia as a result of the reductions in number and thickness of trabeculae and cortical thickness; important for resistance to fatigue fracturing of bone (Hamrick et al., 2008; Hughes et al., 2016; Seeman, 2003). Together these studies reveal a negative impact of caloric restriction on bone health in non-exercising human and animal models. Clearly, it is important to evaluate the physical activity performed and resultant energy expenditure during IET to provide insight for nutritional recommendations to IET soldiers to promote optimal bone adaptation and health.

Dietary protein deficits may also negatively impact bone health. It has been reported that the average American aged 19-30 years old consumes approximately 1.3g/kg of protein which is higher than the recommended dietary intake of 0.8 g/kg body weight (Fulgoni, 2008). However, as noted above, the 0.8g/kg body weight has been reported to be inadequate for physically active individuals (Elango et al., 2010; Humayun et al., 2007; H Kato et al., 2016a). It has been

postulated that higher dietary intake of protein could be detrimental to bone health due to an increase in urinary calcium excretion (Jajoo, Song, Rasmussen, Harris, & Dawson-Hughes, 2006; Pasiakos, 2015; Remer & Manz, 1994). Studies have shown that higher protein intake increases urinary excretion of calcium, although higher protein intake also increases absorption of calcium from the gut (Kerstetter, O'Brien, & Insogna, 2003; Pasiakos, 2015). One study (Kerstetter, O'Brien, Caseria, Wall, & Insogna, 2005) assessing the effects of controlled low and higher protein diets on calcium balance and bone health reported that while there was an increase in calcium excretion with higher protein diets, there was no difference in net calcium balance or bone health in healthy women. The increase in calcium absorption in the intestines seems to offset the increased calcium loss in urine; (Kerstetter et al., 2005; Kerstetter et al., 2003) while lower protein diets were shown to produce low blood calcium levels and hyperparathyroidism. (Kerstetter, O'Brien, & Insogna, 1998; Kerstetter et al., 2003).These findings suggest that lower protein diets could be detrimental to bone health due to decreased blood calcium and increased calcium resorption from bone in response to elevated parathyroid hormone (Kerstetter et al., 1998; Kerstetter et al., 2003). Finally, an 18-month study in older Caucasian adults reported no difference in bone mineral density or other bone parameters in individuals with consistent activity levels and whey protein supplementation (Kerstetter et al., 2015). This body of research indicates that increasing protein intake is not detrimental to bone health and may actually have a beneficial effect on both calcium balance and bone health.

Type 1 collagen is the primary substance that makes up osteoid; the organic, un-mineralized element of bone. Type I collagen is secreted by osteoblasts in the formation of new bone (Zaitseva, Shandrenko, & Veliky, 2015). Bone-type 1 collagen formation is a dynamic, nutritionally modulated process (Babraj et al., 2005). Research using an infusion and bone

biopsy technique reported that type 1 collagen synthesis rates increased rapidly in response to intravenous infusion of a carbohydrate, fat, and complete amino acid mixture (Babraj et al., 2005). This dynamic response of type 1 collagen synthesis provides important evidence for a powerful influence of acute feeding on bone health. Essential amino acids as well as non-essential/ conditionally essential amino acids from dietary intake have been shown to be important for formation of and incorporation into, type 1 collagen in bone of rats (Jim, Jones, Ambrose, & Evershed, 2006). Lysine, an essential amino acid present in whey protein, was found to increase osteoblast proliferation and alkaline phosphatase activity in osteopenic human osteoblasts (Torricelli, Fini, Giavaresi, & Giardino, 2003). Lysine administration increased proliferation in osteoblasts from healthy bone (Torricelli et al., 2002), suggesting a possible role for lysine in the maintenance of the osteoblast cell lineage. One study (Bihuniak & Insogna, 2015) in older males reported that the addition of 45g of whey protein to their usual diet increased markers of bone formation and resorption at 9 months, and only resorption at 18 months of supplementation. IGF-1, which stimulates osteoblast activity, was increased in the serum of the whey protein group (Bihuniak & Insogna, 2015). In vitro and animal model studies have revealed that whey protein stimulates osteoblasts and has an inhibitory effect on osteoclasts (Y Takada et al., 1997). One of the beneficial effects of whey protein on the formation of bone is its influence on the maturation of osteoblasts. One study examined MC3T3-E1 pre-osteoblastic cells that were cultured in various concentrations of whey protein. The authors reported that proliferation and differentiation (maturation) of the MC3T3-E1 pre-osteoblastic cells were stimulated by whey protein (Yukihiro Takada, Aoe, & Kumegawa, 1996). Another cell culture model also reported that whey protein stimulation resulted in a dose-dependent increase in osteoblast differentiation and growth (Xu, 2009). Osteoblasts cultured in a whey protein-

enriched media also exhibit enhanced proliferation and an increased alkaline phosphatase activity; the latter plays an important role in the mineralization of new bone. Whey protein has also been reported to impact osteoclasts through the increased gene expression of osteoprotgerin (OPG) and receptor activator of nuclear factor-κ B ligand (RANKL). Osteoblasts cultured in a whey protein-enriched media also exhibit increased expression of both genes; although osteoprotgerin was increased to a greater extent than RANKL, resulting in a significant increase in the OPG/RANKL ratio (Xu, 2009). Osteoprotgerin is a competitive ligand for the RANKL binding receptor. When RANKL binds to its receptor it stimulates osteoclast maturation and function. Increases in OPG would displace RANKL and reduce the number of active osteoclasts, resulting in a reduction of the amount of bone resorption (Xu, 2009). Whey protein has been shown to be beneficial for bone health by reducing osteoclast number, which may be dose dependent in response to whey protein administered increases (Y Takada et al., 1997). Moreover, authors from the aforementioned study reported that the amount of bone area resorbed was reduced in whey protein cultured samples compared to those that lacked whey protein (Y Takada et al., 1997). Collectively, these findings suggest that whey protein could have beneficial effects on bone health by increasing the amount of bone formation and decreasing the amount of bone resorption. However, it should be noted that mechanistic in-vivo data in humans is lacking in relation to the effects of whey protein on bone health in young, healthy, active individuals.

The Impact of Whey Protein on Performance and Body Composition

The role of whey protein in adaptation to physical training has been extensively investigated in strength athletes. However, soldiers engaged in IET must successfully meet a unique combination of strength, functional, and endurance demands that stem from physical

training as well as occupational training (Henning, Khamoui, & Brown, 2011; JJ Knapik, Rieger, Palkoska, Van Camp, & Darakjy, 2009a). The current model of organized physical training is the Physical Readiness Training program (PRT) which consists of alternating days of performing cardiovascular training and muscular strength and endurance related exercises (JJ Knapik et al., 2009a). Required occupational training demands consist of job-specific duties such as load carriage, obstacle courses, combative exercises, ruck marching, land navigation (day and night), range and weapons skills, and other activities (Henning et al., 2011; JJ Knapik et al., 2009a). The combination of the multiple types of physical activity combined with long hours standing on hard surfaces create a very physically demanding environment. Training data indicate total distance covered during 15 weeks of One Station Unit Training (a type of Army IET training) were approximately 620 miles (J. Sefton, McAdam, et al., 2016). This high level of physical demand requires optimal nutrient intake to improve performance and to enable physiological adaptation to the demands of training.

The scientific literature collectively suggests that protein supplementation, especially whey protein, is beneficial for improvements in muscular strength and gains in muscle mass in response to training (Cermak et al., 2012; P. Cribb, Williams, Carey, & Hayes, 2006a; Jean Farup, Rahbek, Vendelbo, et al., 2014; Naclerio & Larumbe-Zabala, 2015). Whey protein in conjunction with resistance training was shown to be more beneficial than casein in muscle mass and strength gains after a 10 week resistance training program (P. Cribb et al., 2006a). Another study (Jean Farup, Rahbek, Vendelbo, et al., 2014) examined the effects of whey protein supplementation in comparison with a carbohydrate-matched placebo. These authors reported that whey protein supplementation during 12 weeks of resistance training significantly increased muscle and tendon cross sectional area, maximal voluntary contraction, and rate of force

production (Jean Farup, Rahbek, Vendelbo, et al., 2014). Whey protein hydrolysate has also been reported to be beneficial for stimulating gains in muscle cross sectional area and type II muscle fiber hypertrophy in comparison to a carbohydrate energy-matched placebo in response to concentric resistance training (J Farup, Rahbek, Riis, et al., 2014). Obese subjects who began an exercise program and consumed a 65g whey concentrate or 60g soy isolate shake before lunch had significant reductions in body fat percentage (9.2% whey, 3% soy), with the whey protein group also showing significant increases in lean body mass (4.1kg whey, 0.4kg soy-non-significant) (Tahavorgar, Vafa, Shidfar, Gohari, & Heydari, 2014). A similar study in obese individuals examined the effects of whey protein in conjunction with resistance training, or in conjunction with strength/cardiovascular training for 16 weeks (Arciero, Baur, Connelly, & Ormsbee, 2014). All groups experienced significant reductions in abdominal and visceral adipose tissue in relation to baseline. Both exercise groups also experienced non-significant increases in percent lean body mass (Arciero et al., 2014). It is important to note that caloric intake was restricted in these aforementioned studies, but lean mass losses were attenuated or prevented with whey protein consumption. Therefore, research suggests that during periods of energy restriction whey protein may be used to meet the increased protein needs for optimization of muscle mass to promote optimal adaptation (Arciero et al., 2014; E. Helms et al., 2014a). This effect has been demonstrated in non-obese individuals as well. Hydrolyzed whey concentrate was found to improve whole body nitrogen balance following 2 hours of cycling exercise, primarily by reducing muscle protein resorption (Howarth, Moreau, Phillips, & Gibala, 2009). Thus, whey protein could be beneficial for preservation of muscle mass and reductions in fat mass during voluminous exercise and energy-restricted conditions for soldiers engaged in IET.

Whey protein has also been reported to be beneficial for performance in endurance athletes. Two studies (M Hansen et al., 2015a; M. Saunders, Kane, & Todd, 2004a) examined the effects of whey protein on endurance performance and recovery. The first study conducted in elite male orienteering runners reported improved 4 km run time during a 1 week high intensity and high volume training camp, even though participants consumed adequate protein and carbohydrate at levels typically recommended for athletes (M Hansen et al., 2015a). This suggests that the improved recovery was due to the effects of the whey protein. The second study was conducted with cyclists performing exhaustive exercise. The authors reported that time to exhaustion was increased when consuming whey protein plus carbohydrate in comparison to carbohydrate alone (M. Saunders et al., 2004a). In both studies, creatine kinase levels (a marker of muscle damage) were found to be significantly lower in the whey protein/carbohydrate supplement group in comparison to those that consumed only the carbohydrate supplement (M Hansen et al., 2015a; M. Saunders et al., 2004a). Animal studies report similar results when assessing of the impact of whey protein supplementation on performance and recovery. One study (Chen, Huang, Chiu, Chang, & Huang, 2014) in endurance-trained mice revealed that endurance performance and grip strength were significantly increased, whereas creatine kinase levels were decreased following endurance swim training. Together these results suggest that whey protein supplementation can be beneficial for promoting recovery, improving performance, and preventing exercise-induced muscle damage when subjects engage in high volume endurance training.

Intramuscular Mechanisms Induced by Whey Protein Consumption

Rapid digestion and an increase in blood amino acid content is important for increasing muscle protein synthesis after exercise (West et al., 2011). It has been suggested that increases in plasma amino acid concentrations are more important for stimulating muscle protein synthesis than intracellular essential amino acid content (Bohé, Low, Wolfe, & Rennie, 2003). Whey protein is rapidly digested when consumed following exercise, rapidly increasing plasma amino acid content and muscle protein synthesis (Hulmi et al., 2010). In comparison to other proteins, such as soy protein isolate and casein, whey protein hydrolysate was found to significantly increase blood essential amino acid content at 30 and 60 minutes post exercise (J. Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009a). Blood leucine, an essential amino acid that stimulates anabolic processes, was significantly higher in the whey group compared to the soy isolate and casein group at 30 minutes post-ingestion. Furthermore, the leucine content in the blood remained higher in the whey protein group than in the casein group at 60 minutes' post-ingestion. Muscle protein synthesis was also increased post-exercise after whey hydrolysate supplementation in comparison to soy isolate and casein (J. Tang et al., 2009a). Other studies (Boutrou et al., 2013; Dangin et al., 2001; West et al., 2011) have reported rapid digestion of whey protein coupled with a high capacity for increasing blood amino acid content. Some research has theorized the existence of a post-exercise anabolic window (Aragon & Schoenfeld, 2013). The theory suggests essential amino acid delivery to exercised muscle must occur within a 0-2 hour window in order to optimize muscle protein synthesis and the adaptive response to resistance training (Aragon & Schoenfeld, 2013). In line with this theory, the rapid digestibility of whey protein may be beneficial for lean mass and performance enhancement. While this theory is still debated (Aragon & Schoenfeld, 2013; Schoenfeld, Aragon, & Krieger, 2013),

immediate post-exercise protein intake seems to be most beneficial after training sessions performed in the fasted state (Aragon & Schoenfeld, 2013). IET soldiers may benefit from protein supplementation, as morning physical training is performed in the fasted state before breakfast.

Whey protein also modulates the intramuscular mammalian target rapamycin which is a key regulator of muscle protein synthesis (Bodine et al., 2001). Increases in mTOR activity are related to hypertrophy of skeletal muscle and preservation of muscle from atrophy (Bodine et al., 2001). mTOR acts to phosphorylate p70 ribosomal S6 kinase1 and eukaryotic initiation factor 4E binding protein which are necessary for stimulating the assembly of ribosomal subunits and initiation factors for the initiation of protein translation (Laplante & Sabatini, 2009; Nicklin et al., 2009). Amino acid levels in cells must be maintained to meet the anabolic demands of the cell. In periods of low amino acid availability the cell must increase breakdown of intracellular protein components to replenish amino acid pools (Onodera & Ohsumi, 2005). This can lead to reductions in skeletal muscle mass due to increased breakdown of muscle proteins. As previously mentioned, essential amino acids (chiefly leucine) are an important and necessary stimulator of muscle protein synthesis (Dickinson et al., 2011; Tipton, Gurkin, Matin, & Wolfe, 1999; Volpi, Kobayashi, Sheffield-Moore, Mittendorfer, & Wolfe, 2003). The consumption of whey protein has been shown to activate mTOR signaling and subsequently increase muscle protein synthesis, possibly due to the high leucine levels found in whey protein (Bodine et al., 2001).

Whey protein consumption may also improve endurance performance through changes in energy substrate storage and other biochemical adaptations. It has been reported that muscle and liver glycogen stores, both critical for endurance performance, were significantly higher in rats consuming whey protein in comparison to casein after aerobic swim training (Morifuji, Sakai,

Sanbongi, & Sugiura, 2005). Whey protein has also been reported to improve the biochemical response to endurance training and improve endurance performance in mice (Chen et al., 2014). A human study has reported that peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) and AMPK mRNA expression were upregulated in cyclists exposed to high volume and intensity cycling exercise when consuming a whey protein and carbohydrate mix in comparison to carbohydrate intake alone (K. Hill, Stathis, Grinfeld, Hayes, & McAinch, 2013). PGC-1α is a potent regulator of mitochondrial biogenesis and AMPK is necessary for maintenance of mitochondrial content (K. Hill, Stathis, et al., 2013; O'Neill et al., 2011). Stimulation of both are key in promoting adaptation to endurance training, thus whey protein may be beneficial for endurance performance by elevating expression of these proteins (K. Hill, Stathis, et al., 2013; O'Neill et al., 2011).

Whey protein has also been shown to positively influence fat mass via tricarboxyl acid cycle stimulation. A metabolome-wide interrogation study reported that whey protein ingestion increased the urinary excretion of tricarboxyl acid cycle precursors and intermediates (Lillefosse et al., 2014), which reduce the presence of tricarboxyl acid cycle substrates known to stimulate new fatty acid synthesis (Martin & Vagelos, 1962). A similar rodent study reported that citrate excretion increased in mice fed a high fat diet and whey protein. Citrate has been demonstrated to be the most stimulatory tricarboxyl acid cycle intermediate in fatty acid synthesis, acting as a rate-limiting step in the acetyl CoA carboxylase reaction (Martin & Vagelos, 1962). Whey protein may also reduce fat mass through the manipulation of beta-3 adrenergic receptors (B_3-AR). B_3-AR are present on the surface of white and brown adipocytes and can be stimulated through ligand binding and/or via sympathetic nerve activity (Pilvi et al., 2008). Given that B_3-AR ligands increase lipolysis and thermogenesis there is recent pharmacological research

emphasis in developing B_3-AR agonists to combat obesity (McAllan et al., 2013; Pilvi et al., 2008; Sawa & Harada, 2006). Whey protein in conjunction with calcium supplementation has been reported to increase the mRNA expression of B_3-AR in mice that were fed high fat diets along with the whey protein supplement in comparison to mice fed a high fat diet alone (Pilvi et al., 2008). This finding was corroborated in an eight week feeding study in which three separate groups of mice were fed either: a) a low fat diet as a control; b) a high fat diet; and c) a high fat diet with whey protein supplementation. This study demonstrated that B_3-AR mRNA expression tended to be greater in the high fat whey-supplemented group and low fat diet group compared to the high fat diet group (McAllan et al., 2013). Thus, whey protein could possibly have beneficial effects on energy expenditure and whole-body adiposity.

Influence of Whey Protein on Immune System Function

Physical illness such as respiratory infections are the leading cause of outpatient medical visits. Physical illness are prevalent in IET settings as a consequence of large numbers of people living in close quarters and a combination of immunosuppressive factors (Korzeniewski, Nitsch-Osuch, Konior, & Lass, 2015). Physical illness has negative impacts on force readiness, soldier training, and medical costs. Thus, it is important to understand the factors that contribute to the risks of illness and develop strategies to combat them. High volumes of physical activity, (Jin, Paik, Kwak, Jee, & Kim, 2015) psychological stress, (Cohen, Tyrrell, & Smith, 1991; Dhabhar, 2014; Moreno-Villanueva & Bürkle, 2015) lack of sleep, (Wilder-Smith, Mustafa, Earnest, Gen, & MacAry, 2013) and inadequate nutrition (Kramer et al., 1997; P. Li, Yin, Li, Kim, & Wu, 2007) are all possible immunosuppressive factors that affect soldiers engaged in IET . One possible strategy to help combat immunosuppression and increased risk of illness is through

whey protein supplementation. The amount and amino acid composition of protein has been reported to influence immune system function (Jose & Good, 1973). Skeletal muscle has an increased demand for essential amino acids for musculoskeletal adaptations during high loads of physical activity, possibly reducing the available pool of amino acids for immune regulation and leading to post-exercise immunosuppression and overtraining (Bounous & Molson, 1999). Whey protein has a high essential and branched chain amino acid content that is essential for immune health. Thus, whey protein could be beneficial in providing additional substrates for both the musculoskeletal and immune systems.

The immune system consists of the innate and adaptive systems. The innate immune system is made up of a lineage of leukocytes that rapidly respond to infections and secrete cytokines (proteins released that communicate and interact with other cells) to recruit other immune cells to destroy pathogens. The innate immune system is the most rapid responder, is non-specific, and can occasionally lead to tissue damage (Parkin & Cohen, 2001). The adaptive immune system is the system most impacted by whey protein. The adaptive system functions by sensing pathogens and foreign chemical compounds that are introduced to the body. Adaptive system immune cells create/have complementary receptors called antibodies that bind to cellular structures on the membrane of pathogens which allows them to specifically bind to and destroy the pathogen (Bonilla & Oettgen, 2010). The primary cells of the adaptive immune system include B-lymphocytes, T-lymphocytes, and natural killer cells (Chaplin, 2010). B-lymphocytes produce antibodies that can bind to the antigen and promote destruction. They can also function to recruit complement proteins to help destroy the pathogen. T-Cells are responsible for cell-mediated immunity and respond to specific antigens. The three primary T-cell types include helper T-cells, killer T-cells, and regulatory T-cells (Powers, 2014). T-helper cells release

lymphokines that recruit other immune cells and promote proliferation, differentiation, and function of killer T-cells and B-cells. Killer T-cells recognize specific protein sequences on the surface of the antigen and use perforin to create holes in the membrane to destroy the pathogen (Hall, 2015). Regulatory T-cells function to prevent immune cells from attacking the body itself (Powers, 2014). It is important to note that once T-cells bind to antigens they undergo proliferation and differentiation, and are then released into circulation to increase the immune response to the pathogen (Hall, 2015). The adaptive immune system is made up of lymphocytes that have specific immunologic responses to bacterial and viral infections that promote destruction of pathogens and maintenance of homeostasis.

Nutrition is an important regulator of the immune system as it provides nutrients needed for optimal lymphocyte function (Bassit et al., 2002; Jose & Good, 1973; B. Koch, Schroder, Schafer, & Schauder, 1990). For instance, past research has demonstrated mice fed an amino acid-deficient diet devoid in phenylalanine-tyrosine, threonine, valine, tryptophan, leucine, isoleucine, and methionine cysteine present reductions in cellular and innate immune responses to tumor cells (Jose & Good, 1973). Research has also reported that leucine deficiency depresses the immune response when all other amino acid intake is normal. Increased leucine intake results in stimulation of the immune response (Jose & Good, 1973). This finding is reflected in the oxidation and transamination of leucine in the lymphocyte response to pathogens. Specifically, leucine transport, transamination, and oxidation, were found to increase by 267%, 194%, and 122%/182% respectively (122% in first step and 182% in the second step of leucine oxidation) in response to antigen stimulation of lymphocytes (B. Koch et al., 1990). Moreover, cellular investigations of leucine-stimulated immune cell activity suggest that leucine is necessary for stimulatory response of T-cells (Ananieva, Powell, & Hutson, 2016). The mechanism whereby

leucine modulates immune cell activity is likely due to its effects on mTOR signaling which is necessary for lymphoid progenitor differentiation into functioning T-cell lineages (Delgoffe et al., 2009; Delgoffe et al., 2011). Leucine, as well as other branched chain amino acids, have also been reported to be especially important for immune health in athletes. Ingestion of 6 g of branched chain amino acids was found to help maintain blood glutamine levels and increase proliferation of peripheral blood mononuclear cells leading up to either an Olympic triathlon or a 30 km run (Bassit et al., 2002). A study in triathletes (Bassit, Sawada, Bacurau, Navarro, & Rosa, 2000) reported that branched chain amino acid supplementation (6 g Leucine, 2 g valine, 2 g isoleucine) was sufficient to maintain blood glutamine levels and increase the lymphocyte response to the training. Furthermore, these findings were correlated to an approximately 34 % reduction in the symptoms of sickness in the branched chain amino acid group in comparison to the placebo group. The aforementioned findings are physiologically-relevant because high volumes and intensities of exercise have been shown to increase susceptibility of endurance athletes to infection, and this risk has been related to the reduction of plasma glutamine (Bassit et al., 2000).

