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The Relationship Between Diet, Body Composition, and Grip Strength in Professional Cheerleaders

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ABSTRACT

THE REALTIONSHIP BETWEEN DIET, BODY COMPOSITION, AND GRIP STRENGTH IN PROFESSIONAL CHEERLEADERS

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Background: Studies suggest that there is a relationship between 24-hour and within-day energy balance and body composition. In sports with value placed on weight and appearance, an increased prevalence of energy deficiency has been found, which has multiple health and performance implications. In particular, low energy availability is associated with higher body fat percent and lower muscle and bone mass, all of which negatively influence performance. This study assessed professional cheerleaders on dietary intake, within-day and 24-hour energy balance, protein consumption, body composition, and handgrip strength. Professional cheerleaders have not been previously studied on these factors.

Objective: To assess dietary intake, body composition, and grip strength of professional cheerleaders on an active roster and investigate relationships between these factors. Our investigation focused on assessing if long periods of energy balance deficits are associated with reduced grip strength and higher body fat percent, and if protein consumption patterns are associated with grip strength and body composition.

Methods: The study population consisted of 19 women, ages 18-32 yr. (mean = 25.4 yr.), who were interviewed to obtain a one-day recall of dietary intake and energy expenditures to determine dietary/nutrient intake and hourly energy balance using the USDA Nutrient Database for Standard Reference and a relative intensity activity scale (NutriTiming® LLC). Multi-current, 8-mode segmental bioelectric impedance analysis was used to predict body composition, and handgrip strength was assessed using a hand dynamometer.

Results: Dietary inadequacies in energy ($p<0.001$) and carbohydrate ($p<0.001$) were significantly below recommended values. Subjects with the lowest body fat percent had significantly higher energy intakes ($p=0.011$), spent more time in an anabolic state ($p=0.048$), less time in a catabolic state ($p=0.048$), had more eating opportunities of up to 30-grams protein ($p=0.015$), and consumed more their protein while in a positive energy balance ($p=0.025$). Participants with higher body fat mass consumed less total energy ($p=0.012$), had more severe energy balance deficits ($p= 0.032$), and spent more time in a catabolic state ($p=0.048$).

Conclusion: Adequate energy intake that results in less time in a catabolic state and more frequent consumption of moderate amounts of protein (~30 grams/meal) was associated with lower body fat percent and increased muscle mass in professional cheerleaders. It appears from these data that “dieting” behaviors resulting in large energy balance deficits with longer periods in a catabolic state appeared counterproductive, as this was associated with greater body fat percent, lower muscle mass, and lower grip strength.

THE RELATIONSHIP BETWEEN DIET, BODY COMPOSITION, AND GRIP STRENGTH IN PROFESSIONAL CHEERLEADERS

by

Moriah Bellissimo

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ABBREVIATIONS

AA	Anorexia Athletica
ACSM	American College of Sports Medicine
AEE	Activity Energy Expenditure
AN	Anorexia Nervosa
ANOVA	Analysis of Variance
BFM	Body Fat Mass
BF%	Body fat percent
BIA	Bioelectrical Impedance Analysis
BMD	Bone Mineral Density
BMI	Body Mass Index
BN	Bulimia Nervosa
DEXA	Dual Energy X-ray Absorptiometry
DSM-V	Diagnostic and Statistical Manual of Mental Disorders V
EA	Energy Availability
EAT-40	Eating Attitudes Test
EB	Energy Balance
ED	Eating Disorder
ED-NOS	Eating Disorder Not Otherwise Specified
FAT	Female Athlete Triad
FFM	Fat Free Mass
FHA	Functional Hypothalamic Amenorrhea

g/gm	Gram/grams
IOC	International Olympic Committee
kcal	Kilocalories
kg	Kilogram
LBM	Lean Body Mass
LH	Leutenizing Hormone
NCAA	Nationcal Collegiate Athletic Association
RED-S	Relative Energy Deficiency in Sport
REE	Resting Energy Requirement
TEF	Thermic Effect of Food
U.S.	United States
yr.	Years

CHAPTER I

INTRODUCTION

Physical activity increases the requirement for energy, placing a high demand on athlete eating behaviors to satisfy need. It is necessary that athletes adequately supply total energy requirements for their bodies to maximally benefit and recover from training to improve and advance in their athletic careers (Joint Consensus Statement, 2009). However, studies have found a greater prevalence of energy deficiencies among athletes, at both amateur and elite levels. Furthermore, low energy deficiencies have been found more often in female sports than male (Bratland-Sanda & Sundgot-Borgen, 2012). Low energy availability in an athlete can lead to significant health risks, both short- and long-term, and also impaired performance; athletes participating in sports/activities with a focus placed on appearance or weight are found to be at a higher risk for energy deficiencies (Mountjoy et al., 2014). In professional cheerleading, appearance and body shape are of high importance, placing these athletes at high risk for developing eating behaviors aimed at weight loss. These athletes' drive for ideal physique can cause unnecessary dieting in lean and normal weight individuals, which may result in unhealthy dietary strategies (Dulloo et al., 2015).

While there is existing research on common sports injuries in cheerleader populations, there is a scarcity of nutrition-related research (Foley & Bird, 2013; Hardy et al., 2015). There is a need, therefore, to perform research to assess these athletes from a nutrition-perspective to determine whether this population would be considered nutritionally at-risk. Comparisons can be made between a population's current dietary intake and accepted ranges to help better understand these athletes' intakes. Furthermore, comparing the athletes' estimated energy expenditure to energy intake can enable an analysis of energy surpluses and/or deficits.

A commonly used practice for assessing athletes is measuring body composition to help understand components of an athlete's body weight. It can also be beneficial to track an athlete's body composition over time and see trends that develop or how an athlete's body composition changes in season and out of season. Past research has found relationships between dietary/nutrient intakes and body composition in a variety of athletes (Deutz et al. 2000; Reed et al., 2014, Melin et al., 2014). In order for lean body mass to be synthesized and maintained, an athlete must consume adequate energy and energy substrates (Hall et al., 2012). By assessing both body composition and nutrient intake in athletes, these relationships can be explored.

Analyzing nutrient intake for one time period (i.e., one day) offers a snapshot of nutritional status. A more long-term indicator of nutritional status is handgrip strength, which can give a measure of muscle strength and function (Norman et al., 2011). Just as an inadequate energy intake can decrease handgrip strength, adequate energy intake has been shown to improve handgrip strength (Flood et al., 2014).

This study's importance stems from the lack of nutrition-related research on professional cheerleaders and investigated if these athletes have dietary inadequacies that could lead to negative health consequences. Importantly, the findings from this study may provide information for guiding professional cheerleaders to eating behaviors that meet their energy requirements while simultaneously improving body composition. Therefore, the purpose of this study was to examine the relationships between diet, body composition, and grip strength among professional cheerleaders.

Hypotheses:

1. More time spent in an energy balance deficit of > -300 kcal will be associated with a higher body fat percent
    - Null (H1): More time spent in an energy balance deficit of > -300 kcal will not be associated with a higher body fat percent.
2. Subjects with higher protein intakes will have higher handgrip strength.
    - Null (H2): Subjects with higher protein intakes will not have higher handgrip strength.
3. The participants will have energy substrate inadequacies as compared to the joint consensus statement intake recommendations.
    - Null (H3): The participants will not have energy substrate inadequacies as compared to the joint consensus statement intake recommendations.

CHAPTER II

LITERATURE REVIEW

This literature review focuses on the aspects of sports nutrition that are applicable to professional cheerleaders. Although there has been no research conducted on professional cheerleaders' dietary intake or patterns, similarities can be drawn from characteristics shared with other sports. As research publications on low energy availability in athletes exist, a thorough review of affects and diagnoses of low energy availability is included. Furthermore, to better understand if this population was at risk for restrictive dieting or energy deficiency, research into those populations at risk and related pressures were included. Finally, nutrient recommendations for these athletes were investigated to make comparisons with their actual intake and handgrip strength was researched for its relationship with energy intake.

Low Energy Availability

There are short- and long-term health risks associated with energy deficiency, which also affects performance. Health and performance can be affected in athletes who have chronic low energy availability by the development of associated nutrient deficiencies, chronic fatigue, and increased risk of infection from a weakened immune system. Musculoskeletal injuries have been associated with women who exhibit the elements of the Female Athlete Triad (FAT), in addition to a slowed healing and recovery time (Nattiv et al., 2007). Physiological and medical complications ensue as well. Cardiovascular, gastrointestinal, endocrine, reproductive, skeletal, renal, and central nervous systems can all be affected negatively. Furthermore, women who are amenorrheic are infertile with associated hypoestrogenemia will suffer additional complications related to the reproductive and skeletal system. Low bone mineral density (BMD) increases the fracture risk, and low BMD may result in later higher risk of osteoporosis. Psychological issues are also associated with eating disorders (ED), including low self-esteem, depression and anxiety disorders (Mountjoy et al., 2014).

