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Relationship Between Daily Protein Distribution and Body Composition in Elite Gymnasts

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Acceptance

This Thesis, Relationship Between Daily Protein Distribution and Body Composition in Elite Gymnasts, by Julie A. Paszkiewicz, was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee members, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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Abstract

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Body Composition in Elite Gymnasts

Julie Ann Paszkiewicz

Background: Daily nutrient/energy intakes are typically evaluated as a total 24-hour intake rather than as the amounts consumed per eating opportunity. Evidence suggests that smaller, frequent meals containing persistent levels of energy and protein may be more beneficial for achieving a lower body fat and higher fat-free mass than equal intakes consumed in larger and less frequent amounts. This may be due to a better-maintained energy balance (EB) that is achieved with smaller and more frequent intakes.
Objective: The objective of this study was to determine the relationship between hourly EB and protein intake with body composition.
Methods: Using a software program that provides hourly and 24-hour energy and nutrient intakes and hourly energy expenditures, a secondary analysis of previously collected 3-day food diaries was used to examine the relationship between hourly EB and body composition. The food and activity diaries provided information on time of food/beverage consumption and hourly energy expenditure, enabling an hourly analysis of EB. Body composition, including fat mass, fat-free mass, and bone density were examined via dual-energy x-ray absorptiometry. SPSS was used for statistical analysis, and included descriptive statistics, correlational analyses, t-tests, and regression analyses.
Results: Existing data from elite female gymnasts (N=40) were assessed using an IRB-approved protocol. Higher protein consumption was significantly associated with lower bone mineral density (BMD) in the gymnasts at the arms ($r= -0.535$; $p< 0.001$), legs ($r= 0.0523$; $p= 0.001$), trunk($r= -0.517$; $p=0.001$), spine ($r= -0.472$; $p=0.002$), and pelvis ($r= -0.539$; $p< 0.001$). Other dietary factors assessing energy and protein intakes were not significantly associated with body composition. The assessed gymnasts spent the majority of the day in hourly energy balance deficits exceeding -400 kcal. A t-test comparing subjects with higher (n=22) vs. lower (n=18) fat-free mass (FFM), using the statistical mean as the cut point, found that higher protein intakes were significantly associated with lower FFM ($p=.007$). Subjects with more hours spent in an EB surplus had significantly higher FFM/kg ($p=.008$) and lower body fat % ($p=.008$).
Conclusion: These findings suggest that higher protein intakes may compromise BMD, a finding likely exacerbated by the long periods of time spent in EB deficits. More hours in an EB surplus was associated with positive outcomes, including higher FFM/kg and lower body fat percent. These data suggest that higher protein intakes may be consumed by gymnasts with the greatest EB deficits, perhaps as a way of minimizing (unsuccessfully) weight and fat-mass. As virtually all hours of the assessed groups were in an EB deficit, it is possible that consumed protein was used to satisfy energy needs rather than being used anabolically to support or enlarge the muscle mass. Future studies should consider addressing this issue, perhaps by assessing more heterogeneous groups where at least a proportion of the population sustains a reasonably good EB during the assessment period.

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By:

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Abbreviation List

BMD	Bone Mineral Density
DEXA	Dual-energy X-ray Absorptiometry
DRI	Dietary Reference Intakes
EB	Energy Balance
FFM	Fat Free Mass
FM	Fat Mass
LBM	Lean Body Mass
PI	Protein Index (g of protein consumed/ kg of body mass)
RDA	Recommended Daily Allowance
SD	Standard Deviation
UL	Upper Limit

Chapter I

Introduction

Meal frequency has been assessed and has been found to impact body composition. Individuals consuming smaller, more frequent meals appear more able to maintain a desirable body fat percentage and muscle mass. Eating less frequent with larger meals results in hyperinsulinemia, which is associated with increased synthesis of fat stores (Choi et al. 2012; Drummond et al. 1998).

Protein consumption may impact body composition. Consumption of sufficient protein may improve body composition through the maintenance and enlargement of fat-free mass, and there is evidence that protein consumption is an important factor in a more favorable waist circumference (Loenneke et al. 2012). The current DRI for protein for non-athlete, healthy adults is 0.8g/kg or an estimated 46g per day for reference weight for females (57.5kg), and an estimated 56g protein/day for reference weight for males (70kg). One study examining the diet of metabolically obese but normal weight adults using information from the Korean National Health and Nutrition Examination Survey found higher dietary protein intake was related to a more desirable body composition (Choi et al. 2012).