Interleukin 6 (IL-6) is a cytokine that is produced in a variety of cells including neurons of the central nervous system, immune cells, and skeletal muscle (Agorastos et al., 2014). Overall, IL-6 is a key player in the initial response of the immune system. It functions to coordinate the initial response of neutrophils and macrophages, serve as the mediator between the innate and adaptive immune response, as well as initiate the acute phase response (APR) (Kaplanski, Marin, Montero-Julian, Mantovani, & Farnarier, 2003). IL-6 binds to a soluble or membrane-bound IL-6 receptor which initiates an intracellular signaling cascade (Reihmane & Dela, 2014). The IL-6 receptor consists of three main structures: 1) the alpha subunit, which

binds directly to IL-6; 2) two membrane proteins that anchor it to the cell; and 3) a beta subunit called gp130, which extends across the membrane and transduces the signal to initiate the intracellular outcome of IL-6 binding (Reihmane & Dela, 2014; Tanaka, Narazaki, & Kishimoto, 2014). Classical IL-6 signaling occurs when IL-6 binds to the membrane bound IL-6 receptor and initiates intracellular effects (Kaplanski et al., 2003). This receptor is found primarily in immune cells. Most non-immune cells typically express only the gp130 beta subunit and interact with the soluble form of the IL-6 receptor in a process called trans-signaling (Choy & Rose-John, 2017; Kaplanski et al., 2003). The soluble IL-6 receptor circulates in the blood under normal conditions. During trans-signaling, IL-6 is released into circulation, it binds to the soluble receptor in the serum and migrates to the tissues where it binds to the gp130 subunit and initiates intracellular signaling (Kaplanski et al., 2003). The influence of IL-6 on cells varies greatly and depends on the method of signaling (classical or trans), the physiologic context of IL-6 release, and the tissue type (Schmidt-Arras & Rose-John, 2016). IL-6 acts on most cells via trans-signaling, as most express the gp130 beta subunit that binds the soluble IL-6 receptor (Agorastos et al., 2014).

The innate immune system detects pathogens (i.e. virus or bacteria). Neutrophils invade the infected area and begin to phagocytize the pathogens (Nordenfelt & Tapper, 2011). During this process neutrophils proteolyze the membrane bound IL-6 receptors and release the soluble IL-6 receptor (McGreal et al., 2010). The trans-signaling rate increases, playing a critical role in initiating the inflammatory process and transitioning to the adapted immune response (Kaplanski et al., 2003). IL-6 trans-signaling attracts monocytes and stimulates differentiation into macrophages, which have a much higher phagocytic capacity than neutrophils (Hall, 2015; Mitani et al., 2000). Neutrophils and macrophages then release IL-6 (Y. Li, Hsieh, Tang, Liao, &

Yeh, 2009; Schmidt-Arras & Rose-John, 2016). IL-6 plays a primary role in activation of the APR to pathogens and serves as the mediator of the transition from innate to acquired immune response (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Past research has revealed that knockout of the IL-6 gene results in an impaired APR infection and tissue damage (Kopf et al., 1994).

IL-6 then circulates to the liver where it stimulates the synthesis of APR proteins such as C - reactive protein (CRP), serum amyloid A, and fibrinogen (Schmidt-Arras & Rose-John, 2016). These proteins initiate the inflammatory response to pathogens by improving the functionality of immune cells to adhere to pathogens, recruit other immune cells, and activate the compliment system (Gabay & Kushner, 1999). More directly, IL-6 increases the function and differentiation of B and T cell lymphocytes (Dienz & Rincon, 2009; Yang et al., 2016). IL-6 stimulates CD4 T-Cells to differentiate into specific T-helper cells that interact with B-Cells to stimulate the secretion of antibodies (Dienz & Rincon, 2009; Yang et al., 2016). IL-6 also prevents the differentiation of CD4 T-Cells into T regulatory cells that inhibit the immune response (Dienz & Rincon, 2009; Yang et al., 2016). The primary influence of IL-6 on B-Cells is stimulating naïve B-cells to differentiate into anti-body secreting B-cells (Yoshizaki et al., 1984), and has been reported to be required for B-Cell differentiation (Klashman, Martin, Stevens, & Martínez-Maza, 1991).

Exercise induces a similar immune system response, especially under intensities producing large disruption in homeostasis. One study using plyometric training induced a significant increase in IL-6, CRP, and leukocyte count in the blood (Chatzinikolaou et al., 2010). Another study found IL-6 increased significantly within 30 minutes post exercise and return to baseline one day following an ironman triathlon, followed by a significant increase in CRP and

serum amyloid A (Suzuki et al., 2006). The acute phase response to exercise is aimed at restoring homeostasis and focusing on repairing exercise damaged tissues, which may differ depending on exercise type. Endurance exercise elicits an APR to restore tissue that has been damaged due to free radical production (Aoi et al., 2004), whereas ballistic or eccentric exercise results in mechanical disturbances in the muscle that lead to muscle damage (Chatzinikolaou et al., 2010). In either case, macrophages and neutrophils are the first responders to the damaged tissue, migrating from the blood into the tissue and begin phagocytizing the damaged tissue (A. Koch, 2010; Tidball & Villalta, 2010). The initial responders then release IL-6 and other cytokines, to initiate the inflammatory response. The increase in IL-6 stimulates release of metalloproteinases and recruitment of macrophages to the damaged tissue, which function to breakdown connective tissue and promote remodeling (Kossakowska et al., 1999; Reihmane & Dela, 2014). IL-6 plays an important role in repair and adaptation of skeletal muscle by stimulating proliferation of myogenic stem cells that function to repair skeletal muscle (Mitani et al., 2000). Knockout of IL-6 has been shown to reduce the ability of muscle to hypertrophy in response to overload (Tidball & Villalta, 2010). It is important to note that although IL-6 is released in response to muscle damage, it is a lower grade response that may be prolonged for a one to four days (Pedersen, Steensberg, & Schjerling, 2001). This is a lower response compared to the APR of the immune system to a pathogen.

IL-6 is also released in response to exercise that does not result in muscle damage and serves non-inflammatory roles during exercise. IL-6 is responsive to glycogen content and energy sensing (Keller et al., 2001). IL-6 has been shown to serve as a signaling mechanism to the liver, insuring substrate is available in contracting skeletal muscle for energy metabolism. It may also function to increase glucose output from the liver (Reihmane & Dela, 2014). IL-6 also

circulates to adipose tissue to stimulate lipolysis and subsequent release of free fatty acids to be circulated to the tissues for metabolism (Pedersen et al., 2001). Furthermore, IL-6 acts to promote glucose uptake at the muscle, further supporting its role in energy metabolism during exercise (Pedersen et al., 2001). Exercise induced increase in IL-6 is dependent upon intensity and duration of exercise (Gholamnezhad, Boskabady, Hosseini, Sankian, & Rad, 2014). The largest response in IL-6 occurs during long duration endurance exercise, such as marathon running. Shorter duration, higher intensity exercise seems to have an effect on post-exercise IL-6 levels, but to a lesser extent than long duration endurance exercise (Reihmane & Dela, 2014). IL-6 response to moderate volumes of exercise training seems to be acute, and decline within 24-48 hours of post training (Chatzinikolaou et al., 2010; Suzuki et al., 2006). In contrast to homeostatic release of IL-6, in overtraining models IL-6 has been reported to remain elevated for 2 weeks and is likely the result of macrophage and neutrophil penetration into damaged tissue (Gholamnezhad et al., 2014).

In summary, IL-6 serves a wide range of roles in the body that are inflammatory, anti-inflammatory, and energy sensing. Under normal conditions IL-6 increases with exercise but subsequently decreases during the following 24-hour period. In individuals experiencing overtraining or muscle damage, it may persist as low-grade inflammation. This should be considered in interpretation of the results. It is likely that elevated levels of circulating IL-6 would be reflective of the status of the immune system instead of a training reaction due to the acute nature of the IL-6 response to an exercise bout. However, exercise training that results in muscle damage or overtraining can result in persistent, low-grade inflammation and release of IL-6. Thus, reductions in circulating IL-6 levels between groups may be reflective of either

improved immune function or improved recovery if training volume is high enough to result in overtraining.

Clearly nutrition is necessary to support adequate immune response to pathogens and proper functioning of the immune system (P. Li et al., 2007). Essential amino acid intake and, more specifically, branched chain amino acids are necessary for supporting optimal humoral and cellular responses to pathogens. Whey protein has been widely recognized for its high essential and branched chain amino acid content, both beneficial for lymphocyte and neutrophil immune health. Thus, whey protein could be beneficial from an immune perspective as soldiers engaged in IET experience a wide range of stressors that could impair the immune system.

Conclusions and Purpose Statement

Optimal health and performance of soldiers is a multifaceted topic that must focus on optimization of nutrition, training, and physical health to improve force readiness and to reduce the financial burden of medical discharge and attrition (A. Medicine, 2015). Currently, not enough is known about the energy requirements of IET or current nutritional intakes of IET soldiers to optimize nutritional recommendations. Furthermore, the nutritional and supplemental research has been completed predominately in endurance or power athletes whereas tactical athletes have demands that include functional, endurance, strength and precision requirements. Whey protein has a high biological value due to its high essential amino acid content, rapid digestibility, and absorbability (Bos et al., 2000; Boutrou et al., 2013; Moore & Soeters, 2015). Whey protein has shown promise as a supplement to improve muscular adaptation to training (Cermak et al., 2012; Naclerio & Larumbe-Zabala, 2015), reduce fat mass (Naclerio & Larumbe-Zabala, 2015), increase in lean mass (Cermak et al., 2012; Jean Farup, Rahbek, Vendelbo, et al.,

2014; Naclerio & Larumbe-Zabala, 2015), improve bone health (Yukihiro Takada et al., 1996; Y Takada et al., 1997), and improve in immune health (Beaulieu, Dupont, & Lemieux, 2006; Middleton, Jelen, & Bell, 2004; Pacheco & Sgarbieri, 2005). There is no research study to date that has supplemented soldiers during IET with whey protein and examined these health and performance benefits.

Chapter 3 Methodology:

An overview of the methodologies employed in this dissertation is presented in this chapter. Detailed methodologies, sample sizes and participant demographics for each specific project are provided in subsequent chapters.

Design

Study design for investigating training volume and dietary intake alone was non-experimental, descriptive. The study design for investigating whey protein supplementation was a double-blind, placebo-controlled, clinical trial.

Overall Methods

Participants were divided into two supplement groups, a whey protein group or a carbohydrate control group based on platoon, The bottom floor of the barracks (platoons 1 & 2) were given one supplement and the top floor (platoons 3 &4) were given the other supplement. This protocol was designed to simplify distribution and to prevent IET soldiers from switching supplements. The nutritional profile and amino acid content of both supplements were third-party tested by Covance Laboratories, Inc. (Madison, WI, USA) to verify identity, purity, potency, and composition of the packets. One WP serving provided 293 total kcals; consisting of 38.6 g of protein [Power Crunch® ProtoWhey® (BioNutritional Research Group; Irvine, CA, USA) as agglomerated, partially hydrolyzed (12.5% degree of hydrolysis) 80% whey protein

concentrate (Hilmar® 8360; Hilmar Ingredients, Hilmar, CA USA), 19.0 g carbohydrates, 7.5 g fat, and 20.1 g and 9.5 g of essential and branched chain amino acids. One CHO serving provided 291 total kcals, 0.5 g protein, 63.4 g carbohydrates, 3.9 g of fat, and 0.1 g and 0.0 g essential and branched chain amino acids.

IET soldiers were asked to ensure that the entire supplement packet was emptied into the shaker bottle and to consume the entire serving. Each week members of the research team visited the company participating in the study multiple times to speak with the drill sergeants, assess any problems, and to evaluate the study progression and compliance.

Measures for body composition, anthropometry, and performance and blood draws were collected during weeks one and nine of training. The initial measures were conducted on the morning of day 3 of IET. Measures were collected before morning breakfast to ensure IET soldiers were in an overnight fasted state. Participants provided a urine sample for urine specific gravity testing to insure they were properly hydrated. Those in a dehydrated state (urine specific gravity greater than 1.03) were provided with water and re-tested to ensure hydration prior to testing. IET soldiers providing blood had blood draws taken by a phlebotomy trained member of the research team

Consenting

The Auburn University Institutional Review Board (protocol number:15-502 MR 1512), Army Institutional Review Board, and the Director, Research & Analysis Directorate Army Center approved the study procedures for IET . Participants were briefed on the study by members of the research team on the third day of IET training. IET soldiers interested in participating were asked to fill out a Warrior Research Center health questionnaire and IRB informed consent form.

IET soldiers that preferred not to submit blood samples were allowed to participate, and IET soldiers providing blood samples signed an extra consent sheet as required by the IRB. Participants were healthy male, 18-35-year old's engaged in army IET. Participants who participated in supplementation had to be at least 18 years of age, healthy with no apparent disease or MSI, participating in IET, free from allergy to milk or whey proteins, and not supplementing or have supplemented within the past 3 months. Inclusion criteria for individuals who participated in assessment of training volume and dietary analysis were apparently healthy males participating in IET, at least 18 years of age.

Measures

Urine specific gravity: Hydration was assessed using a handheld refractometer (Manual, Atago, Tokyo Japan). Urine specific gravity below 1.03 was considered properly hydrated (Fukuda et al., 2016)

Blood Samples: Blood draws were taken from the antecubital vein via 21 gauge, Safety-Lok needle kits (Benton, Dickinson and Company, Franklin Lakes, NJ. USA) by research team members certified by the Auburn University phlebotomy training course. Blood was collected in 10 ml serum separator vacutainer tubes (BD Vacutainer; Franklin Lakes NJ, USA) and placed on ice in a cooler (Yeti Coolers LLC, Austin TX, USA) until centrifugation the same morning of collection. The blood samples were centrifuged at 3,500xg for 10 minutes at room temperature. Samples that were not fully separated were centrifuged again under the same conditions. Serum was extracted from separated blood and frozen at -80 degrees (Kendra Laboratory Products, Asheville, NC. USA) until analysis. Testosterone (American Laboratory Products Company, Salem, NH, USA, sensitivity: 0.022 ng/mL, CV: 2.9%), cortisol (American Laboratory Products

Company, Salem, NH, USA, sensitivity: 0.4 μg/dL, CV: 4.8%), IGF-1,(American Laboratory Products Company, Salem, NH, USA, sensitivity: 0.091 ng/mL, CV: 10.5%), C-terminal cross-links of type 1 collagen (CTX, Immunodiagnostic Systems, Gaithersberg, MD, USA, CV: 5.2%) and IL-6 (Invitrogen, Carlsbad, CA, USA, sensitivity: 0.3 pg/ml, CV: 7.1%), were measured using Enzyme Linked Immunosorbent Assays (ELISA), according to manufacturers' instructions. Plates were at respective wavelengths using a multispectral spectrophotometer (BioTek Eon, Winooski, VT, USA). All samples were analyzed in duplicate and each participant's pre- and post-intervention samples were analyzed on the same plate. All optical densities were within the detectable range of the assay. IL-6 had four individuals whose concentrations could not be used due to being outside the normal physiologic range for the four compartment logistic regression models and were removed from the analysis. Serum concentrations of each optical density was calculated as per manufacturer instructions using either regression or a four-parameter logistic regression. P1NP was evaluated using chemilumnescence.

Anthropometrics: Height and weight was recorded with IET soldiers wearing only Army issued physical training shorts, socks and underwear using a Health-O-Meter professional scale (Model 500KL, Sunbeam products INC. Boca Raton FL. USA). Participants stood on the scale with their back to the height rod. Then the rod was lowered until the headpiece rested on the crown of the participant's head to measure height (cm). Weight was recorded in kilograms to the tenth position.

*Skinfolds***:** Skinfold measures were taken using a baseline skinfold caliper (Fabrication Enterprises, White Plains, NY USA) as per the American College of Sports Medicine's protocol for 7-site skinfold measures (seven-sites: chest, mid-axilla, abdomen, suprailiac, subscapular,

triceps, and thigh) (G. Dwyer & Davis, 2008a). The American College of Sports Medicine 7-site body density calculation (A. C. o. S. Medicine, 2010) was used to estimate body fat percentage:

1. Body Fat Percent = ((457/body density)-414.4.2)/100
2. Body Fat Mass (BFM) = Body Fat Percent * Weight (kg)
3. Fat Free Mass (FFM) = Body Mass – BFM

*Bioelectrical impedance***:** Body composition was assessed with IET soldiers in a fasted and hydrated state using an ImpediMed DF50 bioelectrical impedance device (ImpediMed Ltd, Brisbane, Australia). Participants laid on a table for approximately four minutes for equilibration of body fluids. Hair was shaven from the right hand and foot to promote optimal electrode contact with the skin. Two electrodes were placed on the hand and two electrodes were placed on the foot. Raw values (R-resistance, Xc-reactance) were collected and entered in the formula below to calculate FFM and FM.

1. FFM = $(\text{Height})^2$ / Resistance * 0.734+ Body Mass * 0.116 + Reactance * 0.096 + 1 * 0.878 - 4.03
2. Fat Mass = Body Mass – FFM

*Circumferences***:** Circumferences were measured at the neck, umbilicus, and hip using a Gullick measuring tape (Fabrication Enterprises, White Plains, NY. USA). The neck measurement was taken at the largest portion of the laryngeal prominence. Umbilicus measures were taken at the level of the umbilicus and hip measures were taken at the largest portion of the gluteus maximus (G. Dwyer & Davis, 2008a).\

Physical activity data

IET soldiers wore Actigraph wGT3X monitors (Actigraph, Pensacola, FL, USA) in one week increments. Monitors were initialized using Actilife software version 13.1.1 (Actigraph, Pensacola, FL, USA) and given to IET soldiers each Sunday. Participants were asked to wear the monitors at all times (except in the shower), around the waist, on the right side of the body. Monitors were given to 12 (two shakes per day) or 20 (one shake per day) soldiers and collected after one week. Upon collection monitors were given to 12 or 20 new soldiers each week. Data were downloaded and age, height, weight, ethnicity, and hand dominance, were entered for each subject. Data were evaluated using Actilife software version 13.1.1 (Actigraph, Pensacola, FL, USA) and were used to estimate physical activity levels, training volume, active energy expenditure, and training intensity. Freedson cut-points were used to divide activity into four categories: sedentary, low, moderate, and high intensity (Migueles et al., 2017; Sasaki, John, & Freedson, 2011) . Values were then averaged for day, week, and phase of training in total minutes and percent of day in each level of activity intensity.

Diet logs

Dietary intake was recorded after each meal on three non-consecutive days (Tuesday, Thursday, and Saturday) during the first full week of IET. Food menus from the cafeteria-style dining facility serving the IET soldiers were used to create meal specific diet logs (Appendix A) containing meal specific food items and serving sizes for each food item. Study staff provided guidance on identifying foods and quantifying portions prior to their first recorded meal. Items on the salad and fruit bars were measured in units relating to hand size whereas items explicitly listed on the dining facility menu were measured in serving sizes (e.g., Lasagna-scoop). To assist participants a document was provided that contained written and visual representations of the

relationships between food portion sizes relative to the hand. The IET soldiers were asked to circle the food item and portion they consumed. A member of the research team met the participants at the company barracks immediately following each meal to administer and obtain the diet logs.

Macronutrient and select micronutrient data for the dining facility foods were accessed from the Army Joint Culinary Center of Excellence (JCOE) website (Army, 2012). Food items not found on the JCOE menu were retrieved from the US Department of Agriculture (USDA) nutrition data base (United States Department of Agriculture, 2013). Dietary intakes and nutrient information were entered into customized excel spreadsheets (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA), and checked by two researchers to ensure data accuracy. Dietary intake calculations were completed using R statistical software (R Core Team, 2015) and R Studio (RStudio, 2014). R programming packages, dplyr (Wickham, Francois, Henry, & Müller, 2017), tidyr (Wickham & Henry, 2017), reshape2 (Wickham, 2007) ez (Lawrence, 2016), car (Fox & Weisberg), vars (Bernhard, 2008), ggplot2 (Wickham, 2009). Total calorie, protein, fat, carbohydrate, cholesterol, and sodium intakes were obtained for each meal and each day. Dietary intakes were then averaged across each day of diet logs to calculate daily averages. Participants who completed at least two full days of diet logs were used in calculation of average daily intakes and energy balance. However, for the statistical comparison of dietary intake at each meal, only participants who completed all three meals were used in the analysis.

Injury and Illness

Each platoon maintains logs of injury (diagnosed by Licensed, Certified Athletic Trainers) and illness that require medical attention. These records were provided to the primary

investigator. A member of the research team spoke with each IET soldier who sustained an injury or illness during training to record: supplement group, illness/injury type, training days missed, health professional visited, injury type, mechanism of injury, and injury date. Data from those who sustain injuries that require them to discontinue IET was used in analysis up to the point of their cessation of training to evaluate injury by treatment interactions.

Performance

Drill sergeants conducted performance assessments and provided this information to the primary investigator. The performance measures included the 1-1-1 Army initial fitness test (1-mile run, 1-minute pushup, and 1-minute sit-up test), and the Army Physical Fitness Test (APFT-2 mile run, 2-minute pushup, and 2-minute sit-up test) (DOD US Army, 1998)

Push-up test. The participants were in in a forward leaning position.

1. The body was positioned in a straight line from the ankles to the shoulders and must be maintained throughout the push up.
2. Feet were no more than 12 inches apart
3. Participant lowered himself until his elbows are at least parallel to the ground and must extend his elbows fully to complete a repetition.

*Sit up test***.** Participants began with their backs on the ground with their knees bent at a 90-degree angle with feet held by a fellow IET soldier.

1. Participants' raised themselves upward until the base of the neck positioned superior to the vertical axis of the spine in the upright position.

2. Participants' lowered themselves until the scapulae have made contact with the ground
3. Feet were no wider than 12 inches apart
4. Hands behind the head
5. Heels and feet must maintain contact with the ground

Each correctly performed repetition in the sit-up and push-up test, the scorekeeper stated the number performed. Failure to maintain proper form listed above resulted in the repetitions not being counted towards the participant's total.

Run test. Each IET soldier was assigned a number to be worn during the run test. The cutoff time for passing the run test was 8:31 (511 seconds), any slower than this is considered failing. A supervisor and a scorekeeper administered the run test and recorded the results. Once the participant completed the test and crossed the finish line, the supervisor stated the time and the scorekeeper recorded it. The test was performed on a flat surfaced running track.

Statistical Analysis

Descriptive analysis was used to evaluate training volume and energy balance (chapter 4). Data is presented as mean ± standard deviation. The study consisting of one shake per day, regression analysis was used to determine if phase of training was a significant predictor of time spent in training.

Analysis of variance (ANOVA) was used compare diet and serum markers between groups and across time of the intervention. The assumption of normality of residuals testing was

completed for all variables using shapiro-wilks, komolgorov smirnov tests, and residual QQ plots were used to visually inspect the data. Data was square root transformed and normality was recalculated for any variable for which more than 75% of the levels were non-normally distributed. An a *priori* alpha level of 0.05 was set for determination of significant effects. Maulchy's test of sphericity was used to evaluate equality of variance and Levene's test was used to evaluate homogeneity of variance. If sphericity was violated a Greenhouse-Geisser correction was used. Group by time interactions were further evaluated using paired samples t-test to evaluate simple main effects of time and independent samples t-tests were used to evaluate simple main effect of group.