Female Athlete Triad

The FAT refers to the relationship between energy availability, menstrual function, and BMD. The FAT categorizes these elements on a continuum ranging from optimal energy availability, eumenorrhea, and optimal bone health to low energy availability, functional hypothalamic amenorrhea, and osteoporosis (Nattiv et al., 2007).

Energy Availability

Energy availability (EA) is calculated as energy intake minus energy expenditure because fat free mass is more metabolically active than other tissues, a higher proportion increases metabolic needs. Athletes may alter energy availability by restricting energy intake or increasing energy expenditure through increased training time, volume, or both. It appears that low energy availability is the key factor affecting menstrual function and bone health (Nattiv et al., 2007).

Menstrual Dysfunction

Menstrual function ranges from eumenorrhea (normal) to amenorrhea (no menses) on the FAT continuum, with amenorrhea being divided into primary amenorrhea, secondary amenorrhea, and oligomenorrhea (Nattiv et al., 2007). Primary amenorrhea is defined as no menarche by the age of 15 years, secondary amenorrhea is defined by the absence of three consecutive cycles post-menarche, and oligomenorrhea is defined as a cycle length greater than 45 days. Eumenorrhea cycle lengths are typically between 21 and 35 days (Mountjoy et al., 2014). Melin et al. (2014) studied 40 elite level female endurance athletes and found that 60% were diagnosed with menstrual dysfunction. Of the 24 athletes diagnosed with menstrual dysfunction, eight were found to have low/reduced current energy availability.

Bone Mineral Density

Typically in healthy athletes, BMD is higher than the general public (athletes in weight-bearing sports display a BMD 5-15% higher than non-athletes). Physical activity and adequate energy availability promote bone health and development by stimulating bone formation-promoting hormones and sustaining eumenorrhea along with estrogen production, which inhibits bone resorption. However, low energy availability damages bone health as the body ceases normal menstrual function in order to conserve energy as well as lowering production of estrogen, and its mediation of bone resorption. A down-regulation of hormones that promote bone formation is also observed in athletes with low EA.

Osteopenia (low BMD) is defined as a Z-score between -1.0 and -2.5 while osteoporosis is defined as a Z-score ≤ -2.5. The American College of Sports Medicine

(ACSM) describes low BMD as associated with a history of nutritional deficiencies, hypoestrogenism, stress fractures, and/or other secondary clinical risk factors for fractures, along with the indicated Z-score range. Low BMD and osteoporosis can be a result of bone mineral loss in adulthood, but can also result from insufficient bone development in childhood and adolescence. All of the factors comprising the FAT pose significant health risks to athletes (Nattiv et al., 2007).

Several issues have been raised about the limitations and misleading title of the FAT. Perhaps the greatest limitation is that the title includes only 'females', neglecting males who are also impacted by low energy availability. It also addresses only athletes, omitting to address the non-athletes who are equally affected by the same problems. Moreover, the term *triad* does not acknowledge current research regarding numerous negative outcomes and indicates only three issues, while ignoring others, including weakened immune system, altered blood values, altered hormone production, poor gastrointestinal health, and psychological changes. Furthermore, some athletes may suffer from only two of the three issues, which would disqualify them from being diagnosed as having FAT. Lastly, the title 'FAT' does not acknowledge the key underlying element: *low energy availability* (Mountjoy et al., 2015).

The International Olympic Committee (IOC) reevaluated the FAT and its components, renaming the syndrome relative energy deficiency in sport (RED-S). The title encompasses additional factors that have been linked by scientific evidence and clinical expertise beyond the three elements of the FAT (menstrual status, bone health, and energy availability) and acknowledges that both males and females are affected. RED-S affects aspects of physiological functions, such as metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular, endocrine, gastrointestinal, hematological, and psychological health (Mountjoy et al., 2014). Optimal performance is impaired through decreased endurance, increased risk of injury, decreased training response, decreased coordination, impaired judgment, decreased concentration, irritability, depression, decreased glycogen stores, and decreased muscle strength (Mountjoy et al., 2015).

As suggested by the name, RED-S labels the underlying issue as inadequate energy availability resulting from disordered eating (DE) or an increase in exercise load. The IOC links weight-sensitive sports in which leanness and/or weight are emphasized with a higher prevalence of ED. Within the elements of RED-S, DE is placed on a continuum similar to that of the FAT continuum beginning with appropriate eating to supply adequate energy for normal metabolic processes and exercise and ending with clinical ED, distorted body image, and medical complications.

Hormonal and metabolic imbalances are another aspect of RED-S. One health goal for female athletes is to achieve eumenorrhea with regular cycles occurring between 21 and 35 days. Primary, secondary, and oligomenorrhea are all causes for concern. Primary amenorrhea has been estimated at 22% prevalence in collegiate cheerleaders, divers and gymnasts while secondary amenorrhea prevalence has been estimated to be as high as 69% in collegiate dancers (Mountjoy et al., 2014). Low energy availability and exercise stress can affect normal hormone levels dealing with menstruation, such as leutenizing hormone (LH). Disruption of normal LH pulsatility can alter menstrual function by affecting the hypothalamic hormone gonadotropin-releasing hormone output. When this condition occurs, it is known as functional hypothalamic amenorrhea (FHA).

Other hormones, which can be affected by low energy availability, include the following: insulin, cortisol, growth hormone, ghrelin, leptin, peptide tyrosine-tyrosine, as well as the metabolic substrates glucose, fatty acids and ketones. Further functional complications of low EA include frequent viral illnesses, injuries, reduced responsiveness to training, and bone tissue deterioration related to a history of nutritional deficiencies, hypoestrogenism, stress fractures, and the measured Z-score range (Mountjoy et al., 2014).

Female Athletic Populations at Risk for Eating Disorders/Disordered Eating

In research publications focusing on athletic populations, with similar characteristics to professional cheerleading, there is a high prevalence of DE and diagnosed ED. These include dancers, gymnasts, divers, and swimmers. The prevalence of ED and DE in many of these populations has been assessed, and the prevalence of ED has been reported in the general public. In general public females (non-athletic), the prevalence is estimated at 1% for AN, and 1-2% in BN. There is a higher prevalence of non-specified eating disorders of 3-6% in the general population. The National Eating Disorders Association estimates ED or DE affects 35 million Americans (Prah, 2006). Males in the general public are estimated to have 10-20 times lower prevalence rates of ED than females (Byrne & McLean 2002), although other estimates find males to be 1 out of every 6 people diagnosed with an ED (Prah, 2006). ED are considered a psychological disorder, and anorexia nervosa has the highest mortality rate of any mental illness (Prah, 2006).

Sundgot-Borgen and Torstveit (2004) investigated the prevalence of ED among elite athletes versus the general population and found similar results to Byrne and McLean (2002). In this Norwegian study, 2,462 male and female elite athletes and aged-matched non-athletes from the general public participated in a screening questionnaire to find those at-risk of having an ED. Those athletes who were found to be at risk for an ED subsequently participated in a clinical interview for diagnosis. Results showed that male and female athletes had a higher percentage at risk for ED than controls (21% vs. 14% in females and 9% vs. 4% in males). Following the clinical interview using DSM-IV criteria for diagnosis (AN, BN, ED-NOS, AA), 20% of the female athletes were diagnosed with an ED while 9% of female controls were diagnosed. The study also separated the athletes into different sport groups for further analysis. In female aesthetic (gymnastics, dancing, figure skating, aerobics, and diving) and weight class sports a prevalence of ED was reported at 42% and 30%, respectively. These represented the highest and second highest prevalence percentages among all female athletes. The study found a higher rate of ED among elite athletes than the general population sample, higher in females than males, and higher in athletes competing in sports with an emphasis on leanness and weight management. Additional studies have found a consistently high prevalence of ED in female endurance and aesthetic sports (Schaal et al., 2011). For professional cheerleaders, these data are relevant as they are at the sport's most elite level with high pressure to maintain an 'ideal' physique.