In addition to total protein intake, the pattern of protein consumption is important for determining optimal protein utilization. Studies suggest that, depending on body size, the maximal 1-meal threshold for protein metabolism appears to be between 20-30g of intact protein during a single meal, with any additional protein consumed contributing to energy availability but not resulting in any additional benefit in muscle protein synthesis (Burd et al. 2009; Paddon-Jones et al. 2009). One study, with 17 participants (mean age = 35±3 yr), found that subjects provided 30 g protein servings rather than 90 g protein servings at equal intervals had equally effective muscle protein synthesis (Symons et al. 2009). There is additional evidence to suggest that athletes may not optimally distribute protein consumption. A study of Canadian athletes found that female athletes were over-consuming recommended amounts of protein at dinner, the final meal consumed for the day, with an average intake of 40 g. In male athletes, protein consumption was high for both lunch and dinner at 41g and 57g respectively (Erdman et al. 2013).

Higher protein consumption at appropriate time intervals may be essential for development and maintenance of desirable levels of fat-free mass (Evans et al. 2012; Arciero et al. 2013). Consuming smaller amounts of protein more often during the day may positively impact the rate of muscle synthesis and, therefore, result in increased muscle mass with relatively lower body fat (i.e., lower body fat percentage) (Mamerow et al. 2014).

Studies suggest that protein requirements for athletes differ than those of the general population, with athletes requiring more. The joint position statement from the American Dietetic Association, Dietitians of Canada and the American College of Sports Medicine on nutrition and athletic performance recommends between 1.2 g/kg and 1.7 g/kg per day (Rodriguez et al. 2009). The position statement notes that this amount can be consumed through food only, and does not require additional supplemental protein. Further, the position statement states that before protein can be used to impact LBM energy balance must be satisfied, meaning adequate caloric intake to ensure the subject will not be in an energy deficient state is required for protein to impact body composition. However, some studies disagree with this range. A 2014 review article recommends that for a beneficial effect on FFM, protein intake should be in the range for 2.3-3.1 g/kg of FFM for resistance-trained athletes, a level significantly higher than the recommended intake established by the American College of Sports Medicine and the American Academy of Nutrition and Dietetics (Helms et al. 2014; Rodriguez et al. 2009). However, this recommendation does not provide any guidance for distribution of protein throughout the day. The study notes that to maintain athletic performance, reductions should be made in carbohydrate and fat to create a caloric deficit if attempting to lower body weight. However, the authors caution against a lower limit of fat intake less than 20% of total kcal because a further reduction of dietary fat can be detrimental to overall heath as well as sport performance.

While protein ingestion is known to be beneficial for muscle synthesis and contributes to lower central abdominal fat, studies suggest that the traditional assessment of 24-hour protein intakes (e.g., 0.8g/kg or 1.5 g/kg) may not be sufficient to determine optimal levels of consumption (Burd et al. 2009; Loenneke et al. 2012).

Rationale for Study

These studies suggest that the pattern of protein consumption may be more relevant to body composition than the total amount of protein consumed in a day. However, research examining this relationship is limited, with scant information on the relationship between protein consumption and energy balance throughout the day as they related to body composition. In addition, the research on protein intake patterns and the importance of proper distribution of protein throughout the day has often focused on the elderly and the prevention of sarcopenia rather than on younger athletes. These researchers have stated the need to continue to examine the effects of consuming protein throughout the day in various additional population groups.

Purpose

It is the purpose of this study to assess the relationship between protein consumption and energy balance patterns as they relate to body composition in elite female gymnasts. This study will assess if the frequency of protein consumption is more associated with higher muscle mass than total daily protein intake, and will make this assessment in the context of the energy balance during the time of consumption. This information is relevant for female gymnasts and other athletes because of the potential positive impact on performance achieved by maintaining a healthy body composition. In addition, since body composition is important to athletes, learning the effect of protein consumption patterns on establishing a lean body composition may decrease the frequency of unhealthy dietary patterns.

Hypotheses

- Consumption of several small, protein containing meals and snacks throughout the day results in lower body fat percentage when compared to larger less frequent meals.
    - Null hypothesis: Consumption of several small, protein containing meals and snacks throughout the day has no impact on body fat percentage when compared to larger less frequent meals

- Consumption of 20-30 grams of protein three times during the day is associated with greater muscle mass than consumption of the same or more protein provided in fewer meals.
    - Null hypothesis: Consumption of 20- 30 grams of protein three times during the day is not associated with muscle mass compared with consumption of the same or more protein provided in fewer meals
- Consumption of protein when the athlete is + or – 400 Kcal of energy balance is associated with higher muscle mass than when protein is consumed in an energy balance deficit exceeding -400 kcal.
    - Null hypothesis: Consumption of protein when the athlete is + or – 400 Kcal of energy balance is not related to muscle mass when compared to protein consumed in an energy balance deficit exceeding -400 kcal

Chapter II

Review of Literature

Female athletes participating in appearance-focused sports are more likely to practice poor nutrition habits and are predisposed to disordered eating and associated bone density deficits (Torstveit et al. 2008). In young female athletes, this potential energy and nutrient deficiency during times of intense training is especially detrimental because of the additional nutritional needs associated with growth. Snacking may be essential to meeting these energy demands. However, the type of snack that is optimal for pregame performance is not clear (Sacheck et al. 2014). Studies have shown that young female athletes consume an inadequate, yet similar, caloric load and macronutrient distribution as their non-athlete counterparts (Soric et al. 2008; Michopoulou et al. 2011). These intakes are likely to result in deficiencies during periods of intense training.

