

The effect of egg consumption on inflammatory markers, psychological measures, dietary intake and quality, and body composition in NCAA division I female collegiate gymnasts

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Diet & Exercise

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2019

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ACKNOWLEDGEMENTS

“Without counsel plans fail, but with many advisers they succeed.” ~Proverbs 15:22

First, I would like to dedicate this thesis to my lord and savior for being with me through this process and for giving me peace when I most needed it most. To my gymnastics team that made this possible, you all are my sisters and I thank you for your contribution to the knowledge of sports nutrition. To my major professor, Dr. Ruth Litchfield, thank you for showing me what authentic hard work is. You have provided nothing but great support to me along the way. In addition, thank you to Jeanne Stewart for her patience and guidance in the lab. I would also like to thank my fellow lab mates, Jessica Young and Julia Lavalley, and dear friend, Cat Rudolph, for all of their help during this journey. Last but not least, thank you to my mom for being my number one supporter. You are my rock.

ABSTRACT

Aesthetic sports, like gymnastics, requires attention to post-exercise recovery methods to attenuate repetitive overuse of the joints during high-intensity training. Essential nutrients are often supplemented in a condensed fashion through the use of post-workout sports drinks and bars. An increased interest in whole food consumption post-exercise has been seen as these promoted products are often high in added sugars and fat, which has increased the interest of whole food consumption post-exercise. Eggs are known as the standard reference protein containing all the essential amino acids and have been investigated in several population groups. Further, research is lacking in the collegiate athlete population. This study examined the effects of whole egg consumption on markers of inflammation, psychological measures, dietary intake and quality, and body composition post-exercise in division I collegiate female gymnasts. Collegiate female gymnasts (n=11), ages 18-22 years old, were recruited for voluntary participation in the study. Participants were quasi-randomly assigned to consume two boiled eggs post-exercise or to continue their normal dietary intake (while limiting egg consumption; control) from August to April (pre-season to post-season). In August, December, and April, dietary intake, diet quality and anthropometric measures were completed. Biochemical indices, including a blood lipid panel, complete blood count (CBC) with differential, and inflammatory markers were measured at the same time points with an additional measure in October. SPSS (v25) and SAS (v9.4) were used for statistical analyses. Following this nutritional intervention, there were no significant changes in CRP and IL-6 but sIL-6R significantly decreased in the no egg group from September to October. Further, results demonstrated a significant relationship between sIL-6R and poor sleep quality during this same time point in both groups. From August to April, dietary energy,

carbohydrates and fat were significantly reduced in egg group participants. Among anthropometric measures, body fat percentage saw a reduction from August to December. In conclusion, results from this pilot study were suggestive that there may be a relationship between post-exercise whole egg consumption and improvements in inflammation, psychological measures, anthropometrics, and diet quality. However, due to the small sample size and variability in the data, additional research is needed to confirm or refute the study's findings.

CHAPTER 1: INTRODUCTION

Background

Collegiate female gymnasts often train in the sport of gymnastics much of their life, where demands further advance at the Division I collegiate level. This training is year-round with official training sessions beginning early fall and ending after the competitive season in the spring. This includes 9-months of varying bouts of volume and intensity to prepare the gymnasts for their competitive season where the use of periodization (i.e. variations in training to optimize the desired performance of an individual/ team) is seen. Though gymnastics changes from an individual sport to that of a team sport at the college level, the high intensity training regimens make recovery vital to prevent injuries.

Gymnastics emphasizes having a high lean muscle mass to be able to perform powerful vertical movements. The pressures of maintaining the ideal body image, weight and ability to perform can often be encountered, making excessive fat mass a disadvantage in the sport. Furthermore, recovery and adequate nutrition are at the forefront of performance success. Improper recovery can result in chronic overuse injuries, inflammation, and changes in body composition. During these high intensity training periods, ingestion of a protein source that is rich in essential amino acids following exercise has been shown to help in increasing muscle mass, recovery, and sustaining immune function. Common protein-rich, post-exercise recovery products promoted and marketed in collegiate athletics consist of sports drinks and bars, like whey and casein, for muscle recovery. Though these sources are composed of complete proteins, it is recommended that individuals obtain essential amino acids from normal dietary intake rather than supplements.

The purpose of this thesis was to investigate the potential benefits of the consumption of a whole food protein source, whole eggs, in collegiate female gymnasts post-exercise for the following: 1) chronic inflammation resulting from high levels of training, 2) mood, stress and recovery, 3) dietary intake and quality, and 4) anthropometric measures. In evaluating previous research and examining nutrition and recovery in this population group, it was proposed that post-exercise whole egg consumption would reduce markers of inflammation, promote positive psychological indicators of mood, stress, and recovery, improve dietary intake and quality, and assist in maintaining lean muscle mass. The findings of this study will contribute to the long-term goal of advancing post-exercise nutritional knowledge related to aesthetic sports (i.e. gymnastics), chronic inflammation, psychological measures of mood, stress and recovery, and body composition.

Specific Aims, Hypotheses, and Impact

Aim 1: To determine if egg consumption attenuates the inflammatory markers: CRP, IL-6, and sIL-6R.

Approach: Four blood draws were conducted throughout the study to evaluate measures at the start of preseason (September), one month into preseason (October), post-preseason (December), and post-competitive season (April).

Hypothesis 1: In comparison to the control group, intervention participants will demonstrate lower markers of inflammation (i.e. CRP, IL-6 and sIL-6R).

Aim 2: To determine the effect of egg consumption on psychological indicators of mood, stress, and recovery.

Approach: Distribute the REST-Q and POMS (August, twice in October, December, and April), PSS (end of October), ACSI-28 (midseason between February and March), and CSAI-2 (January and between February and March) surveys throughout the 9-month study (See Table 3.2).

Hypothesis 2: During the intense training period (i.e. October), intervention participants will experience improvements in recovery and coping skills, as well as an attenuation in total mood disturbance, stress, and anxiety.

Aim 3: To determine if egg consumption improves dietary quality within intervention participants in comparison to the control group.

Approach: Participants were quasi-randomly assigned to consume 2 boiled eggs post-exercise (5x/week; intervention) or to continue their normal dietary intake (control). All participants completed a 5-day food record that was distributed at three time points in the study (Baseline (August), post-preseason (December), and post-season (April) to analyze dietary intake and diet quality via Healthy Eating Index scores.

Hypothesis 3: Healthy Eating Index scores among intervention group participants will be higher than control participants.

Aim 4: To determine if egg consumption decreases body fat percentage among female collegiate gymnasts.

Approach: At baseline (August), post-preseason (December), and post-season (April), anthropometric measures were completed via the use of the BOD POD ®, an air displacement method.

Hypothesis 4: In comparison to the no egg group, intervention participants will have a significant decrease in body fat percentage at 4 months and 9 months' post-baseline.

Impact: This study further examines the effects of post-exercise egg consumption within an athletic population group composed of female collegiate gymnasts. Findings will advance the knowledge on use of whole eggs to combat inflammation and their potential benefits in athletes as a viable, nutrient-dense, and inexpensive source of protein. Current research is moving towards the examination of whole foods for post-exercise recovery and recommendations have directed athletes more towards obtaining essential amino acids from normal dietary intake, rather than from supplementary products. Furthermore, this study will provide research on the use of whole-foods as a means to see beneficial changes among the indicated parameters.

Thesis Organization

This thesis will first discuss the review of literature regarding concepts of sport specialization, the female athlete triad, injuries, inflammation, and current nutrition among collegiate female gymnasts. It also will include an evaluation of current whole food recovery approaches to nutrition. Following the review are descriptive details of the methods utilized within two manuscripts. Lastly, an overall conclusion, reference section, and appendices are provided.

CHAPTER 2. REVIEW OF LITERATURE

Early Sports Specialization

Media frequently highlights youth as a means of success in several sports, including that of gymnastics, where the demands on both the athlete's time and level progresses to an advanced phase of the sport once they reach the NCAA level (Malina, 2010). This training often remains to be year-round with official training sessions beginning in the fall semester (in August or September) and ending after the spring semester (around April). The approximate 9-month training period poses varying bouts of volume and intensity to prepare a gymnast for their training season where periodization is utilized (Brooks, 2003; Gamble, 2006). Gamble further explains this training method as variations in the training loads and volumes that an athlete or team utilizes to optimize successful performance (Gamble, 2006). This training regimen assists in the transition from pre-season to in-season training. Though the sport becomes a team sport at the collegiate level, training intensities are individualized due to the modifications gymnasts may have to make and the number of events they train each day due to factors such as illnesses and injuries.

The participation rate in NCAA female sports has steadily increased from 34% to 44% over the past 30 years and gymnastics is predominantly known as one of the high-risk sports due to the dangerous skills that have to be performed (dthomas, 2013; Meeusen & Borms, 1992; Tenforde et al., 2017). Its aesthetic qualities emphasize having a high lean muscle mass and low body fat mass to attain higher power vertical movements. The pressures of maintaining the ideal body image, weight and performance can be encountered from coaches, fans, teammates, and judges making excessive fat mass a disadvantage (Sundgot-Borgen & Garthe, 2011). It has been suggested that with these desired bodily

attributes, realistic weight limits and balanced diets should be encouraged to not compromise normal physiological functions (Wilmore, 1991).

The Female Athlete Triad

Stipulations for the appearance aspect put female athletes at an increased risk for conditions like the female athlete triad. The female athlete triad is a condition that is common in young athletic women and is comprised of three components: abnormal menses, decreased bone mineral density, and inadequate energy intake (Nazem & Ackerman, 2012).

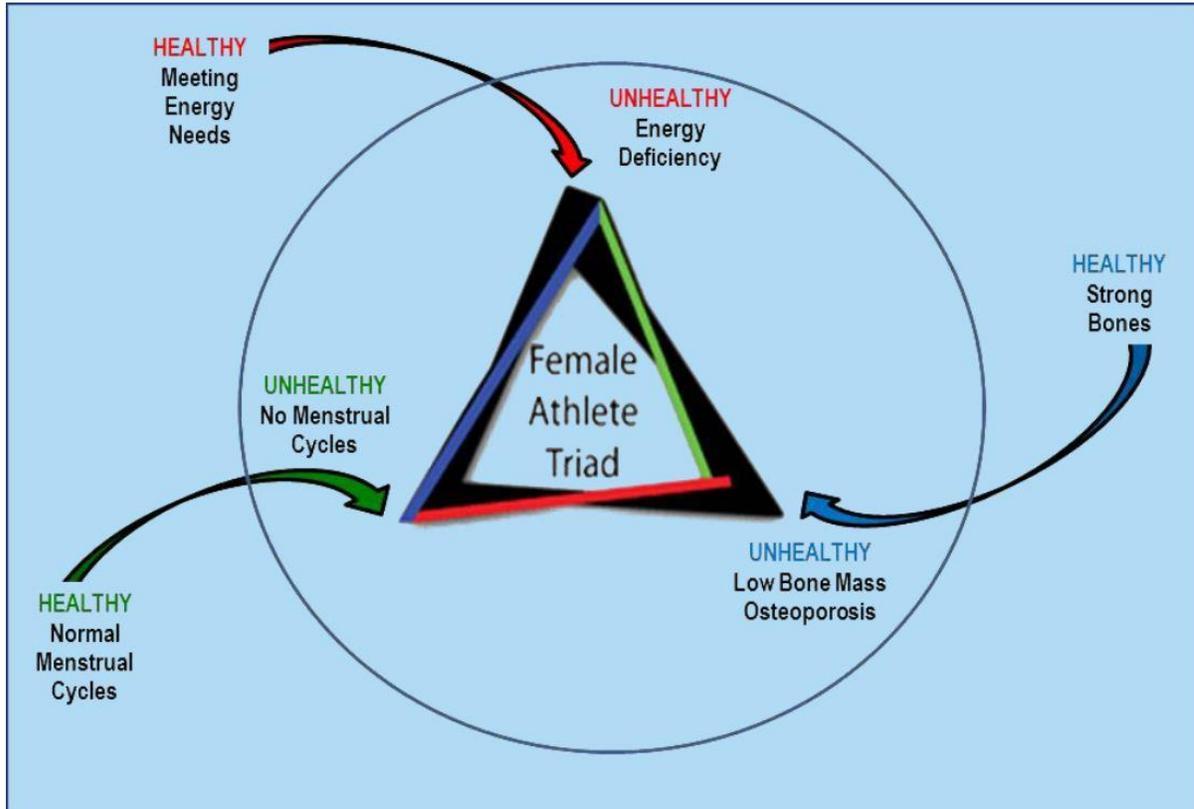


Figure 2.1. Visual Model of the Female Athlete Triad Components (“What is the Triad?,” 2017)

Abnormal Menses

Abnormal menses is one of the prime indicators for examining if a female athlete is at risk of developing the female athlete triad. Abnormal menses is operationally defined as missing a menses for 3 months or longer. Subcomponents of this condition consist of primary and secondary amenorrhea. In primary amenorrhea, it is classified as normally developing but having no menstrual cycle by the age of 15 or no menses following breast development (if breast development happened before 10 years of age)(Nazem & Ackerman, 2012). If an individual has had a menstrual cycle but has an absence of menses thereafter, this is classified as secondary amenorrhea (Nazem & Ackerman, 2012). Pertaining to the female athlete, functional hypothalamic amenorrhea (FHA) has been deemed the most common type of amenorrhea amongst this population as it has been associated with exercise and stress (Gordon, 2010; Nazem & Ackerman, 2012a). Estrogen stores are lacking in this condition, which ultimately may be what leads to a decline in bone mineral density as well (Meczekalski, Podfigurna-Stopa, & Genazzani, 2010; Nazem & Ackerman, 2012).

Low Bone Mineral Density

An additional subcomponent female athletes run the risk of attaining is low bone mineral density (BMD) as a result of menstrual dysfunction and low energy availability. The prevalence of stress fractures can further increase in response with low dietary calcium being noted as one key contributor (Thomas, Erdman, & Burke, 2016a). There are associations between low dietary calcium intake and restrictive eating. Evaluation of sixty-five healthy 18-25 year olds revealed negative correlations of cognitive restrictive eating scores and markers of BMD, suggesting restrictive eating is related to decreases in ones BMD (Nickols-Richardson, Beiseigel, & Gwazdauskas, 2006).

Z-scores are utilized to determine BMD status via examining and comparing an individual's dual-energy x-ray absorptiometry (DEXA) results to their age-matched peers (Nazem & Ackerman, 2012). Z-scores for females prior to menopause are as follows according to the International Society For Clinical Densitometry: ≤ 2.0 SD is defined as "below expected range for age" and ≥ 2.0 SD is defined as "within the expected range for age" ("2015 ISCD Official Positions - Adult - International Society for Clinical Densitometry (ISCD)," n.d.). In contrast to this scoring, athlete z-score definitions slightly differ when utilizing the guidelines of the American College of Sports Medicine (ACSM). This is due in part to the fact that athletes involved in high impact/loading sports have usually have a higher BMD than the normal population (Tenforde & Fredericson, 2011). In the ACSM guidelines, clinical risk factors in conjunction with a z-score between -1 to -2 defines low BMD in athletes (Nattiv et al., 2007a). Further, osteoporosis is defined as a z-score ≤ 2.0 in this population group (Nattiv et al., 2007a). Clinical risk factors can include having a history of stress fractures, low energy availability, and/or menstrual dysfunction (Nattiv et al., 2007a). Loading of the bones via exercise has been shown to be effective at maintaining adequate BMD, with athletes that endure a greater amount of loading having the highest BMD (Arasheben, Barzee, & Morley, 2011). Repetitive loading, concomitantly with higher BMD, is seen in female gymnasts (Taaffe & Marcus, 2004), but the benefits of higher BMD suggest to be greater among female athletes who have a normal menses, and further adequate energy availability (A. B. Loucks, 1990).

Low Energy Availability

Lastly, low energy availability is postulated to be the cause of amenorrhea and low bone mineral density in the female athlete triad (Nazem & Ackerman, 2012). Low energy

availability is the amount of energy necessary for adequate physiological functioning and is operationally defined as subtracting energy expenditure from the dietary energy, divided by kilograms of lean muscle mass (Nattiv et al., 2007a). Changes in the menstrual cycle and bone mineral density have been seen with energy availabilities <30 kcal/kg lean muscle mass (LMM)(Reed, De Souza, & Williams, 2013). This reduction in energy intake may lead to negative physiological consequences due to essential macronutrients, like protein, carbohydrates, and fat, also being deficient. Further, a deficiency in these essential nutrients can cause a decreased ability to conduct muscle protein synthesis, maintenance of lean muscle mass, inhibition of tissue repair, and decreased immune function (Manore, Kam, & Loucks, 2007).

Gymnastics is considered a high-risk sport for the female athlete triad. The sport often encourages a lean aesthetic body type and low energy intake is the common method seen as a means to lose weight to achieve a lean muscle mass (LMM). Moreover, research has shown that higher energy deficits are associated with a higher body fat percentage (Deutz, Benardot, Martin, & Cody, 2000). Such evidence was demonstrated in a study that analyzed the relationship between energy deficits and body composition in 42 female elite gymnasts and 20 runners. It was concluded that there was an inverse relationship with body fat percentage and calorie deficits greater than 300 kilocalories, and also with the number of hours the energy deficit is experienced by the athlete (Deutz et al., 2000). Concerning energy availability, there is a lack of research specific to tracking this condition over the course of the preseason and competitive season training months of collegiate female gymnasts. However, energy availability has been examined by Reed and colleagues in Division I collegiate female soccer players, a population not perceived as having low energy availability

(Reed et al., 2013). The study assessed measures of energy intake and energy expenditure pre-, mid-, and post-season of nineteen, 18-21 year old female soccer players. In the participants that had low energy availability, as classified as < 30 kcal/kg LMM, results demonstrated low energy availability from the preseason to midseason. Energy availability rebounded back above the classified threshold (i.e. 30 kcal/kg/LMM) into the post-season, suggesting low energy availability may be seasonal in this population, and further, also reversible. With one specific participant with low energy availability, it was noted that concomitantly, they also demonstrated decreases in lean muscle mass and weight, reported heavily tracking their dietary intake, and were amenorrhoeic as well. Further, Reed et al. concluded that these findings suggests low dietary intake is a major factor for an athlete having low energy availability (Reed et al., 2013). Moreover, sufficient physiological functioning (i.e. optimal energy balance and overall health) has been explored and found in individuals with energy availabilities >45 kcal/kg LMM (Anne B. Loucks, 2013; Thomas et al., 2016a).

Further, attention to nutrition is vital for the health and performance of collegiate female athletes as these outcomes extend even more to the aesthetic sport sub-population, like gymnasts, where it has been shown that female collegiate athletes in aesthetic sports reported more muscle and skeletal injuries than any other sports (Beals & Manore, 2002).

Injuries

During the 2009-2010 and 2013-2014 academic and collegiate gymnastics seasons, the NCAA Injury Surveillance Program (NCAA ISP) collected data from 11 gymnastics

programs that included 28 team seasons. Results from this epidemiological study showed that 3 in 10 injuries were classified as overuse (Kerr, Hayden, Barr, Klossner, & Dompier, 2015). It is important to note that in a sport where there is high loading and high frequency of impact rates, increased joint cartilage damage can result in changes in the bone complex and conditions such as osteoarthritis to be present in this population (Amoako & Pujalte, 2014).