A meta-analysis and systemic review was conducted by Arcelus et al. (2014) to investigate the prevalence of ED amongst dancers by collecting 33 studies published between 1985-2012; in these studies, the researchers utilized structured interview or distributed questionnaires. First, the review sought to find the prevalence of ED in all dancers compared to non-dancers in studies using diagnostic interviews. In non-dancers there was an ED prevalence of 12.0%, with 2.0% AN, 4.4% BN, and 9.5% ED-NOS. Overall, ED for males ranged from 0-13.6%, and females ranged from 7.4-50%. Secondly, ballet dancers were analyzed separately from all other dancers, and among ballet students, there was an ED prevalence of 16.4%. Three studies included professional ballet dancers, which assessed the lifetime prevalence of ED and found a prevalence of 15.78-82.6%. The study then investigated research that utilized questionnaires. Seven studies used the EAT-40 (eating attitudes test) questionnaire to compare all dancers and non-dancers and found mean ranges between 17.9-51.2% for dancers and a range of 2.37-22.9% in non-dancers. The study found that all dancers were at a higher relative risk than non-dancers of suffering from an ED, and nearly one-fifth of ballet dancers (16.4%) were found to have an ED, the majority of which were classified as ED-NOS (Arcelus et al., 2014). In professional cheerleading, the majority of activity is spent in synchronized dances, thus the findings of this study may pertain to professional cheerleaders.

Byrne and McLean (2002) explored the pressures athletes experience to maintain a certain body shape. A lean body shape is valued in sports with high aesthetic scores, such as ballet, dance and cheerleading, while low body weight is valued to enhance performance in sports with weight emphasis, such as lightweight rowing and long distance running. The pressures to conform to an unrealistic appearance can foster an ED or DE in an athlete. Byrne and McLean (2002) also compared thin-build, normal-build and non-athletes' sociocultural pressure to have a lean body shape by estimating drive for thinness and body dissatisfaction. Both male and female athletes reported significantly higher scores than non-athletes in drive for thinness. Within those athletes, thin-build athletes, which included ballet, gymnastics, lightweight rowing, long distance running, diving and swimming, scored a significantly higher drive for thinness than normal build athletes in sports such as tennis, volleyball, hockey and basketball. All females reported significantly higher values in pressure to be lean, drive for thinness, body dissatisfaction, and ED. Female, thin-build athletes felt the most pressure to conform to an ideal shape, which included participants of ballet, gymnastics, lightweight rowing, long distance running, diving and swimming; the perceived pressure to be thin led to a higher prevalence of eating problems (Byrne & McLean, 2002). Professional cheerleading displays similar characteristics to sports classified as thin-build sports in this study.

Populations at Risk: Factors Contributing to Eating Disorders/Disordered Eating

Many factors comprise the development of ED, some issues being cultural, familial, individual and genetic. Athletics maintains unique sport-specific risk factors such as dieting to enhance performance, pressure to lose weight, frequent weight cycling, overtraining, and inappropriate coaching behaviors to induce weight loss or body shape (Mountjoy et al., 2014).

Several studies have investigated the causes or factors for the development of ED or DE behaviors. The absolute reason remains unclear, but the cause is multifactorial. High achievement orientation, obsessive-compulsive tendencies and perfectionism are all common psychological tendencies of those with clinically diagnosed ED. However, many athletes display these characteristics and some would argue that these traits are necessary for successful competition and careers (Sundgot-Borgen & Tortsveit, 2004). Furthermore, dancers have been found to have a greater eating psychopathology than non-dancers; Ringham et al. (2006) found dancers were more similar to eating-disorder individuals than to controls in their eating pathology. Goodwin et al. (2014) investigated two factors that have been investigated as possible causes of ED development: perfectionism, or high standards, and self-evaluative perfectionism (self-criticism). These elements were investigated for their roles in ED etiology. The researchers found that self-criticism was a predictor for eating psychopathology, which included eating restraint, eating concern, weight concern and body shape concern, and this trait was not mediated by perfectionism. Findings also showed that perfectionism predicted eating psychopathology, but this relationship was fully mediated by self-criticism (Goodwin et al., 2014). These results demonstrate the powerful influence of self-criticism on ED development. Prah (2006) also points out low self-esteem, perfectionism, and a need for control as personality traits that increase risk for ED.

Bratland-Sanda and Sundgot-Borgen (2013) compiled three categories of risk factors: predisposing, trigger, and perpetuating factors. Predisposing factors include genetic factors, which lend a person more likely to develop ED behaviors such as low self-esteem and sociocultural factors (i.e. peer pressure). Trigger factors include negative comments regarding body weight, shape, size, or traumatic events such as an injury or loss of a coach. ED then may be maintained by perpetuating factors such as initial success following weight loss or positive affirmation from coaches about weight loss.

It is clear that the cause of ED is multifactorial and includes a mix of biological, psychological, and social factors. For instance, fashion models who are often held up as having ideal physiques, are thinner than 98% of women in America. In addition to a society that praises thinness, activities that promote a lean or thin build add even more of a burden to be thin (Prah, 2006).

Drive For, and Misconceptions of, Weight Loss

Body weight change occurs when there is a difference between energy output and energy intake. If there is a greater energy output than energy intake, it will result in a change of energy storage and body weight loss, as the body compensates for lower energy levels (Hall et al., 2012). However, this principle does not clarify what components of body weight are lost. Three components of energy balance and imbalance exist: energy intake (calories consumed), energy output, and energy storage, which equals energy intake minus energy output. Energy output can be categorized in three parts: resting energy expenditure (REE), thermic effect of food (TEF), and activity energy expenditure (AEE). REE is variable in each individual and is influenced by body mass, body composition, and recent energy imbalance. A larger body size contains more metabolic tissue, and lean tissue is more metabolically active than adipose tissue, two factors that influence REE. TEF is the obligatory use of energy to metabolize ingested food and can vary depending on macronutrient composition. Proteins require the most energy to metabolize, followed by carbohydrates then lipids. AEE is the most individualized factor of energy expenditure and depends upon one's volume of exercise (Hall et al., 2012). Energy expended from ingested macronutrients is used for, but not limited to, body maintenance, reproduction, and exercise. Stored energy is in the form of triglycerides, glycogen, and proteins. Triglycerides are the primary source of energy storage in the body, with smaller amounts of stored glycogen and protein. Due to the association of protein and glycogen to water, their metabolism results in rapid weight shifts as water balance changes. In addition, body weight fluctuates within a day based on hydration status and gastrointestinal tract content, showing the ambiguity of using weight as a metric. Instead, assessing body composition can supply accurate data for individual assessment and change.

Energy intake, output, and storage are constantly changing over time. A steady body weight is achieved through energy balance (i.e., matching intake and output); however, this ideal is difficult to achieve in the short-term because many fluctuations occur within one day. Past studies have found that steady body weight is achieved by matching energy intake and output over a longer period of time (Hall et al., 2012). For a person to become obese, his/her intake must exceed his/her output, therefore creating a positive state of energy storage. For a person to lose weight, he/she must have a greater energy output than intake. If a negative state of energy balance persists, the body makes alterations both actively and passively to lower REE, TEF, and AEE to diminish weight loss and create a state of energy balance.

There are two phases of weight loss. The first comes rapidly within days or the first few weeks of beginning a weight loss regimen. A second phase then follows with a slower velocity of weight loss, which can continue for up to two years. The first phase of rapid weight reduction is comprised of losses in glycogen, protein, and a small proportion of fat. Water balance is also affected in this phase, as water is a byproduct of the metabolism of glycogen and protein. Over time, hormonal and neurological regulatory mechanisms signal a reduction in resting energy expenditure to match energy intake; this reaction slows rates of protein breakdown and, eventually, glycogen stores are depleted. In the second phase of weight loss, adipose tissue triglycerides are the main source of energy, and the rate of weight loss decreases. Resting energy expenditure, thermic effect of food, and activity thermogenesis can be reduced. Also, body mass has been reduced from the initial starting point, so there is a smaller amount of metabolic tissue requiring energy. Eventually weight loss ceases as each of these elements reaches equilibrium with energy intake. Recent research has found weight loss to equal 2,208 kcal/pound in phase one and 2,986 kcal/pound in phase two, exposing the invalidity of 3,500 kcal/pound (Thomas et al., 2014).

The epidemic increase in the prevalence of obesity in affluent countries is now well known, and this increase is generally attributed to two main causes, that is, genetics and a changing food environment: year-round accessibility of energy dense foods with decreased demands for physical activity. The increase in overweight and obese individuals has paired with an increase in dieting. Not only are those with excess body fat dieting but, due to the pressures of media, family, and society, many normal/healthy weight individuals diet to lose weight, possibly from fear of obesity. A debate that is gaining attention is whether dieting makes a lean person fatter.