Nutritional Concerns of Female Athletes

Energy restriction, with or without the presence of a clinical eating disorder, is necessary for the negative effects of the female athlete triad to be initiated (Marquez & Molinero, 2013). Low energy availability may be the result of greater energy expenditure associated with training exceeding the energy consumed through diet. This type of energy intake inadequacy is not always associated with disordered eating patterns or eating disorders. At particularly high risk of relative energy deficiency are athletes participating in appearance-focused or endurance sports (Melin et al. 2014).

Young females participating in sports while experiencing menstrual dysfunction, which is directly related to low energy availability, may experience losses in BMD and increased risk of skeletal injury (Nattiv et al. 2007). A cross sectional analysis of 44 elite female runners found a high incidence (63.9%; n=23) of menstrual dysfunction (Pollock et al. 2010). A subset of this study (n=7) had participated in two sequential yearly bone density scans and had baseline questionnaire data from the time of the first scan that was further analyzed. The analysis of this subset found that the athletes with oligo-/amenorrhea experienced bone loss. They found this to be particularly troubling in young athletes because, during a time in development when bone density should be increasing, they were experiencing BMD losses. They found that this loss in BMD was associated with low energy availability, which may be related to increased training time.

Female athletes might be more likely to spend time in a lower energy availability state than their male counterparts. In a study by Baker et al. (2014), dietitians monitored the 24-hour dietary intake of both male and female athletes and found that females tended to under consume required energy. The athletes observed were 29 skill/team sport athletes (22 male, 7 female) between the ages of 14-19. The male athletes' carbohydrate and protein more closely met recommendations than the female athletes. The authors noted that the athletes had access to a variety of both food and drinks throughout the day, suggesting that access was not an issue and the energy balance inadequacies witnessed were based on self-selected energy intakes.

In a study by Doyle-Lucas et al. (2010), female ballet dancers were more likely than recreationally active females to experience menstrual irregularities. This study recruited 15 dancers (age 24. +/- 1.3 yr.) and 15 age equivalent non-dancer controls (age 23.7 +/- 0.9 yr.) to compare differences between the physical and behavioral characteristics of the elite female dancers and the control group. Menstrual irregularities and significantly lower energy availability were more common in the ballet dancers than the controls. On average, the dancers began menses a year later than the controls. However, bone density was not significantly different between the dancers and controls.

Female athletes who are aware of problems associated with low energy balance and bone health may not be more likely to address the problems should they experience any abnormalities (Miller et al. 2012). This study of 191 female Australian athletes found that participants in lean sports were more likely to identify amenorrhea as a risk factor for poor bone health, however, they were less likely to take action when menstrual status was continuously interrupted. Athletes with a history of stress fractures were more likely to avoid taking action when experiencing menstrual abnormalities. This study demonstrates that educational programs may not be sufficient to promote athletes taking action when experiencing unhealthy behaviors, even if they are effective in promoting awareness of relationships between lifestyle patterns and problems associated with the female athlete triad.

Macronutrients and Body Composition

Assman et al. (2013) assessed 262 male and female participants between the ages of 18-25 to assess the effects of protein consumption on body composition. The subjects were assessed on anthropometric measurements and provided dietary records and information on socioeconomic factors from early life. The researchers found that, specifically in women, there was a relationship between higher protein consumption in adolescence and lower body fat in adulthood. This demonstrates the importance for protein consumption to be carefully monitored during puberty to promote a healthy body composition in young adulthood. Given the added physiological demands of participating in a high-level sport, it is important that efforts be made to ensure adequate energy intake and nutrient distribution, possibly by increasing protein while decreasing carbohydrate intake, at the age of puberty.

Another study found that total protein consumed might not reliably predict the impact on body composition (Mamerow et al. 2014). In this study, 8 healthy men and women with an average age of 36.1 participated in a 7-day crossover design with a 30-day washout period. They consumed the same amount of protein, either provided evenly across meals or skewed with lower protein consumed in the morning and higher protein consumed at night. The study confirms the idea that a single large consumption of protein does not favorably impact muscle synthesis. Rather, moderate amounts of protein spread evenly over three-meals/day increases muscle synthesis by 25% when compared to other protein consumption patterns.

In a study on 40 resistance trained men and women (average age 24), some subjects were asked to continue consuming their typical diet and maintaining their same exercise routine. Other subjects were asked to consume a high protein diet, containing 4.4 g protein/ kg body weight, while maintaining their same exercise routine and consuming the same carbohydrate and fat intake as before (Antonio et at., 2014). There were no differences in body composition in subjects with different dietary intakes. The authors noted this is surprising because the subjects did not consume an isocaloric diet. The high protein group increased their caloric intake by approximately 800 kcal/day for eight weeks without increasing training.