Analysis of covariance (ANCOVA) was used to evaluate performance and body composition during performance. ANCOVA has been reported to increase sensitivity to group effects, which was the focus of this investigation. Mean centered initial values for each variable were used as the covariate in the ANCOVA model as this reduces the error rate in ANCOVA models with between group factors.

Cohen's d effect sizes were calculated for each variable across training (pre- vs. post-intervention). Effect sizes were classified as small ($d < 0.2$), medium ($d > 0.21$, $d < 0.5$), or large ($d > 0.51$), and the results are presented as Cohen's d effect size estimate. Mean differences and 95% confidence intervals of the mean differences were calculated.

- Effect Size = mean(post) - mean(pre)/pooled standard deviation
- Pooled standard deviation = Square root((SD (pre)^2 + SD (post)^2)/2)

Chapter 4: Estimation of Energy Balance from Quantification of Training Volume and Dietary Intake across 14 Weeks of Army Initial Entry Training.

INTRODUCTION

Initial Entry Training (IET) is a mentally and physically demanding military training course designed to transform civilians into soldiers. This transformation from civilian to soldier has become more challenging as the ability of IET soldiers to respond and adapt to initial military training has declined over recent decades. The American population has become less active due to changes in lifestyle factors such as increases in screen time in the place of physical activity, reductions in physically active jobs, and reduced active transportation (e.g., walking, riding bikes) (Brownson et al., 2005). Consequently, fitness levels of civilians entering IET are lower as failure rates on the initial fitness assessment have increased, which have been shown to increase occurrence of musculoskeletal injuries (MSI) (Jones et al., 1993; Jones et al., 2002; Lisman et al., 2013; J. Molloy et al., 2012a; Plavina, 2004; Shaffer et al., 1999). MSI are detrimental to soldier health and wellness and subsequently force readiness, and IET injuries alone are estimated to cost the United States approximately $384 million per year (J. Molloy et al., 2012a; Teyhen, 2014).

IET soldiers perform organized physical fitness training (PT) as well as occupational physical activity to improve IET soldier fitness and learn soldiering skills (J Knapik et al., 1990; JJ Knapik et al., 2009a; Sharp, Patton, & Vogel, 1996). PT is designed to expose IET soldiers to progressively increasing levels of physical training to improve endurance and strength (JJ

Knapik et al., 2009a). Occupational physical activity consists of the tactical and survival drills that will enable IET soldiers to carry out his or her duties for the completion of successful missions. These physical and cognitive efforts must be adequately fueled through adequate nutrition in order to optimize IET soldier performance.

Rapid increases in training volume and intensity are known to contribute to higher MSI rates (Garber et al., 2011). Army training doctrine, such as physical readiness training, has sought to periodize fitness training so that volume and intensity are gradually increased (JJ Knapik et al., 2003). However, few investigations have quantified overall training volume that includes occupational physical activity. One study quantified steps and distance covered by soldiers during IET and observed that those who were more active during basic combat training had a higher risk of MSI (JJ Knapik et al., 2011). To date, only one study has investigated intensity of training during basic combat training. These authors reported the average activity of IET soldiers over a seven week period as follows: 144 minutes in low intensity, 93 minutes in moderate intensity, 131 minutes in moderately high and approximately 37 minutes per day in high intensity physical activity (Redmond et al., 2013). One limitation of this study is that monitors were removed at dinnertime, and researchers did not quantify activity during the evening, thus likely underestimated training volume.

Dietary intake must match training volume to fuel physiological demands. Inadequate energy is detrimental to bone health (Ihle & Loucks, 2004; Zanker & Swaine, 2000), immune health (Kramer et al., 1997; P. Li et al., 2007), cognitive performance (Cherif, Roelands, Meeusen, & Chamari, 2016; Green, Rogers, Elliman, & Gatenby, 1994), as well as exercise performance in physically active populations (Logue et al., 2018; Oliver, Laing, Wilson, Bilzon, & Walsh, 2007). Research conducted on the dietary needs of active individuals has primarily

focused on power or endurance training populations. IET soldiers are required to complete strength, power, endurance and functional training which suggests IET soldiers have unique fueling needs due to the large variety in training intensity and duration. IET soldiers likely require the higher protein needs of strength and power athletes as well as the higher carbohydrate and fat intake needs of endurance athletes. Current recommendations for macronutrient intakes for athletic populations participating in large volumes of training are as at least 1.2-2 (optimally at least 1.5) grams per kilogram of body weight per day of protein (Jager et al., 2017; D. Thomas et al., 2016a), 6-9 grams per kilogram of body weight of carbohydrate (Burke, Hawley, Wong, & Jeukendrup, 2011; Kreider et al., 2010; D. Thomas et al., 2016a), and between 25-35% but not below 20% of overall calorie intake from dietary fat per day (D. Thomas et al., 2016a). Protein intake is crucial for provision of amino acid pools to support cellular adaptations of skeletal muscle such as hypertrophy, mitochondrial protein turnover (K. Hill, Stathis, et al., 2013; Rowlands et al., 2011), repair of muscle damage caused by training (M Hansen et al., 2015a; M. Saunders et al., 2004a), bone health (Bihuniak & Insogna, 2015; Heaney & Layman, 2008; Torricelli et al., 2003) and improved performance (D. Thomas et al., 2016a). Carbohydrate and fat intake are key to providing sufficient caloric intake, serving as primary fuels during physical activity. Dietary carbohydrate is stored in skeletal muscle in the form of muscle glycogen, which serves as an important substrate for metabolism during exercise. Diets high in carbohydrate intake are associated with increased power output (Rauch et al., 2005; Simonsen et al., 1991), prolonged time to exhaustion (Bergström et al., 1967), and overall increases in exercise performance (Simonsen et al., 1991). Dietary fat is also an important substrate during exercise as it provides substrates to the muscle mitochondria via beta-oxidation necessary for aerobic metabolism. Studies in which lower fat diets (approximately 15-18% of overall intake) were

consumed reveal impaired endurance performance (Hoppeler et al., 1999; Horvath et al., 2000; Venkatraman et al., 2001) and inability of individuals engaged in high volumes of training to consume adequate energy intake to match caloric expenditure (Horvath et al., 2000; A. Loucks, 2007).

Clearly understanding training volume and nutrition intakes of IET soldiers is critical in ensuring training success. However, few studies to date have examined these areas in the IET environment, and none have examined both factors together in combat arms IET soldiers. Thus, the purpose of this study was to quantify total training volume and dietary intake during as well as estimate energy expenditure and energy balance in IET soldiers. Additionally, we sought to determine how dietary intake of IET soldiers compare to the recommendations of the joint statement of the: Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine (D. Thomas et al., 2016a).

METHODS

The Auburn University Institutional Review Board, Army Institutional Review Board, and the Director, Research & Analysis Directorate Army Center approved the study procedures for Initial Military Training. For inclusion criteria, participants had to be at least 18 years of age, healthy with no apparent disease or MSI, and participating in military training. Interested potential participants from one company received verbal explanation of the study from the study team and provided written consent. One hundred eleven IET soldiers (mean ± SD: age: 19 ± 2 yrs., height: 172.6 ± 5.8 cm, mass: 72.2 ± 12.5 kg) from one company of IET soldiers at Fort Benning, Georgia volunteered for this investigation.

Study design

This study was 14 weeks in duration. Diet analysis and body mass information was collected during the first week of IET. Physical activity data was collected daily beginning at week two and ended during week 14 of IET training.

Body mass measurement

Anthropometric measures were conducted in a fasted state prior to morning physical training and breakfast on the morning of day three of IET. Urine specific gravity was evaluated the morning of testing using a handheld refractometer (Manual, Atago, Tokyo, Japan) to ensure participants were properly hydrated (USG below 1.03) (Fukuda et al., 2016). Height and weight were recorded with IET soldiers wearing only army issued physical training shorts, socks and underwear using a Health-O-Meter professional scale (Model 500KL, Sunbeam Products, Inc. Boca Raton, FL, USA).

Diet Logs

Dietary intake was recorded after each meal on three non-consecutive days (Tuesday, Thursday, and Saturday) during the first full week of IET. Food menus from the cafeteria-style dining facility serving the IET soldiers were used to create meal specific diet logs (Appendix A) containing meal specific food items and serving sizes for each food item. Study staff provided guidance on identifying foods and quantifying portions prior to their first recorded meal. Items on the salad and fruit bars were measured in units relating to hand size whereas items explicitly listed on the dining facility menu were measured in serving sizes (e.g., Lasagna-scoop). To assist participants a document was provided that contained written and visual representations of the

relationships between food portion sizes relative to the hand. The IET soldiers were asked to circle the food item and portion they consumed. A member of the research team met the participants at the company barracks immediately following each meal to administer and obtain the diet logs.

Diet Log Analysis

Macronutrient and select micronutrient data for the dining facility foods were accessed from the Army Joint Culinary Center of Excellence (JCOE) website (Army, 2012). Food items not found on the JCOE menu were retrieved from the US Department of Agriculture (USDA) nutrition data base (United States Department of Agriculture, 2013). Dietary intakes and nutrient information were entered into customized excel spreadsheets (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA), and checked by two researchers to ensure data accuracy. Dietary intake calculations were completed using R statistical software (R Core Team, 2015) and R Studio (RStudio, 2014). R programming packages, dplyr (Wickham et al., 2017), tidyr (Wickham & Henry, 2017), reshape2 (Wickham, 2007) ez (Lawrence, 2016), car (Fox & Weisberg), vars (Bernhard, 2008), ggplot2 (Wickham, 2009). Total calorie, protein, fat, carbohydrate, cholesterol, and sodium intakes were obtained for each meal and each day. Dietary intakes were then averaged across each day of diet logs to calculate daily averages. Participants who completed at least two full days of diet logs were used in calculation of average daily intakes and energy balance. However, for the statistical comparison of dietary intake at each meal, only participants who completed all three meals were used in the analysis. In total, 85 participants completed all three days of diet logs (included in analysis), and 26 participants completed only two days of diet logs.

Physical Activity Assessment

Participants were outfitted with Actigraph wGT3X monitors (Actigraph, Pensacola, FL, USA) to evaluate training volume, training intensity, and active energy expenditure over the 14-week intervention. Monitors were initialized using Actilife software version 13.1.1 (Actigraph, Pensacola, FL, USA). Each week of training a member of the research team met with 12 IET soldiers (3 per platoon) to instruct them to wear the monitors around the waist, on the right side of the body, at all times (awake and asleep), and to only remove for showering. At the end of each week the monitors were collected and age, height, weight, ethnicity, and hand dominance were entered into the Actilife software for each subject. Sasaki Vector Magnitude 3 cut points were used were used to divide activity into three categories: moderate (2690-6166 counts/minute), vigorous (6167-9642 counts/minute) and very vigorous (> 9,642 counts/minute) (Sasaki et al., 2011). A cut-point was added to delineate less than moderate physical activity into sedentary and light intensity physical activity (< 200 counts/minute = Sedentary, 201-2689 counts/minute = Light) which has been used in previous research as a cut point to delineate sedentary time from vector magnitude 3 data (Migueles et al., 2017). Values were averaged for day, week, and phase of training in total minutes and percent of day in each level of activity intensity. The monitors collected data at the recommended 30 Hz sampling rate (Brond & Arvidsson, 2016). Wear time validation was also used to estimate adherence to wearing the monitors. A day was considered valid if wear time was at least 600 minutes (Migueles et al., 2017). A total of 840 data points were eliminated from the analysis. All data was grouped by day and week of training and average time per day was calculated across each week of training. The data is presented in average minutes per day.

Energy Expenditure Estimation

Metabolic equivalents (MET) were assigned to each category of physical activity once minutes per day for each week of training were averaged. The MET assignments are as follows: light = 2 METs (Chomistek et al., 2017), Moderate = 3 METs, Vigorous = 6 METs, Very Vigorous = 9 METs (Sasaki) (Sasaki et al., 2011). We conservatively applied the lowest MET values for moderate, vigorous, and very vigorous intensity physical activity to estimate physical activity energy expenditure (Moderate = 3-5.99 METs, Vigorous = 6-8.99 METs, Very Vigorous > 9 METs) (Sasaki et al., 2011). Once MET values were assigned, weekly active energy expenditure (AEE) was estimated for each participant using the Cooper Institutes MET to calorie conversion:

Calories/minute = MET Value x 3.5 x Participant body weight (kg) ÷ 200

Total energy expenditure was the sum of REE and AEE. Energy balance was estimated by subtracting each participant's caloric intake from the estimated total energy expenditure; negative or positive values indicate a caloric deficit or surplus, respectively. Resting energy expenditure (REE) was estimated using the Modified Harris-Benedict equation (REE) (Roza & Shizgal, 1984):

REE = 88.362 + (13.397 x body weight (kg)) + (4.799 x Height (cm)) - (5.677 x age (yrs.)

Data Presentation and Statistical Analysis

Given that this study was largely observational, food intake, physical activity and energy balance data were presented as mean ± standard deviation values, and no statistical testing was performed for these metrics.

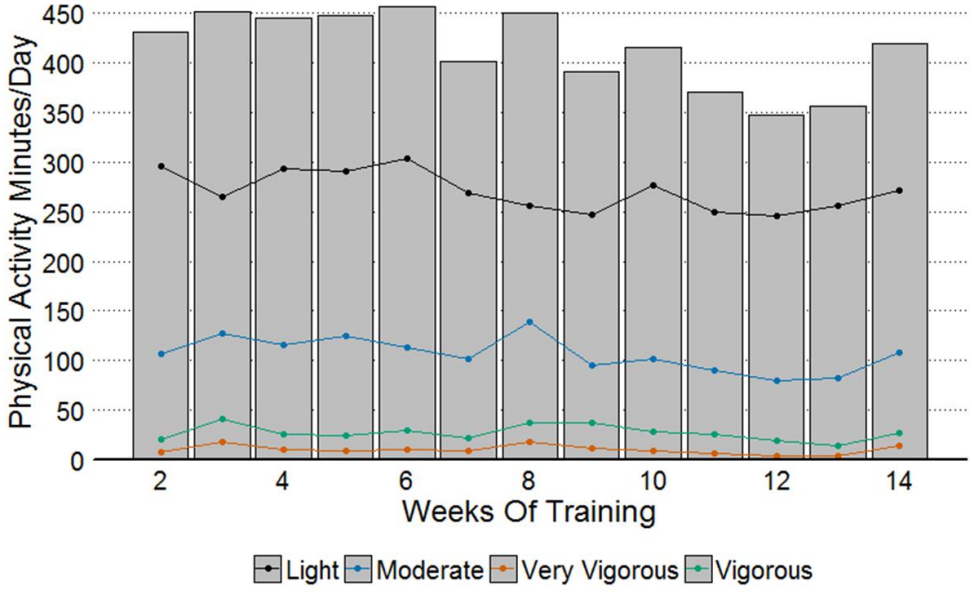
Dietary intake across meals was evaluated using repeated measures ANOVA with paired samples t-tests as follow-up analyses. Normality of residuals was tested using the Shapiro-Wilks and Komolgorov-Smirnov test. Sphericity was evaluated using Maulchy's test. During tests for normality of residuals for dietary intake one meal was determined to be a statistical outlier and was removed from the analysis because the participant's calorie intake was approximately 3,500 calories for one meal, which was 2.5 times greater than his next closest meal (1,427 calories). Linear regression was used to determine if a relationship existed between select dependent variables. For probability testing, statistical significance was set at $p<0.05$.

RESULTS

Physical activity data

Physical activity data are presented in Figure 3. On average IET soldiers spent approximately 273 minutes in light, 107 minutes in moderate, 26 minutes in vigorous, and 10 minutes in very vigorous intensity physical activity per day during 13 weeks of IET. The activity monitors were not worn (non-wear time) an average of 322 minutes per day as estimated by Actilife software and was inversely correlated with time spent in light intensity (Moderate Correlation: -0.51) and moderate intensity (Small Correlation: -0.33) exercise. Therefore, on weeks where wear time was lower, physical activity levels may be underestimated. IET soldiers averaged 13,569 ± 5,197 steps per day during IET.

Figure 3: Summary of training volume during each week IET



Legend: Summary of training volume across IET. Total physical activity (Sum of time spent in: light, moderate, vigorous, and very vigorous intensity) is represented by the columns and time spent in each classification of physical activity is represented by the lines. Data is presented in average minutes per day during each week of training.

Dietary Intake

Average macronutrient intake is presented in Figure 4. IET soldiers consumed on average 2,643 ± 639 calories per day. Intake ranged from 1,211 to 4,228 calories per day. When comparing energy intake across meals, statistical comparison using repeated measures ANOVA revealed a significant main effect of meal with caloric intake is highest at breakfast with an average intake of 1012 ± 273 calories, followed by dinner 813 ± 225 calories, and lunch 769 ± 201.2 calories. There was a significant difference between all meals for calorie intake, breakfast and dinner ($t = 8.29$, $p < 0.01$), breakfast and lunch ($t = 10.72$, $p < 0.01$), dinner and lunch ($t = 2.31$, $p = 0.02$). Protein intake averaged 38.1 ± 11.2 grams for breakfast, 33.7 ± 9.9 grams for

lunch, and 41.1 ± 11.0 grams for dinner. There was a significant effect of meal on dietary protein intake ($F = 24.18$, $p < 0.001$). Follow-up paired t-tests revealed a significant difference between breakfast and dinner ($t = 2.84$, $p < 0.01$), breakfast and lunch ($t = 3.95$, $p < 0.01$), dinner and lunch ($t = 7.14$, $p < 0.01$). Carbohydrate intake averaged 137.3 ± 39.7 grams for breakfast, 104.5 ± 28.2 grams for lunch, and 101.8 ± 30.3 grams for dinner. Statistical analysis reveal a significant effect of meal. Follow-up paired t-tests revealed a significant difference between breakfast and dinner ($t = 9.03$ $p < 0.01$), breakfast and lunch ($t = 8.89$, $p < 0.01$), but no significant difference between dinner and lunch ($t = 0.95$, $p = 0.34$). Fat intake averaged 34.6 (± 11.2) grams for breakfast, 25.9 (± 9.2) for lunch, and 27.8 (± 11.2) grams for dinner. Statistical analysis revealed a significantly higher intake of carbohydrate at breakfast. Statistical analysis reveal a significant effect of meal. Follow-up paired t-tests revealed a significant difference between breakfast and dinner ($t = 5.67$, $p < 0.01$), breakfast and lunch ($t = 7.67$ $p < 0.01$), but no significant difference between dinner and lunch ($t = 1.61$ $p < 0.01$).

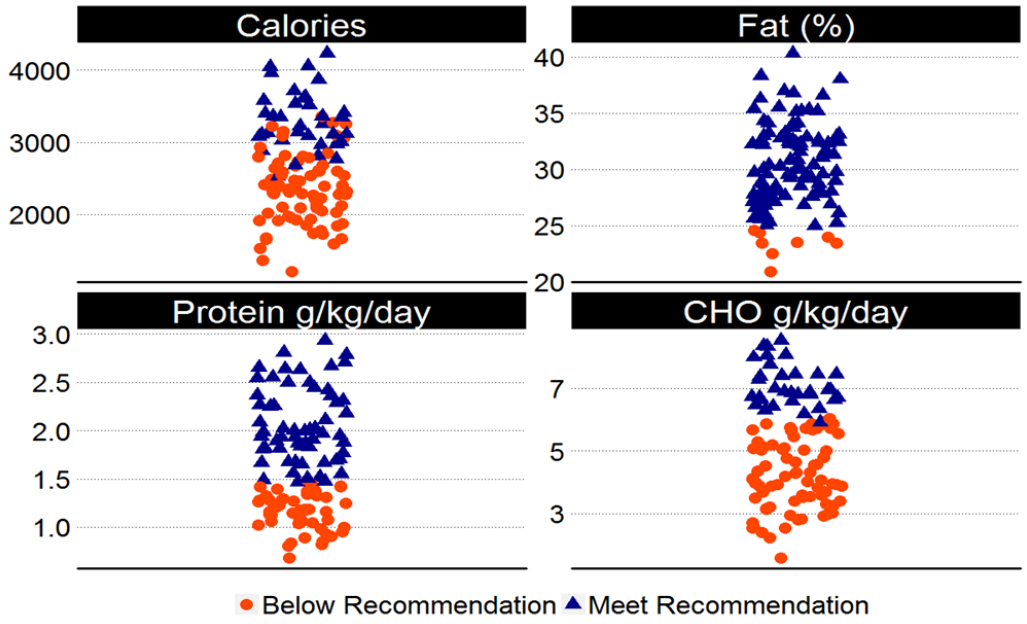
Energy Balance

Energy balance was estimated using the most conservative MET value associated with each classification of physical activity (Moderate = 3 METs, Vigorous = 6 METs, Very Vigorous = 9 METs). On average 69.6% of IET soldiers were classified as being in negative net energy balance.

Average energy balance was -540 ± 895 kcal/day during the 13 weeks in which training volume was measured. Resting energy expenditure averaged 1,777 ± 185 kcal/day and active energy expenditure averaged 1,406 ± 298 Kcal kcal/day. Figure 4 reports the descriptive statistics for energy expenditure and balance across each week of training. Regression analysis

revealed body weight was a significant predictor for negative energy balance (adj. $R^2 = 0.55$, $p < 0.001$). Specifically, for every 1-kilogram increase in body mass, energy balance became more negative by 53 calories.

Figure 4: Summary of training volume during each week IET



Legend: Summary of dietary intake of IET soldiers in comparison to recommendations for active individuals. Recommendations are based on the Joint Position Statement of the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada, for active individuals.

DISCUSSION

This study evaluated the dietary intake, training load, and balance between estimated energy expenditure and energy intake during U.S. Army IET soldiers. Our primary finding was that IET soldiers expended approximately 540 more calories per day than they consumed based on our assessment of metabolic training load. It is likely that our estimation of energy

expenditure is low based on three important considerations: 1) we applied conservative MET assignments to moderate, vigorous, and very vigorous intensity physical activity; 2) estimation of BMR using the Modified Harris-Benedict equation was also a conservative estimate of non-physically active energy expenditure. It assumes the participant is immobile and in a supine position, whereas over half of sedentary time for IET soldiers is standing time which has been reported to increase energy expenditure by as much as 10% (Levine et al., 2000); 3) IET soldiers frequently carry loads (10-80 pounds) which increases energy expenditure and was not considered in our calculations (K Simpson et al., 2013a) . Therefore, the imbalance between energy expenditure and nutrition intake may be higher than reported here. In addition to preventing optimal performance and recovery, inadequate energy intake can negatively affect bone turnover (formation and resorption), and may predispose active individuals to MSI (Barrack, Van Loan, Rauh, & Nichols, 2010; Ihle & Loucks, 2004; Wright, Loucks, & Kiens, 2011; Zanker & Swaine, 2000) which is one of the most costly challenges facing the US armed forces today (J. Molloy et al., 2012a; Teyhen, 2014).