Osteoarthritis is a multifaceted type of condition that is associated with articular cartilage defects, as well as changes within the bone matrix of the joint (Altman et al., 1986; Amoako & Pujalte, 2014b). There are differing opinions as to the cause of osteoarthritis such as risk factors like obesity, genetics, recreational activities, race, and gender that could incline a person to develop OA. All the more specifically with joint injury, it is thought that improper technique, excessive training, or overload can disrupt and damage the cartilage of the joint (Amoako & Pujalte, 2014; Lau et al., 2000; Oliveira, Pina, Reis, & Roman, 2018).

Mechanistically, joint overload in athletes occurs when fast movements do not allow for joint fluid to be distributed appropriately, thereby stressing the joint's framework (Amoako & Pujalte, 2014a). Due to the repeated micro trauma that can occur in an athlete, damage can additionally be done to tendons, muscles, or bones making overuse injuries the consequence (Brenner & American Academy of Pediatrics Council on Sports Medicine and Fitness, 2007). Though recent research of OA has viewed the condition as the result of expanded weight on a specific joint, it is being theorized that inflammation may play a key role (Berenbaum, 2013). Further, continuous joint degeneration, cartilage loss, and progression in joint injuries is suggesting that individuals with OA have chronic low-grade inflammation as the root cause (Jäger et al., 2017a; Thomas et al., 2016a)(Sokolove & Lepus, 2013).

Inflammation

Inflammation is a normal physiological response within the body that is completed by the immune system when stimuli, such as pathogens and injuries, occur (Libby, 2007). Pertaining to chronic diseases, inflammation has been one of the underlying contributors. Common tissue-associated damage with inflammation includes redness, swelling, heat, and pain (Libby, 2007). Redness develops as a result of hyperemia as excess blood pools at the site of injury. The increased blood flow from the body's hyperemic state is what causes warmth in the area, followed by the pain experienced from changes surrounding the blood vessels and the nerve endings that are near (Libby, 2007). Pathophysiologically, the body reciprocates by also recruiting phagocytic blood leukocytes to dispense of the pathogenic substances eliciting inflammation. Further, because of the nature of inflammation, it provides an understanding of the etiologies of many chronic diseases, including diabetes, cardiovascular disease, and overweight/obesity. Additionally, its mechanisms also correspond to the state of inflammation experienced in athletes undergoing high caliber training.

Anti-Inflammatory Effects of Exercise

Exercise has been postulated as an activity that can provide beneficial anti-inflammatory effects. A study by Albert et al measured levels of C-reactive protein (CRP), an inflammatory cytokine, and self-report of strenuous physical activity within both adult men and women (Albert, Glynn, & Ridker, 2004). Overall, the results demonstrated an inverse relationship between CRP and exercise. Further, as levels of strenuous exercise increased, CRP was shown to decrease. This decrease was the greatest amongst individuals reporting the highest amount of intense exercise per week (Albert et al., 2004). Exercise has also

demonstrated promising benefits in individuals with chronic diseases, like type 2 diabetes. In a 2014 study, participants performing 12 weeks of circuit training saw decreases in both CRP and Interleukin-6 (IL-6) (Kim, 2014). IL-6 is another inflammatory cytokine that has anti-inflammatory effects through a signaling pathway called classic signaling. However, its counterpart, the trans-signaling pathway, can stimulate negative physiological effects.

Pro-Inflammatory Effects of Exercise: Interleukin-6

Research has also shown the negative effects of strenuous exercise in association with the IL-6 trans-signaling pathway. In response to exercise, the skeletal muscle has been deemed as the location for IL-6 production. Additionally, it has been suggested to be produced in substantial amounts following an exercise bout (Robson, 2003). Robson-Ansley et al was the first to demonstrate that increased release of IL-6 around the tendons, brain and muscles in healthy and trained male runners, could be the possible cause for impaired exercise performance and neural functioning that furthermore, induced fatigue (Robson-Ansley, de Milander, Collins, & Noakes, 2004).

As of recently, work investigating the response of IL-6's receptor, sIL-6R, has suggested that prolonged exercise training could be related to overreaching symptoms (Cullen, Thomas, Webb, Phillips, & Hughes, 2017). More specifically, Cullen and colleagues found in highly trained endurance athletes positive associations between sIL-6R and sleep quality over a prolonged training period. During intense periods of training, an athlete can begin to display signs and symptoms related to decrements in their performance. This can include feelings of fatigue, staleness, soreness, sleep disturbances, appetite loss, decreased immune function, and several more as the literature has greater than 125 identified signs and symptoms (Roy, 2015). These signs and symptoms indicate that an athlete may be

experiencing a state of overreaching, or more severely, overtraining (Roy, 2015). Further, overtraining employs that intense bouts of exercise training are occurring and recovery periods are lacking (Roy, 2015). Usually before decrements in performance arise, psychological indicators emerge (Rearick, Creasy, & Buriak, 2011). Methods to analyze signs and symptoms of overtraining have frequently been completed through the use of mood, stress, and recovery surveys. Two reliable surveys in particular that have been able to assess overtraining, as well as the well-being of an athlete, are the Profile of Mood States or POMS (McNair, Lorr, Droppleman, & Educational and Industrial Testing Service, 1971) and the Recovery-Stress Questionnaire or REST-Q (Kellmann & Kallus, 2001). The POMS assesses mood, while the REST-Q assesses multiple components (i.e. disturbed breaks, fatigue, fitness/injury, fitness/being in shape, burnout/personal accomplishment, self-efficacy, and self-regulation) related to internal and external stress, recovery, and current mood. Both surveys have been shown to be related to one another and are sufficient instruments that can be utilized to assess in combination with physiological measures of inflammation (Rearick et al., 2011).

Dietary Intake and Inflammation

Dietary constituents have been proposed as a means to lower cytokine concentrations. Bishop et al has shown that plasma IL-6 was greater following a low carbohydrate diet (i.e. less than 10% of energy coming from carbohydrates) in trained male cyclists, suggesting that during extended cycling, carbohydrate intake prior to exercise influences the inflammatory response (Bishop, Walsh, Haines, Richards, & Gleeson, 2001). Further, it proposes that a greater amount of carbohydrates was needed to attenuate inflammation. Though there is work addressing carbohydrate intake's effects on the IL-6 inflammatory complex, there are also

several pieces of work evaluating the effects of post-exercise recovery via the use of protein. Protein has been proposed as a dietary constituent that could mitigate the inflammatory response elicited by IL-6. More specifically, it has been of interest to promote positive muscle protein balance to prevent inherent damage from elevated levels of IL-6 (Hennigar, McClung, & Pasiakos, 2017). In 8 highly trained males consuming glutamine and glutamine-rich protein, Hiscock and colleagues have seen a reduction in IL-6 (Hiscock et al., 2003). Kerasiotti et al had similar findings as both measures of plasma IL-6 and CRP decreased post-exercise in recreationally active males consuming experimental high protein cake (Kerasiotti et al., 2013). Lastly, attenuation of the IL-6 response has been demonstrated by Shroer et al following load cycling and consumption of whey protein (Schroer, Saunders, Baur, Womack, & Luden, 2014).

Nutrition

Nutrition is part of the multifactorial system that make up training recovery (Brooks, 2003a). Though its stressed as being important for athletes, it has been defined as a weakness at the collegiate level (Brooks, 2003a). Among gymnasts, inadequate nutrition due to decreased energy intakes could potentially be a factor in causing chronic overuse injuries. Additionally, nutrition has been postulated to be one of the most important measures to assist in gymnast recovery to help support physical and immune health with protein as the focus (Beelen, Burke, Gibala, & van Loon L, 2010a; Heaton et al., 2017a).

Protein for Recovery

Protein is an essential macronutrient to consume post-exercise, as it supports the remodeling and repair of muscle tissues, as well as metabolic adaptations (Beelen, Burke,

Gibala, & van Loon L, 2010). Following an exercise bout, more specifically, one that is resistance-based, there is an anabolic window where muscle growth or muscle protein synthesis can occur. The process of muscle protein synthesis requires a stimulus that comes from post-exercise protein intake. If the athlete's body is in a state of negative nitrogen balance, the result is a reduced stimulus for muscle growth. Further, this can cause a loss of lean muscle mass, as well as it can negatively affect strength and performance (Koral & Dosseville, 2009; Mettler, Mitchell, & Tipton, 2010; Sundgot-Borgen & Garthe, 2011).

Post-Exercise Protein Timing

Guidelines point towards timing, quality and supplementation with other macronutrients are important facets of dietary protein intake (Phillips, 2012). Regarding timing, studies have found that after a single resistance training session, muscle protein synthesis is stimulated for at least 24 hours (Burd et al., 2011). Further, Phillips et al has found/recommended that protein consumption be immediately to 2 hours post-exercise for adequate recovery and muscle protein accretion (Phillips, 2012). Past studies have demonstrated increases in muscle fiber size, as well as muscle strength, when protein was consumed post-exercise (L. L. Andersen et al., 2005; Esmarck et al., 2001; Hartman et al., 2007).

Post-Exercise Protein Needs for Athletes

When considering how much protein an adult's body needs, it varies as it depends on the type of population group it will serve. The Recommended Dietary Allowance (RDA) suggests that adults consume at least $0.8 \text{ g body weight (BW)}^{-1}\text{-day}^{-1}$ to support nitrogen balance (Institute of Medicine (U.S.), 2005). Though this recommendation may be adequate for a relatively large portion of healthy adults, it should also be individualized when

considering the role physical activity plays with lean muscle mass. It is also important to note that this individualized approach is applicable to all sports as each sport has different training exercises and energy restrictions where there is a wider range in protein recommendations (Vliet, Beals, Martinez, Skinner, & Burd, 2018).

Energy restriction and protein are two interrelated components. In the sport of gymnastics, energy restriction may be an athlete's route to achieve a desirable lean muscle mass and further, lose weight (Deutz et al., 2000). Though this is the solution, it's important that that during times of decreased energy, protein intakes are higher in this population to protect their lean muscle mass (Jäger et al., 2017). Gymnastics is primarily composed of anaerobic, resistance exercises that assist in lean muscle mass maintenance but ensuring adequate protein intake in combination with exercise could help achieve this desired outcome. Current recommendations suggest that physically active, healthy adults consume $1.2 - 2.0 \text{ g} \cdot \text{kg body weight (BW)}^{-1} \cdot \text{day}^{-1}$ of protein (Jäger et al., 2017; Thomas et al., 2016). For athletes, the most recent American College of Sports Medicine Joint Position Statement, it is suggested that athletes consume 20-30g of protein or ~10g of a protein source that contains all the essential amino acids post-exercise (Thomas, Erdman, & Burke, 2016). Specific to strength-trained females, a novel study estimated protein needs for muscle protein synthesis and found protein requirements were at the upper end of the ACSM position stand (i.e. 1.2-2.0 g·kg/d) (Malowany et al., 2018).

The ACSM suggested protein recommendations would seem adequate to support the training regimens of collegiate female gymnasts but in a sport where energy deficits are commonly seen, higher protein intakes may be needed for the purposes of maintaining lean muscle mass. In one study evaluating the influence of dietary protein on lean muscle mass

and performance, there were three diets tested within healthy resistance-trained athletes: mixed diet with 15% protein and 100% energy, hypoenergetic diet with 60% ad libitum energy intake with 15% protein (1.0 g/kg), and hypoenergetic diet with 60% ad libitum energy intake with 35% protein (2.3 g/kg). The greatest maintenance of lean muscle mass on a hypoenergetic diet was shown with participants consuming 35% protein or 2.3 g/kg over a short term of 2 weeks. Although this demonstrated that during an energy deficit, higher protein intakes may be able to maintain muscle stores, this study was short-term, as well as noted that fatigue was “worse than normal” for those consuming more protein (Mettler et al., 2010). More recently, research has shown intakes as high as 1.6 – 3.1 g/kg body weight/day have been seen for recommendations to obtain lean muscle mass (Jäger et al., 2017).

Though past research has indicated the potential need for more protein during an energy deficit, confirmation of these recommendations is needed. Moreover, it is inconclusive as to how much protein an aesthetic female athlete population would need to maintain lean muscle mass during periods of energy deficits long term.

Post-Exercise Protein Sources

Milk Protein and Isolated Milk Protein Constituents

The question then is which protein source is best? Recommendations point to sources of high-quality protein that contain all the essential amino acids. This can include lean meats, dairy protein, and eggs (Phillips, 2012). We see in the past literature that milk protein has been the focus due to its high-quality protein, calcium, and vitamin D (Josse & Phillips, 2012). Furthermore, it has recovery benefits post-exercise. In a study by Hartman et al, the long-term effects of milk consumption was examined in young men, ages 18-30 years old,

and compared to soy and carbohydrate consumption. The 12-week study was composed of 5 days of resistance training a week that required participants to consume milk immediately post-exercise, as well as 1 hour post-exercise. It was concluded that following this training regimen promoted lean muscle mass and assisted in muscle mass gains and fat loss (Hartman et al., 2007). Pertaining to the gender of interest, females, a study by Josse et al in young, healthy women followed this protocol that was utilized by Hartman et al. The purpose of Josse et al's work was to test whether fat-free milk decreased fat mass and increased lean muscle mass over the course of a 12-week resistance training regimen. When compared to carbohydrate intake post-exercise, fat-free milk consumption was shown to parallel this hypothesis. Further, a favorable change in body composition was observed due to increases in lean muscle mass in response to resistance exercise. In investigating the overall influence of milk consumption in concert with resistance training on body composition in female athletes, Josse et al concluded that overall body composition can be improved (i.e. loss in fat, maintenance or increases in lean muscle mass, and adequate bone mineral density) (Josse & Phillips, 2012).

When observing isolated milk protein constituents, like whey, severable pieces of work related to post-exercise recovery are available (Breen et al., 2011; Bukhari et al., 2015; Burke et al., 2012; Pennings et al., 2011, 2011; Phillips, Tang, & Moore, 2009; Soop et al., 2012; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009; Witard et al., 2014; Yang et al., 2012). Pertaining to females, work by West et al found that muscle protein synthesis was similar to men following whey protein intake immediately and 26 hours post-resistance exercise (West et al., 2012).

Consequences of Nutrition Recovery Products

Though milk and isolated milk protein constituents have exhibited physiological benefits following exercise, the concern within collegiate athletics is the added sugar amounts within promoted sports products. These products for example include that of Gatorade Protein Recovery drinks that are commonly seen at collegiate universities in both the weight rooms and practice facilities. Though these drinks contain adequate amounts of protein, they are considerably high in sugar. For example, one 330mL bottle of a Chocolate Gatorade[®] Protein Recovery Drink contains 20g of sugar (“Whey Protein Shake - Chocolate | Gatorade Protein,” n.d.). According to the 2015-2020 Dietary Guidelines for Americans, it is recommended that no more than 10% of an individual’s calories come from added sugars (“2015-2020 Dietary Guidelines - health.gov,” n.d.). In accordance with a 2,000 calorie diet, this equates to 200 calories or 12 teaspoons of sugar. In other words, this Gatorade product is supplying 5 teaspoons, or close to half of the maximum amount of added sugar intake in a day.

Research has also shown that when added sugars are consumed in excess, unwanted weight gain and potentially, obesity, can occur (CDC, 2018). What does this mean for an athlete? And more specifically, one that is involved in an aesthetic sport, like collegiate gymnastics? It is important to note that following an intense exercise bout, carbohydrates in combination with protein, like that found in several sports products, can play a tremendous role in replacing glycogen stores and assisting with maintaining a positive net protein balance, and further, recovery (Thomas et al., 2016b). However, the focus of this argument is towards the education of the athlete and whether they can discern if their body needs a high protein, but energy dense, sports product. This can vary from day to day as the intensities and

volumes of a workout can change throughout the preseason and season months, as well as what the state of each athlete's body is in (i.e. are they modifying their workouts due to illness, injury, or other reason? Are they training every event each day?)

At the collegiate level, many programs utilize these post-exercise recovery products as they are commonly perceived as healthful. What is often obscured through these messages are perception of the high concentrations of both sugar and fat that are in these sports drinks and bars (Maher, Wilson, Signal, & Thomson, 2006; Pettigrew, Rosenberg, Ferguson, Houghton, & Wood, 2013; Thomas et al., 2016). Another factor to consider with liquid protein sources are how satiating they are. In comparison to whole food sources, they may be less satisfying (Vliet et al., 2018). With these factors in mind, the desire to have more whole food products as a means for exercise recovery has been put forth. The most recent joint statement from the American College of Sports Medicine notes whole-foods should be the primary source of protein, with dietary supplemental protein serving as an alternative when a whole-food source is not available (Thomas et al., 2016b).

Eggs

A whole food source of interest includes that of eggs. Eggs are an inexpensive whole food item that are predominantly known as the standard reference protein. This is due in part because of their high essential amino acid content and high protein digestibility. Eggs are also hypocaloric (i.e. one large egg is 70 kilocalories) and contain several bioactive components that could be beneficial to human health. They contain both iron and phosphorous and are a rich source of B vitamins: 1, 2, 8, and 12, choline, as well as vitamins

A, D, E, and K (Anton, Nau, & Nys, 2006). The egg yolk contains protein like the egg white, but is primarily composed of lipids.

Egg Nutrients

Protein

Eggs are known as the standard reference protein, operationally meaning that compared to other protein sources, they rank highest for both their essential amino acid profile and high rate of digestibility (Layman, 2009). Eggs consist of all 9 essential amino acids: threonine, lysine, valine, isoleucine, leucine, methionine, tryptophan, phenylalanine, and histidine. Essential amino acids cannot be synthesized by the body, indicating the need for them to be acquired through dietary intake. Approximately 40% of amino acids ingested into the body are related to the muscle (Layman, 2009). Within the muscle, one amino acid in particular, leucine, is known to elicit muscle protein synthesis when present. Further, eggs can provide a high-quality protein source that can contribute to the maintenance and growth of lean muscle mass to support function of power, strength, and energy (Layman, 2009).

Lipids

Approximately 65% of the egg yolk is lipid with it containing about 65% triglycerides, 29% phospholipids (consisting of 86% being phosphatidylcholine and 14% phosphatidylethanolamine), 5% cholesterol, and <1% of free fatty acids (Anton et al., 2006). Within the yolk itself, over half of a whole egg's protein can be found in addition to its lipid constituents. Other bioactive components include that of choline and the carotenoids, lutein and zeaxanthin (Anton et al., 2006a).

Choline

Choline is synthesized within the body at the site of the liver but relying on de novo synthesis of the nutrient is not enough to meet the body's needs (Folate, Vitamins, & Choline, 1998). There are both water-soluble and lipid-soluble forms of choline. Water-soluble forms include free choline, phosphocholine, and glycerophosphocholine (Wiedeman et al., 2018). Fat-soluble forms include sphingomyelin and phosphatidylcholine (Wiedeman et al., 2018). Regarding dietary intake of the nutrient, a substantial amount of dietary choline can be found in animal-based foods, like liver, eggs, beef, fish, pork and chicken (Wiedeman et al., 2018). Phosphatidylcholine is the predominant type of choline found in these foods (Wiedeman et al., 2018). One of the listed animal sources, whole eggs, have been recognized as one of the primary dietary choline constituents within the American diet. One egg contains nearly 147 mg of choline, resulting in approximately two eggs meeting 50% of the daily dietary choline adequate intake recommendation (Wallace & Fulgoni, 2017).