A study comparing the results on body composition using energy restricted diets containing differing amounts of protein and carbohydrate found protein to be more effective in reducing body fat (Evans et al. 2012). This study on middle aged men and women found that there were no significant differences in weight loss, fat mass, or lean mass.

Energy Balance and Body Composition

Maintaining a steady energy balance throughout the day is important to maintaining a healthy body weight and favorable FFM (Arciero et al. 2013). In a study of 30 overweight individuals, it was found that eating six higher protein containing meals per day resulted in lower body fat and higher lean body mass when compared to those eating the same amount of protein over three meals a day. All subjects had isocaloric intakes, highlighting the importance of macronutrient intake and eating frequency. As greater eating frequency is likely to better sustain energy balance throughout the day, this demonstrates the importance of not only what is consumed but the importance of frequency of intake for overweight subjects.

Favorable results of eating frequency were found with patients who have non-insulin dependent diabetes (Jenkins et al. 1992). Thirteen subjects consumed the same energy load on two separate days, once eating every four hours and once eating every hour. When the subjects ate every hour, they experienced lower concentrations of blood glucose and insulin. While this is a short study and does not provide information about long-term frequent eating patterns, it does show that when food is consumed evenly throughout the day insulin and blood glucose respond favorably. Avoiding hypoglycemia and hyperinsulinemia help to prevent the glucose and insulin spikes associated with higher body fat.

A study of 13 middle-aged overweight or obese men, with an average age of 51 years and an average BMI of 31.3 kg/m^2, showed the impact eating frequency has on insulin (Leidy et al. 2010). In this study, the subjects consumed either a normal protein or high protein meal plan, both of which contained the same amount of total energy. These diets were consumed as three-meals or six-meals/day on four separate days and in a random order. There did not appear to be a relationship between protein and eating frequency on body composition. However, in both protein groups there were larger insulin fluctuations with three meals than six meals.

An assessment of 48 male and 47 female non-obese subjects, Drummond et al. (1998) found that eating frequency was inversely related to lean body weight in men but not in women. The age of the subjects ranged from 20-55 years old and the BMI ranged from 18-30. They were recruited from a workplace setting and, therefore, had different levels of physical activity and fitness. The authors mentioned that physical activity appears to have an important influence on the relationship between eating frequency and body weight. It is noteworthy that the subjects did not have an increase in energy intake when they increased eating frequency.

A study on 24 young male, normal weight subjects found a relationship between eating frequency and adiposity (Chapelot et al. 2006). For this study, 12 subjects who normally consume three meals/day and 12 subjects who normally consume four meals/day, were recruited and asked to change their dietary pattern for 28 days by either adding or omitting the fourth meal. They provided three separate 3-day food diaries during this time. In order to prevent the added fourth meal being consumed as a snack, the lunch intake was decreased in the group who added the fourth meal. After the completion of the study it was found that the subjects who switched from four meals/day to three meals/day experienced an increase in fat mass. Energy intake was increased in lunch and dinner in this group but did not increase enough to compensate for the missing fourth meal. The results from this study suggest that eating more frequently is important for maintaining lower fat mass.

A study of 330 middle aged men between the ages of 45-64 found a relationship between eating frequency and body composition (Ruidavets et al. 2002). This study determined that when energy intake was held stable, higher eating frequency was associated with lower body fatness. Cofounding variables such as physical activity, smoking habits, age, educational level, and restrained diet or energy intake did not alter the relationship between body fatness and eating frequency. In this group, the subjects who ate five or more times were leaner than those who ate only once or twice a day, having both a lower BMI and waist to hip ratio.

The age and hormonal status of the subjects may impact the effectiveness of altering meal frequency and impacting body composition for women. In a study assessing 64 pre-and 50 post-menopausal women who did not report low energy intakes, it was found that there was no significant relationship between body fatness and eating frequency in premenopausal women. In post-menopausal women, however, it was found that there was higher body fatness with increased eating frequency (Yannakoulia et al. 2007). It is important to note that the subjects in this study were not assessed if they were considered to be low energy reporters to avoid underreporting.

Protein intake and Bone Density

A study of postmenopausal women found that high protein intake was related to lower BMD following weight loss (Campbell & Tang, 2010). This study included results from two different protocols, both of which were designed to be calorically restrictive with different amounts and types of protein. The results suggest that the higher protein groups had lower BMD. In addition, the subjects consuming protein from animal sources had lower BMD when compared to the subjects consuming vegetable proteins.

A contrasting study by Jesudason et al. (2013) found that, during weight loss, protein intake had no clinically significant effect on BMD in 323 postmenopausal women. The women were placed on isocaloric diets, with subjects instructed to have diets that contained at least 20 grams of protein difference between groups. For all subjects there was a decrease in BMD, but the authors noted that was to be expected in postmenopausal women. The differences in BMD were not found to be clinically significant between the two protein consumption groups.