Quantification of training volume during IET revealed Soldiers averaged approximately 3,180 (± 320) minutes of physical activity per week with 1,202 (± 291) of those minutes being moderate to vigorous physical activity across 14 weeks of IET. For comparison, research indicates only 49-53% of civilian adults 18-34 reported at least 150 minutes of moderate or 75 minutes of vigorous and only 31% reported participating in 300 minutes of moderate or 150 minutes of vigorous physical activity per week (Center for Disease Control and Prevention, 2015). Furthermore, only 27.1% of adolescents participate in more than 1 hour per day of moderate or vigorous physical activity (Center for Disease Control and Prevention, 2015). Thus, IET soldiers complete substantially more physical activity than the general civilian population of

the same age range. Also noteworthy was our observation that physical activity was slightly higher during the first three weeks compared to the overall cycle average for IET training. IET soldiers experience rapid increases in physical activity upon entry to IET compared to civilian life, which likely increases the risk of MSI. To combat this disparity, there are several voluntary pre-conditioning programs provided to new U. S. Army recruits. Discussions with training and recruiting command Cadre suggest most recruits do not take advantage of these programs or guidance provided on how to prepare for the rigors of military training. A different approach for pre-conditioning of recruits may be necessary to prepare them for the rigors of IET.

Previous research reported that IET soldiers spent on average 144 minutes per day in light, approximately 93 minutes in moderate, and 37 minutes per day in vigorous intensity exercise (K Simpson et al., 2013a). Differences found in the current study are likely due methodological differences in activity monitoring. The previous study distributed activity monitors at morning formation and collected them at dinner, missing any evening physical activity(K Simpson et al., 2013a). Additionally, differences in algorithms used to classify activity counts into physical activity may account for the differences. The prior study used Freedson cut points which are based on vertical axis counts (Freedson, Melanson, & Sirard, 1998; Migueles et al., 2017), whereas our study used Sasaki vector magnitude cut-points which are based on a vector magnitude estimate calculated from all three axes (Sasaki et al., 2011). While vertical axis counts have been validated for recording ambulatory physical activity (walking and jogging), they tend to underestimate activities that do not necessarily occur in the vertical axis (such as shoveling) (Matthew, 2005; K. B. Watson, Carlson, Carroll, & Fulton, 2014b). Comparison of models using vector magnitude (tri-axis) and vertical axis only have reported higher physical activities when using vector magnitude whereas vertical axis tends to report larger amounts of

time in sedentary activity (Keadle, Shiroma, Freedson, & Lee, 2014; K. Watson, Carlson, Carroll, & Fulton, 2014a).

IET soldiers consumed an average of 2,644 calories, 114 grams of protein, 352 grams of carbohydrate, and 89 grams of fat per day based upon entry-level data analysis. A 2002 study of dietary intakes in IET soldiers found they consumed on average 3,000 calories per day, whereas a 2012 study found IET soldiers consumed on average 1,975 calories (78 grams of protein, 240 grams of carbohydrate, and 77 grams of fat) (L. Margolis et al., 2012a; Williamson, 2002). Previous research has reported factors such as not having enough time to eat and command climate can influence nutritional behaviors (Jackson et al., 2013). Special care to protect feeding times needs to be considered especially when the influx of IET soldiers increases due to force expansion or yearly fluctuations. This may further reduce the time allotment for meals due to increased demand on dining facilities. Differences between studies of dietary intake may be due to methodologies employed in collecting nutritional data. The first study conducted in 2002 used a highly accurate food photography method whereas the other studies used food frequency questionnaires and diet logs. Additionally many doctrine changes to the IET environment have been made during this time period. Early in 2012 and 2013 the U.S. Army began using strategies for food education and selection to encourage IET soldiers to consume less energy dense food items. Soldiers were encouraged to select foods lower in fat content and increase consumption of vegetables, fruits and complex carbohydrates (TRADOC, 2013; US Army Food Service, 2012). While this nutritional education is important for development of long-term soldier health, care should be taken when explaining these strategies to IET soldiers who are exposed to very high training volumes and need energy dense food items to match caloric expenditure.

On average IET soldiers consumed more than the lower limit of 1.2 g/kg for protein. However, approximately 44% of IET soldiers consumed less than the recommended 1.5 g/kg of protein intake per day which is thought to be a more accurate estimation of protein needs for strength and endurance training (H Kato et al., 2016a; Lemon et al., 1992a; D. Thomas et al., 2016a). For carbohydrate intake, approximately 50% of IET soldiers did not consume more than 5 g/kg and 70% did not meet the lower limit of the recommendations for individuals participating in high levels of physical activity (6 g/kg) (D. Thomas et al., 2016a). These findings are important as we apply research findings clinically to develop fueling recommendations for the IET population. Currently all IET soldiers receive the same portion and number of servings. Individual soldier body composition, fitness and activity level should be considered as we work towards optimization of health and performance.

This investigation is a first step at evaluating energy balance in the IET environment and represents only one training company at one location. One limitation to this study is our assumption that entry-level dietary intake values were reflective of eating habits throughout the 14-week duration. Previous research suggests that calorie intake during IET can increase between 200 (L. Margolis et al., 2012a) and 700 (Williamson, 2002) calories per day from pre to post training. Hence, assuming this increase in caloric may be able to account for the caloric deficit for some participants across training. Additionally, energy expenditure was estimated based on the average physical activity performed by the entire company of IET soldiers. While many of the activities performed at IET are in the group setting, ideally, investigations should assign each IET soldier a monitor to wear daily throughout training to track individual energy expenditure and training load. Finally, more precise methods to track dietary intake would improve outcomes. There are known inherent errors when using food logs such as portion

estimation error, therefore methodologies in which trained researchers evaluate dietary intake may improve accuracy (R. Hill & Davies, 2001).

CONCLUSION

IET Solders are not obtaining adequate calories and nutrients to meet training needs, which may directly impact soldier performance and injury frequency. IET soldiers undergo rigorous training; adequate nourishment will help optimize soldier health and performance, thus improving Army force strength and readiness.

Chapter 5: Effect of whey protein supplementation on physical performance and body composition in Army initial entry training soldiers

INRODUCTION

Army initial entry training (IET) is a physically demanding program consisting of large volumes of strength and endurance activities (J. J. Knapik, Rieger, Palkoska, Van Camp, & Darakjy, 2009b). IET is the first step in a soldier's military career. The goal of IET is to transform civilians into soldiers who have the strength and endurance needed to meet the demands of deployment and remain healthy throughout a rigorous military career. An increasing percentage of soldiers report to IET with low levels of physical fitness, which is a primary contributor to the high rates of musculoskeletal injuries found in our military (J. M. Molloy, Feltwell, Scott, & Niebuhr, 2012b). To help combat this problem the Army has implemented strategies such as the Army Performance Triad which focuses on improving sleep, exercise, and nutrition (A. Medicine, 2015). The Army has also changed approaches to physical training during IET (21-20, 1998; United States Army Training and Doctrine Command, 2003), and is currently piloting extending length of IET to improve proficiency, health, and performance.

Nutritional intake is a key component of the physiological adaptation to training (D. T. Thomas, Erdman, & Burke, 2016b). Inadequate nutritional intake can result in a negative energy balance, which can adversely impact exercise performance and adaptation (A. B. Loucks, 2004b). Investigations quantifying training have revealed that IET soldiers spend approximately 6-7 hours per day performing light to very vigorous physical activity (McAdam et al., 2017;

Kathleen Simpson et al., 2013b). Dietary intake of IET soldiers has been reported to be between 1,900 (Lee M Margolis et al., 2012c) and 2,600 (McAdam et al., 2017) calories per day, which may be inadequate to meet the demands of training (McAdam et al., 2017). The latter study reported IET soldiers on average consumed 540 kcals less than the estimated energy expenditure per day during IET (McAdam et al., 2017). Optimal protein intake for individuals participating in large volumes of training has been reported to be 1.5 to 2 g/kg of bodyweight per day (Hiroyuki Kato, Suzuki, Bannai, & Moore, 2016b; Lemon, Tarnopolsky, MacDougall, & Atkinson, 1992b). Importantly, energy restriction like that noted during IET may increase these needs (E. R. Helms, Zinn, Rowlands, & Brown, 2014b). Previous research (E. R. Helms et al., 2014b) and organizational recommendations (D. T. Thomas et al., 2016b) suggest intakes between 2-3 g/kg may be necessary during periods of energy restriction . Collectively these dietary and training volume reports suggest soldiers in IET are functioning in a negative energy balance and may need to increase protein intake to support adaptation to training (Kingsbury, Kay, & Hjelm, 1998; Kathleen Simpson et al., 2013b).

Protein supplementation may be a strategy to help combat nutritional deficits during military training (Kingsbury et al., 1998). One study to date has examined the use of protein supplementation in United States IET (Flakoll, Judy, Flinn, Carr, & Flinn, 2004). Marine recruits were supplemented with 10 grams of casein (protein source derived from milk) once daily throughout 54 days of basic training. Results revealed all participants still experienced losses in lean mass. However, the protein group had a 33% reduction in total medical visits and a 37% reduction in medical visits due to musculoskeletal injury (Flakoll et al., 2004). Mixed supplement shakes have also been employed to help optimize nutritional intake during non-IET military training. A study reported the addition of a mixed supplement (45% carbohydrate, 40%

fat, and 15% protein) given to soldiers undergoing section commanders battle course in the United Kingdom resulted in the attenuation of muscle mass loss and physical performance decrements that were experienced by those who did not consume supplementation (M. B. Fortes et al., 2011b).

Whey protein (WP) is rapidly digested, has a high content of branched chain and essential amino acids, and has bioactive peptides that are exclusive from other protein sources (Patel, 2015; J. E. Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009b). Research suggests these characteristics result in an improved ability to stimulate muscle protein synthesis; thereby improving net protein balance and improving recovery through skeletal muscle adaptation and repair (J. E. Tang et al., 2009b). WP has been reported to improve strength (Jean Farup, Rahbek, Vendelbo, et al., 2014; Naclerio & Larumbe-Zabala, 2015), increase lean mass (Jean Farup, Rahbek, Vendelbo, et al., 2014; Naclerio & Larumbe-Zabala, 2015), and facilitate recovery of force production between repeated bouts of exercise in resistance-trained individuals (Buckley et al., 2010). WP supplementation has also been reported to improve run time, cycle time to exhaustion, and reduce serum creatine kinase levels, suggesting WP facilitates recovery in subjects engaged in high-volume endurance training (M. Hansen, Bangsbo, Jensen, Bibby, & Madsen, 2015b; M. J. Saunders, Kane, & Todd, 2004b).

Military training requires a unique combination of strength and endurance-related activities in the form of structured physical training, as well as in required military (functional) tasks such as ruck marches and land navigation (J. J. Knapik et al., 2009b). Increasing intake of high quality protein during IET through WP supplementation may assist in recovery and adaptation while also providing additional calories to help match the energy demands of training. Thus, the purpose of this study was to examine the effects of WP supplementation on physical

performance and body composition during Army IET. We hypothesized that the WP would result in greater improvements in body composition and physical performance metrics compared to an energy-matched carbohydrate (CHO) control group.

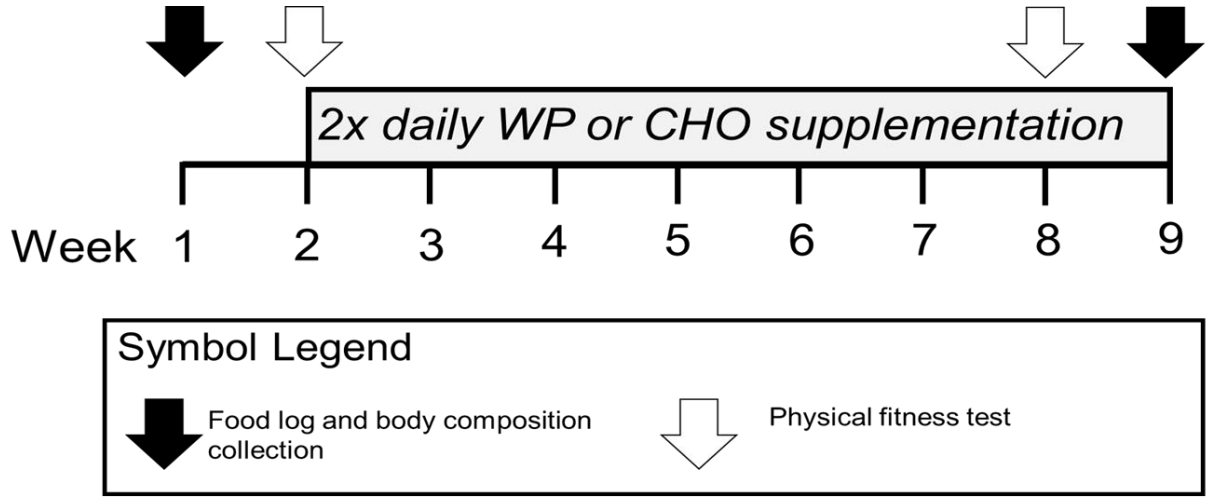
METHODS

Study Design

This study employed a repeated measures, double-blind, parallel groups study design. The Auburn University Institutional Review Board, Army Institutional Review Board, and the Director, Research & Analysis Directorate Army Center approved the study. Inclusion criteria were healthy IET males at least 18 years of age, with no apparent disease or musculoskeletal injury, no allergy to milk or WP, and not supplementing or have supplemented with WP or any other ergogenic aid within the past three months. Each potential participant received verbal explanation of the study and written consent was obtained from those wishing to participate.

A total of 69 healthy male subjects from one training unit at Fort Benning Georgia, consented to participate and completed the study. Figure 5 illustrates the timeline of measurements.

Figure 5. Summary of measures collected during IET



Legend: This figure illustrates the logistics of the training intervention. More detail regarding can be found in the methods section.

IET soldiers from two platoons received WP, and two platoons of the same training unit received a taste and calorie-matched CHO placebo supplement. Two servings per day were administered by drill sergeants; one after morning physical fitness training, and the second serving before bedtime. The nutritional profile and amino acid content of both supplements were third-party tested by Covance Laboratories, Inc. (Madison, WI, USA) to verify identity, purity, potency, and composition of the packets. One WP serving provided 293 total kcals; consisting of 38.6 g of protein [Power Crunch® ProtoWhey® (BioNutritional Research Group; Irvine, CA, USA) as agglomerated, partially hydrolyzed (12.5% degree of hydrolysis) 80% whey protein concentrate (Hilmar® 8360; Hilmar Ingredients, Hilmar, CA USA)], 19.0 g carbohydrates, 7.5 g fat, and 20.1 g and 9.5 g of essential and branched chain amino acids. One CHO serving provided 291 total kcals, 0.5 g protein, 63.4 g carbohydrates, 3.9 g of fat, and 0.1 g and 0.0 g essential and branched chain amino acids. A member of the research team delivered and checked the supplement supply weekly to assess for compliance. Participants also self-reported any missed servings and a researcher asked each participant if any servings had been missed at each assessment. Additionally: 1) IET soldiers are required to live in barracks and are under continual supervision of drill sergeants; 2) daily physical fitness and military training were tightly regulated by unit leadership and were scheduled from the time the soldiers wake up until the time they go to bed; 3) IET soldiers were not allowed to smoke or consume alcohol, and all meals were consumed in the dining facility or at unit provided meals in the field; and 4) no food was allowed in the barracks, and no beverages other than water were allowed outside of the schedule meal times at the IET dining facility.

Dietary analysis

Dietary intake was evaluated using diet recalls collected immediately following each meal on three days (Tuesday, Thursday, and Saturday) during weeks one and nine of IET. A more detailed methodology has been described in our previous work (McAdam et al., 2017). Briefly, participants were provided a diet log sheet immediately after the meal that was pre-filled with food offerings obtained from the battalion dining facility. Participants were asked to circle the food or drink item and amount consumed. Nutritional information for food items was collected from the Army Joint Culinary Center of Excellence (JCOE) website (Army, 2012). Information for food items not found on the JCOE menu were retrieved from the US Department of Agriculture nutrition data base (United States Department of Agriculture, 2013). Dietary intake was calculated by multiplying the portion of food consumed by the nutritional value of that food using R statistical software (Team., 2015) for each meal and summed across each day. Participants who did not complete more than two days of diet logs were excluded from the dietary intake analyses. A full day was determined as completing a diet log for each of the three meals for that day. Overall 27 in the WP and 28 in the CHO group were included in the dietary analysis.

Body Composition and Performance Measures

Anthropometric measures were conducted after an overnight fast, prior to morning physical training and breakfast on the third day of the IET training cycle (pre-intervention) and at the same time of day during week nine (post-intervention) of IET training. Urine specific gravity (USG) was evaluated the morning of testing using a handheld refractometer (Manual, Atago, Tokyo, Japan) to ensure participants were properly hydrated (USG below 1.03). Those with USG

above 1.03 were provided with water and re-tested to assure proper hydration before proceeding to the remaining assessments.

Height and weight. Height and weight were recorded using a Health-O-Meter professional scale (Model 500KL, Sunbeam products INC. Boca Raton, FL. USA) with IET soldiers wearing only Army issued physical training shorts, socks and underwear. Weights were collected to the nearest 0.1 kg.

Body Composition. Skinfolds were measured by three trained technicians using skinfold calipers (Fabrication Enterprises, PO Box 1500 White Plains, NY. USA) as per the American College of Sports Medicine (ACSM) protocol for 7-site skinfold measures (chest, mid-axilla, abdomen, suprailiac, subscapular, triceps, and thigh) (G. B. Dwyer & Davis, 2008b). Duplicate measures from the right side of the body were taken in sequential order and averaged, and a triplicate measure was assessed on a given site if the first two readings differed by ±2.0 mm. The ACSM 7-site body density calculation was used and body composition calculated as follows:

- Body Fat Percent = ((457/Body Density)-414.2) / 100
- Fat Mass (kg)= Body Fat Percent * Body Mass
- Fat Free Mass (kg)=Body Mass-Fat Mass

Based upon within day test-retest measurements performed on 10 male volunteers, the intraclass correlation for the 7-site sum of skinfolds was 0.99, 1.00, and 0.99 for each of the three technicians that performed all skinfold measurements in the current study. Additionally, the measurements exhibited a high degree of correlation (r = 0.91) between all three technicians.

Performance assessments. The Army Physical Fitness Test (APFT) was performed during weeks two and eight of the intervention (21-20, 1998). The APFT consisted of a 2-minute push-up test, 2-minute sit-up test, and 2-mile run test performed in order, with approximately 10

minutes of rest between test portions. The assessment process was supervised by experienced drill sergeants and cadre specifically trained to administer the APFT. Results were then supplied to the research team. A more detailed description of the APFT is previously described (21-20, 1998; J. M. Sefton, Lohse, & McAdam, 2016b).

Statistical Analysis

Shapiro Wilk's and Kolmogorov Smirnov tests were used to test the assumption that residuals were normally distributed across all levels of each dependent variable. Additionally QQ plots were used to visualize residuals for each level of the variables. Square root transformations were performed and used in the analysis for dependent variables in which normality was violated for more than 75% of the measures. Maulchy's test of sphericity was used to test the assumption of equality of variances. Greenhouse-Geisser corrections were used if sphericity was violated. Levene's Test was used to evaluate the assumption of homogeneity. Statistical analyses were completed using R statistical software (Team., 2015) and R Studio (RStudio., 2014). R programming packages included: dplyr, tidyr, reshape2 ez, car, vars, ggplot2. An *a priori* alpha level of 0.05 was used as the criteria for determination of significant effects.

Dietary intake data was evaluated using a two-way (training x supplement group) mixed factorial analysis of variance (ANOVA). If group by time interactions were detected, paired samples t-tests were used to evaluate simple main effects of time, and independent samples t-test were used to evaluate group differences at pre and post-intervention.

Physical performance (push-ups, sit-ups, and run time) and body composition (body weight, FFM, FM) measures were evaluated using analysis of covariance (ANCOVA). Mean centered baseline values for each variable were used as the covariate in the model, which reduces the error

rate in ANCOVA models containing within-subjects factors (Schneider, Avivi-Reich, & Mozuraitis, 2015). ANCOVA improves the sensitivity of detecting group effects (Schneider et al., 2015), which was our primary research interest. Post hoc, we conducted a correlation test to determine whether initial FFM was associated with the change in FFM across training.

Cohen's d effect sizes were calculated for each variable across training (pre- vs. post-intervention). Effect sizes were classified as small ($d < 0.2$), medium ($d > 0.21$, $d < 0.5$), or large ($d > 0.51$), and the results are presented as Cohen's d effect size estimate. Mean differences and 95% confidence intervals of the mean differences were calculated and included in Table 4.

- Effect Size = mean(post) - mean(pre)/pooled standard deviation
- Pooled standard deviation = Square root((SD (pre)^2 + SD (post)^2)/2)

RESULTS

A total of 69 IET soldiers participated in the study (WP; n = 34, age = 19 ± 1 yr., ht. = 173 ± 6 cm, wt. = 73.4 ± 12.7 kg; CHO; n= 35, age = 19 ± 1 yr., ht. = 173 ± 5 cm, wt. = 72.3 ± 10.9 kg). All dependent variables were non-significant for violation of assumption of normality of residuals except for FM. Statistical differences were found for all time points and groups for FM except for post-intervention in the CHO group (WP: Pre-W = 0.929 p = 0.03074, Post-W = 0.92, p = 0.013;0 CHO: Pre-W = 0.93, p = 0.040, Post-W = 0.94, p = 0.055). FM was square root transformed and normality of residuals was re-tested and the data was normally distributed.

<u>Dietary intake</u>

Ten participants provided less than two days of diet log data and were removed from the diet analysis. First, we analyzed dietary intake on only what was consumed at the dining facility

at post-intervention (Post NS) to determine if consuming the supplements decreased intake at the dining facility in either supplement group. There were no significant differences across the intervention or between groups for intake of calories, or any of the macronutrients. We then compared dietary intake with the supplement nutrition added to total dietary intake at post-intervention (Post SI). Calorie ($F = 8.04$, $p < 0.001$) and fat intake ($F = 18.50$, $p < 0.001$) increased across the intervention. There were significant group by time interactions for protein ($F = 95.97$, $p < 0.001$) and carbohydrate intake ($F = 14.68$, $p < 0.001$). Follow-up t-tests revealed the WP group increased protein ($t = 14.28$, $p < 0.001$) and carbohydrate ($t = 3.7$, $p < 0.001$) intake across IET; however the CHO group only increased carbohydrate intake ($t = 0.37$, $p = 0.71$).

These results indicate soldiers in both the WP and CHO groups increased calorie, carbohydrate, and fat intake across IET; whereas only the WP group increased protein intake across IET when consuming supplementation. Dietary intake is summarized in Table 2 and significance is denoted.