The role of dietary choline extends to function as a precursor to betaine, phospholipids, and the neurotransmitter, acetylcholine (Folate et al., 1998). Choline assists phospholipids in both developing cell membrane structures and eliciting its signaling functions (Folate et al., 1998). It also forms the methyl donor betaine, that has a significant position with reducing the risk of cardiovascular disease (Folate et al., 1998). In this process, the formation of betaine has been shown favorable changes by lowering high homocysteine concentrations (Dudman, Tyrrell, & Wilcken, 1987; Varela-Moreiras, Ragel, & Pérez de Miguel, 1995; Wendel & Bremer, 1984; Wilcken, Dudman, & Tyrrell, 1985; Wilcken, Wilcken, Dudman, & Tyrrell, 1983). Further, choline intake is suggested to be associated with cardiovascular risk (Folate et al., 1998). In addition, choline forms acetylcholine that is

essential to neuromuscular control. It functions to relay signals to induce an essential physiological components for athletes: muscular contractions (Anton, Nau, & Nys, 2006; Jäger et al., 2017). Lastly, choline has been suggested to play a role in reducing the inflammatory response in healthy adults (Detopoulou, Panagiotakos, Antonopoulou, Pitsavos, & Stefanadis, 2008a).

More recently, choline has been a nutrient of concern as it is under-consumed by the American population (Wallace & Fulgoni, 2017). Current adequate intakes (AI) of choline are not optimal across several gender and life-stage group populations in the US (Wallace & Fulgoni, 2017). In individuals ≥ 2 years, approximately 11% meet the AI for choline and with adults, ages 19 and older, they were most likely to meet the AI when eggs were consumed (Wallace & Fulgoni, 2017).

Carotenoids

Eggs are also a significant source of the carotenoids: lutein and zeaxanthin. Both antioxidant nutrients are known to have benefits relating to both eye and brain health. Within the eye, lutein and zeaxanthin are centralized within the retina, making adequate consumption vital to preventing age-related macular degeneration (AMD): the number one cause of blindness within older adults (Eisenhauer, Natoli, Liew, & Flood, 2017).

These carotenoids are also bioactive nutrients that are suggested to be inversely related to inflammation (Jing et al., 2018; Kaulmann & Bohn, 2014; Suzuki et al., 2010). Further, they extend to elicit anti-inflammatory effects with chronic low-grade systemic inflammation in the body (Kaulmann & Bohn, 2014). This favorable relationship has been shown in various populations, including that of young male and female adults, as well as postmenopausal women (Hozawa et al., 2007; Wood et al., 2014)

When consumed in the diet, eggs have yielded positive results with absorption of the nutrients, lutein and zeaxanthin, because of their high bioavailability (Blesso, 2015). This is due in part to the fat content within the yolk (Blesso, 2015). Regarding dietary intake, there are no specific recommendations published regarding lutein and zeaxanthin intake, but past work has recommended 6 mg of both nutrients for men and women to prevent conditions like AMD (Rasmussen & Johnson, 2013).

Egg Yolk Controversy

Further, one of the underlying controversies in the past has been the cholesterol content of the yolk (“2015-2020 Dietary Guidelines - health.gov,” n.d.). Past studies have contradicted the beneficial nutrients of whole eggs like the studies conducted by Herron et al and Chakraborty et al. Herron et al showed that within pre-menopausal women, additional cholesterol through the diet did not alter the LDL/HDL ratio, an important biochemical parameter for coronary heart disease risk (Herron et al., 2002). Within this study, the common terms at the time, hypo- and hyper-responders, were used to classify an individual’s response to cholesterol by identifying differences in pre-menopausal women that were in a dietary cholesterol challenge (Herron et al., 2002). Whole eggs were used a means to assess the changes in cholesterol levels among Caucasian and Hispanic women (n=51) that participated in the cross-over trial consisting of consuming 640 mg cholesterol via eggs or 0 mg cholesterol (Herron et al., 2002). Chakoraborty et al’s study in young, healthy Indian participants on a vegetarian diet found no changes in cholesterol variables but, in contrast, saw that consuming one egg per day demonstrated differences in total cholesterol and LDL in hyper-responders at week 4 and the total cholesterol/HDL ratio at 8 weeks post-baseline

(Chakrabarty et al., 2004). It is important to note that the total cholesterol/HDL ratio is also an important biochemical parameter for coronary heart disease risk. Though cholesterol has been a controversial topic in the past, the nutritional benefits of whole eggs in various population groups has been recognized. As of recently, changes to the 2015-2020 Dietary Guidelines have been made for dietary cholesterol recommendations. It has been recognized that there is a difference between dietary cholesterol and blood cholesterol (“2015-2020 Dietary Guidelines - health.gov,” n.d.). Further, the stipulation that eggs are “bad” for one’s health has been minimized.

Whole Egg Consumption in Various Population Groups

Type 2 Diabetes Mellitus, Metabolic Syndrome, and Overweight/Obesity

Over the past decade, research has been conducted in several population groups investigating the benefits of whole-egg consumption, including that of individuals with the following conditions: type 2 diabetes mellitus (T2DM) (Ballesteros et al., 2015) and overweight/obesity (Vander Wal, Marth, Khosla, Jen, & Dhurandhar, 2005). In addition, studies have been conducted more recently in both individuals that are healthy (Missimer et al., 2017) and in that of resistance-trained men (van Vliet et al., 2017a).

Ballesteros et al participants with T2DM, ages 35-60 years old, were randomized to consume either one egg a day or an oatmeal-based breakfast. The egg group participants demonstrated a reduction in the inflammatory markers, aspartate aminotransferase (AST) and tumor-necrosis factor-alpha (TNF- alpha). One important thing that was noted was that there was no effect seen on lipoprotein and glucose metabolism in this population group throughout the duration of the study (Ballesteros et al., 2015). Further, a reduction in

inflammation has also been seen within individuals with metabolic syndrome (C. J. Andersen, Blesso, Lee, & Fernandez, 2013; C. J. Andersen, Lee, Blesso, Carr, & Fernandez, 2014; Blesso et al., 2013). The metabolic syndrome has been associated with metabolic diseases, like the metabolic syndrome (C. J. Andersen et al., 2014). In Anderson's past work, he has demonstrated that whole egg ingestion can modulate inflammation when participants with metabolic syndrome had weight loss in combination with moderate carbohydrate intake (Blesso et al., 2013). In a later study though, Anderson found reductions in inflammation were not significant but that peripheral blood mononuclear cell inflammation can be altered in response to cholesterol homeostasis (C. J. Andersen et al., 2014).

An additional effect of eggs seen is its ability to induce satiety in both overweight and obese, as well as healthy individuals (Vander Wal et al., 2005). Among the participants with the condition of being overweight or obese, satiety was tested in a breakfast consisting of eggs and compared to an isocaloric equal-weighted bagel-based breakfast. The egg-based breakfast induced greater satiety and significantly reduced short-term food intake leading to the conclusion that eggs could potentially help sustain a calorie deficit to assist in weight loss (Vander Wal et al., 2005). Similar results were seen in healthy female and male participants who participated in a randomized, crossover intervention study assessing a two-egg breakfast versus an oatmeal breakfast. The egg breakfast denoted an increase in satiety through the measurement of plasma ghrelin, also known as the hunger hormone, while not affecting cardiovascular disease risk biomarkers (i.e. lipoprotein/high-density lipoprotein ratio). Furthermore, there was no increase in plasma glucose, triglycerides, or liver enzymes (Missimer et al., 2017).

Athletic Population

Muscle protein synthesis is an additional factor that has been evaluated by van Vliet recently in resistance-trained men. The study concluded that eggs are a better source of post-exercise protein due to being in its natural form, than that of an isolate protein source, like whey or casein. When egg consumption was compared to egg whites, whole eggs elicited a greater myofibrillar muscle protein synthesis response within this population group (van Vliet et al., 2017). Both the whole-eggs and egg whites were matched for protein content (18g) with fat content being greater in the whole eggs (17g). Due to the additional macro- and micronutrients within the egg yolk, it was further seen that this stimulated muscle anabolism differently than the being only protein dense, like the egg whites. Though this was the only recent study found relating directly to whole-egg consumption in an exercising, athletic group of men, as well as contained a limited small sample size (n=10), it demonstrates promise towards eggs being a beneficial whole food for post-exercise recovery. Lopez et al has similar agreements with this population group and concluded that eggs are a suitable food for active groups, like athletes, in moderate amounts (López Sobaler, Aparicio Vizueté, & Ortega, 2017).

Summary

Eggs have notably been a controversial food, but are now being recognized as a nutrient-rich and bioactive dietary staple that would be beneficial for several population groups. Regarding its anti-inflammatory constituents, nutrition interventions are of interest and are applicable to the aesthetic athletic population, like collegiate female gymnasts (C. J. Andersen, 2015). Current research is examining whole-foods for post-exercise recovery as

there is a shift in direction for athletes towards obtaining essential amino acids (EAAs) from traditional food intake, rather than supplement products (Jäger et al., 2017b).

van Vliet and colleagues examined the dietary influence of whole eggs on muscle protein synthesis in resistance-trained men as previously mentioned. Following exercise, a greater stimulation of muscle protein synthesis with the consumption of whole eggs was found when compared to egg white ingestion (van Vliet et al., 2017b). A primary nutrient of interest, choline, has additionally been evaluated within the United States. It was shown that eggs are associated with usual choline intake in Americans (Wallace & Fulgoni, 2017).

The benefits of choline extend to suggested anti-inflammatory properties, mood, nervous system functions (i.e. an essential component of the neurotransmitter acetylcholine, and memory (Anton et al., 2006a; Zeisel, 2008). Given the intensity of training and neuromuscular action needed to perform the powerful skills in gymnastics, choline may be of benefit to this population. It has been previously shown that exercise is positively correlated with serum choline uptake (Buchman, Jenden, & Roch, 1999). Buchman and colleagues reported a 24% decrease in circulating choline among marathon runners following a race, suggesting there is increased choline uptake and/or metabolism by tissues during exercise (Buchman et al., 1999). Though the population group studied differs from that of gymnastics, where power is emphasized over endurance, findings suggest the intensity of the exercise is linked to the uptake of choline (Buchman et al., 1999).

The benefits of whole egg consumption in recovery and athletic performance could potentially expand to collegiate gymnasts. Further, the goal of this study was to examine post-exercise egg intake on markers of inflammation, mood, stress and recovery, dietary intake and diet quality, and body composition in collegiate female gymnasts.

CHAPTER 3. METHODS

Participants

Recruitment

Division 1 collegiate female gymnasts from a Midwestern university served as research participants for this study. Participants were sent a study recruitment letter via email communication prior to preseason training. The study recruitment letter can be found in **APPENDIX B**. Interested participants attended an informational meeting to receive an explanation of the study protocol, sign an informed consent document (**APPENDIX A**) and receive instructions for completing the 5-day Food Record and satiety scale. Exclusion criteria for participation included: egg allergies, asthma, coronary artery disease, depression, diabetes, hypertension, migraines, cancer or cancer-related complications, or any autoimmune disorder (i.e. rheumatoid arthritis, lupus, inflammatory bowel disease, multiple sclerosis).

Table 3.1. Descriptive Data of All Participants at Baseline. Values are displayed as mean \pm standard deviation (SD).

Parameter (n=11)	Values
Age (years)	19.6 \pm 1.3
Height (cm)	158.2 \pm 5.8
Weight (kg)	56.8 \pm 5.5
BMI (kg/m ²)	22.7 \pm 1.5
Body Fat (%)	18.1 \pm 2.9
Fat-Free Weight (kg)	46.6 \pm 4.3
Total Cholesterol (mg/dL)	195.5 \pm 34.3
LDL Cholesterol (mg/dL)	104.2 \pm 28.6
Triglycerides (mg/dL)	97.7 \pm 45.7
HDL Cholesterol (mg/dL)	71.9 \pm 14.2
C-Reactive Protein (mg/L)	0.01 \pm .01
Interleukin-6 (pg/mL)	1.1 \pm 1.3
Interleukin-6 Receptor Alpha (pg/mL)	315.0 \pm 78.5
Free Choline (nmol)	0.6 \pm 1.3

Through recruitment, thirteen division I collegiate female gymnasts between the ages of 18-22 years old elected to participate in the study throughout the training and competitive seasons (August-April). See Table 3.1 for the descriptive data of all participants at baseline.

Quasi-Random Allocation

Participants were quasi-randomly assigned to either the intervention (egg; $n=7$) or the control group (no egg; $n=6$; see Figure 3.1). Over the course of the study, attrition occurred as two participants from the egg group were removed from the final data due to injuries. The protocol that was assigned to the participants (egg or no eggs) was thoroughly explained by a member of the research team. Egg participants were strongly encouraged to consume boiled eggs immediately after a weight-lifting workout or practice workout. All participants were informed of the potential risk associated with the study's design. The experimental procedures were approved by the Institutional Review Board at the University. See **APPENDIX A** for IRB approval documents.

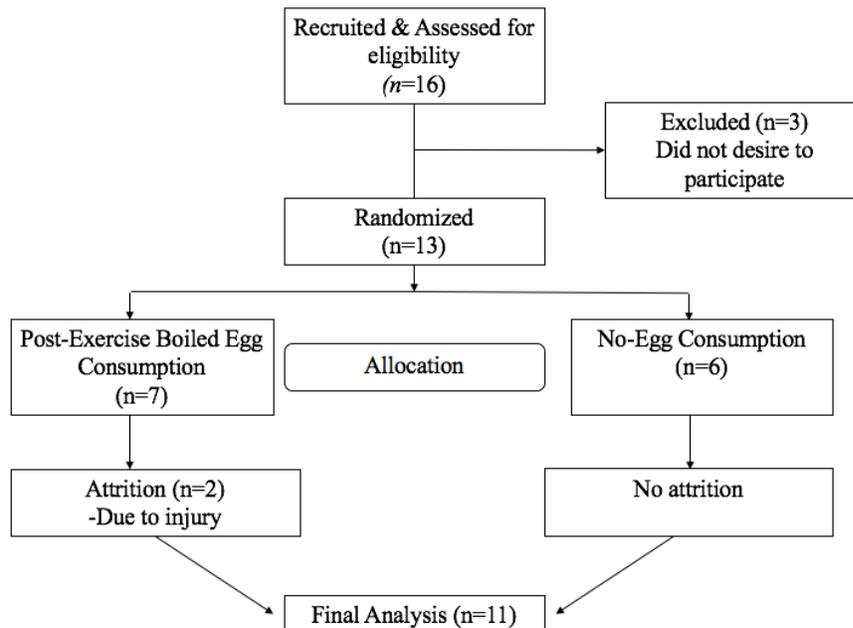


Figure 3.1 Study Allocation Flow Chart.

Study Design

The egg study was a quasi-randomized trial consisting of post-exercise egg consumption, anthropometric screenings, 5-day food records and satiety scales, blood serum analysis, and the following surveys: Health Status Update, RESTQ, Profile of Mood States (POMS), Perceived Stress Scale (PSS), Competitive State Anxiety Index-28 (CSAI-28), Athletic Coping Skills Inventory (ACSI-28), and Weekly Health/ Training Questionnaire. Data collection time points are shown in Table 3.2.

Table 3.2 Study Timeline.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April
5-Day Food Record	*				*				*
Anthropometrics	*				*				*
Blood Analysis		*	*		*				*
Health Status Update	*	*	*	*	*	*	*	*	*
RESTQ©		*	*		*				*
POMS		*	*		*				*
PSS			*						
ACSI-28								*	
CSAI-2						*		*	
Egg Consumption		*	*	*	*	*	*	*	*

Boiled Egg Consumption

SunnyFresh® Eggs ASAP vacuum-packed packages of two boiled eggs were provided to intervention participants. Eggs were available in the gymnastics locker room for post-exercise consumption. See Table 3.3 for nutrient information regarding the SunnyFresh® Eggs ASAP.

Table 3.3. SunnyFresh® Eggs ASAP Nutrient Composition.

Intervention	SunnyFresh® Eggs asap
Energy (kcal)	140
Total Fat (g)	9
Saturated Fat (g)	3
Trans Fat (g)	0
Cholesterol (mg)	330
Sodium (mg)	110
Total Carb (g)	1
Fiber (g)	0
Total Sugars (g)	1
Added Sugars (g)	0
Protein (g)	11
Vitamin D (mcg)	2
Calcium (mg)	44
Iron (mg)	1
Potassium (mg)	110

Anthropometric Screenings

Prior to each anthropometric screening, all participants were instructed to fast (minimum 10 hours) and wear appropriate clothing (i.e. spandex, sports bra, hair cap, no jewelry) for BOD POD® measurements. BOD POD is an air displacement measuring device used to assess body composition. Weight, body fat percentage, and fat-free mass (i.e. lean muscle mass) were collected at baseline (August), post-training (December), and post-competitive season (April).

5-Day Food Records and Satiety Scales

Detailed instructions and 5-day food record and satiety scales were provided to participants to record their dietary intake Monday through Friday at three time points (baseline, post-training and post-competitive season). Both Food Processor Nutrition Analysis Software (ESHA, version 11.3.285, Salem, Oregon) and the Self-Administered 24-hour (ASA24-2016) Dietary Assessment Tool (National Cancer Institute, version 2016,

Bethesda, MD) were used to analyze the nutrient content of the participants' food records. The five-day average of measured nutrients for each of the three time points was used for final analyses (baseline (August), post-preseason training (December), and post-competitive season (April)). In addition, food record information was also entered into ASA24-2016 to output Health Eating Index (HEI)-2015 scores for both the egg and no egg group at the same time points.

Satiety was captured via the Satiety Labeled Intensity Magnitude Scale (SLIM)(Cardello, Schutz, Leshner, & Merrill, 2005) via self-report ratings within the food records prior to each meal (i.e. breakfast, lunch, and dinner). The scale was a rating from greatest imaginable hunger (-100) to greatest imaginable fullness (100), with a score of "0" indicating a feeling of being neither hungry nor full.

Healthy Eating Index (HEI) Scores

Overall diet quality among both the egg and no egg group participants was measured using HEI-2015 scores ("Healthy Eating Index (HEI) | Center for Nutrition Policy and Promotion," n.d.). The US Department of Agriculture (USDA) and National Cancer Institute (NCI) developed this tool to assess diet quality in relation to the US Dietary Guidelines for Americans (DGAs). The components of the HEI-2015 are shown in **Table 3.4**. Scores from 0 to 100 are assigned, with 100 indicating the highest compliance to the DGAs ("Healthy Eating Index (HEI) | Center for Nutrition Policy and Promotion," n.d.).

Table 3.4. HEI-2015 Components & Scoring Standards (“How the HEI Is Scored | Center for Nutrition Policy and Promotion,” n.d.)