Research conducted on 32 men and 7 women, with the average age of 21, determined that a high protein diet produced no change in BMD after 31 days (Cao et al. 2014). The diets consumed in this study provided two to three times the DRI for protein, but calcium and vitamin D were consumed at recommended levels. However, given the short nature of the study it is not possible to speculate on the long-term bone health following a diet this high in protein. These authors suggest that it is important, for maintaining calcium homeostasis, that calcium and vitamin D are consumed at recommended levels when protein is consumed in excess of the DRI. The size of the study sample was small and the subjects were mostly males. These factors make it difficult to determine how applicable the results are to female athletes.

A study on 560 premenopausal women between the ages of 14-40 showed no relationship between high protein diets and low BMD (Beasley et al. 2009). This study utilized longitudinal data from two separate cohort studies, both lasting five years. Protein intake was divided into low, medium and high protein intakes. The results found that those consuming higher protein diets do not develop lower BMD than those in the lower protein groups. However, this relationship may be dependent on age. The authors found suggestive, but not significant, evidence that women younger than 30 had a higher BMD with higher protein intake, while women over 30 had a inverse relationship between high protein intake and BMD. This study provided limited data related to the subjects chronic physical activity status.

A study of 32 young female swimmers between the ages of 11-15 found had similar BMDs when compared to non-athletes (Czeczelewski et al. 2011) They found that, while the subjects were in the normal BMD range, both groups consumed high levels of protein intake and low calcium levels. The authors noted that this combination (high protein and low calcium) of these two nutrients might negatively impact BMD on this young female population.

Chapter III

Methods

This research is a secondary analysis of previously existing data, and was approved by The Institutional Review Board of Georgia State University. Data analyzed were previously obtained as part of a project collecting information on the United States national gymnastics team. The gymnasts who participated were elite and highly competitive athletes from several training gyms across the country. This study performed a secondary analysis on the previously collected three-day food diaries and anthropometric measures.

Anthropometric Data

Body composition data were obtained using a dual energy x-ray absorptiometry (DEXA), including fat mass (FM), fat free mass (FFM) and bone mineral density (BMD) measurements. In addition to a total BMD value, results for the BMD at arms, legs, trunk, ribs, pelvis, and spine were measured. In addition to the body composition data acquired from DEXA, subjects' height and weight were collected using a standard physician scale.

Dietary Analysis

Gymnast files included 3-day food diaries. The food diary form was included in an information booklet, which contained information about serving sizes, including pictures to assist the athletes in providing the most accurate reporting possible. The food diaries were completed on three consecutive typical training days. When the initial data collection was performed all participating gymnasts had a personal interview by a Registered Dietitian to review the completed food diary. Information about what time foods and beverages were consumed was provided on the food diary form, which was used in the analysis. The software program used to analyze the food diary data was NutriTiming®, which provides information on the time spent in caloric energy balance, deficit, and surplus throughout the day. In addition to dietary intake patterns, daily activity was reported, which was input into NutriTiming® to provide hourly and end of day energy balance. NutriTiming® provided data informing what percentage of the diet was composed of protein, carbohydrate, and fat.

Data Analysis

All statistical analyses were performed using SPSS (version 20.0, SPSS, Inc., Chicago, IL). Demographic and anthropometric variables were described using frequency statistics. Correlations, t-tests, and ANOVA were used to assess the hypothesis.

Chapter IV

Results

Demographics

Forty female gymnasts completed the food diaries and were included in the results, shown in table 1.

Table 1. Subject Characteristics (N= 40)				
	Min.	Max.	Mean	Std. Dev.
Age (yr)	12	22	15.0	2.0
Weight (kg)	19.4	61.1	46.5	8.9
Height (cm)	129.5	185.4	151.8	8.9
Body Fat (%)	5.2	28.4	14.6	4.6
Fat Free Mass (kg)	17.3	55.6	39.5	7.2

The average body fat percentage was 14.63, which is considered a healthy body fat percentage (Rodriguez et al. 2009). However, the low end of the body fat percentage, 5.2%, is below 12%, which is the body fat percentage that is considered to be healthy for females. Menstrual status was part of the health history questionnaire administered at the time of data collection, and all subjects reported that they had amenorrhea.

Energy Balance

Thirty-eight of the subjects completed all three days of the food diary, while one of the subjects completed two days and one completed only one. However, since the three days were analyzed as one average day, all food diaries were used in the analyses. The protein index is the ratio of grams of protein consumed compared to kg of body weight. Characteristics of the three-day food diary analysis are shown in table 2.