Table 2. Summary of dietary intake during IET

Group	Variable		Pre NS	Post NS	Post SI
WP	Energy	(kcal/d)	2825 ± 611	2930 ± 681	3516 ± 681*
		(kcal/kg/d)	40.3 ± 12.7	40.8 ± 11.1	49.0 ± 11.7*
CHO		(kcal/d)	2624 ± 740	2766 ± 542	3348 ± 542*
		(kcal/kg/d)	37.5 ± 13.7	38 ± 9.3	46.0 ± 9.9*
WP	PRO	(g/d)	122 ± 25	124 ± 29	201 ± 29*#
		(g/kg/d)	1.7 ± 0.5	1.7 ± 0.4	2.8 ± 0.5*#
CHO		(g/d)	112 ± 32	113 ± 21	114 ± 21
		(g/kg/d)	1.6 ± 0.6	1.5 ± 0.4	1.6 ± 0.4
WP	CARB	(g/d)	371 ± 84	392 ± 96.6	430 ± 97*
		(g/kg/d)	5.3 ± 1.7	5.5 ± 1.6	6.0 ± 1.6*
CHO		(g/d)	349 ± 95	368 ± 85	495 ± 85*#
		(g/kg/d)	5.0 ± 1.7	5.0 ± 1.4	6.8 ± 1.5*#
WP	FAT	(g/d)	98 ± 27	100 ± 30.9	115 ± 31*
		(g/kg/d)	1.4 ± 0.5	1.4 ± 0.5	1.6 ± 0.5*
CHO		(g/d)	90 ± 31	98 ± 23.2	106 ± 23*
		(g/kg/d)	1.3 ± 0.6	1.4 ± 0.4	1.5 ± 0.4*

Legend: all data are food log data that are self-reported, averaged to one-day daily average intakes, and presented as means ± standard deviation values. Abbreviations: Pre NS, pre-intervention when not supplementing; Post NS, post intervention values not including supplement; Post SI, post intervention values including supplement nutrition data; PRO, dietary protein; CARB, dietary carbohydrate; FAT, dietary fat; WP, whey protein group (n = 27); CHO, carbohydrate-placebo group (n = 28). *, indicates Post > Pre ($p < 0.05$), #, indicates group differences at a given testing time point ($p < 0.05$).

Body Composition

After controlling for initial body weight (week one) there was no statistically significant difference between groups in body mass at post-intervention (week nine) ($F = 0.93$, $p = 0.34$), or initial weight by group interaction (2.05, $p = 0.16$).

There was a significant difference in FM post-intervention after controlling for initial FM ($F = 4.63$, $p = 0.04$). There was no significant initial FM by group interaction ($F = 1.30$, $p = 0.26$).

There was no statistically significant difference in FFM between the groups post-intervention when controlling for initial FFM ($F = 0.70$, $p = 0.41$). There was no initial FFM by group interaction ($F = 1.90$, $p = 0.17$). Post-hoc testing revealed a significant inverse correlation between change in FFM across training with initial FFM ($r = -0.45$, $p < 0.001$), indicating that individuals with lower initial FFM were likely to have larger gains in FFM during IET. Body composition data is summarized in Table 3 and effect sizes are summarized in Table 4.

Table 3. Summary of body composition during IET

Variable	Group	Pre-Intervention	Post-Intervention
Body weight (kg)	WP	73.4 ± 12.7	72.2 ±10.5
	CHO	72.3 ±10.9	73.2 ±7.9
FFM (kg)	WP	60.0 ±7.9	64.2 ±7.5
	CHO	60.1 ±7.3	63.7 ±6.1
Fat Mass (kg)	WP	13.5 ±6.1	8.9 ±4.2 *
	CHO	12.2 ±6.1	9.5 ±3.9 *

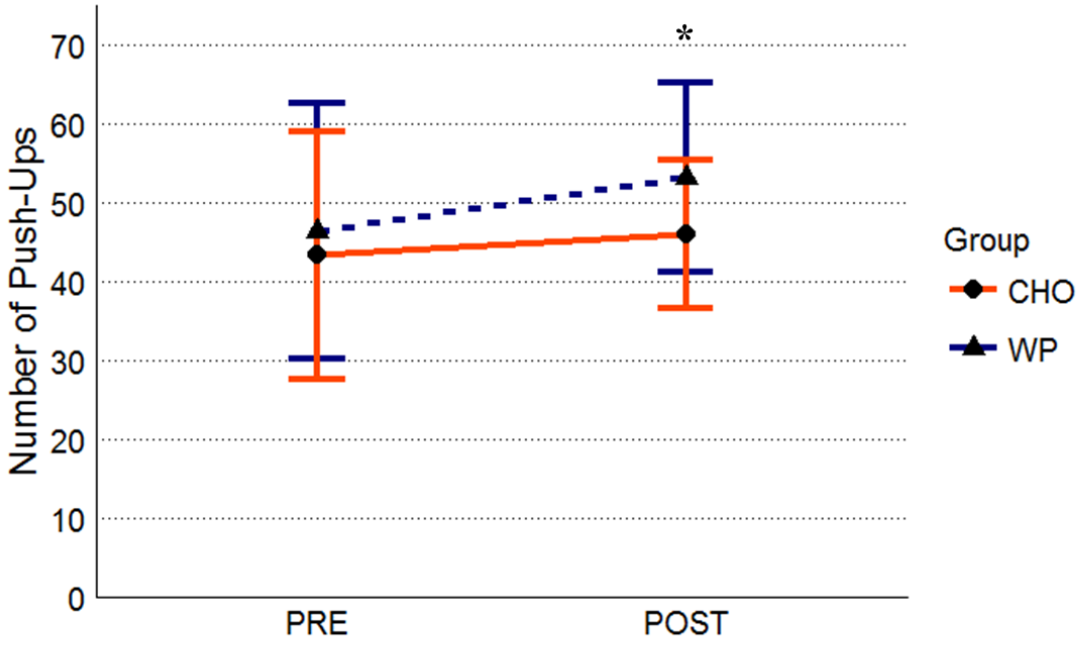
Legend: all data are presented as means ± standard deviation values. Body composition was assessed using 7-site skinfolds as described in the methods. Abbreviations: kg, kilograms; FFM, fat-free mass; WP, whey protein group (n=34); CHO, carbohydrate-placebo group (n=35); Symbols: *, indicates a significant group difference at post training ($p < 0.05$)

Physical Performance

After controlling for initial push-up performance there was a significant group difference at post-intervention ($F = 10.02$, $p < 0.001$). There was no significant initial push-up by group interaction ($F = 0.25$, $p = 0.62$). There was a difference of 6.87 push-ups on average between

groups at post-intervention, with the WP performing more pushups (53 ± 12) compared to the CHO (46 ± 9 push-ups) group at post-intervention.

Figure 6. Summary of push-up differences between groups during IET

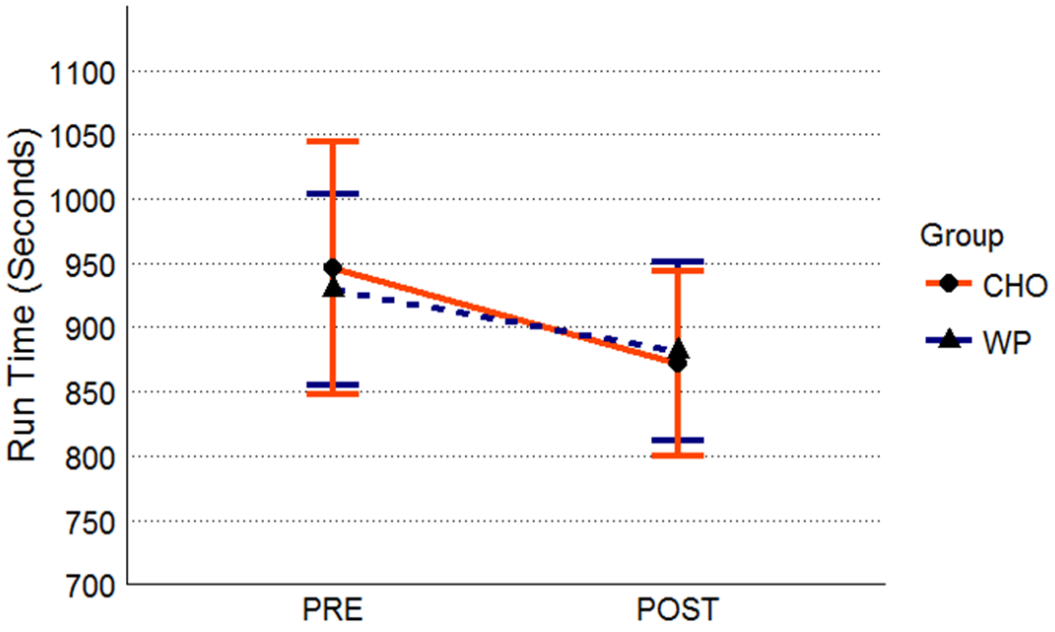


Legend: all data are presented as means ± standard deviation values. Abbreviations: WP, whey protein group (n=34); CHO, carbohydrate-placebo group (n=35). Symbols: *, indicates WPH > CHO at POST.

After controlling for initial sit-up performance there was no significant group difference at post-intervention (F = 0.02, p = 0.90). There was no significant initial sit-up by group interaction (F = 0.42, p = 0.52). WP group performed 50.4 ± 14.3 sit-ups pre- and 59.7 ± 11.0 post-intervention. The CHO group performed 51.8 ± 13.3 sit-ups pre and 60.7 ± 9.2 sit-ups post-intervention.

After controlling for initial run performance there was no significant group difference at post-intervention (F = 3.6, p = 0.06). There was no significant initial run by group interaction (F = 2.89, p = 0.09). Post-intervention run performance was 881.3 ± 69.1 seconds in the WP group and 871.8 ± 71.8 seconds in the CHO group.

Figure 7. Summary of two-mile run time differences between groups



Legend: all data are presented as means ± standard deviation values. Abbreviations: WP, whey protein group (n=34); CHO, carbohydrate-placebo group (n=35)..

Effect sizes for all body composition and performance variables are reported in Table 4.

Table 4. Summary of effect sizes

Variable	Group	Mean Difference	95% CI	Units	Effect Size	Classification
FFM	WP	4.2	(3.1, 5.4)	(kg)	0.44	Medium
	CHO	3.6	(2.3, 4.9)	(kg)	0.42	Medium
FM	WP	-4.5	(-5.8, -3.2)	(kg)	-0.67	Large
	CHO	-2.7	(-4, -1.3)	(kg)	-0.4	Medium
PU	WP	6.8	(2.9, 10.7)	(push-ups)	0.41	Medium
	CHO	2.6	(-0.7, 6)	(push-ups)	0.18	Small
SU	WP	9.3	(5.4, 13.2)	(sit-ups)	0.62	Large
	CHO	8.9	(6.3, 11.5)	(sit-ups)	0.68	Large
Run	WP	-48.3	(-63, -33.6)	(seconds)	-0.56	Large
	CHO	-74.2	(-96.5, -51.9)	(seconds)	-0.74	Large

Legend: Mean differences along with 95% confidence intervals for the mean difference (lower limit, upper limit); cohen's d effect sizes are calculated for pre- to post-intervention changes in sit-up (SU), run time (Run), push-up performance (PU), fat-free mass (FFM) and fat mass (FM). Values in the positive direction indicate an improvement in the metric (i.e., a decrease in run time, an increase in push-up number, an increase in FFM, and a decrease in fat mass). Abbreviations: WP, whey protein group (n=34); CHO, carbohydrate-placebo group (n=35).

DISCUSSION

This project examined how eight weeks of WP influenced physical performance and body composition across eight weeks of IET compared to a carbohydrate control group. IET is expected to increase all measures of physical performance (Sonna et al., 2001). This study revealed WP supplementation significantly improved push-up performance in IET soldiers compared to a CHO control. These findings are supported by previous literature indicating that

WP supplementation is beneficial for strength performance (Buckley et al., 2010; Jean Farup, Rahbek, Vendelbo, et al., 2014; Naclerio & Larumbe-Zabala, 2015). This finding is practically relevant with soldiers in the WP group performing on average 7 more push-ups relative to those in the CHO post-intervention after controlling for initial push-up performance. The push-up test is a requirement for graduation from IET, and 7 additional push-ups could enable an IET soldier to successfully graduate (21-20, 1998). The Army uses the push-up test because it is applicable to soldiering tasks such as lifting/carrying equipment or climbing over walls. Therefore, these improvements in strength may also translate into improvements in performance in other areas of a soldier's career beyond IET graduation. Conversely, IET soldiers who do not pass the APFT are required to receive additional training and possibly repeat the training program. This leads to higher training costs and delayed integration of IET soldiers into operational units for military service. Proper preparation is crucial as the pool of physically ready soldiers has been diminished by repeated deployments (Larson et al., 2018), musculoskeletal injury, and a small pool of available recruits resulting from poor health and fitness of the U.S. general population (Bornstein et al., 2018).

WP had a significant effect on FM. The WP group lost an additional 1.8 kg of FM and had a larger effect size ($d = 0.67$) than the CHO ($d = 0.40$) group. This finding coupled with the statistical group differences in FM suggests WP supplementation may have augmented FM loss in IET soldiers. Decreasing FM is an important practical benefit. Several studies have reported that WP supplementation promotes fat loss in college-aged men over several months of resistance training compared to other protein supplements (P. J. Cribb, Williams, Carey, & Hayes, 2006b; C. M. Lockwood et al., 2017b). Moreover, rodent studies have suggested that WP mechanistically elicits lipolytic effects (Roberts et al., 2014). Conversely, a recent study in

college-aged men (the same age group as IET soldiers) failed to demonstrate this fat loss effect when comparing WP to CHO and other protein supplemented groups (soy and WP concentrate) over a 12-week resistance training period (Mobley et al., 2017). IET differs from traditional resistance training as it is comprised of strength endurance and cardiovascular training. Here, our results agree with the former studies (P. J. Cribb et al., 2006b; C. M. Lockwood et al., 2017b) and suggests WP has a beneficial effect on FM.

There was no statistically significant benefit to consuming CHO compared to WP on run performance. There were large effect sizes regardless of supplement type. Previous research suggests that additional intake of carbohydrate optimizes endurance performance (Bergström et al., 1967). The additional amino acids provided by WP may have helped improve endurance performance by optimizing recovery. IET is physically demanding with participants consistently performing large volumes of training (McAdam et al., 2017; Kathleen Simpson et al., 2013b). Studies in endurance athletes engaged in large volumes of training reveal supplementation with WP improved recovery and reduced markers of muscle damage (M. Hansen et al., 2015b; M. J. Saunders et al., 2004b). Thus, it is possible that WP helped maintain run performance relative to CHO supplementation by promoting recovery of IET soldiers.

The lack of differences in FFM changes between groups was contrary to our hypothesis. It is notable that approximately 90% of the soldiers in the current study gained FFM. A previous study in IET soldiers reported only 36% of non-supplemented males gained FFM (L. M. Margolis et al., 2012b). The authors suggested that a calorie deficit could be the reason for the small percentage of IET soldiers who gained FFM. We have previously reported IET soldiers exist in an estimated 540-calorie energy deficit across training when no supplementation is provided (McAdam et al., 2017). In the current study we supplied two WP or CHO servings per

day totaling approximately 580 additional daily calories during IET. Thus, in light of previous research our findings may suggest that additional calorie intake itself may be beneficial for increasing FFM during IET.

Self-reported diet logs indicated participants consumed a relatively high protein diet relative to body weight (1.5-1.7 g/kg) during the first week of training when no supplementation was provided. Protein intakes at these levels have been shown to be adequate for nitrogen balance and optimization of FFM response to resistance exercise training (Lemon et al., 1992b; MA Tarnopolsky et al., 1992). Thus, the high protein intake in both groups may have been adequate to support an optimal FFM response to IET training. The negligible between-group differences in FFM changes could also have been due to a variation in training experience and fitness levels prior to IET. A recent meta-analysis and regression study (Morton et al., 2018) revealed that protein supplementation is likely not as effective in untrained individuals compared to individuals who regularly participate in resistance training. The initial FFM in the soldiers in this study ranged from 47.0 to 80.0 kg across both groups, suggesting that there was a heterogenic distribution of muscle mass prior to training. Post hoc analysis indicated low initial FFM inversely correlated with change in FFM across training ($r = -0.45$, $p < 0.001$) indicating soldiers with low initial FFM experienced more robust increases in this variable with training. These findings suggest more research is needed in determining how baseline fitness and body composition levels affect the physiological responses to WP supplementation.

Limitations

Our participants exhibited a large range of body composition and fitness levels. While this study had a large sample ($n = 69$), future investigations should use sample sizes enabling

participants to be divided into subgroups based on these variables to determine if supplementation has a differential effect between these individuals. Skinfolds were used to assess body composition, which may introduce variability in our measure. Although, there was a high intraclass correlation between the researchers, future investigations should use methodologies such as bioelectrical impedance or DEXA, which have less variation in measures. It should be noted that the fitness testing was collected by unit drill sergeants. As a result of multiple testers, interrater variability could influence our findings. However, drill sergeants are highly experienced and trained to administer these fitness assessments and do so multiple times each month. The APFT is the Army fitness test of choice. Thus, we chose it as an assessment because of its applicability to the Army. Additionally, tight training schedules during IET combined with large numbers of IET soldiers precluded the use of additional performance measures.

CONCLUSIONS

Twice daily supplementation of approximately 39 grams of WP resulted in improved push-up performance and had a significant reduction in FM in comparison to CHO supplementation. There was no statistical difference in FFM, sit-up, or run performance between WP and CHO supplementation groups.

Chapter 6: Evaluation of Training Volume and Once Daily Whey Protein Supplementation in IET

INTRODUCTION:

The goal of military Initial Entry Training (IET) is to mentally and physically prepare new recruits to be capable of immediate integration into active duty units. Physical fitness is key for the success of our soldiers, as they must perform physically strenuous daily tasks (Sharp, Patton, & Vogel, 1998). Physical preparation during IET is also key for force health, as lower fitness is related to increased risk of musculoskeletal injury (MSI) (C. Milgrom et al., 2000; Moran et al., 2008). MSI costs the US Army approximately 10 million limited duty days and approximately 384 million dollars per year to replace soldiers that have separated from the service (Teyhen, 2014). Physical preparation of IET soldiers has become a challenging task due to decreased recruit fitness upon entry to IET (J. Molloy et al., 2012a). The strenuous military environment has been reported to have negative effects on performance and body composition. Past research in US Army IET has revealed that IET soldiers participate in at least six to seven hours of daily physical activity ranging from low to very vigorous in intensity (McAdam et al., 2017; K Simpson et al., 2013a). Recent investigations of dietary intake in IET soldiers report that they consume between 1,900-and 2,600 calories per day, an estimated 540 calories less than their daily energy expenditure (L. Margolis et al., 2012a; McAdam et al., 2017). Studies in US Army and Marines have reported that IET military members on average lost 1-3 kg of fat free mass (FFM) across training, and only 30% of Army IET soldiers gained FFM (L. Margolis et al.,

2012a). Other military training environments in the US and Australia have shown the negative impacts on FFM may also be related to reductions in physical performance (M. Fortes et al., 2011a; Nindl, Barnes, et al., 2007).

The negative effects of the military training environment may be assessed, in part, by serum biomarkers markers. Acutely, one study (Nindl, Alemany, et al., 2007b) found that military training resulted in reductions in serum testosterone and Insulin like Growth Factor 1 (IGF-1). These are anabolic hormones that are positively related to body composition and performance due to their ability to stimulate muscle protein synthesis (Kraemer & Ratamess, 2005; Nindl, Alemany, et al., 2007a). One study (Friedl et al., 2000) in Army rangers revealed decreases in testosterone and IGF-1 can be modulated by dietary intake. Testosterone and IGF-1 were significantly decreased when training in an energy deficit. However, during periods of increased nutritional intake testosterone and IGF-1 levels were restored (Friedl et al., 2000). Military training has also been shown to increase markers of stress and catabolism. Cortisol is a hormone that results in the breakdown of skeletal muscle and increases in response to military training (M. Fortes et al., 2011a; Friedl et al., 2000). The catabolic effect of cortisol can be balanced by an increase in testosterone to promote muscle remodeling. Imbalances in the cortisol/testosterone ratio, whether decreases in testosterone or increases in cortisol, have been shown to be associated with reductions in performance (Kraemer et al., 2004). Studies in Army Rangers, Australian basic training, and United Kingdom sections commanders battle course have all reported that military training results in elevated cortisol levels and a reduction in the testosterone cortisol ratio (Diment et al., 2012; Drain, Groeller, Burley, & Nindl, 2017; Friedl et al., 2000). Military training has also been reported to increase the release of interleukin-6 (IL-6), a marker of inflammation and immune response. IL-6 is a key player in stimulating the

inflammatory response to exercise induced muscle damage and pathogens (Suzuki et al., 2006; Tanaka et al., 2014). Chronically elevated levels of IL-6 have been related to overtraining and may represent inadequate recovery (Gholamnezhad et al., 2014). Four intensive days that culminated in a three day march in Norwegian military training resulted in significant increases in IL-6 (Gomez-Merino, Chennaoui, Burnat, Drogou, & Guezennec, 2003). Another study revealed that IL-6 was elevated across four weeks of in military training in the French military (McClung et al., 2013). These studies suggest that IL-6 may be a valuable marker for monitoring training stress and recovery. To date no investigations examining IL-6 have been conducted in the Army IET environment.

Nutritional supplementation may have an important influence on the physical and hormonal response to military training. One study (M. Fortes et al., 2011a) in non-IET training in the United Kingdom reported that the addition of a mixed supplement was able to negate the decrease in performance and FFM during eight weeks of training. Additionally, our previous work suggests that consuming two whey protein (WP) shakes per day resulted in significantly higher push-up performance and supplementation overall resulted in a higher percentage of participants gaining fat free mass across IET in comparison to a previous investigation of non-supplemented IET soldiers (L. Margolis et al., 2012a; McAdam et al., 2018). WP with small amounts of casein, has been shown to increase IGF-1 levels in individuals involved in strength training (Willoughby, Stout, & Wilborn, 2007). These authors found that protein increased muscle mass and IGF-1 across 10 weeks of training in comparison to a carbohydrate placebo (Willoughby et al., 2007). Another study (Ballard, Clapper, Specker, Binkley, & Vukovich, 2005) found that 6 months of protein supplementation resulted in increases in serum IGF-1 levels in individuals involved in concurrent strength and endurance training. The effects of protein

supplementation on IGF-1 levels are thought to be mediated by an increased supply of amino acids that are able to stimulate IGF-1 gene transcription via increases in insulin signaling (Willoughby et al., 2007). WP has also been reported to increase serum testosterone in comparison to soy, as well as reduce serum cortisol levels in comparison to soy and carbohydrate (CHO) supplementation in response to resistance training (Kraemer et al., 2013). Collectively, these studies suggest that WP may be beneficial for improving the physiologic and physical response to strenuous training.

The goal of the current study is to build upon our previous research in IET soldiers to determine if consuming only one WP shake per day can improve performance, body composition, and endocrine responses to IET. We hypothesized that WP would be beneficial for push-up performance and body composition. Additionally, we hypothesized that WP would be beneficial for improvements in the testosterone to cortisol ratio (T:C) and IGF-1 responses to training due to improvements in anabolic status of the body as well as reductions in cortisol and IL-6 due to improved recovery during IET.