Dulloo et al. (2015) investigated years of prospective research, and they found that people who are at a normal, healthy body weight and choose to diet have an increased risk of future weight (fat) gain compared to those in a normal weight range who do not diet. Studies have shown these results in many different age groups and populations. In pre-adolescent and adolescent boys and girls, dieting predicted future weight gain (Field et al., 2003). Girls who were encouraged by their parents to diet prior to 11 years of age had greater increases in BMI percentiles from 9 to 15 years than girls who did not diet (Balanttekin et al., 2014), and a ten-year longitudinal study found female adolescents who dieted increased their BMI by 4.6 units compared to 2.3 units for girls who did not diet (Neumark-Sztainer et al., 2012). Other studies have found participants who were originally at a normal weight and repeatedly attempted to lose weight had twice the risk of a major weight gain (greater than ten kilograms) than people who did not attempt weight loss. Countering this evidence of normal weight individuals, in men and women who were initially overweight, there was no increased risk for major weight gain corresponding to attempts at weight loss (Korkelia et al., 1999). Therefore, the question arises: what determines and attributes to an excess of weight (mostly fat) gained after attempted weight loss in normal weight individuals?

These results correspond not only with weight, but also in alterations of body composition. In one study of U.S. Army Rangers, following an 8-9 week training period, which included energy deficits, and a 5-week recovery period, all subjects (n=8) had an increase in body weight and fat mass (Nindle et al., 1997). Another study of U.S. Army Rangers who went through four repeated cycles of energy deficits and refeeding, then five weeks of recovery, gained an average of four kilograms excess fat mass-an increase of 40% in fat mass from pre-training levels (Friedl et al., 2008). In a study of elite Finish male athletes, those who participated in sports where weight cycling is common, had greater BMI increases from the age 20 to 60 years than did athletes in sports without weight cycling (Saarni et al., 2006). Dulloo et al. (2015) explain the increase of fat mass seen in multiple populations through a desynchonization of fat and fat-free mass recovery and prolonged hyperphagia following a restricted/starvation period.

As weight is lost, the body has a decrease in both fat mass and fat free mass (FFM). In a study re-analyzed by Dulloo et al. (2015) assessing the affects of semi-starvation in male volunteers, the amount of fat gained in excess of the baseline after ceasing food restriction, was greater depending on initial adiposity: the leaner participants gained more fat post-dieting than did the participants with initially higher body fat. The hyperphagic response following a starvation period was found to peak at four weeks and persisted until FFM had been fully recovered. Thermogenesis has been found to be suppressed during weight lost and remain suppressed during weight recovery, as a function of depleted fat mass. The result of this function is that fat mass recovery is accelerated. As it takes longer to restore FFM, fat mass storage continues at the same rate, therefore increasing the amount of fat mass in individuals compared to starting values, at which time the individuals' body composition has been altered with an increased body fat percent. These negative effects on body composition have been explained through control systems in place in the body. In lean dieters, a greater proportion of protein mass is lost, and the extent to which fat is excessively stored post-dieting is correlated with a negative relationship between initial body composition values (lower adipose tissue initially, the more fat is stored). This research shows that lean dieters are at increased risk of excessive fat gains following dieting, and in sports where weight cycling is common, athletes will do more harm to his/her appearance than benefit from repeated dieting.

Weight cycling and repeated dieting in lean individuals has also been linked to increased risk factors for metabolic and cardiovascular disease. Zhang et al. (2005) found men with a BMI of <25 kg/m^2 with weight fluctuations displayed components of metabolic syndrome such as high blood pressure, hypertriglyceridemia, decreased high-density lipoprotein cholesterol, and high fasting glucose. Yatsuya et al. (2003) found Japanese men aged 40-59 years with a normal weight and BMI below 25 kg/m^2 but with weight fluctuations had higher insulin concentrations than did men of a normal weight and BMI but with fewer weight fluctuations. Weight gain and weight fluctuations were found to increase the risk of metabolic syndrome in a seven-year follow-up study in adults (Vergnaud et al., 2008). In a study of non-obese young women, repeated weight cycling led to significant decreases in lean body mass, serum triiodothyronine, serum total thryoxine, and resting energy expenditure; there were also significant increases from baseline in systolic and diastolic blood pressure and increased serum triglycerides (Kajioka et al., 2002). These elevations in risk factors for metabolic syndrome result in increased risk for cardiovascular disease as well as renal disease through increased cardiac load and vascular injury (Montani et al., 2015).

Many people have misconceptions about weight loss. In a 1958 report, Wishnofsky reported the energy content of weight change equal to 3,500 kcal/pound of adipose tissue based on a review of the literature and analysis of research. Today, this hypothesis is widely believed and applied to weight loss strategies, but it operates on several assumptions: that energy intake remains consistent, weight loss is not affected by energy output, the weight loss exhibited will be derived from adipose tissue, and one pound weight loss will remain constant as a deficit of 3,500 calories continues (Thomas et al., 2014). Current research has enlightened researchers about these assumptions and shows the dynamic adaptations the body makes during times of decreased energy intake.

Thomas et al. (2014) explain some of these misconceptions and the science that disproves them. For example, obesity has been attributed to a low metabolism. This idea originated from an estimated REE being divided by total body weight; due to the large body mass of an obese person, an invalid low metabolic rate was calculated. However, energy balance is not achieved by matching energy intake with an individual's weight in pounds/kilograms, but by matching individual energy intake and expenditure. To further clarify the misconception of a 3,500 kcal decrease in intake equaling one pound of weight loss, Thomas et al. (2013) showed that this theory gives the impression of permanent weight change from short-term intervention and describes a linear relationship between body weight and decreased energy intake, which is now known to be untrue as the body makes compensatory changes to reduce energy output by reducing muscle mass to lower metabolic needs and energy expenditure. Furthermore, an idea of "small lifestyle changes" has risen in nutrition consultation to prevent obesity. Many of these small changes have been based on the 3,500-kcal/pound rule (1 lb = 3,500 kcal), which has been shown to be inaccurate; therefore, these small lifestyle changes give unrealistic expectations to people about projected weight loss.

Extreme dieting and eating are virtually expected in sports with an aesthetic or weight-class element (Bratland-Sanda & Sundgot-Borgen, 2013). In turn, these elements cause participating athletes to strive for leaner bodies for enhanced performance and/or appearance. At equivalent weights, muscle is denser than fat, taking up less space than fat. It would be advantageous for these athletes to preserve muscle mass and decrease fat mass to increase an athlete's strength to weight ratio, thus creating a leaner and more powerful athlete, even at the same weight (Benardot, 2013). However, misconceptions of weight loss have caused this to be a difficult outcome to achieve.

Nutrient Recommendations for Athletes

The joint consensus statement by the American College of Sports Medicine, Academy of Nutrition and Dietetics, and Dietitians of Canada suggests a carbohydrate intake for athletes of 6-10 g/kg, a protein intake of 1.2-1.7 g/kg, and a fat intake ranging from 20-35% of total energy intake (Joint Consensus Statement, 2009). In a study of U.S. Women's National Artistic Gymnasts, average reported energy intake was significantly lower than estimated energy requirement (Jonnalagadda et al., 1998); in a women's NCAA division I soccer team assessed in preseason, midseason, and post-season, the athletes' average carbohydrate gm/kg intake (5 gm/kg) was below recommendations in midseason and post-season while average protein gm/kg (1 gm/kg) intake in post-season fell below the recommendations (Reed et al., 2014).

Athletes may be interested in losing weight to enhance performance, and this can be done in a healthy manner by lowering energy intake to gradually reduce weight; however, some athletes may seek weight loss by following a restrictive diet, defined as < 30 kcal/kg of fat-free mass in a day (Bratland-Sanda & Sundgot-Borgen 2013). In a study of elite level female endurance athletes, eight of 40 participants were found to have energy intakes below 30 kcal/kg FFM (Melin et al., 2014). This study examined the macronutrient distribution of professional cheerleaders, and if the athletes' dietary intakes matched the recommendations of the joint consensus statement, as well as investigated if any of the participants were on a restrictive diet.

Studies have shown that energy balance is correlated with body composition, with greater energy deficits being related to higher body fat percent. Deutz et al. (2000) found a significant relationship between daily energy deficits > 300 kcal and a higher body fat percent in female Olympic rhythmic and artistic gymnasts and elite-level middle and long distance runners. In the study, there was a positive relationship between energy deficits and higher body fat percent, and a negative relationship between energy surpluses and lower body fat percent. Additionally, hours spent in an energy surplus of > 300 kcal was also positively associated with higher body fat percent. They found that athletes who spent the most time in optimum energy balance (± 300 kcal) had the lowest body fat percent. Due to similarities in size and sport of the gymnasts and professional cheerleaders, this study used ± 300 kcal as optimal energy balance. Hall et al. (2012) show the importance of using new dynamic models to assess energy requirements. Investigating macronutrient distribution and total caloric intake in real time energy balance will identify if the athletes are performing in energy deficits, energy surpluses, or in energy balance.