Table 2. Subject Energy Balance Characteristics (N=40)

	Min.	Max.	Mean	Std. Dev.
Kcal consumed	-1592	2416	1313	665
Kcal expended	1331	2679	2199	285
Net energy balance	-1635	495	-803	563
Ending energy balance	-3304	1035	-1231	1102
Hours optimal (+/- 400kcal)	0	24	6.9	9.1
Hours surplus (+400 kcal)	0	16	0.7	2.7
Hours deficit (-400 kcal)	0	24	16.2	9.9
Hours anabolic	0	24	3.3	6.8
Hours catabolic	0	24	20.7	6.8
Highest energy balance	-1669	1076	-440.8	709.8
Lowest energy balance	-3304	204	-1515.7	978.1
Ratio of catabolic hours to PI	.00	35.3	18.1	9.3
Ratio of optimal EB to PI	.00	19.7	4.8	6.5
Ratio of anabolic hours to PI	.00	16.1	1.9	3.8

EB= energy balance, PI= protein index (g pro consumed/ kg body weight)

The energy balance relationships were determined based on a 24 hour time period. Because the three-day food diaries were recorded on three consecutive days, the ending energy balance from the previous day was used as the starting point for the next day, which provided variations in the beginning energy balance, with the average being -503 kcal (SD 625 kcal). Relationships between body fat percentage and energy balance are shown in table 3.

Table 3. Body Fat Percentage and Energy Balance (N=40)

		Body Fat (%)	EB_net	EB_Kcal/ kg_ total	EB_opt
Body Fat (%)	R	1.000	-.326	-.380	.077
	P	.	**.040**	**.016**	.636
EB_net	R	-.326	1.000	.728	.695
	P	**.040**	.	**<.001**	**<.001**
EB_ Kcal/kg_ total	R	-.380	.728	1.000	.391
	P	**.016**	**<.001**	.	**.013**
EB_ opt	R	.077	.695	.391	1.000
	P	.636	**<.001**	**.013**	.

EB_net= net energy balance, EB_kcal/kg_total= total energy balance consumed/ kg BW, EB_opt= time spent in optimal energy balance (+/- 400 kcal) R=correlation, P=probability

Optimal energy balance is defined as +/- 400kcal of perfect energy balance, where the calories consumed match perfectly with the energy expenditure. On average, most of the gymnasts spent more time in an energy balance deficit of at least -400 kcal EB than in a surplus of 400 kcal EB or within ±400 kcal EB. We found a significant inverse relationship with body fat percentage and net energy balance (r=-.326; p= .040) and total energy balance per kg of body weight consumed (r=-.380; p=.016). In general, higher energy intakes are associated with lower body fat percent.

Table 4. Body Fat Percentage and Anabolic and Catabolic Energy Balance

		Body Fat (%)	EB_ana	EB_cat
Body Fat (%)	R	1.000	-.028	.028
	P	.	.863	.863
EB_ana	R	-.028	1.000	-1.000
	P	.863	.	.
EB_cat	R	.028	-1.000	1.000
	P	.863	.	.

EB_ana= time spent in an anabolic energy balance, EB_cat= time spent in a catabolic energy balance, R= correlation, P= Probability

The gymnasts spent more time in a catabolic state as opposed to an anabolic state. However, there were no significant relationships between body fat percent and time spent either in a catabolic or anabolic state.

Bone Mineral Density

BMD values were measured during the DEXA scans for the head, arms, legs, trunk, ribs, pelvis, spine, as well as a total, overall BMD. The results of the descriptive BMD values are show in table 5.

Table 5. Subject Bone Mineral Density Values (g/cm2) (N=40)

	Min	Max	Mean	Std. Dev.
Arms	0.70	1.19	0.91	0.11
Legs	0.97	1.45	1.24	0.12
Trunk	0.76	1.31	0.98	0.11
Ribs	0.63	0.88	0.74	0.06
Pelvis	0.89	1.44	1.21	0.14
Spine	0.82	1.45	1.16	0.17
Total	0.94	1.32	1.15	0.10

Relationships between protein consumption and BMD were found to be statistically significant at the arms ($r= -0.535$; $p< 0.001$), legs ($r= -0.523$; $p= 0.001$), trunk($r= -0.517$; $p=0.001$), spine ($r= -0.472$; $p=0.002$), and pelvis ($r= -0.539$; $p< 0.001$). Diet composition and BMD are shown in Appendix A. The relationship between protein and BMD illustrate that the higher protein consumptions were associated with significantly lower BMD. This relationship further demonstrates the need for understanding between protein consumption and BMD, especially while in a consistent energy balance deficit.

Body Composition

Body composition and energy balance are detailed in appendix B. The relationships between body composition and macronutrients are not statistically significant. A t-test comparing subjects with higher (n=22) vs. lower (n=18) FFM, using the mean as the cut point, found statistically significant differences in protein (g/kg) consumption. The higher protein group had a statistically significant lower FFM.