HEI-2015¹ Components and Scoring Standards

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruits ²	5	≥0.8 cup equivalent per 1,000 kcal	No Fruit
Whole Fruits ³	5	≥0.4 cup equivalent per 1,000 kcal	No Whole Fruit
Total Vegetables ⁴	5	≥1.1 cup equivalent per 1,000 kcal	No Vegetables
Greens and Beans ⁴	5	≥0.2 cup equivalent per 1,000 kcal	No Dark-Green Vegetables or Legumes
Whole Grains	10	≥1.5 ounce equivalent per 1,000 kcal	No Whole Grains
Dairy ⁵	10	≥1.3 cup equivalent per 1,000 kcal	No Dairy
Total Protein Foods ⁴	5	≥2.5 ounce equivalent per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins ^{4,6}	5	≥0.8 ounce equivalent per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids ⁷	10	(PUFAs + MUFAs) / SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 ounce equivalent per 1,000 kcal	≥4.3 ounce equivalent per 1,000 kcal
Sodium	10	≤1.1 grams per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

¹ Intakes between the minimum and maximum standards are scored proportionately.

² Includes 100% fruit juice.

³ Includes all forms except juice.

⁴ Includes legumes (beans and peas).

⁵ Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.

⁶ Includes seafood; nuts, seeds, soy products (other than beverages), and legumes (beans and peas).

⁷ Ratio of poly- and mono-unsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

Blood Serum Analysis

Blood serum analysis were conducted at the start of preseason (September), one month into preseason (October), post-preseason (December), and post-competitive season (April). Following an overnight fast (10-hour minimum), blood samples were obtained by antecubital venipuncture at four time points (see Table 3.2.). A blood lipid panel and complete blood count (CBC) with differential were completed by QUEST Diagnostics. After each blood analysis time point, samples sat for 30 minutes to allow for clotting. They then were centrifuged for 15 minutes, followed by aliquoting serum that was stored at -80 °C for

analysis that took place the following summer. C-reactive protein (CRP), interleukin-6 (IL-6), and soluble interleukin-6 receptor (sIL-6R) were analyzed via sandwich enzyme-linked immunosorbent assays (ELISA) kits (Human Quantikine ELISA Kits, R&D Systems, Minneapolis, MN, USA). In addition, choline was measured via the Choline/Acetylcholine Assay Kit (ab65345) (abcam, Cambridge, United Kingdom). All assays were completed in duplicate and followed according to the kit's instructions. A Synergy® 2 Multi-Detection Microplate Reader (Biotek Instruments, Inc., Winooski, VT, USA) was utilized for the sample readings.

Surveys

Health Status Update

Participants were distributed a survey every month (i.e. August – April) to record their last menses, intake of contraceptive pills, or devices, medications, supplements and current injury (s) and duration of injury. See **APPENDIX D**.

REST-Q

Measures of recovery and stress were assessed in all participants using the Recovery-Stress Questionnaire (REST-Q) (Pearson Assessment and Information, Frankfurt, Germany) at 5 time points throughout the study: beginning of preseason training (September), 1-month into training (October), post-training season (December), and post-competitive season (April). Six components related to stress and recovery were evaluated from the questionnaire: fatigue, sleep quality, disturbed break (i.e. how restful breaks from training were), fitness/injury (i.e. how the athlete felt that their current fitness/training level may increase their risk for injury), fitness-in-shape (i.e. how fit the athlete felt), and self-regulation (i.e.

examined the following psychological skills: goal setting, self-efficacy, imagery, attention, and activation regulation). See **APPENDIX D**.

Athletic Coping Skills Inventory (ACSI-28)

The ACSI-28 is a highly validated assessment tool developed by Smith et al, consisting of seven categories related to sports performance (Smith, Schutz, Smoll, & Ptacek, 1995). These include: “Coping With Adversity, Peaking Under Pressure, Goal Setting/Mental Preparation, Concentration, Freedom from Worry, Confidence and Achievement Motivation, and Coachability (Smith et al., 1995).” Scoring entails a series of questions addressing these components. Participants were required to use a scale ranging from “1-Almost Never” to “4-Almost Always” (See **APPENDIX D**). This 28-question survey was distributed mid-season between February and March to examine how the gymnasts felt about their performance during the competitive season thus far.

Competitive State Anxiety Inventory (CSAI-2)

A strong relationship between anxieties in relation to athletic performance has been found through the use of the Competitive State Anxiety Inventory (CSAI-2). Martens further developed the CSAI to become the CSAI-2 by focusing on competitive sport via incorporating measures of cognitive and somatic anxiety (Martens, Vealey, & Burton, 1990). Further, this study utilized the CSAI-2 at the beginning of the competitive season (January), as well as mid-season between February and March. Participants were asked to answer the 27-question survey that addressed feelings right at the moment they were completed it. The scale provided asks both cognitive and somatic questions ranging with as scale ranging from “1-Not at all” to “4-Very Much So.” See **APPENDIX D**.

Perceived Stress Scale (PSS)

Past research has utilized the PSS for assessing stress in relation to burnout and injuries among athletes (Chiu et al., 2016). Within this present study, the PSS was utilized to examine the effects of stress within the collegiate female gymnasts. The surveys were distributed and completed twice during the most intense training month of the preseason, October: once at the beginning of the month and once at the end of the month. The survey asks ten questions about feelings within the last month. Each question was answered on a scale of: “0-never” to “5-very often”. See **APPENDIX D**.

Profile of Mood States (POMS)

The POMS has been shown to be a workload- and recovery-sensitive questionnaire (“(15) Profile of Mood States is Sensitive to Changes in Workload and Perceived Recovery,” n.d.). The assessed psychological states of stress via six subcomponent categories of mood: tension, depression, anger, confusion, vigor, and fatigue. This survey was completed in conjunction with the REST-Q at the following time points: beginning of preseason training (September), 1-month into training (October), post-training season (December), and post-competitive season (April). For each question, a 5-point scale is assigned ranging from 1:”not at all” to 5:”extremely”. See **APPENDIX D**.

Weekly Health/Training Questionnaire

The weekly health/training questionnaire was distributed weekly throughout the 9-month study. The questionnaire was created to track the number of events the gymnasts were currently training, how many hours were spent training in the week, how many practices had to be modified, indicate any illnesses and/or injuries, and the corresponding intensities for both their lifting and gymnastics practices. See **APPENDIX D**.

**CHAPTER 4. EFFECT OF WHOLE EGG CONSUMPTION ON INFLAMMATION
IN COLLEGIATE FEMALE GYMNASTS DURING PRE-SEASON AND
COMPETITIVE SEASON TRAINING**

A paper to be submitted to the American College of Sports Medicine: Medicine in Sports and
Science

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Abstract

Attenuating the effects of high-intensity training is critical for post-exercise recovery. Recovery products in the form of sports drinks and bars are often supplemented to provide essential nutrients in a condensed fashion. These products are commonly high in sugar, impelling interest in the consumption of whole foods. One such food, whole eggs, has been examined among different population groups, but is lacking information among the athletic population. Collegiate female gymnasts (n=11; ages 18-22 years old) were recruited and participated in a quasi-experimental intervention of either consuming 2 hard-boiled eggs post-training (intervention) or continue normal dietary intake (control) throughout preseason and competitive season training. RESTQ, POMS, and blood samples were collected in September, October, December, and April. Blood lipid panels (cholesterol, HDL, LDL, TG),

complete blood count with differential, choline, markers of inflammation (CRP, IL-6, sIL-6R), lutein, zeaxanthin, albumin and total protein were analyzed. Statistical analyses included SPSS (v25) and SAS (v9.4). No significant treatment over time effects for CRP, IL-6, sIL-6R, total protein, albumin or zeaxanthin. Overall significant time effects were found for sIL-6R, total protein, albumin and zeaxanthin ($p < 0.05$). Time effects only remained in sIL-6R and albumin after multiple comparisons. Specifically, sIL-6R and albumin decreased between September and October ($p < 0.05$) in the no egg group. Significant correlations between sIL-6R and confusion (0.89), sleep and CRP (-0.92), IL-6 (0.85), and sIL-R (0.94) in the egg group, and CRP and IL-6 with sleep (0.88) and depression (0.90), were noted in the no egg group in October. Additional research is warranted to address inflammation and markers of mood, stress, and recovery following extensive training periods in collegiate female gymnasts.

KEY WORDS (4-6) - collegiate gymnasts; whole egg; inflammation; recovery

Introduction

The training of Division I collegiate female athletes tends to begin prior to grade school intensifying until college. Training often occurs year-round with official training beginning early fall and ending after the competitive season in the spring. Periodization (Brooks, 2003b; Gamble, 2006a), variations in training volume and intensity, is used throughout the fall training season to optimize the desired performance of an individual/team for the competitive season. Recovery is essential to maintain this high intensity training regimen and prevent negative physiological effects.

Nutrition, specifically protein, has been postulated as one of the most important recovery factors to promote physical and immune health (Beelen et al., 2010a; Heaton et al.,

2017b). Post-exercise protein ingestion is vital to achieve a positive net protein balance for skeletal muscle remodeling and repair. Positive protein balance occurs when muscle protein synthesis is activated through exercise and when high-quality sources of protein are available to provide the amino acids needed to facilitate muscle remodeling and repair (Beelen et al., 2010a).

Common post-exercise recovery products in collegiate athletics consist of sports drinks and bars containing protein required for muscle recovery. There is a perception of ‘health’ with these products, which obscures the high fat and sugar concentrations (Maher et al., 2006; Pettigrew et al., 2013; Thomas et al., 2016a). The most recent joint statement from the American College of Sports Medicine notes whole-foods should be the primary source of protein, with dietary supplemental protein serving as an alternative only when a whole-food source is not available (Thomas et al., 2016a).

Current research is examining whole-foods for post-exercise recovery. Recommendations are directing athletes to obtain essential amino acids (EAAs) from traditional foods, rather than supplement products (Jäger et al., 2017b). van Vliet and colleagues examined the influence of whole eggs on muscle protein synthesis in resistance-trained men. Following exercise, a greater stimulation of muscle protein synthesis with the consumption of whole eggs compared to egg white ingestion was noted (van Vliet et al., 2017b). Eggs are also a major contributor to choline intake in Americans (Wallace & Fulgoni, 2017). The benefits of choline extend to potential anti-inflammatory properties, mood, nervous system functions (i.e. neurotransmitter acetylcholine), and memory (Anton et al., 2006b; Zeisel, 2008).

In response to exercise, skeletal muscle produces Interleukin-6 (IL-6) (Robson, 2003). IL-6 is a multifunctional cytokine known to generate acute reactions, support red blood cell production, and regulate immune system responses (Robson, 2003). This cytokine has two signaling pathways: classic signaling (anti-inflammatory) and trans-signaling (pro-inflammatory) (Jones, Horiuchi, Topley, Yamamoto, & Fuller, 2001). The trans-signaling, or pro-inflammatory pathway, is comprised of IL-6, soluble interleukin-6 receptor (sIL-6R), and gp130. Further, the physiological implications of IL-6 via the trans-signaling pathway depends on the concentrations of these pro-inflammatory constituents (Robson, 2003). IL-6 is produced within the central nervous system and has the potential ability to cross the blood-brain barrier (Banks, Kastin, & Gutierrez, 1994). Recent work investigating the response of IL-6's receptor, sIL-6R, has suggested prolonged exercise training could be related to overreaching symptoms (Cullen et al., 2017, p. 6). Overreaching can occur during intense training and includes feelings of fatigue, staleness, soreness, sleep and mood disturbances, appetite loss, and decreased immune function; the literature has identified more than 125 identified signs and symptoms (Roy, 2015).

Methods to attenuate, decrease or minimize the IL-6/sIL-6R complex are limited but suggest attenuation of the inflammatory response and fatigue (Nishimoto et al., 2000). Micronutrients consumed prior to exercise have been proposed as a means to lower IL-6 concentrations. Choline has been shown to influence IL-6 and/or IL-6 receptors, and ultimately the inflammatory response. In healthy adults, Detopoulou and colleagues were the first to investigate the relationship between dietary choline with markers of inflammation. Results suggest a reduction in both in CRP and IL-6 in those consuming ≥ 310 mg/d of choline (Detopoulou, Panagiotakos, Antonopoulou, Pitsavos, & Stefanadis, 2008b).

At the macronutrient level, carbohydrates have been suggested to attenuate high concentrations of IL-6. Bishop et al demonstrated a low carbohydrate diet increased plasma IL-6 two-fold in trained male cyclists. Further, a greater amount of carbohydrate was needed to attenuate the response (Bishop et al., 2001). Protein has also been proposed as a means to mitigate the IL-6 inflammatory response pre- and/or post-prolonged, strenuous exercise. More specifically, during recovery, protein has been of interest to prevent muscle damage from elevated IL-6 via promoting positive muscle protein balance (Hennigar et al., 2017). Hiscock et al, reported a decline in IL-6 among 8 highly trained males consuming glutamine and glutamine-rich protein (Hiscock et al., 2003). Similar findings were reported by Kerasioti et al, where a decrease in plasma IL-6 and CRP among recreationally active males post-exercise was observed after consuming an experimental high protein cake (25). Finally, Schroer et al reported whey protein may attenuate the IL-6 response following exercise bouts of load cycling (Schroer et al., 2014).

Each of these studies examined the effect of protein consumption on IL-6 throughout exercise, but there is no research examining the effect of a protein in a whole food form, like whole eggs, on inflammation following extended training periods in collegiate female athletes. As IL-6 is located within the muscle and protein is an essential macronutrient for muscle repair and recovery, the purpose of this pilot study was to investigate the impact of a high-quality protein, whole eggs, on markers of inflammation in highly trained Division 1 collegiate female gymnasts.

Methods

Participants

Division I collegiate female gymnasts at a Midwestern university served as research participants for this study. Potential participants were contacted prior to the training season through email communication. Those interested in participating agreed to attend an informational meeting to explain the study, obtain informed consent, and receive instructions for completing a 5-day food record and satiety scale. Exclusion criteria for participation included the following health conditions: egg allergies, asthma, coronary artery disease, depression, diabetes, hypertension, migraines, cancer or cancer-related complications, or any Auto-immune disorder (i.e. rheumatoid arthritis, lupus, inflammatory bowel disease, multiple sclerosis). Thirteen division I collegiate female gymnasts between the ages of 18-22 years old elected to participate in the study throughout the training and competitive seasons (August-April). See **Table 1** for descriptive data of all participants at baseline.

Table 1. Baseline descriptive data of all participants

Parameter (n=11)	Values*
Age (years)	19.6 ± 1.3
Height (cm)	158.2 ± 5.8
Weight (kg)	56.8 ± 5.5
BMI (kg/m ²)	22.7 ± 1.5
Body Fat (%)	18.1 ± 2.9
Fat-Free Weight (kg)	46.6 ± 4.3
Total Cholesterol (mg/dL)	195.5 ± 34.3
LDL Cholesterol (mg/dL)	104.2 ± 28.6
Triglycerides (mg/dL)	97.7 ± 45.7
HDL Cholesterol (mg/dL)	71.9 ± 14.2

*Displayed as means ± standard deviation.

The study utilized a quasi-experimental design where participants either consumed eggs (n=7) or no eggs (n= 6) (see Figure 1). Two participants in the egg group were removed from the final data set due to one career-ending and one season-ending injury reducing the sample

size to 11 participants. Egg participants were strongly encouraged to consume the eggs immediately after either a weight-lifting or practice workout. All participants were informed of the potential risks associated with the study design. The experimental procedures were approved by the Institutional Review Board at Iowa State University.

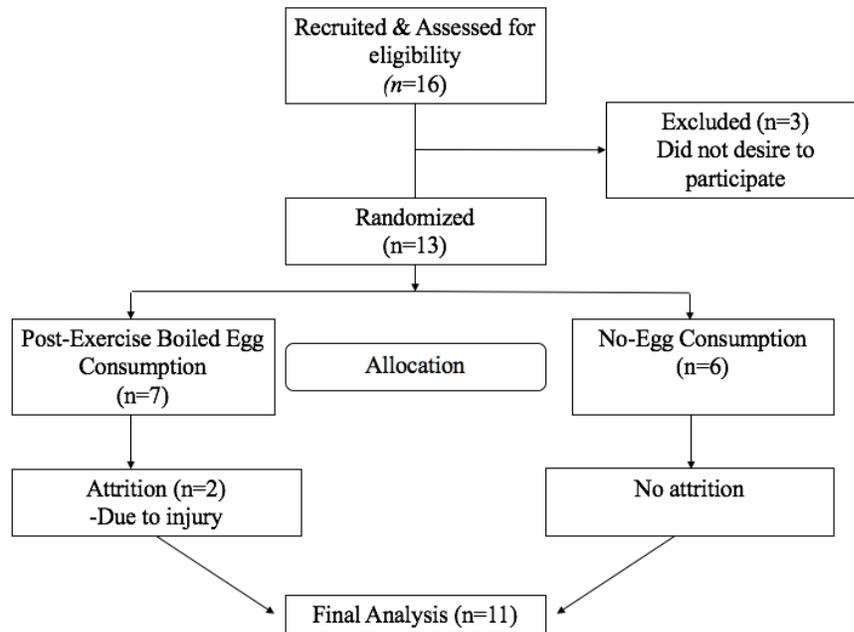


Figure 1. Study Randomization

Study Design (Table 2)

The study design was a quasi-randomized study. Blood serum analysis of blood lipids, inflammatory markers (C-reactive protein [CRP], interleukin-6 [IL-6], and soluble interleukin-6 receptor [sIL-6R], and choline were conducted at the start of preseason (September), one month into preseason (October), post-preseason (December), and post-competitive season (April). Additionally, health status updates were distributed to the athletes to document their menstrual status, vitamins, minerals, and/or medication usage,

current injury(s), and duration of injury (if applicable) each month throughout the training- and competitive-season.

Table 2. Study Timeline for Each Measure.

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April
Blood Analysis		*	*		*				*
Health Status	*	*	*	*	*	*	*	*	*
RESTQ©		*	*		*				*
POMS		*	*		*				*
Egg Consumption		*	*	*	*	*	*	*	*

RESTQ= Recovery-Stress Questionnaire

POMS= Profile of Mood States Questionnaire

Intervention

Boiled eggs were provided in vacuum-packed packages of two (SunnyFresh® Eggs ASAP) to intervention participants in the study. Eggs were distributed to participants immediately following training (i.e. post weight-lifting or post-practice workout). Intervention participants consumed the eggs five times each week for a total of 10 eggs per week for 30 weeks.

Recovery-Stress Questionnaire (REST-Q)

Stress and recovery rates were assessed using the Recovery-Stress Questionnaire (Pearson Assessment and Information, Frankfurt, Germany) at 5 time points throughout the study: start of training (September), 1-month into training (October), post-training season (December), and post-competitive season (April). An additional time point was included at the end of October to quantify the high-intensity training period. The REST-Q questionnaire evaluated six components: fatigue, sleep quality, disturbed break (i.e. how restful breaks from training were), fitness/injury (i.e. how the athlete perceived that their current fitness/training level may increase the risk of injury), fitness-in shape (i.e. how well the

athlete perceives their fitness level), and self-regulation (i.e. psychological skills like goal setting, self-efficacy, imagery, attention, and activation regulation).