Currently, there is a growing body of evidence supporting the intake of moderate amounts of protein throughout the day. Symons et al. (2009) found that consuming more than 30 grams of protein in one meal did not further stimulate muscle synthesis. Through a meta-analyses conducted by Leidy et al. (2015), ingestion of 30 grams of protein at each meal showed improvements in appetite, management of body weight, and/or cardiometabolic risk factors. Mamerow et al. (2014) found a 25% increase in muscle synthesis in a population that consumed ~30 grams protein three times a day versus a population with a skewed consumption of protein, beginning with a small amount (~10 grams) for breakfast and ending with a larger amount of protein at dinner (~60 grams). Snijders et al. (2015) also found benefits of ingesting 30 grams of protein prior to sleeping, finding an increased muscle mass and strength. Due to the current research regarding the increased muscle synthesis associated with moderate protein intake throughout the day, variables were created investigating the affects of ingesting protein in 30-gram increments. If a participant consumed more than 30 grams of protein in one sitting, the grams of protein excess of 30 grams were not counted.

Handgrip Strength

In a systematic review by Norman et al. (2011), handgrip strength was found to be a good indicator of nutritional status. Reduced food intake results in a compensatory loss of whole body protein, as the body works to be energy-efficient. Current hypotheses regarding the results of malnutrition and effects on muscle function include decreased muscle synthesis, increased protein breakdown, decreased metabolic pathways such as muscle glycolysis, creatine phosphate, and oxidative phosphorylation, and increased intracellular calcium leading to impairment in the conversion of free energy and cell membrane potential (Norman et al., 2011). The majority of lost protein comes from muscle mass, which causes a decrease in muscle strength. This loss of muscle mass can be perceived visually as muscle atrophies; however, muscle function can be reduced before muscle structure and body composition alterations can be detected, hence the benefit of testing muscle function. One study found that a short-term starvation period of obese women resulted in a significant decrease in handgrip strength measurements, but there were no measured differences anthropometrically, suggesting that muscle function varies according to decreased nutritional intake. Furthermore, handgrip strength has a high test, re-test, and inter-rater reliability; the test is also economical, fast, and easy to administer (Flood et al., 2014).

In postmenopausal women, decreased handgrip strength has been associated with low BMD and the amount of protein consumed per kilogram of body weight in a day (Kim et al., 2012; Filion et al., 2012). Other studies have found handgrip strength to be associated with BMD in the hand, while also a predictor for BMD in distant skeletal sites in adolescents (Norman et al., 2011). Although BMD was not assessed in this study, handgrip strength may indicate a necessity for measuring bone health in professional cheerleaders. In this study, handgrip strength served as another measure of nutritional status along with a one-day dietary recall.

In summary, the importance of adequate energy intake for health and athletic performance suggests the need to investigate a population that presents with characteristics that place them at high risk of inadequate energy consumption. Inadequate energy consumption can lead to numerous negative health and performance consequences, many of these issues presenting themselves after an athlete has completed his/her career.

CHAPTER III

METHODS

Participants

The study included 19 female professional cheerleaders currently on one professional team roster. The Institutional Review Board at Georgia State University reviewed the study protocol, and each participant signed a written informed consent. All members of the professional cheerleading team were included as potential participants; there were no exclusion criteria for the subjects. The subjects voluntarily agreed to participate in the study after the protocol had been discussed with them and after they signed the approved informed consent for this study.

All cheerleaders were in their competitive season at the time of the data collection. The mean age of the cheerleaders was 25.4 ± 3.5 years (range 18-32 years), mean height was 164.1 ± 5.0 cm (range 156.2-174.2 cm), and mean weight was 57.5 ± 4.7 kg (range 50.2-66.1 kg). Race and ethnicity were not collected from the participants. Table 1 describes the demographic descriptions of subjects in this study.

Table 1: Descriptive statistics for age, height, and weight (N=19)

	Mean ± SD	Range
Age (yr.)	25.4 ± 3.5	18-32
Height (cm)	164.1 ± 5.0	156.2-174.2
Weight (kg)	57.50 ± 4.7	50.2-66.1

Procedures

The athletes were assessed during their competitive season using the following protocols:

a) **Weight and body composition:** Assessed with an InBody 230 multi-current 8-mode Bioelectrical Impedance Analyzer (BIA). The BIA requires that volunteer subjects wipe their hands and feet with a disposable electrolyte cleaning cloth (provided), and then stand on the scale and hold the handles for approximately 20 seconds. For the purpose of this study, the measured values were weight, lean body mass, total body water, dry lean mass, body fat mass, skeletal muscle mass, BMI, and percent body fat. The BIA is non-invasive and poses no risk or discomfort to the subjects.

b) **Diet/fluid intake, energy expenditure, and energy balance**: The day prior to the current day was assessed via interview. The interviewer asked volunteer subjects to recall their food and beverage intake for the previous day and to provide an estimate of activity intensity using the Harris-Benedict Equation and 13-point MET Value Relative Energy Expenditure Scale. Common household items, such as a baseball equaling one cup, were referenced to aid in the accuracy of measurement. Energy intake was analyzed using the USDA Food Nutrient Database for Standard Reference, ver 27. As menstrual status is associated with energy intake availability, the questionnaire contained a single question regarding current menstrual status as regular, irregular, or no periods. There was no risk or discomfort associated with completing this questionnaire via interview. Total protein was measured in grams and gm/kg. Total protein consumed per meal up to 30 grams was also calculated. In this variable, amounts greater than 30 grams of protein consumed in one meal were not counted in total, the 30-gram protein total was further analyzed to find if the protein was consumed in a positive or negative energy balance.

c) **Grip strength test:** Assessed using a hydraulic hand dynamometer (Baseline 12-0246 Hi-Res Hydraulic), which requires the volunteer subjects to grip an aluminum handle and squeeze with maximum force three times per hand. Measurement averages and sums were used to assess individual grip strengths, which have been associated with energy intake availability. Grip strength results were analyzed using percentiles grouped by age and gender. Values were measured for the dominant hand, non-dominant hand, and combined sum averages for both hands. Rankings for individual handgrips are weak, normal, and strong. Rankings for the combined sum of dominant and non-dominant hands are poor, below average, average, and above average. There was no risk or discomfort associated with completing this procedure.

d) **Height**: Measured using a portable Martin-type anthropometer. Volunteers were asked to stand straight with no socks or shoes on for the measurement. There was no risk or discomfort associated with this measurement.

The estimated total time for each day that data were collected was estimated to be less than one-hour per athlete. The assessments were taken one time during the competitive season with each athlete.

Data Analyses

Data from the dietary recall was analyzed using NutriTiming (NutriTiming® Nutrient and Energy Analysis 2.1, NutriTiming LLC, 2014). Statistical analyses were performed using SPSS (version 20.0, SPSS, Inc., Chicago, IL). Descriptive statistics and correlation coefficients were used to examine the relationships between diet, body composition, and handgrip strength.

CHAPTER IV

RESULTS

<u>Dietary Intake</u>

The energy and macronutrient intake reported by the professional cheerleaders is shown in Table 2. Mean total energy intake was lower than energy expenditure (1,481.8 ± 294.1 vs. 2,199.4 ± 360.3, respectively). On average, the greatest proportion of the diet came from carbohydrate (47.2%), followed by fat (29.7%) then protein (23.1%). Grams per kilogram of body weight for each macronutrient category were also calculated with the following means: 3.1 gm/kg carbohydrates, 1.5 gm/kg protein, and 0.9 gm/kg fat. The average carbohydrate intake for gm/kg was significantly lower than the recommended 6-10 gm/kg ($p<0.001$). The average kcal/kg of LBM was 33.3 ± 6.7 (range, 24.1-51.8 kcal/kg LBM).

Table 2: Descriptive statistics for dietary intake (N=19)

	Mean ± SD	Range
Kcal In	1482 ± 294.1	1054-2218
Kcal Out	2199 ± 360.3	1707-3035
Kcal(in)/kg	26.0 ± 5.8	17.9-42.4
CHO (gm/kg)	3.1 ± 0.9	1.9-5.1
CHO (%)	47.2 ± 9.0	28.5-64.9
Prot (gm/kg)	1.5 ± 0.4	0.8-2.5
Prot (%)	23.1 ± 6.6	11.8-33.9
Fat (gm/kg)	0.9 ± 0.3	0.4-1.6
Fat (%)	29.7 ± 7.4	17.8-45.5
Kcal per kg LBM	33.3 ± 6.7	24.1-51.8

Two groups were created for participants with energy intakes greater than or equal to 30 kcal/kg LBM or less than 30 kcal/kg LBM. Independent samples t-tests were used to analyze relationships between energy intake and body composition in the two groups. Participants with an intake >30 kcal/kg LBM had lower body weight (p=0.024) (Table 3). Also, participants with an intake <30 kcal/kg LBM had significantly lower intakes of carbohydrate grams and calories (p=0.027) and fat grams (p=0.022), calories (p=0.021), and fat gm/kg (p=0.028) but did not have significant differences in total protein intake.