T-tests were used to determine differences between FFM and energy balance surplus and deficits. Less time spent in an energy balance surplus was associated with a significantly higher body fat percentage (p=0.008). More time spent in an energy balance surplus was related to significantly higher FFM (p=0.008). The t-tests compared more (N=4) or less (N=36) time spent in an energy balance surplus with FFM and body fat percentage, using the mean as the cut off. T-tests examining the difference between time spent in an energy balance deficit and body composition found no differences between time spent in an energy balance deficit and FFM or body fat percentage.

Table 6. Fat Free Mass and Energy Balance (N=40)

		Fat Free Mass (kg)	**EB_net**	**EB_ kcal/kg_ total**	**EB_opt**
Fat Free Mass (kg)	R	1.000	-.209	-.214	-.129
	P	.	.196	.184	.428
EB_net	R	-.209	1.000	.728	.695
	P	.196	.	**<.001**	**<.001**
EB_ kcal/kg_total	R	-.214	.728	1.000	.391
	P	.184	**<.001**	.	**.013**
EB_opt	R	-.129	.695	.391	1.000
	P	.428	**<.001**	**.013**	.

EB_net= net energy balance, EB_kcal/kg_total= total energy balance consumed/ kg, EB_opt= optimal energy balance, R= correlation, P= probability

Table 6 shows the relationships between FFM and EB.

There were no significant relationships between fat free mass and net energy balance, total energy balance consumed per kg of body weight, and time spent in an optimal energy balance. Table 7 shows the relationship between time spent in anabolic and catabolic energy balance and fat free mass.

Table 7. Fat Free Mass and Anabolic and Catabolic Energy Balance (N=40)

		Fat Free Mass (kg)	EB_ ana	EB_cat
Fat Free Mass (kg)	R	1.000	-.226	.226
	P	.	.161	.161
EB_ana	R	-.226	1.000	-1.000
	P	.161	.	.
EB_cat	R	.226	-1.000	1.000
	P	.161	.	.

EB_ana= time spent in anabolic energy balance, EB_cat= time spent in catabolic energy balance, R= correlation, P= probability

There were no significant relationships between time spent in an anabolic energy balance or a catabolic energy balance.

Table 8. Fat Free Mass/ kg and Energy Balance (N=40)

		FFM/kg	EB_net	EB_Kcal/kg _ tot	EB_opt
FFM/kg	R	1.000	.327	.382	-.080
	P	.	**.039**	**.015**	.622
EB_net	R	.327	1.000	.728	.695
	P	**.039**	.	**<.001**	**<.001**
EB_kcal/ kg_ total	R	.382	.728	1.000	.391
	P	**.015**	**<.001**	.	**.013**
EB_opt	R	-.080	.695	.391	1.000
	P	.622	**<.001**	**.013**	.

FFM/kg= Fat Free Mass/ kg body weight, EB_net= net energy balance, EB_kcal/kg_tot= total energy balance consumed/ kg body weight, EB_opt= hours spent in optimal energy balance, R= correlation, P= probability

There are significant positive relationships between both fat free mass/kg and net energy balance (r=.327, p=.039) and total energy balance consumed/ kg of body weight (r=.382, p=.015). Higher amounts of both net energy balance and total energy balance consumed/ kg of body weight are associated with higher FFM/kg. The relationship between higher energy balance and higher FFM/kg is likely due to the fact the athletes spent majority of the time in an energy balance deficit and higher energy balance can be used to increase lean body mass.

Table 9. FFM/kg and anabolic and catabolic Energy Balance (N=40)

		FFM/kg	EB_ana	EB_cat
FFM/ kg	R	1.000	.029	-.029
	P	.	.859	.859
EB_ana	R	.029	1.000	-1.000
	P	.859	.	.
EB_cat	R	-.029	-1.000	1.000
	P	.859	.	.

FFM/kg= Fat Free Mass/ Kg Body Weight, EB_ana= time spent in anabolic energy balance, EB_cat= time spent in catabolic energy balance, R= Correlation, P= Probability

Table 9 shows the relationship between FFM/kg and anabolic and catabolic energy balance. There were no significant relationships between FFM/kg and the amount of time spent in either an anabolic or catabolic state.

Chapter V

Discussion and Conclusion

Athletes participating in appearance sports may be under consumers of energy and nutrients. While female athletes participating in appearance sports may not need to lose weight, it should not be assumed they have healthy LBM to FM ratios. These athletes may still experience the beneficial effects of protein and caloric distribution in changing body composition to more favorable LBM and decreases in body fat percentage.

While having a desirable range of macronutrient intake is important, focus should be placed on the consumption pattern to secure the most beneficial impact on FFM. Protein consumption appears to have the most beneficial effect on FFM when it is spread evenly throughout the day as opposed to consuming it in large quantities less often.

A systematic review found that, in healthy elderly populations, no clear association between protein and bone health could be determined (Pederson & Cederholm, 2013). While some studies appear to show protein having a protective effect on bone health, others have shown a negative effect. Despite possible negative effects on bone health, no detrimental effects were determined to be a result of protein intake. However they determined, based on available evidence, no UL for protein related to bone health could be determined.