Profile of Mood States (POMS) Questionnaire

The POMS questionnaire served to assess psychological states of stress through six subscale categories of moods. In the literature, this questionnaire has been shown to be sensitive to both workload and recovery (“(15) Profile of Mood States is Sensitive to Changes in Workload and Perceived Recovery,” n.d.). Participants utilized the questionnaire’s 5-point scale, ranging from 1: “not at all” to 5: “extremely”, for each mood listed. The questionnaire was completed in conjunction with the REST-Q.

Blood Serum Analysis

Blood samples were obtained by antecubital venipuncture after an overnight fast (10-hour minimum) at 4 time points throughout the study: start of training (September), 1-month into training (October), post-training season (December), and post-competitive season (April). A private laboratory conducted blood lipid panel and CBC with differential analyses and the carotenoids found in the egg yolk, lutein and zeaxanthin, were measured for compliance through a lab collaborating on the project. Blood samples were also collected for the examination of CRP, IL-6, and IL-6R alpha. Blood samples sat at room temperature for 30 minutes to allow for clotting, centrifuged (15 minutes), aliquoted, and stored at -80 °C.

Sandwich enzyme-linked immunosorbent assays (ELISA) were used to measure serum CRP, IL-6, and IL-6R alpha via commercially available ELISA kits (Human Quantikine ELISA Kits, R&D Systems, Minneapolis, MN, USA). Serum samples for both CRP and IL-6R alpha underwent a 100-fold dilution (1:5 dilution) with the provided diluent. Analyses were completed in duplicate for CRP, IL-6, IL-6R alpha, and choline assays

according to the manufacturer's instruction. Blood serum choline was measured using the Choline/Acetylcholine Assay Kit (ab65345) (abcam, Cambridge, United Kingdom). All results were read on a Synergy® 2 Multi-Detection Microplate Reader (Biotek Instruments, Inc., Winooski, VT, USA).

Statistical Analyses

Statistical analyses were conducted using IBM SPSS Statistics (version 25, IBM, Armonk, NY) and SAS (version 9.4, SAS Institute Inc, Cary, NC). Viable statistical analyses were limited due to the small sample size, which was further compounded by loss of two participants due to injuries in the egg group. A p-value of <0.05 was used for all analyses. Mixed Model tests was used to investigate differences in the inflammatory markers, CRP, IL-6, and IL-6R alpha. Stepdown Bonferroni post-hoc test was run on significant fixed effects found (i.e. treatment, time, and treatment by time). Repeated Measures ANOVA was used for the POMS and RESTQ® to identify differences over time. Bonferroni post-hoc test was utilized to identify where differences occurred. In addition, correlations between POMS, REST-Q and inflammatory markers were completed. The provided figures and table data are displayed as mean values \pm standard errors of the mean (SEM) unless specified otherwise.

Results

The mean participant age was 19.6 ± 1.3 years. At baseline, there were no significant differences in measures of biochemical indices between the egg and no egg groups.

Inflammatory Markers (Table 3)

Table 3 displays the inflammatory indices by group at each time point (CRP, IL-6, sIL-6R, lutein, zeaxanthin, albumin, and total protein). Throughout the season, serum lutein

and zeaxanthin were assayed to monitor compliance with egg consumption and protein status was examined via albumin and total protein. Through mixed model tests, no significant effect of treatment over time was found in serum CRP, IL-6, sIL-6R, total protein, albumin, and zeaxanthin. However, there was an overall significant time effect ($p < 0.05$) for sIL-6R, total protein, albumin and zeaxanthin. Following post-hoc tests, the time effect remained in sIL-6R and albumin only. More specifically, sIL-6R decreased 57.45 pg/mL and albumin decreased 0.27 g/dL between September and October ($p < 0.05$) in the no egg group. Change in sIL-6R and albumin was somewhat variable over time. The greatest decrease in inflammation via sIL-6R occurred between September and October in both the egg and no egg groups. A rebound in the sIL-6R occurred in the egg group between October and December; the no egg group experienced another decline between December and April. A rebound in albumin as also noted in the egg group between October and December.

POMS (Table 4)

Subcomponents of the POMS questionnaire demonstrated no significant time and/or treatment by time effects in depression, anger, fatigue and confusion. Total mood disturbance (TMD) and tension demonstrated significant time and treatment over time effects, respectively ($p < 0.05$). For both measures, time effects remained in the egg group, but was not found significant at any time point. Though confusion was non-significant, trending time and treatment over time interactions were present ($p = 0.053$; $p = 0.056$, respectively). More specifically, these effects were present in the no egg group from September to October ($p < 0.05$) and April ($p < 0.05$).

Table 3. Biochemical Indices Values

Biochemical Indices	September		October		December		April	
	Egg	No Egg	Egg	No Egg	Egg	No Egg	Egg	No Egg
CRP (mg/dL)	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
IL-6 (pg/mL)	1.09 ± 0.15	1.28 ± 0.80	1.76 ± 0.50	0.64 ± 0.21	0.68 ± 0.11	1.47 ± 0.90	0.73 ± 0.21	0.76 ± 0.12
sIL-6R (pg/mL)	306.15 ± 43.26	324.25 ± 34.12 ¹	251.37 ± 19.69	266.80 ± 25.27	322.67 ± 40.48	270.30 ± 24.68	266.48 ± 39.19	269.38 ± 29.84
Lutein (µmol/L)	0.30 ± 0.04	0.42 ± 0.07	0.32 ± 0.05	0.45 ± 0.07	0.28 ± 0.04	0.43 ± 0.06	0.45 ± 0.15	0.51 ± 0.06
Zeaxanthin (µmol/L)	0.09 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.16 ± 0.04	0.16 ± 0.01
Albumin (g/dL)	4.62 ± 0.08	4.62 ± 0.09 ¹	4.20 ± 0.08	4.35 ± 0.08	4.48 ± 0.14	4.55 ± 0.06	4.40 ± 0.13	4.30 ± 0.06
Total Protein (g/dL)	4.62 ± 0.11	7.43 ± 0.11	6.84 ± 0.17	6.87 ± 0.10	7.34 ± 0.21	7.20 ± 0.13	7.06 ± 0.31	6.85 ± 0.13

Displayed as mean ± standard error mean.

¹significant time effect from September to October (p<0.05)

Table 4. Profile of Mood States questionnaire components. TMD= Total Mood Disturbance. Values reported as means \pm standard error mean

POMS	September		October-1		October-2		December		April	
	Egg	No Egg	Egg	No Egg	Egg	No Egg	Egg	No Egg	Egg	No Egg
Tension*	18.00 \pm 1.64	11.83 \pm 0.87	21.00 \pm 2.17	13.67 \pm 0.99	16.20 \pm 1.66	13.60 \pm 0.81	16.20 \pm 1.28	13.50 \pm 1.31	12.60 \pm 0.68	14.00 \pm 1.03
Depression	16.00 \pm 0.32	15.50 \pm 0.34	17.60 \pm 1.17	17.17 \pm 1.40	16.40 \pm 0.87	17.00 \pm 1.38	16.80 \pm 1.32	15.83 \pm 0.65	16.20 \pm 0.97	16.33 \pm 0.95
Anger	12.80 \pm 0.37	12.83 \pm 0.65	14.20 \pm 1.02	14.00 \pm 1.24	15.00 \pm 1.90	13.20 \pm 0.73	13.80 \pm 1.11	13.50 \pm 0.76	13.60 \pm 1.36	13.83 \pm 0.98
Fatigue	13.80 \pm 1.32	9.17 \pm 0.70	14.00 \pm 2.07	11.17 \pm 1.92	15.80 \pm 2.35	12.00 \pm 1.84	14.60 \pm 2.18	10.33 \pm 1.23	10.20 \pm 0.86	9.17 \pm 0.70
Confusion [†]	11.80 \pm 0.97	10.00 \pm 0.63 ^{1,2}	12.60 \pm 0.51	12.00 \pm 0.63	12.60 \pm 0.51 ^c	11.60 \pm 0.40	12.80 \pm 0.80	10.50 \pm 0.89	11.60 \pm 0.24	12.00 \pm 0.52
Vigor	16.40 \pm 2.40	18.50 \pm 2.14	16.20 \pm 2.06	18.33 \pm 3.08	18.60 \pm 2.20	21.00 \pm 4.74	15.80 \pm 3.48	17.33 \pm 3.16	20.80 \pm 2.01	20.83 \pm 3.54
TMD	56.00 \pm 0.71	40.83 \pm 2.32	63.20 \pm 4.26	49.67 \pm 6.56	57.40 \pm 7.35	46.40 \pm 7.72	58.4 \pm 7.30	46.33 \pm 6.23	43.40 \pm 4.03	44.50 \pm 5.24

*Significant time effect ($p < 0.05$)

[†]Trending time effect ($p = 0.053$)

Following simple effects:

¹September and December

²September and April

Superscript letter denote correlations between the following inflammatory markers at the specified time point:

^aCRP

^bIL-6

^csIL-6R

Table 5. RESTQ components. Values reported as means \pm standard error mean

RESTQ	September		October-1		October-2		December		April	
	Egg	No Egg	Egg	No Egg	Egg	No Egg	Egg	No Egg	Egg	No Egg
Fatigue	14.14 \pm 1.44	7.50 \pm 1.18 ¹	11.29 \pm 1.38 ^a	7.67 \pm 2.09	10.71 \pm 1.95	10.40 \pm 1.40	12.50 \pm 1.67	10.33 \pm 1.76	8.57 \pm 1.02	7.67 \pm 0.76 ^a
Sleep Quality	11.71 \pm 1.27	14.50 \pm 1.18	11.29 \pm 0.87 ^{a,c}	13.17 \pm 1.80 ^a	13.71 \pm 1.34	15.80 \pm 1.74	11.67 \pm 1.48	10.83 \pm 2.17 ^a	13.57 \pm 1.29	17.00 \pm 1.46
Disturbed Break	6.29 \pm 0.94 ^a	5.50 \pm 1.38	6.43 \pm 0.81	3.50 \pm 1.15 ^b	7.00 \pm 0.69	6.40 \pm 1.07	4.83 \pm 0.54 ^b	3.67 \pm 1.33	2.57 \pm 1.15	3.67 \pm 0.84
Fitness/Injury	11.71 \pm 0.78	6.67 \pm 1.58	12.14 \pm 1.30	9.50 \pm 2.17	11.14 \pm 1.03	8.40 \pm 1.63	9.57 \pm 1.83	7.50 \pm 1.41 ^c	8.43 \pm 0.84	7.50 \pm 1.65 ^b
Fitness-in-Shape	10.71 \pm 0.42	14.17 \pm 1.66	10.43 \pm 1.19	12.67 \pm 1.02	10.29 \pm 1.51	14.00 \pm 2.35	10.50 \pm 1.09	11.50 \pm 1.80	11.57 \pm 1.49	14.67 \pm 1.31
Self-Regulation	10.86 \pm 0.94	13.17 \pm 1.92	10.86 \pm 1.49	13.33 \pm 1.76	12.71 \pm 0.99	13.20 \pm 2.27	12.33 \pm 0.84	13.50 \pm 2.28	15.86 \pm 2.18 ^c	16.17 \pm 1.35

Significant time effect ($p < 0.05$):¹September and December

Superscript letter denote correlations between the following inflammatory markers at the specified time point:

^aCRP^bIL-6^csIL-6R

No significant correlations were present between the inflammatory markers and POMS total mood disturbance and the subcomponents: fatigue, tension, and vigor. However, sIL-6R exhibited a significant positive correlation with confusion in October. Trends were also present as serum CRP (September) was negatively associated with depression ($r=-0.82$; $p=0.89$) and positively associated with fatigue ($r=0.83$; $p=0.08$). There was also a positive relationship between serum sIL-6R and tension in September ($r=0.84$; $p=0.08$) and anger and TMD in October (Anger: $r=0.05$; $p=0.05$; TMD: $r=0.86$; $p=0.07$). For the no egg group, serum IL-6 was positively correlated with anger in September ($r=0.90$; $p<0.05$) and December ($r=0.90$; $p<0.05$). Positive correlations with serum IL-6 were also present in measures of depression in October ($r=0.90$; $p<0.05$) and December ($r=0.97$; $p<0.05$). There were also several identified trends among measures of fatigue. Fatigue was negatively correlated with CRP in September ($r=-0.79$, $p=0.06$) and sIL-6R in December ($r=-0.78$, $p=0.07$).

REST-Q (Table 5)

Psychosocial factors of the REST-Q Questionnaire demonstrated time interactions and associations relative to inflammation. A significant effect over time was present in measures of fatigue ($p<0.05$). Following a simple effects test with Bonferroni adjustment, the effect remained in the no egg group as fatigue increased from September to December. In addition, the egg group experienced a trend increasing in fatigue from September to April ($p=0.058$). Measures of sleep quality, disturbed break, and self-regulation had main effects of time ($p<0.05$) but no significant treatment by time interactions were indicated, suggesting that the response over time was not within the egg and no egg group. No significant differences were demonstrated in measures of fitness/injury and fitness-in shape.

Within the egg group, sleep quality was negatively correlated with CRP ($r=-0.92$; $p<0.05$) and positively correlated with sIL-6R ($r=0.94$; $p<0.05$) in October. At this time point, IL-6 experienced a positive trending correlation with sleep quality ($r=0.85$; $p=0.067$). In December, disturbed break was negatively correlated with IL-6 ($p<0.05$), and further saw a significant negative correlation in self-regulation in April ($r=-0.90$; $p<0.05$). The no egg group had a significant positive relationship with CRP and sleep in October and December ($r=0.88$; $p<0.05$). CRP was also negatively associated with fatigue in April ($r=-0.81$; $p=0.05$), and showed negative trends in September and October ($p=0.055$; $p=0.052$). Disturbed break was negatively associated with CRP ($r=-0.90$; $p<0.05$) in September and positively associated with IL-6 in October ($r=0.82$; $p<0.05$). sIL-6R and IL-6 were negatively associated with fitness injury measures in December ($r=-0.87$; $p<0.05$) and April ($r=-0.84$; $p<0.05$), respectively. No significant relationships were demonstrated in fitness-in-shape with markers of inflammation in both groups.

Discussion

Throughout this 9-month study, markers of inflammation were measured (i.e. CRP, IL-6, and sIL-6R) to explore the relationship between whole egg intake and the inflammatory response throughout preseason and competitive training. Whole eggs are recognized as the standard reference protein and contain several essential nutrients that may attenuate inflammation (Detopoulou et al., 2008b). Among no egg participants, a significant reduction in sIL-6R from September to October was observed; however, no significant differences in the egg group or the inflammatory markers, CRP and IL-6, were noted. Though this was the

case, observational analyses for both groups demonstrated an overall decrease in both IL-6 and sIL-6R from September to April, which suggests a decrease in inflammation.

The implications of the complexity of IL-6 relates to overreaching symptoms due to increased concentrations of cytokines released pre-, during, and post-exercise, termed ‘cytokine sickness’, or a state of chronic inflammation (Robson, 2003). Past research has identified elevated IL-6 concentrations as a marker of increased fatigue. Within healthy and trained male runners, Robson-Ansley and colleagues were the first to identify this phenomenon where IL-6 was found to surround the tendons, brain and muscles of these participants. Following subjective measures via the Profile of Mood States (POMS) questionnaire, participants in Robson-Ansley’s study exhibited a greater mood disturbance post-exercise (Robson-Ansley et al., 2004). Within this present study, the POMS questionnaire was utilized to examine whether higher levels of disturbed mood were associated with markers of inflammation. Additionally, it explored whether egg treatment could potentially reduce these subjective and objective indicators of inadequate recovery. Observationally, total mood disturbance increased one month into preseason training (baseline to October). In the months following October, the egg participants experienced an attenuation in total mood disturbance (TMD), while the no egg participants TMD was maintained over time. This suggests that the egg treatment, in conjunction with intense training, may have some benefit in reducing disturbed mood; however, due to observational differences at baseline, these results may be null.

sIL-6R’s relationship with perceived stress, mood, and poor sleep quality has also been explored. Positive associations between sIL-6R with stress and mood, as well as negative associations between sIL-6R and sleep quality, have been displayed in highly

trained endurance athletes over an extended training period by Cullen et al (Cullen et al., 2017). Cullen and colleagues work further indicated that this marker may be of benefit in identifying athletes demonstrating symptoms associated with overtraining. Overtraining is an area of concern in athletes where an athlete's overall wellbeing and performance can be influenced by the recovery stress-state. Negative overtraining can result in staleness of the athlete, further eliciting signs of underperformance, muscles soreness, fatigue, overuse injuries, mood disturbances, immune system deficits, and difficulties concentrating (Kenttä & Hassmén, 1998). Overtraining components were examined in this study via the REST-Q that measured stress and recovery. In October, a significant relationship between sIL-6R and poor sleep quality were demonstrated within both the egg and no egg group in October. Both the quality and quantity of sleep can often decrease with changes in the volume of training, especially among athletes experiencing overtraining (Cullen et al., 2017; Hausswirth et al., 2014; Lastella et al., 2018). Within this study, the October training period was noted to be the most intense training phase of the preseason; that very well could have contributed to the decrease in sleep quality in both groups. These results suggest importance of not only evaluating physiological markers in an athlete, but also psychological ones as well. The results also put forward the importance that adequate nutrition may have on the overall wellbeing and success of the athlete during intense training.

In conclusion, the present pilot study suggests there may be a relationship between whole egg intake, inflammation and mental state in collegiate female gymnasts. Our results did not demonstrate significant differences in most of the variables but observationally, there was a reduction in sIL-6R, a pro-inflammatory marker, among the egg group participants throughout the 9-month intervention. It is important to note that these results are only

suggestive because of the small sample size and variability in the measures. First, the small sample size leaves room for error due to the inter-individual variability, attrition, and difficulty in generalizing the effects of egg consumption. Quasi-randomization of the participants was used to assist in eliminating room for error, though not being able to randomize those with an allergy or aversion to eggs was an additional limitation. Second, two boiled eggs/day post-exercise elicited underlying compliance problems. The study's design attempted to address this issue through testing carotenoids (lutein and zeaxanthin which are found in the egg yolk) within each participant where results suggested egg intake may have decreased over time. It is important to note that carotenoid results may or may not be from egg intake as these nutrients can be obtained from other food sources as well. Third, an egg washout period was not included and further could have hindered our results. Applying a crossover trial with alternating periods of egg intake and no egg intake with the participants may have better distinguished the elicited effects of whole-egg intake. Lastly, the use of self-report surveys is confounding. Finally, though a mixed model test was applied within groups (egg and no egg) with stepdown Bonferroni, our results did not have the appropriate power and effect size to use these tests. Further trials are needed to reduce the present confounders and to confirm or refute our findings. Future research should look to address the encountered issues with this pilot study and re-evaluate methods to increase compliance with nutrition interventions.

Acknowledgements

We want to thank the Iowa Egg Council and Egg Nutrition Center for their support and for funding this research study, as well as all study personnel and athletes who made this possible.

Conflicts of Interest and Source of Funding

Funding was received from the Iowa Egg Council and Egg Nutrition Center. The study's sponsor did not participate in designing, conducting, or presenting the study during the publication process. There are no conflicts of interest to declare from the authors.