Table 3: Relationships between restrictive diets (< 30 kcal/kg LBM) and body composition variables

	Kcal per kg LBM	Mean ± SD	*p* value
Lean Body Mass (kg)	>= 30	43.5 ± 3.8	.074
	< 30	47.6 ± 5.6	
TBW (kg)	>= 30	31.8 ± 2.7	.074
	< 30	34.8 ± 4.1	
Dry Lean Mass (kg)	>= 30	11.7 ± 1.0	.075
	< 30	12.8 ± 1.5	
BF Mass (kg)	>= 30	12.4 ± 2.4	.449
	< 30	13.3 ± 2.3	
Skeletal Muscle Mass (kg)	>= 30	23.9 ± 2.3	.073
	< 30	26.4 ± 3.3	
% Body Fat	>= 30	22.2 ± 4.1	.956
	< 30	22.1 ± 4.6	
Weight (kg)	>= 30	55.9 ± 4.1	**.024**
	< 30	61.0 ± 4.2	

n= 13 (>=30) or 6 (<30)

Energy Balance

Daily and hourly energy balance were calculated using NutriTiming®. The average 24-hour net energy balance (-720.5 ± 448.1 kcal) was found to be greater than average ending energy balance (-562.0 ± 450.4 kcal) (Table 4). The majority of the day was spent in a catabolic state (19.8 ± 3.4 hours), while the average time spent in an anabolic state was 4.2 ± 3.4 hours. The average percent of participants in a catabolic state at any given hour was 82%, or 15.6 out of 19 participants. The greatest average energy surplus occurred at 12:00 AM with an energy balance of 100.3 ± 92.4 kcal; the greatest average energy deficit occurred at 11:00 PM with an energy balance of -562.2 ± 449.6 kcal. Figure 1 displays the average hourly energy balance of all subjects. This graph begins at 12:00 AM the day of analysis, and initial energy balance is calculated based on the last time the participant ate the night before analysis. As energy balance carries over from one day to the next, the day of analysis begins with the ending energy balance of the day prior. The average time spent in optimal energy balance (± 300) was 13.3 ± 4.8 hours. The average within-day energy surplus was 184.7 ± 130.3 kcal, and the average within-day energy deficit was -817.2 ± 319.5 kcal.

Table 4: Descriptive statistics for energy balance (N=19)

	Mean ± SD	Range
24 Hr Net	-720.5 ± 448.1	(-1616) - (-17)
Ending EB	-562.0 ± 450.4	(-1387) - 164
Hours Catabolic	19.8 ± 3.4	10 - 24
Hours Anabolic	4.2 ± 3.4	0 - 14
Hours ± 300 EB	13.3 ± 4.8	4.0 - 23.0
Highest EB	184.7 ± 130.3	(-58) - 527
Lowest EB	-817.2 ± 319.5	(-1405) - (-310)

Body Composition

The mean body fat percent was 22.2 ± 4.1%. Lean body mass was found to have a mean value of 44.8 ± 4.7 kg; the mean total body water was 32.7 ± 3.4 kg. Dry lean mass had a mean of 12.1 ± 1.3 kg. Body fat mass had a mean of 12.7 ± 2.4 kg, and skeletal muscle mass had a mean of 24.7 ± 2.8 kg. Table 5 describes the body composition values of subjects in this study.

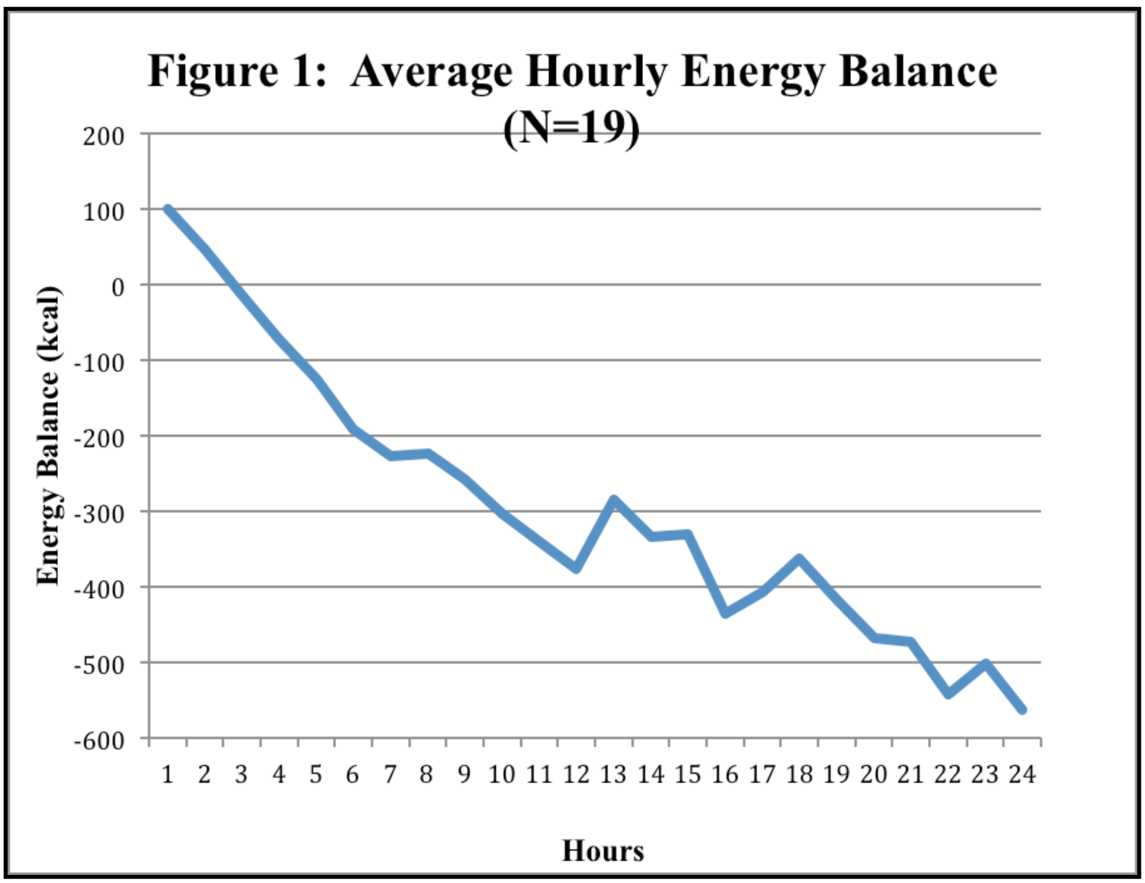

Table 5: Descriptive statistics for body composition (N=19)		
	Mean ± SD	Range
% Body Fat	22.2 ± 4.1	15.5 - 32.1
Lean Body Mass (kg)	44.8 ± 4.7	35.8 - 55.9
TBW (kg)	32.7 ± 3.4	26.2 - 41.0
Dry Lean Mass (kg)	12.1 ± 1.3	9.6 - 15.0
BF Mass (kg)	12.7 ± 2.4	9.3 - 17.1
Skeletal Muscle Mass (kg)	24.7 ± 2.8	19.1 - 31.3

When body fat percent was analyzed using an independent sample t-test and the 50th percentile as a cut-off point, participants with lower body fat percent consumed more total calories (p=0.011), more kcal/kg (p=0.026), spent more time anabolic (p=0.048), less time catabolic (p=0.048), consumed a lower percentage of protein in a negative energy balance (p=0.016), consumed more protein grams and a higher percentage of protein in a positive energy balance (p=0.014, 0.025), and consumed more grams of protein between 0 and 300 energy balance (p=0.030) (Table 6 and 7).