As discussed in the joint position statement from the American Dietetic Association, Dietitians of Canada and the American College of Sports Medicine o nutrition and athletic performance, energy balance must first be satisfied before nutrients can be optimally used to impact body composition (Rodriguez et al. 2009). On average, the subjects spent 16 hours a day in an energy deficient state exceeding -400 kcal EB and 20 hours a day in a catabolic state less than 0 kcal EB. This is problematic considering the athletes will have difficulty recovering from the physical demands of competing and training at an elite level while spending majority of the time in a catabolic state. These values demonstrate a potential reason no significant trends related to macronutrient and body composition was found. This may suggest that protein is being used to satisfy energy needs rather than supporting other protein functions, including sustaining or increasing lean body mass. The relationship between protein intake and body composition may be better studied in a subject pool that does not spend a vast majority of the day in an energy balance deficit.

The results from this study suggest a relationship between higher protein diets and lower BMD. This result parallels previous research supporting the relationship between higher protein consumption lowering BMD (Campbell & Tang, 2010). However, it is not possible to conclude the bone health of these athletes is exclusively the result of the protein consumption. Another study found no relation between protein and BMD over the course of five years (Beasley et al. 2009). The conflicting previous research shows the complicated interactions between dietary and lifestyle factors and BMD. Further research should be conducted to attempt to further understand the complicated relationship between protein intake and BMD. The relationship between BMD and chronic energy deficit needs to be further understood. It may be possible that the decrease in BMD found in this study is related to a high protein diet and exasperated by the chronic energy deficit.

A significant inverse relationship was found between body fat percentage and both net energy balance and total energy balance/ kg of body weight consumed. Both of these relationships show higher energy intakes are associated with lower body fat percentages. Additionally, a significant positive relationship was found between FFM/kg of body weight and both net energy balance and total energy balance consumed/ kg of body weight. The relationship between higher energy balance and higher FFM/kg is likely due to the fact the athletes spent majority of the time in an energy balance deficit and higher energy balance can be used to increase lean body mass. A greater amount of time spent in an energy balance surplus of more than +400 kcal EB was associated with an increase in FFM. This supports other research that shows the beneficial effects of not allowing energy balance to fall into a deficit (Arciero et al. 2013). Eating meals frequently to prevent large variances in energy balance relates to a higher FFM. Frequent eating patterns limit hyperinsulinemic episodes (Jenkins et al. 1992). Preventing time spent in a hyperinsulinemic state is important because more time spent in a hyperinsulinemic state relates to a higher body fat percentage. There was no relationship between an energy balance deficit of more than 400 kcal and body composition. This contradicts other research that shows a decrease in body mass when in an energy deficit. (Carbone et al. 2012). They related energy deficits to decreased body fat mass and decreased fat free mass. The severity and large ranges of energy balance deficit in this study may impact the ability to draw any conclusions about the role of energy balance deficits of more than 400 kcal and body composition.

Limitations of this study are that the nutrition and activity data was self-reported. Additionally, because this study is an analysis of previously collected data, no further follow up was possible. The gymnasts in this study spent majority of the day in a severe energy balance deficit, which may limit the applicability of this research to other populations. There is a considerable range in subject ages, 12-22 yr, which is important because of differences in development at these ages. This study may be better studied with subjects who had more similar developmental patterns. However, since all the subjects reported amenorrhea the limitation from this is likely minimal.

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Appendix A. Protein Index and BMD (N=40)								
		BMD Arms	BMD Legs	BMD Trunk	BMD Ribs	BMD Pelvis	BMD Spine	BMD Total
PI	R	-.535	-.523	-.517	-.539	-.472	-.570	-.570
	P	.000	.001	.001	.000	.002	.000	.000

PI= Protein Index (gm protein / kg body weight). R=correlation. P=probability.

Appendix B. Energy Substrate and Energy Balance (N=40)					
		Protein (%)	Fat (%)	Carb (%)	Sugar (%)
Protein (%)	R	1.000	-.004	-.595	-.428
	P	-	.982	**<.001**	**.006**
Fat (%)	R	-.004	1.00	-.764	-.276
	P	.982	-	**<.001**	.085
Carb (%)	R	-.595	-.764	1.000	.524
	P	**<.001**	**<.001**	-	**.001**
Sugar (%)	R	-.428	-.276	.524	1.00
	P	**.006**	.085	**.001**	-
Pro Consumption per EB	R	-.210	-.056	.139	-.104
	P	.193	.733	.392	.522
Body Fat (%)	R	.212	-.131	**-.043**	-.059
	P	.190	.421	.792	.717
FFM (kg)	R	.061	-.180	.098	-.107
	P	.707	.267	.547	.511
FFM/ kg	R	-.211	.130	**.042**	.060
	P	.192	.423	.796	.712

R= correlation, P= probability