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CHAPTER 5. DIET QUALITY OF FEMALE COLLEGE GYMNASTS FOLLOWING WHOLE EGG CONSUMPTION

A paper to be submitted to the Journal of American College Health

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Abstract

Research has documented the prevalence of aesthetic characteristics, as well as energy deficits that concomitantly can decrease essential nutrients within collegiate female gymnasts, further increasing the risk of one or more components of the female athlete triad. The importance of nutrition in recovery for elite athletes, including collegiate gymnasts has also been documented. Further, the purpose of this paper is to examine the impact of whole egg consumption (post-exercise 5 days/week for 8 months) on anthropometrics, dietary intake, and diet quality during the pre-season and competitive season. Variable measures occurred in August, December, and April and were analyzed via repeated measures ANOVA via SPSS (v25) and HEI via SAS (v9.4) and ASA24-2016. No significant anthropometric measures were found, but energy intake, carbohydrate and fat significantly decreased in the egg group ($p < 0.05$). The present study indicates the need for nutritional interventions to prevent insufficient nutrient intake in collegiate female gymnasts.

Introduction

The sport of gymnastics emphasizes high lean muscle mass to support powerful vertical movements. Maintaining a desired body image and weight, as well as ability to perform, makes extra fat mass a disadvantage in the sport (Sundgot-Borgen & Garthe, 2011a). Athletes with low energy intake are likely to be deficient in essential macro- (i.e. protein, carbohydrate, and fat) and micro-nutrients (i.e. vitamins and minerals)(Cialdella-Kam, Kulpins, & Manore, 2016). Notably, among U.S. elite gymnasts, estimated energy intakes were 20% below the estimated needs (Jonnalagadda, Bernadot, & Nelson, 1998). More recently, Shriver and colleagues documented energy intakes of less than 2,000 kcal/day (Shriver, Betts, & Wollenberg, 2013) among female collegiate athletes.

Past research suggests collegiate athletes lack nutrition knowledge, particularly related to the preparation and purchasing of food (Reed et al., 2013). Reed and colleagues found collegiate female soccer players that were catered dinner consumed significantly more food/energy than when they were responsible for preparing their own lunch. For athletes, not only health, but performance can be impacted by suboptimal dietary intake (i.e. calories). More specifically, female athletes participating in aesthetic sports with pressure related to physical appearance, are at increased risk for the female athlete triad (Nazem & Ackerman, 2012a). The female athlete triad is common among young athletic women and is comprised of three components: abnormal menses, decreased bone mineral density, and low energy availability^{7,8}. Low energy availability is postulated to be the etiology of the female athlete triad.

Energy availability is defined as dietary energy-energy expenditure and the proposed causes of low energy availability are that of disordered eating, as well as insufficient energy

intake (Nazem & Ackerman, 2012a). Implications of insufficient dietary energy may lead to a compromise in macro- and micro-nutrient intake, further decreasing the ability to synthesize muscle protein and maintain lean muscle mass (Manore et al., 2007). Ultimately, this poses an increased risk for immune suppression, menstrual disturbances, hormonal imbalances, iron-deficiency anemia, and musculoskeletal injuries (Cialdella-Kam et al., 2016).

Weight loss and dieting methods that cause fluctuations in weight status are commonly seen in athletes involved in aesthetic sports. These methods are not conducive to the overall wellbeing of an athlete and can cause underperformance (Sundgot-Borgen & Garthe, 2011a). This suggests balanced diets that do not compromise normal physiological functions should be encouraged (Wilmore, 1991). Nutrition interventions are pertinent to improve the diet quality of female college athletes in aesthetic sports (Shriver et al., 2013) and ensuring adequate energy intake post-exercise is just one of the many challenges to be addressed.

The current nutrition approach at the collegiate level consists of promoting sports drinks and bars for both fueling and recovery purposes; however, many of these items are often energy dense and high in added sugars. This has led to a whole foods approach, instead of sports bars and shakes, to supplement the dietary intake of elite athletes (Bytomski, 2017; Thomas et al., 2016a). Nutrition, specifically protein, has been postulated as one of the most important factors to promote both physical and immune health (Beelen et al., 2010a; Heaton et al., 2017b). There are several protein sources including lean meats, Greek yogurt, cottage cheese, and eggs (Bytomski, 2017). Eggs, a whole food of interest, are an inexpensive source of protein. In fact, eggs serve as the standard reference protein as they contain all the essential amino acids in the appropriate proportions to support both the needs of the entire

body, as well as the muscle (van Vliet et al., 2017a). In addition to its high-quality protein content, other bioactive components in eggs of benefit to human health include: iron, phosphorous, choline, B vitamins (1, 2, 8, and 12), and vitamins A, D, E, and K (Anton et al., 2006a).

The purpose of this research was to examine diet quality, energy availability and body composition of Division I collegiate female gymnasts following a nutrition intervention of post-exercise whole egg consumption throughout the preseason and competitive season training months. What are your hypotheses?

Subjects and Methods

Participants

Division I collegiate female gymnasts at a Midwestern university served as research participants for this study. Potential participants were contacted prior to the training season through email communication. Those interested in participating agreed to attend an informational meeting to explain and receive instructions for completing the 5-day food records. Exclusion criteria for participation included the following health conditions: egg allergies, asthma, coronary artery disease, depression, diabetes, hypertension, migraines, cancer or cancer-related complications, or any auto-immune disorder (i.e. rheumatoid arthritis, lupus, inflammatory bowel disease, multiple sclerosis). Thirteen division I collegiate female gymnasts between the ages of 18-22 years old elected to participate in the study throughout the preseason and competitive seasons (August-April). Participants were quasi-randomly assigned to either the intervention (egg; n=7) or the control group (no egg; n= 6). Two participants in the egg group were removed from the final data set due to one career-

ending and one season-ending injury. Egg participants were strongly encouraged to consume the eggs immediately after either a weight-lifting workout or practice workout. All participants were informed of the potential risks associated with the study design and completed an informed consent document. The study was approved by the Institutional Review Board at the University.

Anthropometrics

Weight, body fat percentage (BF %), and fat-free weight (FFW) were collected via BOD POD[®] measurements for all participants at three time points: baseline (August), post-preseason training (December), and post-competitive season (April). The BOD POD[®] device is an air displacement measuring instrument used to assess body composition. All participants were instructed to fast for a minimum of 10 hours and wear appropriate clothing (i.e. spandex, sports bra, hair cap, no jewelry).

Nutrient Analysis and Energy Availability (EA)

Nutrient analysis of the five-day food records at each time point using Food Processor Nutrition Analysis Software (ESHA, version 11.3.285, Salem, Oregon). Energy intake values from five day food records were used to calculate energy availability via the equation: $[(\text{energy intake [EI]} - \text{estimated energy expenditure [EEE]}) / \text{fat-free weight [FFW]}]$ (Thomas et al., 2016a). August and April were the measured EA time points. Further, energy expenditure was set at zero due to August corresponding to baseline, and April as a post-season resting period. Direct energy expenditure measures were not completed for this present study.

Healthy Eating Index (HEI)-2015

The average nutrient intake of each five-day food record was used for each time point in statistical analyses. HEI scores were calculated using the Automated Self-Administered

24-hour (ASA24-2016) Dietary Assessment Tool (National Cancer Institute, version 2016, Bethesda, MD) (“ASA24® Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH,” n.d.). HEI-2015 scores were calculated through SAS (version 9.4, SAS Institute Inc., Cary, NC) via the use of ASA24-2016 to output food record information. The simple HEI-2015 scoring algorithm-per day was utilized to calculate scores for each day within each participant’s 5-day food record. Overall 5-day mean values were then calculated for each person to obtain their mean HEI-2015 scores at each time point (i.e. August, December, and April). The HEI-2015 (“Healthy Eating Index (HEI) | Center for Nutrition Policy and Promotion,” n.d.) was used to measure the overall diet quality among the intervention and control participants. This tool was created by the National Cancer Institute (NCI) and US Department of Agriculture (USDA) to assess diet quality in conjunction with the US Dietary Guidelines for Americans (DGAs). It functions to assign a score from 0 to 100, with 100 indicating compliance with the 2015-2020 Dietary Guidelines (“Healthy Eating Index (HEI) | Center for Nutrition Policy and Promotion,” n.d.). According to the HEI grading scale, receiving a diet score of 80 and above is “good”, 51-80 is classified as “needs improvement”, and less than 51 is poor (Kennedy, Ohls, Carlson, & Fleming, 1995).

Statistical Analysis

All results are shown as mean \pm standard error mean. IBM SPSS Statistics (version 25, IBM, Armonk NY) was used for the calculation of mean values, as well as repeated measures ANOVA with Bonferroni adjustment (run on significant fixed effects of time, treatment, and treatment over time) for anthropometrics and paired t-tests for nutrient analysis. Results were considered significant at $p < 0.05$.

Results

Anthropometrics

The mean age of the 11 participants was 19.6 years old. Anthropometrics including height, weight, Body Mass Index (BMI), body fat percentage (BF%), and Fat-Free Weight (FFW) appear in Table 1. There was no significant difference in anthropometric measures between the egg (n=5) and no egg (n=6) group at any time point. However, within groups, the egg group experienced a significant decline in BF% between August and December.

Table 1. Anthropometric Measures of Division I Collegiate Gymnast Participants

	Collegiate Female Gymnast Participants [†]					
	August		December		April	
	Egg (n=5)	No Egg (n=6)	Egg (n=4)	No Egg (n=6)	Egg (n=5)	No Egg (n=6)
Age	19.6 ± 1.3					
Height	158.2 ± 5.8					
Weight (kg)	54.83 ± 1.37	57.94 ± 2.82	54.59 ± 1.96	58.26 ± 2.43	54.98 ± 1.98	58.42 ± 2.66
BMI (kg/m ²)	21.90 ± 0.65	23.41 ± 0.60	21.78 ± 0.58	23.55 ± 0.53	21.93 ± 0.65	23.61 ± 0.59
Body Fat (%) ^a	16.58 ± 1.30	18.78 ± 1.19	13.76 ± 1.02	17.13 ± 0.93	13.26 ± 1.52	17.80 ± 1.39
Fat-Free Weight (kg)	45.81 ± 2.05	46.98 ± 1.87	47.12 ± 2.09	48.27 ± 1.91	47.74 ± 2.32	47.97 ± 2.11

^a Overall time effect between August and December

[†] Values are displayed as mean ± standard error mean

Dietary Intake

Mean dietary intakes, including energy, energy availability, protein, fat, carbohydrate, Vitamins B12 and D, and choline among the participants appear in Table 2. Mid-season data for these measures (December) were not included due to missing data skewing distribution between the groups.

Table 2. Dietary Intake of Division I Collegiate Gymnast Participants

Dietary Intake	Collegiate Female Gymnast Participants				Current Recommendations
	August		April		
	Egg (n=5)	No Egg (n=6)	Egg (n=5)	No Egg (n=6)	
Calories ^a	2175.65 ± 636.89	1743.91 ± 524.06	1563.50 ± 315.34	1475.18 ± 494.28	3,000 ¹⁸
Energy Availability ^a (kcal/kg FFM)	47.11 ± 5.09	37.37 ± 11.69	32.82 ± 2.95	30.76 ± 9.65	≥ 45 kcal/kg FFM ⁹
Protein (g)	88.31 ± 35.90	71.34 ± 33.11	72.54 ± 15.19	69.86 ± 20.23	-
Protein (g/kg)	1.59 ± 0.59	1.23 ± 0.49	1.31 ± 0.23	1.19 ± 0.32	1.2-2.0 g/kg ⁹
Fat (g)	103.12 ± 48.77	66.02 ± 16.40	59.87 ± 21.86	53.06 ± 13.29	-
Fat (g/kg) ^a	1.87 ± 0.83	1.16 ± 0.34	1.07 ± 0.37	0.91 ± 0.21	-
Carbohydrates (g) ^a	233.37 ± 39.97	223.31 ± 73.52	189.79 ± 30.58	189.70 ± 81.15	-
Carbohydrates (g/kg) ^a	4.25 ± 0.61	3.87 ± 1.25	3.47 ± 0.63	3.23 ± 1.28	6-10g/kg ⁹
Vitamin D (IU)	75.57 ± 81.66	53.32 ± 50.77	94.72 ± 66.54	81.33 ± 47.84	600 IU/d ¹⁹
Vitamin B12 (mcg)	2.81 ± 2.98	3.77 ± 2.73	2.62 ± 1.93	3.97 ± 3.25	2.4 mcg ²⁰
Choline (mg)	230.64 ± 203.28	144.80 ± 87.20	165.94 ± 155.51	192.78 ± 140.40	425 mg/d ²¹
HEI Score	57.89 ± 4.85	62.58 ± 4.82	59.22 ± 3.22	62.62 ± 3.98	58.0 ²²

^a significant (p<0.05) change in the egg group between August and April

No significant differences in dietary intake of any nutrient between the egg (n=5) and no egg (n=6) groups were observed in independent sample t-tests at either time point (August/April). Results of paired t-tests indicate a significant decrease in caloric intake, carbohydrates (both total g and g/kg), and fat (g/kg) between August and April (p<0.05) in the egg group. Further, dietary fat (total g) and choline trended downwards between August and April (p=0.052; p=0.056) in the egg group. The only trend observed in the no egg group, was a decrease in fat intake (total g and g/kg) from August to April (p=0.061; p=0.052). Both groups demonstrated a non-significant decrease in energy availability, protein (total and g/kg), Vitamin D, and Vitamin B12 between time points. Recommended intakes for calories, EA, protein, carbohydrate, fat and vitamin D were not met by either group at either time point.

Healthy Eating Index (HEI)-2015

HEI scores also appear in Table 2. There was no significant difference in HEI score between the egg and no egg groups at either time point or no significant change in HEI score within either group over time.

Comment

Limitations

There were several inherent limitations with this study. First and foremost, the small sample size limits the interpretation of the results. The study experienced attrition that further suggests additional variability in the data and difficulty generalizing diet quality in the population group. Second, the intervention of this study required whole egg consumption for 8 months where compliance problems occurred with egg consumption. Third, two different nutrient analysis software programs were utilized to examine nutrient intake. It may be prudent to utilize the ASA24-2016 software to assess both dietary intake and corresponding HEI scores. Finally, the use of the 5-day food records can yield confounding results due to it being a self-report measure.

Conclusion

Energy Availability

As mentioned previously, one of the potential causes of the female athlete triad components, low energy availability (EA), is insufficient energy intake. In this study, we found energy intake decreased significantly for the egg group participants between the two time points. Recommended EA intake is ≥ 45 kcal/kg FFW (Cialdella-Kam et al., 2016) (Nattiv et al., 2007b; Thomas et al., 2016a) after accounting for the energy expended in exercise. Energy expenditure was not captured in this study; however, we were interested in

exploring the adequacy of EA assuming no energy expenditure needs. Not surprisingly, EA in this population of collegiate female gymnasts did not meet the recommendation of 45 kcal/kg, with the exception of the egg group in August. Thus, the gymnasts were not even achieving adequate EA assuming no energy expenditure.

Similar findings demonstrating a reduction in energy intake and energy availability among collegiate female athletes has been reported in Division I female soccer players as the preseason and season training time period progressed (Reed et al., 2013). The collegiate gymnast participants in this study for the egg and no egg group had EA values post-season (April) of approximately 33 and 31 kcal/kg FFW, respectively. Reed and colleagues study demonstrated that five female collegiate female soccer players with low energy availability had EA <30kcal/kg FFW during the pre- and mid-season. Results for the post-season noted that EA rebounded back in a few participants suggesting that this condition may be seasonal, and further, reversible(Reed et al., 2013). One area of concern is the association between low EA and menstrual dysfunction and low bone mineral density associated with the female athlete triad. These complications are more likely when EA is less than 30 kcal/kg FFW per day and our results were nearing this threshold. Although an athlete may be striving to achieve a particular body shape via an energy deficit for a competitive advantage, the consequences of this practice can potentially lead to deficits in hydration, glycogen, lean muscle mass, and other physiological issues that could impair performance(Thomas et al., 2016a). Recommendations by the American College of Sports Medicine suggest energy deficit practices should take place prior to competitive season training, at a slower rate, and with increased protein intake(Thomas et al., 2016a).

Macro-and Micro-nutrients

Overall energy intake, as well as several macro- and micronutrients, decreased between time points (August and April) in both groups (egg and no egg). A balance of macro- and micro-nutrients is crucial to fuel an athlete's training and recovery. Fat and carbohydrates decreased throughout the competitive season whereas protein intake remained relatively unchanged. Fat is an essential energy source and there are no specific recommendation amounts for athletes. However, the American College of Sports Medicine (ACSM) notes that fat intake should be dependent upon on both an athlete's individual level of training and goals for body composition (Thomas et al., 2016a). In addition, ACSM indicated that athletes should follow the current 2015-2020 Dietary Guidelines that states less than 10% of energy should be from saturated fat and sources of essential fatty acids should be included within the diet ("2015-2020 Dietary Guidelines - health.gov," n.d.; Thomas et al., 2016a). Carbohydrates are an additional energy source and recommendations suggest that during moderate-high intensity exercise programs, classified as lasted 1-3 hours/day, athletes should consume nutrient-rich carbohydrates in the amount of 6-10g/kg/day (Thomas et al., 2016a). The intensity, time, and amount are relevant to the collegiate gymnast participants where both the egg and no group reported average carbohydrate intakes of 4.25 ± 0.61 and 3.87 ± 1.25 g/kg, respectively in August, and 3.47 ± 0.63 and 3.23 ± 1.28 g/kg, respectively in April. Further, carbohydrate intakes were severely low when compared to the recommendations stated by ACSM.

Examination of protein intakes suggested that protein intake was maintained over time. Current recommendations by the American College of Sports Medicine suggest 1.2-2.0 g/kg/d of dietary protein to support muscle protein synthesis and repair(Thomas et al., 2016a). Even higher protein intakes may be needed when there is a reduction in energy intake or during short and intense exercise bouts (2.3 g/kg) (Thomas et al., 2016a). While this

population did not meet the recommended EA, they did meet the protein recommendation albeit on the low end. The maintenance of protein intake in the egg group may have prevented these athletes from falling below the EA threshold (i.e. <30 kcal/kg/FFW) where physiological issues may arise.

Relative to the micronutrients, neither group met the recommended intakes for Vitamin D, whereas both groups met the recommended intake of Vitamin B12 at each time point. Maximization of vitamin D intake is recommended to prevent low bone mineral density and other physiological consequences of the female athlete triad. It is best known for its role in muscle function and the inflammatory response. Further, increased intake has been shown to downregulate pro-inflammatory cytokines (Mousa, Misso, Teede, Scragg, & de Courten, 2016). No changes in vitamin D were found in either group, but it's important to note that intake was severely low in comparison to the Recommended Dietary Allowance (RDA) of 600 IU. This recommendation is for children and adults up to 70 years old ("Office of Dietary Supplements - Vitamin D," n.d.). For athletes, there are no vitamin D recommendation values. Gymnasts train indoor year-round where adequate sun exposure cannot be achieved to acquire vitamin D. Further, recommendations by the endocrine society has suggested individuals have up to 1,500-2000 IU/day for optimal vitamin D levels (Pramyothin & Holick, 2012). Low vitamin D in this study is not a surprising find as in past work examining vitamin D intake across several college sports found only a small percentage met the RDA (Halliday et al., 2011). Moreover, the current finds indicate the need for interventions with sufficient levels of vitamin D via dietary intake and/or dietary intake with added vitamin D supplementation.