METHODS

Study Design and Population

This was a double blind, placebo controlled, 2 x 2 (Group x Time) factorial-repeated measures design. The Auburn University Institutional Review Board, Army Institutional Review Board, and the Director, Research & Analysis Directorate Army Center approved the study procedures for Initial Military Training. Potential participants were given a description of the study. Those wishing to participate gave written consent and were enrolled in the study. Participants were apparently healthy, 18-35-year old males engaged in army IET. Participants

had to be free from MSI, free from allergies to milk or whey protein, and not have been supplementing or have supplemented within the past 3 months.

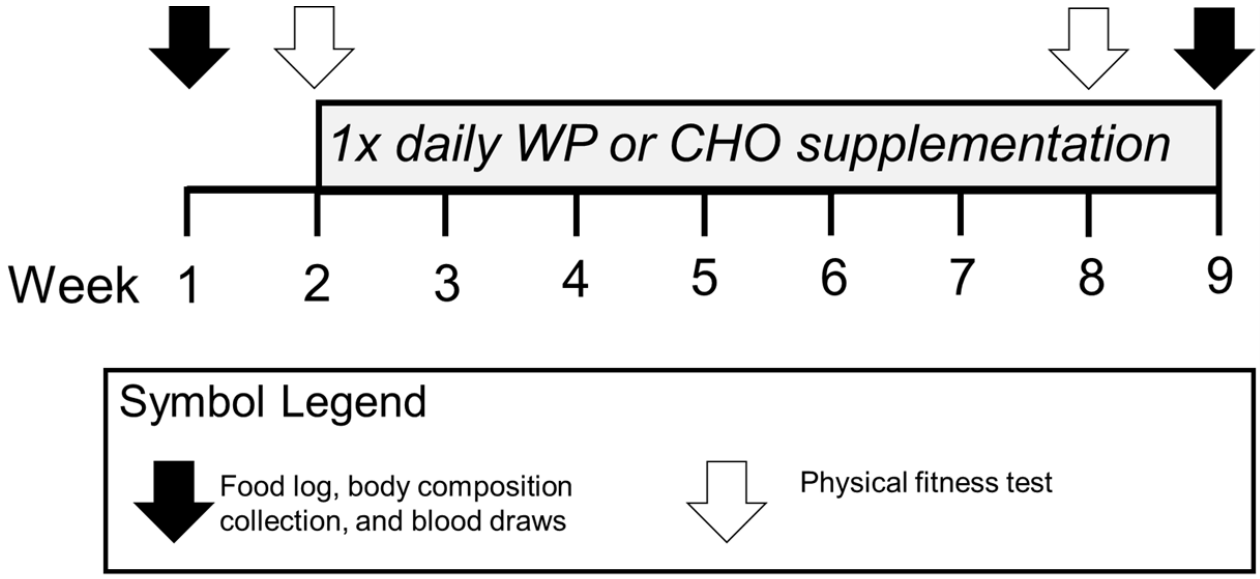
In total 95 participants agreed to participate in the study. 81 total participants completed the study (WP: n = 39, Ht. = 173 ± 8 cm, Wt. = 76.8 ± 12.8 kg, Age = 21 ± 3yrs; CHO: n = 42, Ht. = 175 ± 8 cm, Wt. = 77.8 ± 15.3 kg, Age = 23 ± 4yrs). Four participants were removed due to prior supplementation, five were removed due to missed shakes, four discontinued training due to injury or leaving IET, and one dropped out of the study because he felt he was gaining too much weight. Participants were supplemented with either one whey protein hydrolysate or one taste and calorie matched carbohydrate supplement per day. Macronutrient content of the two supplements are listed in Table 5 below.

Table 5: Nutrition information per supplement

Macronutrient	**WP Supplement**	**CHO Supplement**
Energy (kcal)	293	291
Protein (g)	38.6	0.5
Carbohydrate (g)	19	63.4
Fat (g)	7.5	3.9

All measures of body composition and serum biomarkers markers were collected after an overnight fast before breakfast and morning physical training, during weeks one and eight of training. Performance measures were performed during weeks two and seven of training. Figure 8 summarizes the timeline of measures.

Figure 8. Summary of measures collected during IET



Legend: This figure illustrates the logistics of the 10-week training intervention. More detail regarding can be found in the methods section.

Measures

The independent variables were supplementation group (WP or CHO) and time (training week). Outcome variables were daily training volume, physical performance, body composition, dietary intake, and biomarkers of bone health (P1NP, CTX), anabolic status (testosterone, cortisol, IGF-1), and immune health/recovery (IL-6). Serum and body composition variables were collected during weeks one and eight of training, after an overnight fast, in the morning before breakfast and physical training. Urine specific gravity (USG) testing was completed prior to all serum and body composition assessments. Participants with USG above 1.030 were considered inadequately hydrated, given water to drink and not allowed to proceed with testing until USG was below 1.030. Performance measures were performed during weeks two and seven of training.

Height and weight

Height and weight were assessed using a Health-O-Meter professional scale (Model 500KL, Sunbeam products INC. Boca Raton, FL. USA) and reported in centimeters and kilograms. Participants were asked to remove shoes and wore only Army issued physical training, shorts, socks, and shirts during the measurement.

Body Composition

Body composition was assessed using an Impedimed DF50 device (ImpediMed Ltd, Brisbane, Australia). This measure is sensitive to hydration; therefore, hydration was assessed prior to measurement through urine specific gravity (described above). Participants were asked to lay supine for approximately five minutes to allow for equilibration of body fluids across intracellular and extracellular compartments prior to assessment. Measurements were taken in the supine position. Electrode placement on the hand and ankle were determined as per the manufacturer recommendations. A proximal electrode was placed on the left wrist on the midline of the ulnar styloid process and a distal electrode was placed on the midline 5 cm apart. Another electrode was placed on the midline between the medial and lateral malleolus and a final electrode was placed 5 cm distal to the malleolus on the midline. All application sites were shaved to ensure optimal electrode placement. All electrode placements were performed by the same member of the research team to minimize variability in electrode placement. Raw output was collected from the device; fat free mass (FFM) and fat mass (FM) were calculated using the formulas below:

$$FFM = (Height)^2 / Resistance * 0.734 + 0.116 + Reactance * 0.096 + 1 * 0.878\text{-}4.03$$

FM = Body Mass - FFM

Height was input into the FFM calculations in centimeters and body weight was in kilograms. Both FFM and FM are represented as mean ± standard deviation in kilograms.

Physiological Biomarkers

Blood draws were taken from the antecubital vein via 21 gauge, Safety-Lok needle kits (Benton, Dickinson and Company, Franklin Lakes, NJ. USA) by research team members. Blood was collected in 10 ml serum separator vacutainer tubes (BD Vacutainer; Franklin Lakes NJ, USA) and placed on ice in a cooler (Yeti Coolers LLC, Austin TX, USA) until centrifugation the same morning of collection. The blood samples were centrifuged at 3,500xg for 10 minutes at room temperature. Samples that were not fully separated were centrifuged again under the same conditions. Serum was extracted from separated blood and frozen at -80 degrees (Kendra Laboratory Products, Asheville, NC. USA) until analysis. Testosterone (American Laboratory Products Company, Salem, NH, USA, sensitivity: 0.022 ng/mL, CV: 2.9%), cortisol (American Laboratory Products Company, Salem, NH, USA, sensitivity: 0.4 μg/dL, CV: 4.8%), IGF-1,(American Laboratory Products Company, Salem, NH, USA, sensitivity: 0.091 ng/mL, CV: 10.5%), C-terminal cross-links of type 1 collagen (CTX, Immunodiagnostic Systems, Gaithersberg, MD, USA, CV: 5.2%) and IL-6 (Invitrogen, Carlsbad, CA, USA, sensitivity: 0.3 pg/ml, CV: 7.1%), were measured using Enzyme Linked Immunosorbent Assays (ELISA), according to manufacturers' instructions. Plates were at respective wavelengths using a multispectral spectrophotometer (BioTek Eon, Winooski, VT, USA). All samples were analyzed in duplicate and each participant's pre- and post-intervention samples were analyzed on the same plate. All optical densities were within the detectable range of the assay. IL-6 had four

individuals whose concentrations could not be used due to being outside the normal physiologic range for the four compartment logistic regression models and were removed from the analysis. Serum concentrations of each optical density was calculated as per manufacturer instructions using either regression or a four-parameter logistic regression. P1NP was evaluated using chemilumnescence.

Physical Performance

The Army Physical Fitness Test (APFT) was performed during weeks two and eight of the intervention. The APFT was administered by unit drill sergeants according to the standards of the US Army field manual for physical fitness training (DOD US Army, 1998). The APFT consists of two-minute push-up, two-minute sit-up, and a two-mile run test. Proper technique on push-ups and were monitored by drill sergeants and only counted if the IET soldier lowered himself to parallel in the eccentric phase of the push-up and fully extended the elbows in the concentric phase of the push-up. Proper sit-up form to be counted as valid is for the participant to raise himself upwards until the base of the neck was positioned superior to the vertical axis of the spine and for the scapula to make full contact with the ground. Run performance tests were conducted on a flat road under constant supervision of unit leadership, with one evaluator calling out times and another recording final run times by IET soldier number. Times were announced as IET soldiers crossed the finish line and recorded after the completion of the test.

Daily Physical Activity

Physical activity and energy expenditure were estimated using Actigraph GTX (Actigraph, Pensacola, FL, USA). Each week 20 participants (10 per supplement group) were

asked to wear a monitor on their right hip for one week and not remove the monitor except to shower. Monitors were initialized prior to deployment and physical activity per day was estimated using Actilife software version 13.1.1 (Actigraph, Pensacola, FL, USA). Time spent in each category of physical activity was estimated using Sasaki vector magnitude 3 (VM3) (Sasaki et al., 2011). The following range of counts were used for each category of physical activity: Moderate = 2690 - 6166 counts/minute (3 - 5.99 METs), Vigorous = 6167 - 9642 counts/minute (6 - 8.99 METs), Very Vigorous ≥ 9,642 counts/minute (> 9 METs) (Sasaki et al., 2011). All VM3 counts below 200 counts/minute were classified as sedentary (Migueles et al., 2017), and the difference between sedentary cut points and moderate cut points were classified as low intensity (201-2689 counts/minute). Sampling rate was 30 Hz (Brond & Arvidsson, 2016) and a valid wear day must have a minimum wear time of 600 minutes (Migueles et al., 2017).

Nutritional Intake

Diet logs were completed on three, non-consecutive days during weeks one and eight of IET. Members of the research team met with the participants to fill out the diet logs after each meal and to help answer any questions. Diet log data was entered into excel spreadsheets and reviewed by two researchers for accuracy. The diet data was then imported into R statistical software (R Core Team, 2015) and dietary intake calculations were completed using and R Studio (RStudio, 2014) along with the R programming packages: dplyr (Wickham et al., 2017), tidyr (Wickham & Henry, 2017), reshape2 (Wickham, 2007), ez (Lawrence, 2016), car (Fox & Weisberg), vars (Bernhard, 2008), ggplot2 (Wickham, 2009). Total calorie, protein, carbohydrate, and fat intakes were calculated for each meal and day, and then averaged for training weeks one and week eight. Nutritional data for the dining facility foods were retrieved

from the Army Joint Culinary Center of Excellence (JCOE) website (Army, 2012). Food items not found on the JCOE menu were retrieved from the US Department of Agriculture (USDA) nutrition data base (United States Department of Agriculture, 2013). A detailed description of the diet analysis can be found in chapter 4. Diet logs for three meals was required to be considered valid for dietary analysis. Days in which a participant did not complete all three logs were removed from the analysis. Participants without at least two full days of diet logs each week were removed from the summary of diet logs.

Statistical Analysis

Regression analysis was used to compare training time between groups during training. Additionally, regression analysis was used to compare training volume during phase of training (Red = weeks 1 - 3, White = weeks 4 - 6, and Blue = weeks 7 - graduation). Distribution of residuals of the linear models was visualized using density plots and normality was tested using the Kolmogorov–Smirnov test.

Analysis of variance (ANOVA) was used compare diet and serum markers between groups and across time of the intervention. The assumption of normality of residuals testing was completed for all variables using Shapiro-Wilks, Komolgorov Smirnov tests, and residual QQ plots were used to visually inspect the data. Data were square root transformed and normality was recalculated for any variable for which more than 75% of the levels were non-normally distributed. An a *priori* alpha level of 0.05 was set for determination of significant effects. Maulchy's test of sphericity was used to evaluate equality of variance and Levene's test was used to evaluate homogeneity of variance. If sphericity was violated a Greenhouse-Geisser correction was used. Group by time interactions were further evaluated using paired samples t-

test to evaluate simple main effects of time and independent samples t-tests were used to evaluate simple main effect of group.

Analysis of covariance (ANCOVA) was used to evaluate performance and body composition during performance. ANCOVA has been reported to increase sensitivity to group effects, which was the focus of this investigation. Mean centered initial values for each variable were used as the covariate in the ANCOVA model as this reduces the error rate in ANCOVA models with between group factors.

Cohen's d effect sizes were calculated within groups across training as well as between groups post training. Effect sizes are reported as effect size with the associated upper and lower limits of the 95% confidence interval.

Testosterone violated assumption of normality at all levels (WP = W: 0.78, $p < 0.01$ pre; W: 0.70, $p < 0.01$ post; CHO = W: 0.80, $p < 0.01$ pre; W: 0.70, $p < 0.01$ post). Testosterone was log transformed and re-tested for normality. Only post-intervention was non-normally distributed but ANOVA is robust to partial violations of normality, so we chose to proceed with analysis. P1NP violated assumption of normality for 75 % of the levels of the dependent variable (WP = W: 0.89, $p < 0.02$ pre; CHO = W: 0.89, $p < 0.02$ pre; W = 0.83, $p < 0.01$ post). Log transformed P1NP was re-tested for normality and was normally distributed at all levels. IL-6 violated the assumption of normality at all levels of the dependent variable (WP = W: 0.75, $p < 0.01$ pre, W = 0.77, $p < 0.01$ post; CHO = W: 0.85, $p = 0.03$ pre; W = 0.85, $p = 0.03$ post). IL-6 data were square root transformed and normality was re-tested and all levels were normally distributed.

RESULTS

Training Volume

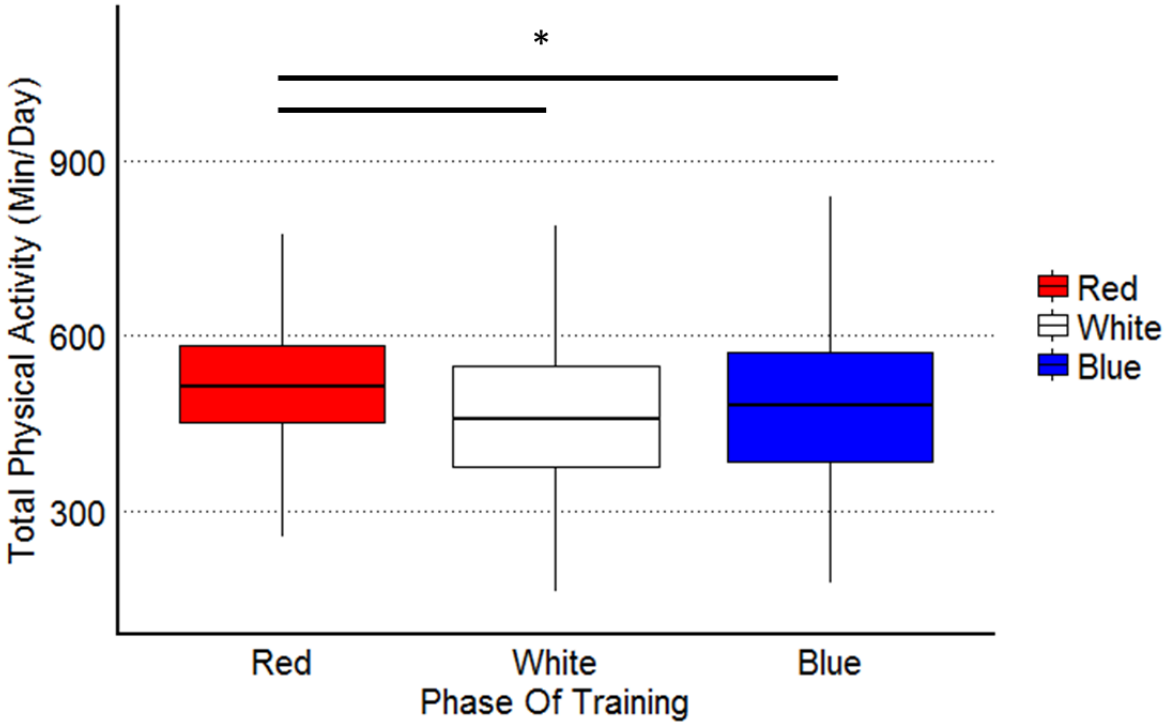
Figure 9 summarizes training volume across each week of IET. Overall IET soldiers performed on average 490 ± 136 minutes of total (light to very vigorous) physical activity per day. IET soldiers spent on average 282 ± 75 minutes in light, 101 ± 39 minutes in moderate, 22 ± 23 minutes in vigorous, and 5 ± 8 minutes in very vigorous physical activity per day. Regression of total training time by phase revealed that red phase (weeks 1-3) was a significant predictor ($F = 28.75$, $p < 0.01$) of training time such that being in red phase (weeks 1-3) resulted in 47 more minutes per day on average of total training time versus white (weeks 4-6) and blue (weeks 7-graduation) phases. Figure 10 summarizes training volume by phase.

Regression analysis revealed that supplement group was not a significant predictor of time spent in light ($F = 1.02$, $p = 0.31$), moderate ($F = 0.18$, $p = 0.67$), vigorous ($F = 0.22$, $p = 0.64$), or very vigorous intensity physical activity ($F = 2.59$, $p = 0.11$). This suggests that both the WP and CHO groups performed the same volume of physical training and may not confound the findings in the dependent variables collected in this study.

Figure 9: Summary of training volume during each week IET

Legend: Summary of training volume across IET. Total physical activity (Sum of time spent in: light, moderate, vigorous, and very vigorous intensity) is represented by the columns and time spent in each classification of physical activity is represented by the lines. Data is presented in average minutes per day during each week of training.

Figure 10. Training volume per phase of IET



Legend: Summary of training volume across IET. Total physical activity (Sum of time spent in: light, moderate, vigorous, and very vigorous intensity). Data is presented in average minutes per day during each week of training. Red phase = weeks 1-3, White phase = weeks 4-6, Blue phase = weeks 7-9
* Symbolizes significant difference, $p < 0.05$

Dietary Intake

First, we analyzed dietary intake on only what was consumed at the dining facility at post-intervention (Post NS) to determine if consuming the supplements decreased intake at the dining facility in either supplement group. There was a significant group by time interaction for overall protein ($F = 18.26$, $p < 0.001$) and protein intake relative to body weight ($F = 16.43$ $p < 0.001$) intake. There were no significant interactions for overall calories ($F = 2.26$, $p = 0.11$) or calories relative to body weight ($F = 2.67$ $p = 0.11$), carbohydrate ($F = 2.88$ $p = 0.09$) or

carbohydrate relative to body weight ($F = 3.01$ $p = 0.09$), or overall fat ($F = 2.33$ $p = 0.13$) or fat intake relative to body weight ($F = 1.95$ $p = 0.17$).

We then compared dietary intake with the supplement nutrition added to total dietary intake at post-intervention (Post SI).There were significant group by time interactions for calories ($F = 34.67$ $p < 0.001$), protein ($F = 110.7$, $p < 0.001$), and CHO ($F = 29.98$, $p < 0.001$), however there were no increases in fat ($F = 0.71$, $p = 0.41$) intakes across the intervention. Additionally, there were significant group by time interactions for dietary data relative body weight for calorie (kcal/kg, $F = 31.92$, $p < 0.001$), protein (g/kg, $F = 96.51$, $p < 0.001$), and carbohydrate (g/kg, $F = 27.83$, $p < 0.001$), however there were no increases in fat (g/kg, $F = 0.76$, $p = 0.39$) intakes across the intervention.

Dietary intake as well as time and group differences for each macronutrient are summarized in Table 6.

TABLE 6. Summary of dietary intake during IET

Group	Variable		Pre NS	Post NS	Post SI
WP	Energy	(kcal/d)	2561 ± 607	2649 ± 718	2942 ± 718*
		(kcal/d)	34.3 ± 10.2	35.5 ± 10.6	39.5 ± 10.9*
CHO		(kcal/d)	2619 ± 603	2752 ± 789	3043 ± 789*
		(kcal/d)	35.1 ± 11.6	37.1 ± 13	41 ± 13.4*
WP	PRO	(g/d)	116 ± 25	126 ± 34*	165 ± 34*#
		(g/kg/d)	1.5 ± 0.4	1.7 ± 0.5*	2.2 ± 0.5*#
CHO		(g/d)	117 ± 26	133 ± 35*	134 ± 35*#
		(g/kg/d)	1.6 ± 0.5	1.8 ± 0.6*	1.8 ± 0.6*#
WP	CARB	(g/d)	336 ± 88	354 ± 101	373 ± 101*
		(g/kg/d)	4.5 ± 1.4	4.7 ± 1.5	5 ± 1.5*
CHO		(g/d)	338 ± 90	357 ± 117	420 ± 117*
		(g/kg/d)	4.5 ± 1.6	4.8 ± 1.9	5.7 ± 2*
WP	FAT	(g/d)	87 ± 23	82 ± 26	89 ± 26
		(g/kg/d)	1.2 ± 0.4	1.1 ± 0.4	1.2 ± 0.4
		(g/d)	91 ± 21	89 ± 27	92 ± 27
CHO		(g/kg/d)	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4

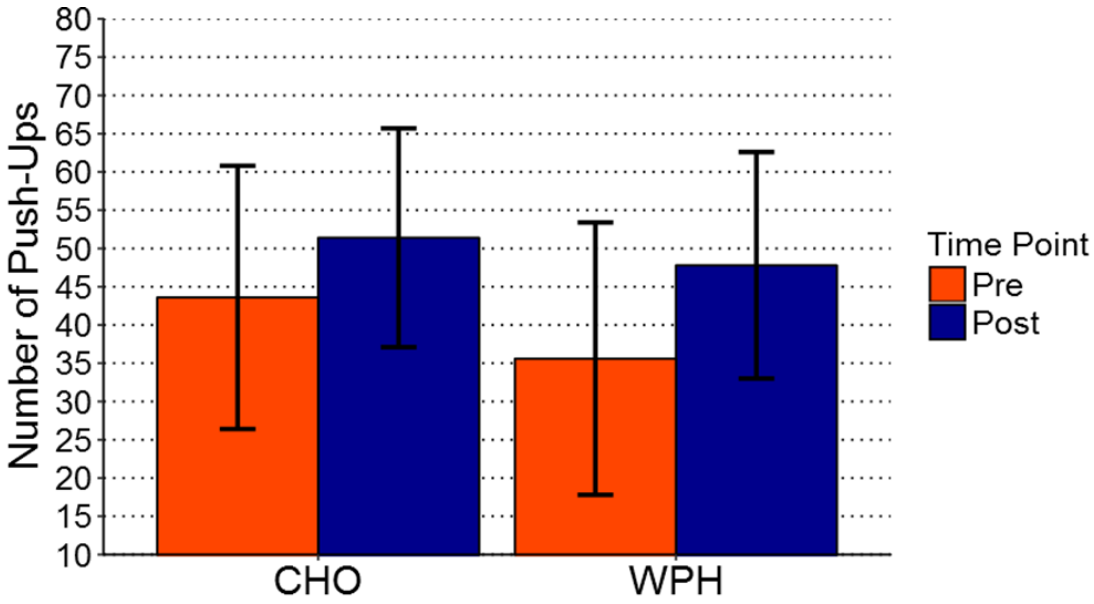
Legend: all data are food log data that are self-reported, averaged to one-day daily average intakes, and presented as means ± standard deviation values. Abbreviations: PRO, dietary protein; CARB, dietary carbohydrate; FAT, dietary fat; WPH, hydrolyzed whey protein group) (n=39); CHO, carbohydrate-placebo group (n=42). *, indicates Post>Pre ($p<0.05$), +, indicates group differences at a given testing time point ($p<0.05$).