Table 6: Body fat percent at the 50th percentile and relationships between energy intake and output and energy balance variables			
	% BF	Mean ± SD	*p* value
Kcal In	>= 20.9	1327.2 ± 189.5	**0.011**
	< 20.9	1653.6 ± 302.0	
Kcal Out	>= 20.9	2143.7 ± 339.7	0.494
	< 20.9	2261.2 ± 392.5	
24 Hour Net Energy Balance	>= 20.9	(-822.00) ± 353.4	0.312
	< 20.9	(-607.2) ± 533.0	
Kcal (in)/kg	>= 20.9	23.2 ± 3.5	**0.026**
	< 20.9	29.0 ± 6.5	
Hours Anabolic	>= 20.9	2.7 ± 2.2	**0.048**
	< 20.9	5.8 ± 4.0	
Hours Catabolic	>= 20.9	21.3 ± 2.2	**0.048**
	< 20.9	18.2 ± 4.0	
n = 10 (>= 20.9%) or 9 (<20.9%)			

Using a one-way analysis of variance (ANOVA) test and dividing participants according to tertiles, the group with the lowest body fat percent (below the 25th percentile) consumed statistically significant higher amounts of total protein (p=0.017), kcal from protein (p=0.017), protein in 30 gm increments (p=0.015), protein in 30 gm increments/kg (p=0.010), and total protein/kg LBM (p=0.029) than the 25th-75th percentiles.

Table 7: Body fat percent at the 50th percentile and the relationship between macronutrients and protein ingestion at various energy balances

	%BF	Mean ± SD	*p* value
Protein (%)	>= 20.9	23.8 ± 7.5	.664
	< 20.9	22.4 ± 5.8	
Protein (gm/kg)	>= 20.9	1.4 ± 0.5	.250
	< 20.9	1.6 ± 0.4	
Fat (%)	>= 20.9	27.0 ± 5.8	.099
	< 20.9	32.7 ± 8.2	
Fat (gm/kg)	>= 20.9	0.7 ± 0.2	**.013**
	< 20.9	1.1 ± 0.3	
Protein (gm) Consumed in Negative EB	>= 20.9	68.7 ± 29.9	.255
	< 20.9	49.7 ± 40.3	
Protein (%) Consumed Negative EB	>= 20.9	87.0% ± 21.7%	**.016**
	< 20.9	51.2% ± 35.7%	
Protein (gm) Consumed in Positive EB	>= 20.9	9.7 ± 16.4	**.014**
	< 20.9	43.0 ± 34.6	
Protein (%) Consumed in Positive EB	>= 20.9	13.0% ± 21.8%	**.025**
	< 20.9	49.3% ± 40.6%	
Protein (gm) Consumed between 0 & 300 EB	>= 20.9	9.7 ± 16.4	**.030**
	< 20.9	35.7 ± 30.2	
Protein (gm) Consumed between 0 & -300 EB	>= 20.9	27.1 ± 16.7	.855
	< 20.9	29.2 ± 31.5	
n = 10 (>= 20.9%) or 9 (<20.9%)			

Table eight displays the result found between body fat percent tertiles and protein intake, there were no significant results found between the tertiles and fat or carbohydrate intake values.

Table 8: Relationship between body fat percent tertiles and protein intake				
		df	F	Sig.
Prot (gm)	Between Groups	2	5.437	**.016**
	Within Groups	16		
	Total	18		
Prot (%)	Between Groups	2	5.407	**.016**
	Within Groups	16		
	Total	18		
Prot 30 gm Intake	Between Groups	2	6.298	**.010**
	Within Groups	16		
	Total	18		
Prot 30 gm/kg	Between Groups	2	7.283	**.006**
	Within Groups	16		
	Total	18		
Total Protein gm/kg LBM	Between Groups	2	6.216	**.010**
	Within Groups	16		
	Total	18		

An independent sample t-test was used to analyze body fat mass using the 50th percentile as a cutoff point; those with a higher body fat mass consumed less total calories (p=0.012), had a greater net energy balance deficit (p=0.032), and consumed less kcal/kg (p=0.003) than those with a lower BFM (Table 9).

Table 9: The relationship between body fat mass and energy intake, output, and balance			
	BF Mass (kg)	Mean ± SD	*p* value
Kcal In	>= 12.45	1328.6 ± 185.0	**0.012**
	< 12.45	1652.0 ± 306.9	
Kcal Out	>= 12.45	2252.6 ± 430.5	0.513
	< 12.45	2140.2 ± 275.8	
24 Hr Net EB	>= 12.45	-924.0 ± 421.0	**0.032**
	< 12.45	-494.4 ± 378.4	
Ending EB	>= 12.45	-742.4 ± 417.1	0.064
	< 12.45	-361.6 ± 418.0	
Kcal(in)/kg	>= 12.45	22.5 ± 2.9	**0.003**
	< 12.45	29.8 ± 5.92	
n= 10 (>=12.45) or 9 (<12.45)			

Skeletal muscle mass (kg) was also analyzed, according to tertiles using a one-way ANOVA test. The group with the lowest skeletal muscle mass (below the 25th percentile) consumed the highest percentage of their diet from protein and statistically significant more than the 25th-75th percentiles (p=0.006). (Table 10)

Table 10: Relationships between skeletal muscle mass tertiles and macronutrient intakes

		df	F	Sig.
Prot (gm)	Between Groups	2	1.410	.273
	Within Groups	16		
	Total	18		
Prot (%)	Between Groups	2	7.444	**.005**
	Within Groups	16		
	Total	18		
CHO (gm)	Between Groups	2	8.774	**.003**
	Within Groups	16		
	Total	18		
CHO (%)	Between Groups	2	4.667	**.025**
	Within Groups	16		
	Total	18		
Fat (gm)	Between Groups	2	.252	.780
	Within Groups	16		
	Total	18		
Fat (%)	Between Groups	2	.114	.893
	Within Groups	16		
	Total	18		

Menstrual Status

A total of 17 participants answered whether their menstrual cycle was regular, irregular, or had no periods; 12 answered that they were eumenorrheic, 3 were oliomennorrheic (~18%), and 2 reported having amenorrhea (~12%). There were no significant results found with menstrual status in regards to body composition, energy balance, or handgrip strength.

Handgrip Strength

The mean for dominant handgrip strength was less than the mean for non-dominant handgrip strength (21.3 ± 3.4 kg and 24.6 ± 4.3 kg, respectively). The mean for combined sum averages was 45.9 ± 7.4 kg (Table 11). For the dominant hand, eight participants placed in the weak category and 11 placed in the normal category. For the non-dominant hand, 14 participants placed in the weak category, and five placed in the normal category. The sum rankings placed 14 participants as having poor handgrip strength, four below average, and one participant was between poor and below average.

Table 11: Descriptive statistics for handgrip strength (N=19)

	Mean ± SD	Range
Non-Dominant Hand	24.6 ± 4.3	17.6 - 31.3
Dominant Hand	21.3 ± 3.4	17.5 - 27.3
Sum Average	45.9 ± 7.4	36.6 - 58.3

Sum averages for handgrip strength were analyzed according to tertiles using a one-way ANOVA and divided into three groups: below the 25th percentile, 25th to 75th percentiles, and above the 75th percentile. Participants above the 75th percentile had statistically significant higher lean body mass (p=0.048), total body water (p=0.049), dry lean mass (p=0.045), and skeletal muscle mass (p=0.049) than the 25th-75th percentiles group. (Table 12)

Table 12: Relationship between grip strength tertiles and body composition				
		df	F	Sig.
Weight (kg)	Between Groups	2	2.881	.085
	Within Groups	16		
	Total	18		
Lean Body Mass (kg)	Between Groups	2	3.702	**.048**
	Within Groups	16		
	Total	18		
TBW (kg)	Between Groups	2	3.666	**.049**
	Within Groups	16		
	Total	18		
Dry Lean Mass (kg)	Between Groups	2	3.790	**.045**
	Within Groups	16		
	Total	18		
BF Mass (kg)	Between Groups	2	.147	.865
	Within Groups	16		
	Total	18		
Skeletal Muscle Mass (kg)	Between Groups	2	3.653	.049
	Within Groups	16		
	Total	18		
%BF	Between Groups	2	.780	.475
	Within Groups	16		
	Total	18		

Results Relative to Hypotheses

1. More time spent in an energy balance deficit of > -300 kcal will be associated with a higher body fat percent
    - Finding: Participants above the 50th percentile for body fat percent (higher body fat) spent more time in a catabolic state.
        i. We reject our first null hypothesis that there would be no association with time spent in an energy deficit of > -300 kcal and body composition.
2. Subjects with higher protein intakes will have higher handgrip strength

- Finding: There was no association with handgrip strength and protein intake.
    i. We found no association between protein intake and low handgrip strength. Therefore, we are unable to reject our second null hypothesis that there would be no association between handgrip strength and protein intake.

3. The participants will have energy substrate inadequacies as compared to the ACSM intake recommendations.
    - Finding: Participants consumed less calories than they expended and consumed on average significantly less carbohydrate gm/kg than are recommended.
        i. We also reject our third null hypothesis as dietary inadequacies were found in the assessed population.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The study participants displayed dietary inadequacies, as defined by the standard for carbohydrate intake, although they fell within the appropriate ranges for protein and fat intake (Joint Consensus Statement, 2009). Despite being within range for two macronutrient groups, most participants exercised and performed in a catabolic state, which will not allow for sustained optimal performance.