Choline

Choline was an additional nutrient of interest as whole eggs are recognized as one of the primary sources of dietary choline. It was demonstrated that choline intake in both groups was lower than the typical intake among adult females (273 mg/d) and about half the current recommendation (425 mg/d)¹⁵. However, the egg group demonstrated the highest dietary choline intake mid-season (December data not shown). Dietary choline is a precursor to the neurotransmitter, acetylcholine that functions to relay signals to induce muscular contractions. Given the intensity of training and neuromuscular action needed to perform the powerful movements in gymnastics, choline may be of benefit to the population. Though dietary choline intake amongst the participants was lacking, it very well could have been related to the decreasing energy intake.

HEI-2015

Lastly, through measurements of overall diet quality, HEI-2015 scores were greater than 50 among both groups of participants. When compared to the general population, the athletes demonstrated score results similar to that of the general population as the mean values for all Americans and adults, ages 18-64 years old are 59.0 and 58.0, respectively (“Healthy Eating Index (HEI) | Center for Nutrition Policy and Promotion,” n.d.). Though we can’t generalize the present study population, past research has by Webber et al examining diet quality demonstrated similar findings amongst a larger group of collegiate athletes (n=138). Average diet quality of the study’s participants was 51.2 ± 8.8 (Webber et al., 2015). Moreover, it is apparent that what are considered some of the “healthiest” individuals are not aligning with the dietary recommendations (i.e. 2015-2020 Dietary Guidelines for Americans).

Overall Conclusion

Our findings within collegiate female gymnasts indicate both inadequate energy intake, energy availability, macronutrient, and micronutrient intake. In addition, diet quality was fair even after following a dietary intervention of boiled egg consumption post-exercise. Further, this suggests that improving both nutritional knowledge and dietary intake among collegiate female gymnasts is needed to ensure adequate physiological functioning, recovery and successful performance.

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CHAPTER 6. CONCLUSIONS

The objectives of this study were to 1) determine the effect of whole egg consumption on 1) markers of inflammation (i.e .CRP, IL-6, and sIL-6R, 2) mood, stress and recovery (i.e. POMS and RESTQ), 3) dietary intake and diet quality, and 4) anthropometric measures of weight, body fat percentage, and fat-free weight.

Inflammation: Results suggest that whole egg consumption post-exercise may have attenuated levels of the pro-inflammatory marker, sIL-6R, specifically one month into preseason and overall from the preseason to the end of the competitive season. Though there were no significant changes seen in CRP and IL-6, egg intake, more specifically its dietary constituent, choline, may need to be higher to see a further reduction in these measures. Moreover, future research should investigate the effect of whole egg consumption post-exercise further to determine its anti-inflammatory benefits.

Mood, Stress and Recovery. Significant relationships between sIL-6R and poor sleep quality were noted one month into preseason for both groups. This finding correlated with the most intense time training period and further suggests that sIL-6R may be an indicator for overtraining. As mentioned previously, we also saw a decrease in inflammation as sIL-6R was reduced during this time period. Further, though sleep quality decreased, there was a counterbalance with inflammation suggesting the egg intervention or other factor may be involved. Future research should consider a crossover trial with egg washout periods to evaluate the effects of nutritional interventions on psychological and physiological stress and recovery measures.

Dietary Intake and Diet Quality: Following a post-exercise nutritional intervention, we found that there was inadequate intake of energy, carbohydrates, and fat between the start

of the preseason and the end of the competitive season (August-April) in the egg group. Further, additional steps are needed to address this issue to prevent inadequate physiological functional and promote proper fueling for training and recovery.

Anthropometrics: During the preseason training months (August-December), the egg group participants had a significant decrease in body fat percentage, suggesting that their lean muscle mass was maintained during the intense training period. It was demonstrated that though energy, carbohydrates, and fat intakes were low, protein was fairly maintained. Furthermore, protein intake may have led to this increase in lean muscle mass. However, this change cannot be entirely attributed to post-exercise egg intake due to several confounding factors.

In conclusion, the results of this study are only suggestive. Additional research is needed to examine the effect of whole egg consumption in collegiate female gymnasts. Future research in this area should consider utilizing a crossover trial that uses randomization to treatment groups and has participants serve as their own control. In addition, an egg washout period should be implemented at baseline and between treatments phase. Lastly, methods to ensure compliance with egg consumption should be considered.

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APPENDIX A. IRB DOCUMENTS

IRB Approval: New

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Institutional Review Board
Office for Responsible Research
Vice President for Research
2420 Lincoln Way, Suite 202
Ames, Iowa 50014
515 294-4566

Date: 7/5/2017

To: Hilary Green
1305 Coconino Rd.
Unit 303

CC: Dr. Ruth Litchfield
1104 HNSB

From: Office for Responsible Research

Title: Effect of Egg Consumption on Inflammatory Markers and Body Composition in Female NCAA Division I Collegiate Gymnasts

IRB ID: 17-309

Approval Date: 7/5/2017 **Date for Continuing Review:** 6/29/2019

Submission Type: New **Review Type:** Full Committee

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University according to the dates shown above. Please refer to the IRB ID number shown above in all correspondence regarding this study.

To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- Use only the approved study materials in your research, including the recruitment materials and informed consent documents that have the IRB approval stamp.
- Retain signed informed consent documents for 3 years after the close of the study, when documented consent is required.
- Obtain IRB approval prior to implementing any changes to the study by submitting a Modification Form for Non-Exempt Research or Amendment for Personnel Changes form, as necessary.
- Immediately inform the IRB of (1) all serious and/or unexpected adverse experiences involving risks to subjects or others; and (2) any other unanticipated problems involving risks to subjects or others.
- Stop all research activity if IRB approval lapses, unless continuation is necessary to prevent harm to research participants. Research activity can resume once IRB approval is reestablished.
- Complete a new continuing review form at least three to four weeks prior to the date for continuing review as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.

Please be aware that IRB approval means that you have met the requirements of federal regulations and ISU policies governing human subjects research. Approval from other entities may also be needed. For example, access to data from private records (e.g. student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. IRB approval in no way implies or guarantees that permission from these other entities will be granted.

Upon completion of the project, please submit a Project Closure Form to the Office for Responsible Research, 202 Kingland, to officially close the project.

Please don't hesitate to contact us if you have questions or concerns at 515-294-4566 or IRB@iastate.edu.

IRB Approval: Modification

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Institutional Review Board
Office for Responsible Research
Vice President for Research
3420 Lincoln Way, Suite 302
Ames, Iowa 50014
515-294-4566

Date: 9/6/2017

To: Hilary Green
1305 Coconino Rd.
Unit 303

CC: Dr. Ruth Litchfield
1104 HNSB

From: Office for Responsible Research

Title: Effect of Egg Consumption on Inflammatory Markers and Body Composition in Female NCAA Division I Collegiate Gymnasts

IRB ID: 17-309

Approval Date: 9/6/2017 **Date for Continuing Review:** 6/29/2019

Submission Type: Modification **Review Type:** Expedited

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University according to the dates shown above. Please refer to the IRB ID number shown above in all correspondence regarding this study.

To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- **Use only the approved study materials in your research, including the recruitment materials and informed consent documents that have the IRB approval stamp.**
- **Retain signed informed consent documents for 3 years after the close of the study, when documented consent is required.**
- **Obtain IRB approval prior to implementing any changes to the study by submitting a Modification Form for Non-Exempt Research or Amendment for Personnel Changes form, as necessary.**
- **Immediately inform the IRB of (1) all serious and/or unexpected adverse experiences involving risks to subjects or others; and (2) any other unanticipated problems involving risks to subjects or others.**
- **Stop all research activity if IRB approval lapses, unless continuation is necessary to prevent harm to research participants. Research activity can resume once IRB approval is reestablished.**
- **Complete a new continuing review form at least three to four weeks prior to the date for continuing review as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.**

Please be aware that IRB approval means that you have met the requirements of federal regulations and ISU policies governing human subjects research. **Approval from other entities may also be needed.** For example, access to data from private records (e.g. student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. **IRB approval in no way implies or guarantees that permission from these other entities will be granted.**

Upon completion of the project, please submit a Project Closure Form to the Office for Responsible Research, 202 Kingland, to officially close the project.

Please don't hesitate to contact us if you have questions or concerns at 515-294-4566 or IRB@iastate.edu.

Informed Consent

ISU IRB # 1	17-309
Approved Date:	06 September 2017
Expiration Date:	29 June 2019

INFORMED CONSENT DOCUMENT

Title of Study: Effect of Egg Consumption on Inflammatory Markers and Body Composition in Division I Collegiate Female Gymnasts

Investigators: Hilary Green (Principal Investigator)
 Ruth Litchfield, Ph.D. (Co-PI)
 Marian Kohut, Ph.D. (Co-PI)
 Jessica Drenth (Co-PI)
 Erin Hinderaker (Co-PI)
 Jessica Young (Co-PI)
 Julia Lavallee (Co-PI)

You are invited to be in a research study examining the role of egg consumption on satiety and immune function among female college athletes. You were selected as a possible participant because you are a member of the Iowa State University gymnastics program.

We ask that you read this document and ask any questions that you may have before agreeing to be in this study. This study is being conducted by a graduate student, Hilary Green, and faculty in the Departments of Food Science and Human Nutrition and Kinesiology at Iowa State University. Please take your time in deciding if you would like to participate. Please feel free to ask any questions at any time.

INTRODUCTION

The purpose of this 9-month study is to explore the impact of egg consumption on inflammatory markers and body composition throughout the pre-season and competitive season within collegiate female gymnasts. This study will explore whether: 1. Daily egg consumption lowers inflammatory markers when the body is put under long term high intensity workout regimens, like that of gymnastics; and 2. Daily egg consumption influences satiety, weight management and body composition; and 3. Stress of training is related to inflammation. If you agree to participate in this study, you will be asked to complete anthropometric screenings [i.e. height, body weight, lean body mass], blood draws, 5-day food records, satiety scales, and stress-related surveys.

If you presently have one of the following health conditions (egg allergies, asthma, coronary artery disease, depression, diabetes, hypertension, migraines, cancer or cancer related complications, or any autoimmune disorder [i.e. rheumatoid arthritis, lupus, inflammatory bowel disease, multiple sclerosis]) you will be excluded from participating in this study. If you have an egg allergy, you would still be permitted to participate in the control arm (no consumption of egg required) of the study.

DESCRIPTION OF PROCEDURES

If you agree to participate, you will be asked to:

- August 2017- complete pre-season physical required for participation in ISU athletics (time commitment about 1 hour)
 - Anthropometrics-height, weight, BOD POD body composition analysis
 - Complete Health Status Update
 - 5-Day Food Record and Satiety Scales
- Consume 2 eggs post-training (after lifting or team workout) each day 5 days/week (Monday- Friday, August - April) OR the option to not consume 2 eggs daily
- September 2017 (time commitment about 40 minutes)
 - Blood draw- 50 ml of blood (a little more than 3 tablespoons) will be collected in 4 tubes; venous puncture for the blood draw may cause emotional and/or physical discomfort for some
 - Complete Health Status Update
 - Surveys (Mood, Stress, Recovery)
- October 2017 #1 (time commitment about 40 minutes)
 - Blood draw- 50 ml of blood (a little more than 3 tablespoons) will be collected in 4 tubes; venous puncture for the blood draw may cause emotional and/or physical discomfort for some
 - Complete Health Status Update
 - Surveys (Mood, Stress, Recovery)
- October 2017 # 2 (time commitment about 5 minutes)
 - Survey (Mood, Recovery)
- November 2017 (time commitment about 15 minutes)
 - Complete Health Status Update
- December 2017 (time commitment about 1 hour)
 - Anthropometrics- height, weight, BOD POD body composition analysis
 - Blood draw- 50 ml of blood (a little more than 3 tablespoons) will be collected in 4 tubes; venous puncture for the blood draw may cause emotional and/or physical discomfort for some
 - Complete Health Status Update
 - 5-Day Food Record and Satiety Scales
 - Surveys (Mood, Stress, Recovery)

- January 2018 (time commitment about 20 minutes)
 - Complete Health Status Update
 - Survey (Competitive State Anxiety)
- February 2018 (time commitment about 20 minutes)
 - Complete Health Status Update
 - Survey (mid-season) (Competitive State Anxiety, Coping Skills)
- March 2018 (time commitment about 15 minutes)
 - Complete Health Status Update
- April 2018 (time commitment about 1 hour)
 - Anthropometrics- height, weight, BOD POD body composition analysis
 - Blood draw- 50 ml of blood (a little more than 3 tablespoons)) will be collected in 4 tubes; venous puncture for the blood draw may cause emotional and/or physical discomfort for some
 - Complete Health Status Update and Training Questionnaires
 - 5-Day Food Record and Satiety Scales
 - Surveys (Mood, Stress, Recovery)
- Weekly time commitment during training / competition (mid-September 2017 – April 2018) (3 minutes to complete Weekly Health/Training questionnaire)

RISKS

Potential risks of the study are emotional and/or physical discomfort during the body composition assessment (BOD POD) and/or blood draw. During the blood draw, the needle stick may cause slight discomfort and there is a small risk of bruising, fainting, and a rare risk of infection. You may also find that the consumption of 2 eggs daily may be challenging mentally and/or physically if you are unaccustomed to consuming eggs.

BENEFITS

If you decide to participate in this study, there are no direct benefits to you. You may benefit indirectly from the information gathered about the body composition and immune response to athletic training.

COSTS AND COMPENSATION

You will not have any costs from participating in this study. You will be compensated for their time to participate in the study. After completing each data collection visit (September, October, December, April), participants will receive \$25. Total compensation for participation is \$100.

You will need to complete a form to receive payment. Please know that payments may be subject to tax withholding requirements, which vary depending upon whether you are a legal resident of

ISU IRB # 1	17-309
Approved Date	06 September 2017
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the U.S. or another country. If required, taxes will be withheld from the payment you receive. You will need to provide your social security number (SSN) and address on the form in order for us to pay you.

PARTICIPANT RIGHTS

Your participation in this study is completely voluntary and you may refuse to participate or leave this study at any time. Your decision whether or not to participate will not affect your current or future relations with Iowa State University or the ISU Athletics program. If you decide to participate, you are free to withdraw at any time without affecting your relationship with Iowa State University or the ISU Athletics program.

CONFIDENTIALITY

Records identifying you will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal government regulatory agencies, auditing departments of Iowa State University, and the Institutional Review Boards (a committee that reviews and approves human subject research studies) may inspect and/or copy study records for quality assurance and data analysis. These records may contain private information. To ensure confidentiality, the following measures will be taken:

- Only research personnel will be able to access data and records during this study.
- Personal identifiers (name) will be removed from your documents and an identification number will be assigned at the same time for all data collected. The principal and co-investigator will be the only ones who have access to the sheet linking your identification to the code.
- All data collected on each subject will be stored in a locked file cabinet and assigned a code number for analytical purposes.
- Any data transferred to a computer will be entered and stored on a secure, password protected, encrypted server accessible only by the research personnel.
- Presentations or manuscripts pertaining to this study will only report group data.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study.

- For further information about the study contact Hilary Green, (910) 728-7063 or via email at hilaryg@iastate.edu or Ruth Litchfield (515) 294-9484 or via email at litch@iastate.edu.
- If you have any questions about the rights of research subjects or research-related injury, please contact the IRB Administrator, (515) 294-4566, IRB@iastate.edu, or Director (515) 294-3115, Office for Responsible Research, Iowa State University, Ames, Iowa 50011.

PARTICIPANT SIGNATURE

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Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given time to read the document, and that your questions have been satisfactorily answered. You will receive a copy of the written informed consent prior to your participation in the study.

Participant's Name (printed) _____

(Participant's Signature)

(Date)

APPENDIX B. STUDY RECRUITMENT LETTER

ISU IRB # 1	17-309
Approved Date	06 September 2017
Expiration Date	29 June 2019

Participants Needed for Research Study:

Egg Consumption and Inflammatory Markers/ Body Composition in Female NCAA Division I Collegiate Gymnasts

You are being contacted because you are a member of the Iowa State University gymnastics program. I am seeking female gymnasts age 18 and over who are collegiate gymnasts at the Division I level. I am a Diet and Exercise student at Iowa State University conducting a study examining the role of egg consumption on satiety and inflammation among female gymnasts.

Participation in this study entails:

- August 2017 (time commitment about 1 Hour): An in-person, scheduled meeting to explain the study, obtain informed consent, receive instructions for the 5-Day Food Record and Satiety Scales, and anthropometric measures (Height, Weight, BOD POD body composition analysis)
 - Voluntary option to consume OR not consume 2 eggs/day, 5 days a week, Monday through Friday throughout the duration of the study (August 2017 – April 2018)
- September 2017 (time commitment about 40 minutes): Blood draw, Surveys
- October 2017 (time commitment about 40 minutes): Blood Draw, Surveys
- October 2017 2nd visit (time commitment about 5 minutes): Survey
- December 2017 (time commitment about 1 hour): Anthropometric measurements (Height, Weight, BOD POD body composition analysis), blood draw, and 5-Day Food Record and Satiety Scales, Surveys
- January 2018 -1st competition (time commitment about 20 minutes): Surveys
- February 2018 – mid season (time commitment about 20 minutes): Surveys
- April 2018 (time commitment about 1 hour): Anthropometric measurements (Height, Weight, BOD POD body composition analysis), blood draw, and 5-Day Food Record and Satiety Scales, Surveys
- August through April (time commitment about 15 minutes each month): Complete Health Status Update
- September through April (time commitment about 3 minutes/week): Weekly Health/Training Questionnaire

Participants will be compensated for their time to participate in the study. After completing each data collection visit (September, October, December, April), participants will receive \$25. Total compensation for participation is \$100.

If you are interested in participating voluntarily, please contact Hilary Green, (910) 728-7063 or via email at hilaryg@iastate.edu or Ruth Litchfield (515) 294-9484 or via email at litch@iastate.edu. A follow up email will be sent to all voluntary participants to schedule an in-person meeting.

Thank you again for considering this research opportunity.

Sincerely,

Hilary Green

APPENDIX C: SCREENING QUESTIONNAIRE**Screening Questionnaire**

Please answer these questions by responding with **Yes** or **No**.

1. What is your age? _____
2. Do you have any allergies to eggs? If yes, assign to control group.
3. Will it be problematic for you to consume 2 eggs/day for 5 days/week for the duration of the study? If yes, assign to control group.
4. Have you ever been diagnosed for any of the following by a health care professional?
 - a. Asthma
 - b. Heart Disease
 - c. Depression
 - d. Diabetes
 - e. Hypertension
 - f. Cancer
 - g. Migraines
 - h. Auto-Immune Disorders (Rheumatoid Arthritis, Lupus, Inflammatory Bowel Disease, Multiple Sclerosis)
 - i. If yes, to any of the above, participant will be excluded from the study.