Performance

Push-up performance was 35.6 ± 17.8 pre- and 47.8 ± 14.8 post-intervention in the WP and 43.6 ± 17.2 pre- and 51.4 ± 14.3 post-intervention in the CHO groups. There was no significant difference post-intervention when controlling for pre-intervention push-up

performance (F = 0. 43, p = 0.51) or interaction with group, when pre-intervention push-up performance was controlled for (F = 0.97, p = 0.33).

Figure 11. Push-up performance during IET



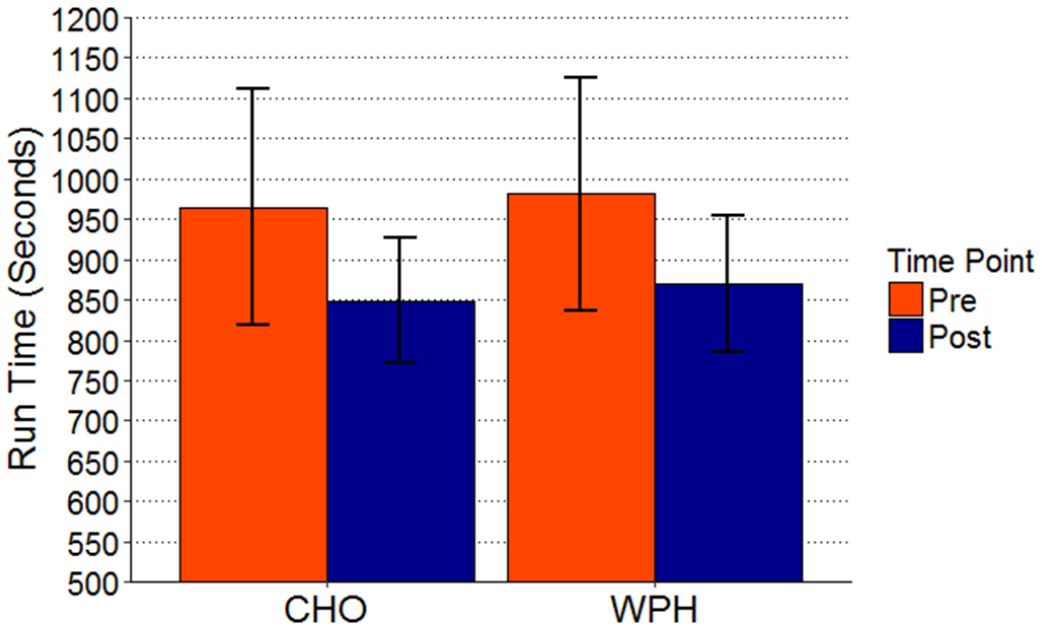
Legend: all data are presented as means ± standard deviation values.
Abbreviations: WP, whey protein group (n=37); CHO, carbohydrate-placebo group (n=18). Symbols: *, indicates WP > CHO at POST.

Sit-up performance was 44 pre- and 59.9 post-intervention in the WP and 51.0 ± 14.0 pre- and 66.2 ± 10.7 post-intervention in the CHO group. There was no significant difference in sit-up performance at post-intervention when controlling for initial sit-up performance (F = 0.64, p = 0.69) or initial push-up by group interaction (F = 0.16, p = 0.69).

Run performance was 981.4 ± 144.2 pre- and 869.6 ± 85 post-intervention in the WP and 964.9 ± 146.3 pre- and 848.6 ± 77.3 post-intervention in the CHO group. There was no

significant difference post-intervention when controlling for pre-intervention run performance (F = 1.04, p = 0.31) or interaction with group, when pre-intervention run performance was controlled for (F = 0.02, p = 0.90).

Figure 12. Run performance during IET



Legend: all data are presented as means ± standard deviation values. Abbreviations: WP, whey protein group (n=37); CHO, carbohydrate-placebo group (n=18). Symbols: *, indicates WP > CHO at POST.

Body Composition

There was no significant difference post-intervention when controlling for pre-intervention body weight (F = 0.13, p = 0.72). There was no significant pre-intervention by group interaction for body weight (F = 0.73, p = 0.186).

There was no significant difference post-intervention when controlling for pre-intervention FFM ($F = 2.79$, $p = 0.099$). There was a significant pre-FFM by group interaction ($F = 9.46$, $p < 0.01$) suggesting that WP was more beneficial for individuals with higher pre-FFM.

FM was 17 ± 7 pre- and 15 ± 5 kg post-intervention in the WP group and 16 ± 7 pre- and 15 ± 6 post-intervention in the CHO group. There was no significant difference between groups post-intervention for FM ($F = 2.55$, $p = 0.114$) when controlling for pre-intervention FM. There was no significant pre-FM by group interaction ($F = 1.777$, $p = 0.186$).

TABLE 7. Summary of body composition between groups during IET

Variable	Group	Pre	Post
Body weight (kg)	WP	76.8 ± 12.8	75.8 ± 11.6
	CHO	77.8 ±15.3	76.9 ± 13.1
FFM (kg)	WP	59.5 ± 8.4	60.7 ± 8.5
	CHO	61.4 ± 10.5	61.5 ± 8.7
Fat Mass (kg)	WP	17.2 ± 6.6	15.1 ± 6.0
	CHO	16.3 ± 6.2	15.4 ± 5.7

Legend: all data are presented as means ± standard deviation values. Body composition was assessed using 7-site skinfolds as described in the methods. Abbreviations: kg, kilograms; FFM, fat-free mass; WP, hydrolyzed whey protein group (n = 39); CHO, carbohydrate-placebo group (n = 42)

Effect sizes for physical performance and body composition are reported in Table 8 below.

Table 8: Summary of effect sizes

Variable	Group	Mean Difference	95% CI	Units	Effect Size	Classification
FFM	WP	1.2	(0.4, 2)	(kg)	0.11	Small
	CHO	0.1	(-0.9, 1)	(kg)	0.01	Small
FM	WP	-2.1	(-3.1, -1.2)	(kg)	-0.26	Small
	CHO	-0.9	(-1.7, -0.1)	(kg)	-0.12	Small
PU	WP	12.1	(9.1, 15.2)	(kg)	0.59	Large
	CHO	7.8	(1.41, 14.2)	(kg)	0.39	Medium
SU	WP	15.8	(11.7, 20)	(kg)	0.91	Large
	CHO	15.2	(11.3, 19.1)	(kg)	0.95	Large
Run	WP	-111.8	(-141.5, -82.1)	(kg)	-0.72	Large
	CHO	-116.3	(-157.7, -74.9)	(kg)	-0.74	Large

Legend: Mean differences along with 95% confidence intervals for the mean difference (lower limit, upper limit); cohen's d effect sizes are calculated for pre- to post-intervention changes in sit-up (SU), run time (Run), push-up performance (PU), fat-free mass (FFM) and fat mass (FM). Values in the positive direction indicate an improvement in the metric (i.e., a decrease in run time, an increase in push-up number, an increase in FFM, and a decrease in fat mass). Abbreviations: WP, whey protein group (n=34); CHO, carbohydrate-placebo group (n=35).

Serum Biomarkers

There was a significant main effect of time for testosterone ($F = 14.06$, $p < 0.01$), however there was no was no significant group by time interaction ($F = 0.03$, $p = 0.87$) or main effect of group ($F = 0.91$, $p = 0.35$). There was a small effect size in the WP ($d = 0.34$, CI = -0.25, 0.94), and medium in the CHO ($d = 0.60$, CI = 0, 1.19) groups across the intervention.

There was no significant group by time interaction for cortisol ($F = 1.58$, $p = 0.22$), main effect of time ($F = 3.64$, $p = 0.06$), or group ($F = 0.70$, $p = 0.41$) for cortisol.

There was a main effect of time for T:C ($F = 10.08$, $p = p < 0.01$), however there was no significant group by time interaction ($F = 0.08$, $p = 0.78$) or group ($F = 0.63$, $p = 0.43$) for the

T:C.There was a main effect of time for IGF-1 (F = 6.62, p = p = 0.01), but no significant group by time interaction (F = 0.78, p = 0.38) or main effect of group (F = 2.44, p = 0.12) for IGF-1. There was no significant group by time interaction for P1NP (F = 0.27, p = 0.61), main effect of time (F = 3.02, p = 0.09), or group (F = 0.25, p = 0.62) on P1NP. There was no significant group by time interaction for CTX (F = 0.16, p = 0.69), main effect of time (F = 0.01, p = 0.91), or main effect of group (F = 0.04, p = 0.83). There was a significant main effect of time for IL-6 (F = 7.63, p = 0.01), but no group by time interaction (F < 0.01, p = 0.95), or main effect of group (F = 0.54, p = 0.47) on IL-6 across the intervention.

Table 9: Summary of serum results across IET

Biomarker	Units	Group	n	Pre	Post	
Testosterone	(ng/dL)	WP	23	540.48 ± 266.78	661.94 ± 463.87	*
		CHO	24	474.72 ± 226.54	552.84 ± 281.71	*
Cortisol	(ug/dL)	WP	23	22.19 ± 7.49	21.63 ± 7.63	
		CHO	24	21.99 ± 4.67	19.24 ± 3.71	
T:C		WP	23	25.12 ± 10.01	33.1 ± 22.57	*
		CHO	24	22.64 ± 11.92	29.3 ± 15.67	*
IGF-1	(ng/dL)	WP	23	230.62 ± 88.36	243.05 ± 85.73	*
		CHO	24	191.94 ± 70.83	217.38 ± 49.38	*
P1NP	(ng/ml)	WP	20	87.29 ± 43.6	90.87 ± 40.72	
		CHO	21	81.43 ± 43.98	87.35 ± 44.65	
CTX	(pg/ml)	WP	23	7038.34 ± 2837.25	6933.21 ± 2376.27	
		CHO	24	7062.25 ± 3500.13	7252.81 ± 3358.63	
IL-6	(pg/ml)	CHO	13	6.66 ± 5.11	4.15 ± 2.55	*
		WP	14	5.52 ± 4.34	3.78 ± 3.92	*

Legend: Serum data is presented as means ± standard deviation values; WP, whey protein group; CHO, carbohydrate-placebo group. *, indicates Post>Pre ($p<0.05$), +, indicates group differences at a given testing time point ($p<0.05$).

DISCUSSION

This project examined how eight weeks of WP versus CHO supplementation influenced physical performance, physiological biomarkers, and body composition across IET. Our primary findings were: 1) training volume was higher in the initial phases of IET in comparison to the later phases, 2) WP had differential effects on FFM depending on the level of pre-intervention FFM, and 3) supplementation during IET had a beneficial effect on physiologic markers of training status.

One important finding from this study is that IET phase was a significant predictor training time. This is in agreement with our previous work that found training volume is high during the initial weeks of training (McAdam et al., 2017; K Simpson et al., 2013a). Red phase had the highest training volume on average in comparison to white and blue phase. Previous research suggests that less than 30% of individuals in the U.S. aged 18-35 participate in 300 minutes per week of moderate or 150 minutes of vigorous intensity exercise (Center for Disease Control and Prevention, 2015). Here, we report that IET soldiers participate in over 400 minutes per day of at least light intensity exercise, and that the amount of time spent in physical activity is greatest during the first three weeks of training. Thus, IET soldiers likely experience rapid increases in training volume as they perform more physical activity in one day than the majority of the US population performs in one week. Rapid increases in running volume in novice runners has been reported to be a significant predictor of incurring training related injuries (ØN et al., 2014). These authors suggested novices should increase training volume no more than 30% over a two week period (ØN et al., 2014). The Army has sought to standardize training volume in organized morning physical training sessions in order to reduce training load and progressively increase volume across the training cycle to help reduce MSI during IET (JJ Knapik et al.,

2009a). However, periodization recommendations should be implemented to IET as a whole. IET consists of large volumes of physical activity outside of the normal organized morning physical training sessions in events such as combatives, obstacle courses, ruck marches carrying loads, and land navigation (K Simpson et al., 2013a). Therefore, strategies to either periodize total training volume or exposing new recruits to pre-IET training programs may be beneficial for reducing MSI during IET. The Army has voluntary pre-IET training programs available, but only a small percentage of recruits regularly participate in these programs.

WP was found to provide more benefit on FFM in individuals who had higher pre-intervention FFM than did the CHO. The WP group consumed approximately 30 more grams and approximately 0.3 more g/kg/d of protein than the individuals in the CHO group. Therefore, it is possible that individuals with higher FFM in the CHO group were not able to consume adequate protein from diet alone to optimize their response to training, which could negatively influence FFM. Previous literature has shown that higher protein intake during energy restricted training may be beneficial for preservation of FFM (E. Helms et al., 2014a). The overall effect of WP on FFM was not significant ($p = 0.099$). However, a low p-value in light of the much larger effect size for WP on FFM (WP = 0.50, CHO = 0.02) and the finding that the WP group on average gained an additional kilogram of FFM, suggests that WP may have a clinically relevant effect on FFM in IET soldiers.

WP had double the effect size on FM (WP = 0.72, CHO = 0.36), and push-up performance (WP = 1.32, CHO = 0.61) in comparison to the CHO group. Numerically, the WP group gained five more push-ups and lost an additional 1.1 kg of FM on average than the CHO group. These are similar responses to our previous work in IET, where the WP group gained seven more push-ups and lost an additional 1.8 kg on average than the CHO group (McAdam et.

al, in review). WP has been shown to have a beneficial effect on strength (P. Cribb et al., 2006a; Jean Farup, Rahbek, Vendelbo, et al., 2014) and FM (P. Cribb et al., 2006a; C. Lockwood et al., 2017a) in similar aged males. The consistency of findings between the two studies in two different units/training commands suggests that WP may have a clinically relevant effect on push-up performance and FM in IET soldiers.

Serum IGF-1, testosterone, and the T:C ratio significantly increased whereas IL-6 decreased across IET. This is in contrast to previous literature on the influence of military training on IGF-1, T:C, and testosterone (Alemany et al., 2008; Nindl, Barnes, et al., 2007). Studies in Army Ranger training have reported that IGF-1 and testosterone decrease in response to training due to large volumes of training and inadequate energy intake (Alemany et al., 2008; Nindl, Barnes, et al., 2007). The decrease in these biomarkers are thought to reflect imbalance between training volume and nutritional intake. This imbalance can be restored by increased nutritional intake (Friedl et al., 2000). Physiologically, IGF-1 and testosterone play important roles in stimulating muscle protein synthesis (Crossland, Timmons, & Atherton, 2017; White et al., 2013) and incorporation of satellite cells into skeletal muscle (Braga, Bhasin, Jasuja, Pervin, & Singh, 2012; Matheny Jr, Nindl, & Adamo, 2010). Furthermore, reductions in testosterone levels in relation to cortisol have been linked to reductions in performance across competitive season in college athletics (Kraemer et al., 2004). Thus, the finding that all of these serum markers increased suggests that supplementation of either CHO or WP, or IET alone without supplementation, promotes a favorable physiologic response in IET soldiers.

In addition to increases in anabolic hormones in response to training, we also report that IL-6 decreased across IET. IL-6 is released post-exercise and plays a variety of roles, one of which is stimulating the inflammatory response to muscle damage (Reihmane & Dela, 2014).

Chronic elevations in IL-6 have been linked to overtraining and muscle damage (Gholamnezhad et al., 2014). Currently, IL-6 has only been investigated in Israeli basic training, (Merkel et al., 2008; Nindl et al., 2012) which revealed a numeric but non-significant decrease in IL-6 in male (Nindl et al., 2012) and female (Merkel et al., 2008; Nindl et al., 2012) recruits across 4 months of basic training. It is important to note that IL-6 also plays a key role in stimulating the immune response to pathogens (Tanaka et al., 2014). Therefore, it is possible that pre-IET levels of IL-6 could have been elevated at pre-intervention due to immunizations or other lifestyle factors that may not occur in Israeli basic training soldiers, thus creating an artificial reduction in IL-6 across US Army IET. More work is needed in US Army IET to establish the IL-6 response to IET in the absence of supplementation. Collectively, the increase in testosterone, IGF-1, T:C ratio, and the decrease in IL-6 suggest that IET soldiers in this cohort had a beneficial response to IET and supplementation of additional calories to the diet via either WP or CHO and were not in a state of overtraining.

One limitation to the performance data is that we were only able to obtain performance data from 75% of the participants. This resulted in more data being collected for the WP group in comparison to the CHO. We were still able to obtain 55 (WP = 37, CHO = 17) data points. Another limitation is that performance data was collected by multiple testers. While inter-rater reliability could influence the findings of this study, it is notable that drill sergeants administered all tests and are very well trained in conducting the APFT as they administer the test often and IET soldier graduation is dependent upon the APFT. Finally, it is notable that blood draws were collected only at the pre- and post-intervention time points due to limited access to the soldiers during the IET period.

CONCLUSION

IET soldiers are immediately exposed to large volumes of physical activity during IET that remains high and tapers in the late stages of training. WP supplementation is beneficial for IET FFM mass in soldiers who enter training with high initial FFM, and may have a clinically relevant influence on push-up performance, FM, and FFM in individuals who have high initial FFM. More research is needed to determine the normal serum markers of overtraining and inflammation, to determine if supplementation improves the response of these variables in comparison to individuals who are not supplemented.

Chapter 7: MSI during IET

INTRODUCTION

Engaging in regular physical activity produces structural changes in bone such as larger shaft diameter and greater cortical thickness, which can help prevent micro-damage and improve resiliency to training-induced injuries (Bennell et al., 1997; Liu et al., 2003). Skeletal muscles also undergo structural changes that may decrease muscle damage during training (Aagaard et al., 2001; Doering et al., 2016; Flann et al., 2011). Overall fitness of the American population has been declining due to increased sedentary activity and decreased life time physical activity (Brownson et al., 2005). This has led to a decline in the fitness of individuals entering military Initial Entry Training (IET) as evidenced by increased failure rates on the IET initial fitness assessment (J. Molloy et al., 2012a). Low physical activity levels prior to and low fitness levels upon entry to IET is an important risk factor for musculoskeletal injuries (MSI) (C. Milgrom et al., 2000; Moran et al., 2008). Research has previously shown that run performance is a predictor of MSI during IET (J. Sefton, Lohse, & McAdam, 2016a; Shaffer et al., 1999; Henri Taanila et al., 2010; H. Taanila et al., 2015). Individuals with lower fitness must exercise at higher relative intensities in comparison to their higher fit counterparts (Ploutz et al., 1994; Turner, 2016). Therefore, during IET individuals with lower fitness will experience a much higher relative training stimulus versus those with higher fitness. Strategies to improve recovery and adaptation of the musculoskeletal system need to be employed to help promote healthy adaptation to training. Whey protein (WP) supplementation has been reported to increase activity of

osteoblasts and reduce that osteoclasts (Yukihiro Takada et al., 1996; Y Takada et al., 1997; Xu, 2009). WP has also been shown to be beneficial for skeletal muscle recovery by improving performance during periods of large volumes of training while reducing serum markers of muscle damage (creatine kinase) (M Hansen et al., 2015a; M. Saunders et al., 2004a).

Based on the evidence that initial fitness is a significant predictor of MSI, we hypothesized that the serum marker of bone formation (P1NP) would be related to higher endurance performance upon entry to IET. Additionally, we hypothesized that there would be group differences in MSI between WP and carbohydrate (CHO) supplementation due to previous research demonstrating positive effects of WP supplementation on bone and skeletal muscle.

METHODS

Each platoon maintains logs of injury (diagnosed by Licensed, Certified Athletic Trainers) and illness that require medical attention. These records were provided to the primary investigator. A member of the research team spoke with each IET soldier who sustained an injury or illness during training. The following variables were noted: supplement group, illness/injury type, training days missed, health professional visited,mechanism of injury, and injury date. Data from those who sustain injuries that require them to discontinue IET were used in analysis up to the point of their cessation of training to evaluate injury by treatment interactions.

Supplementation: two servings per day

Statistical Analysis: Logistic regression was utilized to predict the likelihood of an IET soldier sustaining a MSI. Injury analysis was only performed using individuals who also had

performance, body composition, and missed supplementation data. The number of predictors in each model were limited to one predictor per number of 10 events (injuries) to prevent overfitting (Ranganathan, Pramesh, & Aggarwal, 2017). Models were created for each of the following predictors: initial push-up, initial run performance, initial fat free mass, initial fat mass, initial body weight, age, group, relative calorie (kcal/kg), protein (g/kg), carbohydrate (g/kg), and fat (g/kg) intake during IET. The number of MSI was unusually low in the study group compared to historical data. After combining the MSI data with the body composition and performance data there were only 15 MSI for injury analysis (WP = 10 injured, 24 uninjured, CHO = 5 injured, 30 uninjured). Additionally, when combining MSI data with dietary intake for logistic regression there were only 12 MSI for injury analysis (WP = 8 injured, 21 uninjured, CHO = 4 injured, 26 uninjured). Chi-square analysis was used to compare group differences for medical visits due to MSI and illness. Wilcoxon rank sum test was used to evaluate total medical visits and profile days.

Results: No body composition or performance variable was a significant predictor of MSI. Only Initial push-up ($p = 0.15$) performance and group ($p = 0.13$) had a p value below 0.2. No body composition, performance, or diet value was a significant predictor of having an illness. There were no group differences for total MSI or illnesses, medical visits, or profile days (data and p values are presented in Table 10.