Average total energy intake for all participants was 1482 ± 294 kcals/day while the average energy output was 2199 ± 360. The importance of adequate energy intake to ensure optimum athletic performance is explained in the joint consensus statement (2009). The increased fluid and energy needs that result from physical activity place more of an importance on adequate intake. Jonnalagadda et al., (1998) found that of 33 Olympic gymnasts, 48% were on a self-prescribed diet, and the average daily intake based on 3-day food records was 20% below estimated needs. Participants in this study displayed similar total energy requirements and deficits, but participants were not asked if they were attempting to diet or if the daily intake happened to be low for the day analyzed. Also noted is that a low energy intake, below 1800-2000 calories/day, is cause for major nutritional concern because athletes operate in a negative energy balance, lose weight, and can disrupt proper endocrine function. In a one-sample t-test, the average energy intake was significantly lower than 1800 kcal ($p<0.001$). It has also been documented that inadequate energy intakes negate the benefits of training as the body compensates for the lack of energy by using lean tissue and fat. The loss of lean tissue then results in a loss of strength and endurance in the athletes, as well as numerous negative health consequences as previously listed (Joint Consensus Statement, 2009). It is invaluable for athletes to have adequate energy intake for health and performance. Athletes should be made aware that low-energy intake will not sustain athletic training and performance.

Dietary inadequacies have been noted in multiple female athletic populations (Deutz et al., 2000; Jonnalagadda et al., 1998; Reed et al., 2014). Although limited in the accuracy of dietary recalls, the body of evidence is strong suggesting that many athletes fail to consume adequate energy and fluid intakes related to performance needs. In this study, 6 of 19 participants were found to have an intake < 30 kcal/kg LBM, labeled a restrictive diet. Participants with an intake >30 kcal/kg LBM had a lower body weight (p=0.024). Also, participants with an intake <30 kcal/kg LBM had significantly lower intakes of carbohydrate grams and calories (p=0.027) and fat grams (p=0.021), calories (p=0.022), and fat gm/kg (p=0.028) but had similar intakes of protein. Reed et al., (2014) investigated the nutritional intakes of division I female soccer players in preseason, midseason, and postseason. Those who consumed >30 kcal/kg LBM had significantly lower body fat percent (means: 21.2 ± 1.0% and 27.2 ± 2.4%, p=0.016).

When body fat percent was analyzed using the 50th percentile as a cut-off point, participants with lower body fat percent consumed more total calories (p=0.011), more kcal/kg (p=0.026), spent more time anabolic (p=0.048), less time catabolic (p=0.048). If an athlete does not satisfy energy needs he/she will develop low blood glucose, causing the body to enter into a state of gluconeogenesis and breakdown lean tissue to manufacture glucose (Benardot, 2013). Notably, there was no statistical significance with body fat percent and 24-hour net energy balance or energy output (p=0.312 and p=0.494, respectively). Furthermore, participants with a lower body fat percent consumed a lower percentage of protein in a negative energy balance (p=0.016), consumed more protein grams (p=0.014) and a higher percentage of protein in a positive energy balance (p=0.025), and consumed more grams of protein between 0 and 300 energy balance (p=0.030). There was no difference found in the amount of total protein consumed in grams (p=0.213); the differences were found in how these subjects consumed protein. By consuming protein while in an anabolic state, the body utilizes protein for muscle maintenance and synthesis instead of consuming protein in a catabolic state and the body using protein as an energy source. These results show a positive correlation between body fat percent and energy deficits in these athletes.

According to an assessment of the population organized by tertiles, the group with the lowest body fat percent consumed statistically significant higher amounts of total protein (0.017), kcal from protein (0.017), protein in 30 gm increments (0.015), protein in 30 gm increments/kg (0.010), and total protein/kg LBM (0.029) than the 25^{th}-75^{th} percentiles. The increased ingestion of protein in 30 gram increments could offer positive outcomes to body composition (i.e., decreased body fat mass and increased muscle mass). In addition, some athletes may have a protein intake of 2-3 gm/kg or higher, but if the intake exceeds the utilization amount (~30-35 gm per meal), the protein is not used anabolically (Benardot 2013). If so, the protein will not be used anabolically, and athletes may have inadequate intake of protein. Each individual athlete will perform optimally at a specific body fat percent, unique to other athletes; therefore, a specific body fat percent should not be prescribed for each athlete but rather a healthy range should be recommended. In addition, there is a standard error associated with each body composition assessment tool, and the coaches and athletes should be aware of the estimated error (Joint Consensus Statement, 2009).

There is a common belief that if total energy intake is reduced, increased intake of protein will negate any breakdown of lean tissue and preserve muscle mass. All participants displayed inadequate total in energy intake, and in this population, the group with the lowest skeletal muscle mass had the highest intake of protein, suggesting that protein intake, per se, is not protective of skeletal muscle.

The lower measured values of handgrip strength display the decreased muscle strength and function of the participants, which is indicative of malnutrition. If energy intake is reduced, the body must compensate for the inadequate energy resulting in a loss of whole body protein, mostly from muscle mass. Loss of muscle mass results in weakness and decreased muscle functions (Norman et al., 2011). Participants with the greatest sum average handgrip strength had more muscle mass and variables related to muscle mass (total body water, dry lean mass, etc.). These components of body composition can only be synthesized and maintained through adequate energy intake. The correlation of low handgrip strength and low BMD as noted by Norman et al. (2011) should also be a consideration in this population. Participants may benefit from having a dual energy x-ray absorptiometry (DEXA) scan conducted to measure BMD and screen for osteoporosis.

The nature of professional cheerleading places an emphasis on appearance, similar to other sports. While body weight affects an athlete's speed, endurance, and power, body composition impacts an athlete's agility, strength and appearance. Having a lean body with a greater proportion of muscle mass to fat mass is advantageous in many sports (Joint Consensus Statement, 2009). Inappropriate dieting causing loss of muscle mass and gaining of excess fat mass is both dangerous to health and counterproductive for appearance and performance.

Limitations

The accuracy of the dietary information is contingent upon the participants' memory and honesty. It has been noted in several populations that athletes under- or over-report food records (Sundgot-Borgen, 1993; Jonnalagadda et al., 2000). Micronutrients were not analyzed from this population's nutrient intakes, and they may offer more insight to individual nutritional status. The sample size was of moderate size, and a larger group could be useful in showing results. Also, the generalizability of this population to other cheerleading populations is unknown.

Conclusions

The subjects were found to have dietary inadequacies in total energy intake and carbohydrate intake, and participants spent most of the 24 hours analyzed in a catabolic state. Those participants with lower body fat percent consumed more energy and more kcal/kg, with no difference in energy expended. Furthermore, those participants with lower body fat percent consumed more protein in amounts up to 30-grams and in a positive energy balance state. Subjects with higher body fat mass consumed less total energy and had greater energy deficits. Handgrip strength, which is correlated to nutritional status, revealed that all participants had poor or below average handgrip strength when compared to population, age, and gender-specific values. Six participants (32%) consumed <30 kcal/kg LBM, categorized as a restrictive diet, which is an indicator to screen for ED. Due to the characteristics and pressures of the sport, professional cheerleading should be considered a high-risk sport for the prevalence of ED. Although there is not a defined *ideal* body type/shape/weight for these athletes, the similarities between professional cheerleading compared to other sports with an increased prevalence of ED/DE along with the revealing uniforms worn by the athletes, place these athletes in similar situations to sports with a high prevalence of dieting and energy restriction. As research has warned, improper dieting among lean populations can result in future fat gain and negative health consequences. Eating behaviors and strategies should be modified to consume small/moderate amounts of protein while in a positive energy balance, and energy intake should match real time energy expenditure to optimize athletes' body compositions and sustain optimal performance. The eating behaviors of this studied population warrant further research regarding professional cheerleaders to determine if this sport has a high prevalence of ED, with a possible follow-up need for screening, preventing, and treating ED.

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APPENDIX I

NutriTiming® Data Entry Form

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

NutriTiming Activity Factor Scale

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

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

NutriTiming® Data Entry Form

Name:____ Age: ____Years Date of Birth:____/____/________
(MM / DD / YYYY)

Gender: M or F Height: ____Feet ___Inches Weight: ____ lbs. Menstrual Status: [Normal] [Irregular] [No Periods]

Date Analyzed: ___/____/____ (M / DD / YYYY) Time last meal eaten before date analyzed: _____________

Begin Hour	End Hour	Activity Factor	Activity Descriptions	Food/Drink Descriptions	Food/Drink Amounts