APPENDIX D: SURVEYS

5-Day Food Record

5-Day Food Intake Record

Please keep a record of *everything* you **EAT** and **DRINK** for 5 days- Monday through Friday. Include all meals, snacks, and beverages, the brand of each food (if possible), and the time of day you are eating or drinking.

Please also rate your feeling of satiety using the scale provided, as well as your mood after each meal.

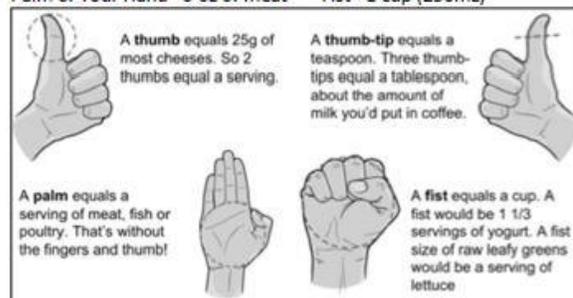
The purpose of filling out these food record is to help better understand **WHAT** you are eating, and **HOW MUCH** you are eating. Please be as honest and accurate as you can.

FOOD/BEVERAGE RECORDING INSTRUCTIONS:

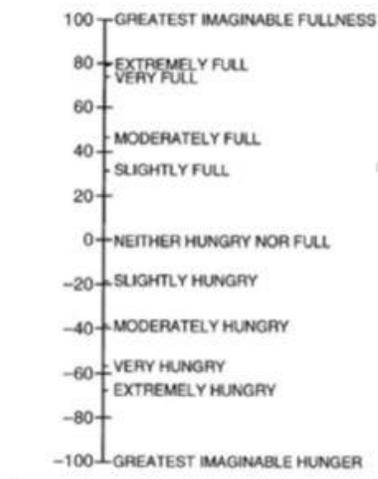
- Record all food and beverages consumed during a 24-hour period. Provide the following:
 - Type of Food Eaten: e.g. tomato soup
 - Brand Name: e.g. Campbell's, Lipton, Hy-Vee
 - Food or Beverage Characteristics:
 - Whole Grain: e.g. white vs. whole wheat bread; white vs. brown rice
 - Fat content: %fat (e.g. skim, 1%, 2% or homo milk), leanness of meat (e.g. extra lean beef), fat claims) e.g. ("low-fat"), was skin removed from poultry?
 - Form: e.g. fresh, frozen, canned, or dried
 - Other Details: e.g. 25% reduced sodium, "diet" products, etc.
 - Time of Day you ate or drank
- Please **MEASURE** and describe the amount of food eaten as best as possible. Diet records are only reliable with accurate measurements.
 - Always estimate portion sizes of food after cooking.
 - Use household measures to specific serving sizes.

1 cup= 250 mL= 8 fluid oz	1 tablespoon(Tbsp)= 15mL
1 ounce (oz)= 30g	1 teaspoon (tsp)= 5mL
 - Measuring cups (examples): Put cup of cooked rice into a measuring cup to record the correct amount before placing it on your plate. Measure your cereal out before pouring it into a bowl, and don't forget to measure your milk as well.
 - Teaspoons/ tablespoons (examples): Measure out butter, margarine, nut butters (e.g. peanut butter, almond butter, etc.), mayonnaise, ketchup, mustard, ground flaxseed, sugar, milk/cream, and other condiments, seasonings, and toppings before adding to your foods or beverages.
 - Count the number of food items if practical: e.g. 10 strawberries, 5 baby carrots, 8 medium-sized shrimp, etc.
 - Fluids: Record amounts in fluid ounces (oz), milliliters (mL), or in cups. Remember 1 cup= 250 mL= 8 fl. oz
 - Use food labels to estimate quantities: Food labels can help you estimate the quantity of food eaten based on weight or volume. For example, write down a 12 oz can of pop, 1/2 of a 5 oz can of tuna, a 1 oz granola bar, etc.
 - Use your hand to estimate portion sizes quickly:

Whole Thumb= 1 Tablespoon Tip of your Thumb= 1 Teaspoon
 Palm of Your Hand= 3 oz of meat Fist= 1 cup (250mL)



3. Record if anything was **ADDED** when preparing the food, such as oil (list specific kind), sauce, butter, margarine, or other condiments or seasonings.
4. For **COMBINATION DISHES** such as lasagna, casseroles, chili, soups, or stews include a description of the main ingredients. E.g. Lasagna: lean ground beef (1/4 cup per piece), mozzarella cheese (1 oz per piece), cottage cheese (1 oz per piece), 1/2 cup tomato sauce, 2 noodles, 1/4 cup spinach.
5. Include **SNACK FOODS** eaten. Don't forget to include candy, chips, cookies, popcorn, ice cream, and beverages such as soft drinks, juice, coffee, or tea.
6. Rate your **SATIETY** and indicate your **MOOD** using the satiety index provided at each meal or snack.



Sample 1-Day Food Record

Below is an EXAMPLE of how to keep accurate records. Include a detailed description and amounts of eat item. Remember to record water, product details, times of day, and indicate your satiety/ mood.

Time of Meal, Snack, or beverage	Type of Food or Beverage (please include brand name if possible)	Amount Eaten (e.g. 1 Tbsp., 1 slice, 1 cup, 1 bar)	Method of Preparation (e.g. baked, fried, broiling, etc.)	How satisfied do you feel after each meal? (Refer to satiety index and insert a number in this column) Please also indicate your mood.
8:00 am	Oatmeal	1 cup	Microwave	50-Moderately Full I felt content this morning.
	Jiff's Peanut Butter	1 Tbsp		
	Sun-Maid Raisins	¼ cup		
	Minute Maid Orange Juice	1 cup		
10:00 am	Carrot Sticks	5 sticks		
	Hy-vee Hummus	2 Tbsp		
12:00 pm	Hy-Vee Whole Wheat Bread w/ Tuna	2 slices/ 3oz		
	Miracle Whip Mayonnaise	2 Tbsp		
	Cucumber Sticks	5 Sticks		
	Yoplait Low-Fat Vanilla Yogurt	¼ cup		
	Hy-vee Low-Fat Milk	1 Cup		
2:30 pm	Honeycrisp Apple	1 Medium		
	Jiff's Peanut Butter	1 Tbsp		
5:30 pm	Tyson's Boneless-Skinless Chicken Breast	5 oz	Baked	
	Steam fresh Broccoli Florets	1 cup	Microwave	
	Uncle Ben's Brown Rice	½ cup	Stovetop	
8:00 pm	Chips Ahoy Chocolate Chip Cookies	3 small cookies		
	Oatmeal			

Health Status Update

Health Status Update

Each month of the study (August – April) we will ask you to provide some information about your health status. This information is important for us to interpret the results of the blood draws occurring throughout the study. Because we are interested in blood markers of inflammation, we need to consider all factors that will influence those markers. For example, injury and anti-inflammatory drugs (naproxen [Aleve, Anaprox, Naprosyn], ibuprofen [Motrin, Advil], will influence blood markers of inflammation. The menstrual cycle also influences inflammatory markers.

Please answer the following questions about any injuries, medications and menstrual status. All information will be kept confidential. Please take your time and complete it carefully and thoroughly. Your answers will help identify any correlations between the information being evaluated (as listed above) and the effect egg consumption has on the inflammatory response/ body composition throughout the pre-season and competitive season.

Date of your last period: _____

Are you currently taking any contraceptives? If Yes, please identify the type of contraceptive in the comments section below.

Comments: _____

Please list any prescribed medications, dietary supplements, or vitamins you are now taking:

Do you have any current injuries? If YES, please provide details on the injury, length of time you have been injured and any medication(s) prescribed in the comments section below. Please feel free to provide any other details about your current injury(s).

Comments: _____

Athletic Coping Skills Inventory (ACSI-28)

ACSI-28

INSTRUCTIONS: A number of statements that athletes have used to describe their experiences are given below. Please read each statement carefully and then recall as accurately as possible how often you experience the same thing. There are no right or wrong answers. Do not spend too much time on any one statement.

		Almost Never	Some- times	Often	Almost Always
1	On a daily or weekly basis, I set very specific goals for myself that guide what I do	①	②	③	④
2	I get the most out of my talent and skills	①	②	③	④
3	When a coach or manager tells me how to correct a mistake I've made, I tend to take it personally and feel upset	①	②	③	④
4	When I am playing sports, I can focus my attention and block out distractions	①	②	③	④
5	I remain positive and enthusiastic during competition, no matter how badly things are going	①	②	③	④
6	I tend to play better under pressure because I think more clearly	①	②	③	④
7	I worry quite a bit about what others think about my performance	①	②	③	④
8	I tend to do lots of planning about how to reach my goals	①	②	③	④
9	I feel confident I will play well	①	②	③	④
10	When a coach or manager criticizes me, I become upset rather than helped	①	②	③	④
11	It is easy for me to keep distracting thoughts from interfering with something I am watching or listening to	①	②	③	④
12	I put a lot of pressure on myself by worrying how I will perform	①	②	③	④
13	I set my own performance goals for each practice	①	②	③	④
14	I don't have to be pushed to practice or play hard; I give 100%	①	②	③	④
15	If a coach criticizes or yells at me, I correct the mistake without getting upset about it	①	②	③	④
16	I handle unexpected situations in my sport very well	①	②	③	④
17	When things are going badly, I tell myself to keep calm, and this works for me	①	②	③	④
18	The more pressure there is during a game, the more I enjoy it	①	②	③	④
19	While competing, I worry about making mistakes or failing to come through	①	②	③	④
20	I have my own game plan worked out in my head long before the game begins	①	②	③	④
21	When I feel myself getting too tense, I can quickly relax my body and calm myself	①	②	③	④
22	To me, pressure situations are challenges that I welcome	①	②	③	④
23	I think about and imagine what will happen if I fail or screw up	①	②	③	④
24	I maintain emotional control no matter how things are going for me	①	②	③	④
25	It is easy for me to direct my attention and focus on a single object or person	①	②	③	④
26	When I fail to reach my goals, it makes me try even harder	①	②	③	④
27	I improve my skills by listening carefully to advice and instruction from coaches and managers	①	②	③	④
28	I make fewer mistakes when the pressure's on because I concentrate better	①	②	③	④

Competitive State Anxiety (CSAI-2)

CSAI-2

ILLINOIS SELF-EVALUATION QUESTIONNAIRE

Directions: A number of statements that athletes have used to describe their feelings before competition are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate *how you feel right now* — at this moment. There are no right or wrong answers. Do *not* spend too much time on any one statement, but choose the answer which describes your feelings *right now*.

		Not At All	Somewhat	Moderately So	Very Much So
1	I am concerned about this competition	Ⓐ	Ⓑ	Ⓒ	Ⓓ
2	I feel nervous	Ⓐ	Ⓑ	Ⓒ	Ⓓ
3	I feel at ease	Ⓐ	Ⓑ	Ⓒ	Ⓓ
4	I have self-doubts	Ⓐ	Ⓑ	Ⓒ	Ⓓ
5	I feel jittery	Ⓐ	Ⓑ	Ⓒ	Ⓓ
6	I feel comfortable	Ⓐ	Ⓑ	Ⓒ	Ⓓ
7	I am concerned that I may not do as well in this competition as I could	Ⓐ	Ⓑ	Ⓒ	Ⓓ
8	My body feels tense	Ⓐ	Ⓑ	Ⓒ	Ⓓ
9	I feel self-confident	Ⓐ	Ⓑ	Ⓒ	Ⓓ
10	I am concerned about losing	Ⓐ	Ⓑ	Ⓒ	Ⓓ
11	I feel tense in my stomach	Ⓐ	Ⓑ	Ⓒ	Ⓓ
12	I feel secure	Ⓐ	Ⓑ	Ⓒ	Ⓓ
13	I am concerned about choking under pressure	Ⓐ	Ⓑ	Ⓒ	Ⓓ
14	My body feels relaxed	Ⓐ	Ⓑ	Ⓒ	Ⓓ
15	I am confident I can meet the challenge	Ⓐ	Ⓑ	Ⓒ	Ⓓ
16	I'm concerned about performing poorly	Ⓐ	Ⓑ	Ⓒ	Ⓓ
17	My heart is racing	Ⓐ	Ⓑ	Ⓒ	Ⓓ
18	I'm confident about performing well	Ⓐ	Ⓑ	Ⓒ	Ⓓ
19	I'm concerned about reaching my goal	Ⓐ	Ⓑ	Ⓒ	Ⓓ
20	I feel my stomach sinking	Ⓐ	Ⓑ	Ⓒ	Ⓓ
21	I feel mentally relaxed	Ⓐ	Ⓑ	Ⓒ	Ⓓ
22	I am concerned that others will be disappointed with my performance	Ⓐ	Ⓑ	Ⓒ	Ⓓ
23	My hands are clammy	Ⓐ	Ⓑ	Ⓒ	Ⓓ
24	I am confident because I mentally picture myself reaching my goal	Ⓐ	Ⓑ	Ⓒ	Ⓓ
25	I'm concerned I won't be able to concentrate	Ⓐ	Ⓑ	Ⓒ	Ⓓ
26	My body feels tight	Ⓐ	Ⓑ	Ⓒ	Ⓓ
27	I'm confident of coming through under pressure	Ⓐ	Ⓑ	Ⓒ	Ⓓ

Perceived Stress Scale (PSS)

Perceived Stress Scale

The questions in this scale ask about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way; rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

0 - never 1 - almost never 2 - sometimes 3 - fairly often 4 - very often

_____ 1. In the last month, how often have you been upset because of something that happened unexpectedly?

_____ 2. In the last month, how often have you felt that you were unable to control the important things in your life?

_____ 3. In the last month, how often have you felt nervous and stressed?

_____ 4. In the last month, how often have you felt confident about your ability to handle your personal problems?

_____ 5. In the last month, how often have you felt that things were going your way?

_____ 6. In the last month, how often have you found that you could not cope with all the things that you had to do?

_____ 7. In the last month, how often have you been able to control irritations in your life?

_____ 8. In the last month, how often have you felt that you were on top of things?

_____ 9. In the last month, how often have you been angered because of things that happened that were outside of your control?

_____ 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Profile of Mood States (POMS)

POMS

Directions: Describe HOW YOU FEEL RIGHT NOW by circling the most appropriate number after each of the words listed below

FEELING	Not at all	A little	Moderate	Quite a bit	Extremely
1. Tense	1	2	3	4	5
2. Angry	1	2	3	4	5
3. Worn Out	1	2	3	4	5
4. Unhappy	1	2	3	4	5
5. Clear Headed	1	2	3	4	5
6. Lively	1	2	3	4	5
7. Confused	1	2	3	4	5
8. Sorry for things done	1	2	3	4	5
9. Shaky	1	2	3	4	5
10. Listless	1	2	3	4	5
11. Peeved	1	2	3	4	5
12. Considerate	1	2	3	4	5
13. Sad	1	2	3	4	5
14. Active	1	2	3	4	5
15. On Edge	1	2	3	4	5
16. Grouchy	1	2	3	4	5
17. Blue	1	2	3	4	5
18. Energetic	1	2	3	4	5
19. Panicky	1	2	3	4	5
20. Hopeless	1	2	3	4	5
21. Relaxed	1	2	3	4	5
22. Unworthy	1	2	3	4	5

23. Spiteful	1	2	3	4	5
24. Sympathetic	1	2	3	4	5
25. Uneasy	1	2	3	4	5
26. Restless	1	2	3	4	5
27. Unable to Concentrate	1	2	3	4	5
28. Fatigued	1	2	3	4	5
29. Helpful	1	2	3	4	5
30. Annoyed	1	2	3	4	5
31. Discouraged	1	2	3	4	5
32. Resentful	1	2	3	4	5
33. Nervous	1	2	3	4	5
34. Lonely	1	2	3	4	5
35. Miserable	1	2	3	4	5
36. Muddled	1	2	3	4	5
37. Cheerful	1	2	3	4	5
38. Bitter	1	2	3	4	5
39. Exhausted	1	2	3	4	5
40. Anxious	1	2	3	4	5
41. Ready to Fight	1	2	3	4	5
42. Good Natured	1	2	3	4	5
43. Gloomy	1	2	3	4	5
44. Desperate	1	2	3	4	5
45. Sluggish	1	2	3	4	5
46. Rebellious	1	2	3	4	5
47. Helpless	1	2	3	4	5
48. Weary	1	2	3	4	5
49. Bewildered	1	2	3	4	5
50. Alert	1	2	3	4	5

51. Deceived	1	2	3	4	5
52. Furious	1	2	3	4	5
53. Efficient	1	2	3	4	5
54. Trusting	1	2	3	4	5
55. Full of Pep	1	2	3	4	5
56. Bad Tempered	1	2	3	4	5
57. Worthless	1	2	3	4	5
58. Forgetful	1	2	3	4	5
59. Carefree	1	2	3	4	5
60. Terrified	1	2	3	4	5
61. Guilty	1	2	3	4	5
62. Vigorous	1	2	3	4	5
63. Uncertain about things	1	2	3	4	5
64. Bused	1	2	3	4	5

REST-Q Sport (Modified)

REST-Q SPORT (MODIFIED)

DIRECTIONS: OVER THE PAST 3 DAYS RATE THE FOLLOWING:

1. **I did not get enough sleep**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
2. **I recovered well physically**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
3. **I fell asleep satisfied and relaxed**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
4. **Parts of my body were aching**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
5. **I could not get rest during the breaks (in practice or training)**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
6. **I was convinced I could achieve my set goals during performance**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
7. **I was tired from work (school)**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
8. **I was in a good condition physically**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
9. **I had a satisfying sleep**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
10. **My muscles felt stiff or tense during sports performance (or training)**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
11. **I had the impression there were too few breaks (in sports practice/training)**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always
12. **I was convinced that I could achieve my performance at any time**
0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

13. I was dead tired after work (school)

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

14. I felt very energetic

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

15. I slept restlessly

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

16. I had muscle pain after performance

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

17. Too much was demanded of me during the breaks

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

18. I was convinced that I performed well

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

19. I was overtired

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

20. My body felt strong

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

21. My sleep was interrupted easily

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

22. The breaks (during practice or training) were not at the right times

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

23. I felt vulnerable to injuries

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

24. I was convinced that I trained well

0.Never 1.Seldom 2.Sometimes 3.Often 4.More Often 5.Very Often 6.Always

Weekly Health/ Training Questionnaire

Weekly Health/ Training Questionnaire

Subject ID# _____ Date _____

How many events are you currently training this month? _____

Estimate how many hours you spent training the previous week. This includes all warm-up, gymnastics training, lifting workouts, etc.

Approximately how many practices did you have to modify your event assignments during the previous week?

In the previous week, did you experience any illness, injury or other issue that affected your ability to train? Please check one or more of the boxes below. If other, please describe.

Illness _____ Injury _____ Other? _____

In the tables below, please indicate the intensity level for each training activity from the previous week:

Lifting

	Monday	Tuesday	Wednesday	Thursday	Friday
Intensity of Training Activity [high, med, low]					

Gymnastics Practice

	Monday	Tuesday	Wednesday	Thursday	Friday
Intensity of Training Activity [high, med, low]					