Table 10: Summary injuries consuming two supplement servings per day

Variable	WP	CHO	P value
Total MSI	11	10	p = 0.9
MSI Visits	1.6 ± 0.8	3.4 ± 4.0	p = 0.4
Profile Days	6.5 ± 4.5	13.2 ± 16.3	p = 0.6
Total Illness	9	9	p = 0.9
Illness Visits	1.4 ± 0.8	1.3 ± 0.5	p = 0.8
Profile Days	3 ± 3.5	2.5 ± 0.7	p = 0.8

Legend: WP, Whey protein; CHO, carbohydrate placebo group; MSI, musculoskeletal injury; Data is presented as mean and standard deviation;

Supplementation: one serving per day

Statistical Analysis: Logistic regression was utilized to predict the likelihood of an IET soldier sustaining a MSI. Injury analysis was only performed using individuals who also had performance, body composition, and missed shake data. The number of predictors in each model were limited to one predictor per number of 10 events to prevent overfitting (Ranganathan et al., 2017). Models were created for each of the following predictors: initial push-up, initial run performance, initial fat free mass, initial fat mass, initial body weight, age, group, relative calorie (kcal/kg), protein (g/kg), carbohydrate (g/kg), and fat (g/kg) intakes during IET. Pre-intervention as well as change scores (Post – Pre) for serum P1NP, CTX, and IGF-1 were analyzed using logistic regression to determine if they were a significant predictor of MSI. Only a subset of participants consented to blood analysis, this reducing the sampling pool to 26 non-injured individuals and 16 injured individuals. Additionally, this figure was reduced to 21 non-injured and 12 injured when change scores were used for bone P1NP biomarker due to lack of available samples at post-intervention. Because of this low number, only one predictor was used per

model. Pre-intervention values as well as change across training in these markers were used in logistic regression models to predict MSI. Chi-square analysis was used to compare group differences for medical visits due to MSI. Wilcoxon rank sum test was used to evaluate total medical visits and profile days. Initial run performance, due to its relationship with risk of injury, was used in a linear regression model to determine if it is a predictor of bone formation and bone resorption at the onset of IET.

Results: Individuals who did not sustain a MSI were on average 1.1 kg heavier, had 1.3 kg greater FFM, performed seven more push-ups, and 19 seconds faster on at the onset of IET. Initial push-up performance was the only significant predictor of MSI (Coefficient = -0.037, z = -1.97, p = 0.048). Every one-unit increase in push-ups was related to a 3.7% reduction in the odds of sustaining a MSI. Because initial push-up was a significant predictor it was included in the models for group and relative macronutrient intakes. When controlling for initial push-up performance, the model that included relative carbohydrate intake was the only model that was a significant predictor of MSI. There were no group differences for total MSI or illnesses, medical visits, or profile days (data and p values are presented in Table 11.

Table 11: Summary injuries consuming one supplement serving per day

Variable	**WP**	**CHO**	**P value**
MSI	10	15	p = 0.5
MSI Visits	4.7 ± 5.7	6 ± 6.1	p = 0.4
Profile Days	23.6 ± 19.8	16.5 ± 14.9	p = 0.8

Legend: WP, Whey protein; CHO, carbohydrate placebo group; MSI, musculoskeletal injury; Data is presented as mean and standard deviation;

No pre-values or change scores for P1NP, CTX, or IGF-1 were significant predictors of MSI. Linear regression analysis revealed that initial run performance was a significant predictor of CTX (Coefficient = -16.675. $F = 6.18$, $p = 0.02$). Initial run performance was not a significant predictor of P1NP ($F = 2.10$, $p = 0.16$).

DISCUSSION

Our analysis revealed: 1) initial push-up performance was the only significant predictor for MSI; and 2) initial run performance was a significant predictor of bone resorption (CTX).

Initial performance has been shown to be a significant predictor of MSI during IET (J. Sefton, Lohse, et al., 2016a). Our previous work found that combined push-ups and sit-ups were correlated with acute MSI and run performance was related to overuse and acute MSI (J. Sefton, Lohse, et al., 2016a). In the current investigation, we did not differentiate between acute and overuse type injuries. Discrepancies in predictors between the studies may be explained by the low number of injuries. The first study (two supplement servings per day) had only 15 MSI and in the second study (one supplement serving per day) there were only 24 MSI. The amount of injuries was reduced even further when filtering out individuals who did not have diet or serum data for regression analysis. The small number of MSI in this study was a limitation to the analysis but also an interesting finding. Our first study in which two supplement shakes were consumed daily had an 18.5% injury rate for IET soldiers. The second study consuming only one shake per day resulted in a 21.7% injury rate. Previous literature suggests that the injury rate in IET ranges from 19-44% (JJ Knapik et al., 2001; Nindl, 2012; Reis, Trone, Macera, & Rauh, 2007; J. Sefton, Lohse, et al., 2016a). The injury rates reported here are on the lower end of that spectrum and may suggest a benefit of additional nutritional intake on MSI. McGinnis et al.

(McGinnis et al., 2018) investigated the data presented here in relation to historical injury data in the same training units as the current study. Historically, non-supplemented individuals who participated in IET in the same unit at the same time of year, were five times more likely to sustain a MSI and were four times more likely to be placed on profile and miss training (McGinnis et al., 2018). Thus, given that this study did not have a non-supplemented group of IET soldiers, a larger study needs to be completed to directly compare WP- or CHO-supplemented versus non-supplemented IET soldiers.

Initial run performance was a significant predictor of bone resorption. Previous work has consistently reported that endurance ability at the onset of training is a significant predictor of injury during IET (C. Milgrom et al., 2000; Moran et al., 2008; J. Sefton, Lohse, et al., 2016a). Therefore, we sought to determine if bone formation, bone resorption, and the ratio of the two were associated with run performance at pre-intervention. We report that bone resorption was significantly associated with run performance; every one second increase (slower) in run performance was associated with a 16.8 unit decrease in bone resorption. Additionally, pre-intervention bone formation was not significantly associated with run performance. This was in contrast to our original hypothesis. Previous literature suggests that exercise, whether endurance or strength in nature, results in increases in bone mineral density and cortical thickness (Eleftheriou et al., 2012; Hughes et al., 2016; Langberg et al., 2000; Siddiqui & Partridge, 2016). Thus, we hypothesized that higher initial fitness would be associated with higher serum markers of bone formation and reduced bone resorption. One consideration is that the relationship may have been confounded by the transition to IET environment. Serum samples were taken on the fourth day of training. This is during one of the most physically and mentally stressful portions of training. Furthermore, IET soldiers experience rapid increases in training volume combined

with energy restriction as they transition to IET. Previous research has reported that marathon running acutely depressed bone formation, which was not restored until 48-72 hours until it peaked at 72 hours. In the same study, bone resorption was reduced acutely (Langberg et al., 2000). Furthermore, individuals exposed to energy deficits experience acute decreases in bone formation and increases in bone resorption (Hughes et al., 2014) and this effect may be exacerbated when combined with exercise (Zanker & Swaine, 2000). It is unclear why bone resorption was higher in more fit individuals, however in light of the aforementioned confounders to bone turnover irrespective of previous endurance training history or ability, it is possible that our results were simply confounded by the stressors of exposure to the IET environment.

Limitations

One limitation of this study was the low number of MSI. This limited the ability to evaluate the interaction of multiple predictors as well as limited the number of observations of each event (injured or non-injured) related to each single predictor. Additionally, a more detailed dietary analysis should be employed in future studies that: 1) provide more accurate results than self-reported diet analysis; 2) provide more detail into the composition of each sub-category of macronutrient. This would provide insight into whether overall macronutrient intake is important for MSI or if the composition of those macronutrients (e.g. essential vs. non-essential amino acids) is a contributor.

CONCLUSION

Supplementation with WP did not have a statistically significant effect on MSI or profile length. The results are limited due to the low number of MSI in this investigation. Push-up performance upon entry to IET was a significant predictor of MSI suggesting that strength may be related to MSI during IET. Bone resorption was inversely related to initial run performance and therefore may relate to MSI occurrence during IET.

Chapter 8: Summary and Future Directions

Our work has provided valuable information on: 1) the physiological demands of IET; 2) the relationship between these training demands and dietary intake of IET soldiers; 3) the physical and physiologic response of IET soldiers to training and WP/CHO supplementation. We were the first researchers to investigate the balance between dietary intake and training/energy balance in the Army IET environment. We found that IET soldiers experience rapid increases in training volume coupled with inadequate energy intake. It is worth noting that we used conservative estimations when calculating energy expenditure and still found that IET soldiers were on average in approximately -540-calorie energy balance. This could be a contributor to the musculoskeletal injury problem in the IET environment. Future investigations need to employ more precise methodologies to more specifically quantify the energy imbalance in IET. Furthermore, these studies need to seek to determine if the energy and diet status of IET soldiers contributes to MSI or influences recovery. In regard to performance, we report that WP seems to consistently improve push-up performance by approximately 5 push-ups more per cycle in comparison to the improvement in with CHO supplementation. For perspective, the CHO group only improved 2.7 push-ups across IET total in the first study. Thus, the WP group had a larger gain in relation to the CHO group, than the CHO group gained across all of IET for push-up. Additionally, WP had larger effect sizes on FFM and FM in relation to the CHO groups. These results were consistent between studies. Change in FFM and FM between groups were on average 0.8 and 1.2 kg more for FFM and 1.1 and 1.8 kg for FM in the WP group in relation to

the CHO. Additionally, the effect of WP on FM was statistically significant in the first study and 0.11 in the second study. Therefore, WP may be an effective strategy for individuals entering IET who need to lose FM.

We were not able to effectively investigate the influence of WP on MSI due to the low numbers of MSI events that occurred during our investigation. It is possible that supplemental nutritional intake was a contributor to the low MSI rates. Work in our lab found those who consumed two supplement servings were less likely to sustain an MSI and less likely to miss training compared to one serving or no supplementation. Future studies should take these findings one step further to directly compare individuals in the same cycle and company with and without supplementation.

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Appendix A: Example food log

Roster Number: _____ **Dinner**

Please circle the food you ate	**Please Circle the portion you ate**							
Lasagna	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Chicken	1/2	piece	1	piece	1.5	piece	_____	piece
Vegetarian Cheese Manicotti	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Orzo-Spinach, tomato, onion	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Mashed potatoes	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Green bean combo	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Squash	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Gravy (chicken)	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Rolls	1/2	roll	1	roll	1.5	roll	_____	roll
Soup	1/2	scoop	1	scoop	1.5	scoop	_____	scoop
Salad bar								
Salad	1	fist	2	fist	3	fist	_____	fist

Tomatoes	2	tomatoes	3	tomatoes	4	tomatoes	_____	tomatoes
Peppers	1/2	handful	1	handful	1.5	handful	_____	handful
Cheese	1/2	handful	1	handful	1.5	handful	_____	handful
Other _____								
Other _____								
Other _____								
Salad dressing _____	1	thumb	2	thumb	3	thumb	_____	thumb
Fitness bar								
Fruit _____ type	1/2	handful	1	handful	1.5	handful	_____	handful
Fruit _____ type	1/2	fruit	1	fruit	1.5	fruit	_____	fruit
Fruit _____ type	1/2	handful	1	handful	1.5	handful	_____	handful
Fruit _____ type	1/2	fruit	1	fruit	1.5	fruit	_____	fruit
Yogurt _____ type	1/2	cup	1	cup	1.5	cup	_____	cup
Other _____								
Drinks								
Milk								

Water								
Juice _____ type	1/2	cup	1	cup	1.5	cup	_____	cup
Other	1/2	cup	1	cup	1.5	cup	_____	cup
Butter	1/2	cup	1	cup	1.5	cup	_____	cup
Honey								
Peanut butter	1	thumb	2	thumb	3	thumb	_____	thumb
Jelly	1	thumb	2	thumb	3	thumb	_____	thumb
Relish	1	thumb	2	thumb	3	thumb	_____	thumb
A1	1	pack	2	pack	3	pack	_____	pack
Hot sauce	1	thumb	2	thumb	3	thumb	_____	thumb
Heinz 57 sauce	1	thumb	2	thumb	3	thumb	_____	thumb
Ketchup	1	thumb	2	thumb	3	thumb	_____	thumb

Appendix B: Data Collection Sheet:

Warrior Research Center

Name:

Sex (male/female)	
Age (yrs)	
Ht (cm)	
Weight (kg)	

Neck (cm)	
Umbilicus (cm)	
Hip (cm)	

Hydration: _____

Skinfolds

	Inputs	Measure 1	Measure 2	Measure 3	Average
1	Chest				
3	Triceps				
4	Subscapular				

Body Density	
Body Fat %	

BIA Device

Z	
Ph	
R	
Xc	

Body Fat %

Notes:

Appendix C: Consent Form

Auburn University, Alabama 36849-5323

Department of Kinesiology
301 Wire Road
School of Kinesiology,
Warrior Research Center

Telephone: (334) 844-4483
Fax: (334) 844-1467

(NOTE: DO NOT SIGN THIS DOCUMENT UNLESS AN IRB APPROVAL STAMP WITH CURRENT DATES HAS BEEN APPLIED TO THIS DOCUMENT.)

INFORMED CONSENT

Evaluation of Energy Expenditure and Impact of Whey Protein Supplementation on Fitness, Injury Rates and Medical Visits during 9 Weeks of Basic Combat Training

You are invited to participate in a research study to determine the energy expenditure required during Basic Combat Training (BCT) and how/if whey protein and carbohydrate supplementation and timing impacts injury rates, medical visits, biomarkers, and physical performance measures in BCT Soldiers during 9 weeks of training. The study is being conducted by Dr. JoEllen Sefton, Director of the Warrior Research Center and Jeremy McAdam, Doctoral Student in the Auburn University School of Kinesiology.

Why is this research being done? Nutrition is an important factor in performance, health, and preventing injury. Therefore, to optimize soldier performance and training, it is important to know what the energy used by the body during BCT so nutritional recommendations can be made for Soldiers during BCT. Furthermore, whey protein and carbohydrate supplementation has been reported to be beneficial for health and performance. The goal of this study is to determine how supplementation affects Soldiers and how much energy is used during the 9 weeks of BCT and the optimal timing of supplementation.

What will be involved if you participate?

1. You agree not to supplement with any other amino acids, creatine, or protein supplementation other than what we provide for you.
2. You also agree not to participate in training outside of what is required by BCT.
3. You will be given time to read the informed consent document and a member of the research team will be available to answer any questions that you may have about the study. If you choose to participate you will sign the consent form. Choosing to, or not to participate will not affect your status in your class, BCT, Fort Benning, training, Auburn University, or the United States Army.
4. If you choose to participate and are determined, as per Army health screening, to be physically healthy/able to participate in BCT, you will be enrolled in the study.
5. You will be given a Warrior Research Center health questionnaire to complete.
6. You will then be asked to report the following morning fasted (before eating in the morning) for pre-intervention measures.
    a. You will be asked to submit a urine sample to record hydration levels.
    b. Height, weight, and circumferences for neck, waist, and hip will be recorded. Next, your body composition will be estimated using a body composition device (Impedimed-DF50) and skin fold calipers.
    a. Body composition device (Impedimed-DF50) -Two electrodes will be placed on your hand and two will be placed on your foot on the same side of the body. This will be used to estimate body water, fat free mass, and bod fat percentage.
    b. You will also have body fat percentage estimated using skin fold calipers and an ultrasound machine.
7. Random selection:
    a. You will be randomly assigned into a group that will either receive supplementation with whey protein or a group that receive a carbohydrate (maltodextrin) drink. You will not be told which group you are in so that you will not have knowledge of group assignment.
    b. Blood draws
        i. Blood draws will be consented separately. You may agree to participate in this study without agreeing to be eligible for blood draws. At the end of this document there will be a separate place to sign if you are willing to submit a blood draw.

ii. You will have approximately 10 milliliters (1.5 teaspoon) of blood drawn from a vein located in the area in front of your elbow. The needle and supplies used are sterile and similar to what is used by your physician's office to draw blood. It is important for you to follow all instructions provided in order to minimize any bruising and/or discomfort you may feel from the blood draw. In the extraordinarily rare event that medical care is needed following the blood draw, you will be instructed to proceed (and provided with directions if needed) to the Consolidated Troop Medical Center (less than 2 miles away) for medical care. The tester will then draw blood using standard blood drawing techniques. This will take 5 min but may last longer if you have any follow-up questions. The blood samples will be refrigerated and used in future analysis.

iii. You will be asked to submit 2 blood samples: one at the beginning of training and one at the end of basic combat training.

c. Finally, participants will be randomly chosen to wear monitors around the waist that will track activity levels and energy expenditure for assigned weeks during BCT.

i. Activity monitors (Actigraph wGT3X-BT monitors) will be assigned to participants to wear on different assigned weeks during the 9 weeks of training. Monitors will be assigned to different Soldiers weekly so that no one Soldier will have to always wear the monitor. These monitors will be worn around the waist and data will be downloaded.

1. The monitors are accelerometers that measure physical activity levels and estimate energy expenditure.

d. The researchers will let you know all information necessary for your participation and group assignment. If you have ANY questions, feel free to ask a researcher.

8. All participants will be asked to submit paper dietary intake logs. This information will be collected for 3 days during weeks 1,4,8.
9. For the duration of the study
    a. You will be asked to consume the assigned supplemental drink (Protein or carbohydrate) at the assigned time (morning or before bed) once daily for the duration of BCT.
    b. We will collect the results of your Army physical fitness tests and 1-1-1 tests, record any injuries, medical visits, and collect diet logs as explained above.
10. On Selected Training events:

a. You may be asked to wear an activity monitor on your chest for the training event. The monitor measures heart rate and rate of movement.

11. Time commitment
    a. Week 1-Day 1 pre-intervention measures about 1 hour
        i. Blood draw (if selected)- 5-7 minutes)
        ii. Body composition- skinfolds/ultrasound (5-7 minutes) BIA device estimation (3-5 minutes), circumference measures (~3 minutes).
        iii. If you do not have blood draws time will be less.
    b. Week 9 - post measures- about 1 hour.
        i. Blood draw if selected 5-7 minutes
        ii. Body composition- skinfolds/ultrasound (5-7 minutes) BIA device estimation (3-5 minutes), circumference measures (~3 minutes).
    c. Diet logs- (15 minutes per day, 3 days for weeks 1,4,8)
        i. ~45 minutes for each of the weeks.
    d. Monitors (5-minute meeting with researcher to calibrate and download data).
        i. 5-minute meeting to be assigned Chest strap monitor on select training events.

Potential risks and discomforts

a. If you will be receiving blood draws there is a risk of pain, bruising where the blood is taken, redness, swelling and infection. There is also a slight risk of fainting when blood is drawn.
b. There is a risk of breach of confidentiality because your roster numbers will be collected during the screening and consenting process. All paper documents and personal history information collected will be locked in a room, in the Neuromechanics laboratory at Auburn University where only the primary investigator and faculty advisor will have access to them.
c. There is a risk of adverse reactions (nausea, vomiting, wheezing) if you are allergic to whey protein or milk proteins.
d. Other than these risks, there are no risks to participants above the risks that are expected due to training during BCT.

What are the possible benefits of participating in this research?

a. Each participant will be given feedback on his or her body fat and lean mass estimations, testosterone/estrogen levels, cholesterol, and blood triglycerides, pre- and post- BCT.

This feedback will be given to you by a member of the research team and will be of no cost to you.

b. Also, there is an overall benefit of providing necessary information to be used to develop dietary recommendations for future Soldiers entering BCT. This information can help prevent injuries, improve performance, and save money on the costs of treating injuries.

Will I have to pay for anything if I take part in this research?

a) No, there will be no cost to you for your participation. Everything you need will be provided to you by the research team.

Will I be paid for my participation in this research?

a) No there will be no payment for participation in this research.

What happens if I am injured as a result of this research?

1. If you incur an injury throughout the study duration, you will be instructed to report to the Certified Athletic Trainer of the Battalion as you normally would for any injury for evaluation during sick call. The Certified Athletic Trainer will evaluate all injuries incurred, document the evaluation, and report them to the principal investigator. If you report to the Certified Athletic Trainer with an injury you will be evaluated, possibly put on profile, treated with given rehabilitation exercises, and followed up with until you are returned to full duty. If at any time you believe you have suffered an injury or illness as a result of participating in this research, please contact JoEllen Sefton at (334) 844-1694 or jmsefton@auburn.edu.

2. **DOD Healthcare Beneficiaries** (e.g., active duty in the military, military spouse or dependent)

    a. If you are injured because of your participation in this research you are entitled to medical care for your injury within the DOD healthcare system, as long as you remain a DOD healthcare beneficiary. This care includes but is not limited to free medical care at Army hospitals or clinics. Medical care will be provided at no cost to you.

How will you protect my privacy and the confidentiality of records about me?

1. Each person who chooses to participate in this study will be given a participant number. A master sheet will be kept that will contain this information. This sheet will be kept

locked in the faculty advisor's office (Dr. JoEllen Sefton) to which she is the only one who has access

2. All other data collected (performance tests, injury information) will be password locked, saved on a compact disc and will be anonymized by the Neuromechanics Laboratory coordinator. Once the database has been anonymized, only persons listed on the Internal Review Board document will be able to view the information. Any information entered into the database will be maintained on a secure computer and backed up daily. All backups will be stored in the locked office of the Neuromechanics Laboratory coordinator.
3. Forms will be maintained in locked storage at all times. The database will be password protected and accessible only by the researchers; the database will be on a single computer locked in a personal office that is only accessible to the Principal investigator and research investigators.
4. Authorized representatives of the following groups may need to review your research and/or medical records **as part of their responsibilities to protect research participants**:
    a. U.S. Army Medical Research & Materiel Command Institutional Review Board
    b. U.S. Army Human Research Protections Office
    c. Auburn University Institutional Review Board
5. Complete confidentiality cannot be promised for military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities.

What if I decide not to participate in this research?

1. Your participation in this research is voluntary. You may decline to participate now or stop taking part in this research at any time without any penalty or loss of benefits to which you are entitled. Deciding not to participate now or withdrawing at a later time does not harm or in any way affect your medical care or training during Infantry Officer Basic Leadership Course Training, future relationships with Auburn University, Fort Benning, or the United States Army.

What could end my involvement in the research?

1. The investigator or study sponsor may withdraw you from participating in this research if circumstances arise which warrant doing so. Non-compliance in: intake of the dietary intake, training outside of standardized BCT, wearing the metabolic monitors, and not

reporting dietary information can also result in you being eliminated from the study. The investigator will make the decision and let you know if it is not possible for you to continue. Your taking part in the study may be stopped without your consent if it is determined by the investigator that remaining in the study might be dangerous or harmful to you.

2. If you discontinue with BCT or are removed from your respective Battalion and Company for any medical, psychological or miscellaneous reason you may be eliminated from participating in the study. This information will be passed on to the investigators
3. During the course of the research, the investigators will tell you of any new findings that might cause you to change your mind about continuing in the study. If new information is provided to you, the investigators will obtain your consent to continue participating in this study.

WHO SHOULD I CALL IF I HAVE QUESTIONS OR CONCERNS ABOUT THIS RESEARCH?

1. If you have questions about the research at any time, you should contact Dr. Sefton at (334) 844-1694 or jmsefton@auburn.edu or Jeremy McAdam at (334) 399-3699 or by email jsm0039@auburn.edu.

SIGNATURE OF RESEARCH PARTICIPANT

I have read the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction.

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

SIGNATURE OF PERSON OBTAINING CONSENT

My signature certifies that the participant signed this consent form in my presence as his/her voluntary act and deed.

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

SIGNATURE OF RESEARCH PARTICIPANT CONSENTING TO ELIGIBILITY OF BLOOD DRAWS

I have read the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction.

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

SIGNATURE OF PERSON OBTAINING CONSENT

My signature certifies that the participant signed this consent form in my presence as his/her voluntary act and deed.

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date