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Protein Intake and Body Composition in Collegiate Female Dancers

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PROTEIN INTAKE AND BODY COMPOSITION IN COLLEGIATE FEMALE DANCERS

By

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Table Of Contents

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Abstract

Protein is often recognized as the most influential macronutrient to lead to positive alterations in body composition. A diet higher in protein is not only beneficial to athletes, but also a potentially effective strategy used to promote weight loss. Recent studies suggest that an increased protein intake with or without strenuous physical exercise can lead to beneficial changes in fat mass and lean mass. This study was conducted to assess the association between protein consumption and body composition in 30 collegiate female dancers. Subjects completed a 3-day dietary food log followed by analysis of body composition (dual-energy x-ray absorptiometry). Subjects were then stratified into one of three categories (low, moderate, high) of protein consumption. Fat mass and lean mass were correlated to the level of protein intake (g/kg) and to each group of protein intake. No associations were noted between protein consumption and total fat mass or lean mass percentage. There were also no significant associations between protein intake and bone mineral density (BMD). Although not significantly different, the high protein group had lower body weight when compared to low protein and moderate protein consuming groups (55.11 $\pm$ 9.22 kg, 60.38 $\pm$ 4.58 kg, respectively). The higher protein groups also had lower fat mass percentage than the moderate and lower level protein groups, although the results were not statistically different. The results of this study demonstrate no association between protein intake and body composition in female collegiate dancers.

Introduction

Proteins are often referred to as the building blocks of the body. Due to several structural and regulatory functions, protein is critical for optimal performance in athletes worldwide (1). According to the American Food and Nutrition Board, the recommended dietary allowance (RDA) for adults is 0.8 g/kg of body weight. This value, however, has stimulated controversy among dieticians and sport nutritionists due to a plethora of evidence supporting that a higher intake may be required for optimal health, body composition, and performance (23, 25, 28, 29). Likewise, the current RDA does not account of physical activity levels which are known to increase the need for protein in the diet (21, 25). Despite the form of exercise, a higher protein intake is required in individuals who regularly engage in exercise in order to recover and regenerate muscle proteins to support movement (2,6,7). Studies show that both endurance athletes and strength/power athletes require protein consumption values between 1.2-1.8 g/kg per day to support the metabolic demands placed on body (2, 6, 21). These demands of the sport and training adaptations between individuals justify the differing values of protein requirements between athletes (26).

Although there is a demand for higher protein consumption in active individuals, athletes that participate in aesthetic sports such as dancing, gymnastics, and cheerleading, commonly under consume macronutrients to maintain a low body weight (3). Caloric restriction has become an increasingly widespread problem for these athletes, and the demands of a lean physical appearance typically influence dancers to restrict their diet in efforts to produce modifications in body composition. Although a decrease in caloric intake may lead to lower body weight, an increase in protein consumption has also been shown to have beneficial effects on body composition, more specifically, fat mass and lean mass (2). Many studies conclusively reveal that higher protein intake combined with physical activity aids in weight loss and also helps prevent weight re-gain (4,6,11,15). In relevance to athletes, combining a high protein diet (1.2-1.8 g/kg) along with physical activity may also lead to an increase in lean mass through increases in lean tissue accruement, and a decrease in total fat mass (5,21).

Background

Proteins are large complex molecules that are involved in numerous metabolic functions in living organisms. Proteins act as catalysts for reactions, act as transporters in the blood, influence fluid balance, serve as buffers, replicate DNA, and have many additional structural roles (5,8). Protein is found mostly in skeletal muscle, but also in bone, blood, skin, and other tissues in the body (5,7). Protein is composed of smaller organic compounds known as amino acids linked together by peptide bonds. Each protein has its own unique amino acid sequence that aids in its classification (4). Amino acids can be classified by essentiality, net charge, polarity, and by structure (5). All amino acids contain a central carbon atom that is bound to one amino group, one carboxyl group, and a differentiating side chain. When classifying by structure, amino acids can be categorized as: Alipathic, Hydroxylic, Sulfur-containing, Carboxyl/Amide, basic, aromatic, or Imino Acid (5). The various structures of the side chains help differentiate between the diverse classes of amino acids. When classifying based on net charge, amino acids are deemed as positively charged, negatively charged, or neutral. (5). Amino acids may also be termed as polar, non-polar, or basic depending on their respective side chains. Polar side chains contain acids, amides, alcohols, and amine functional groups in their structure. Non-polar side chains include hydrocarbon alkyl and aromatic functional groups, where as basic side chains are composed of amine functional groups. When classifying by essentiality, amino acids are labeled as either essential, non-essential, or conditionally essential. Humans are only capable of producing eleven of the twenty amino acids, commonly referred to as non-essential amino acids. Essential amino acids make up the remaining nine amino acids.

Essential amino acids cannot be synthesized from other compounds in the body, so they must be supplied through the diet and supplementation. An increase in dietary intake of essential amino acids can increase muscle synthesis in individuals (18). Conditionally essential amino acids are typically not essential amino acids, but become essential during unforeseen circumstances, such as injuries and infections, resulting from an inability of a non-essential amino acid to be processed (5). More relevant to athletes are the three amino acids known as branched chain amino acids (BCAAs). The three BCAAs (leucine, valine, and isoleucine) play a considerable role in exercise performance by stimulating protein synthesis and reducing protein and muscle breakdown during strenuous exercise (7). BCAAs are essential amino acids, meaning they must be consumed in the form of dietary intake. Protein can be found in both exogenous and endogenous sources. The sources with the highest amount of protein content are animal sources such as meat, poultry, fish, eggs, and dairy products. Plant products are also an exogenous source, and contain some source of protein through legumes, cereals, beans, grains, nuts, and seeds (5). Other endogenous sources of protein are produced by desquamated mucosal cells and digestive enzymes and glycoproteins in the body (5).

Due to the extensive contribution protein has in metabolic functions, much debate has arisen regarding the optimal protein intake for athletes. Strength training athletes typically have a larger protein requirement in comparison to endurance athletes due to the increased muscle fiber breakdown during exercise. Nevertheless, most sports involve a combination of both strength and endurance, so the estimated range for athletes can be generalized over a larger span versus specifically by sport (7). Protein quality is also a determinant in the level of influence protein has on body weight and composition. Protein from varying sources produce differing satiety levels, and also have diverse metabolic effects on the body (4). The highest quality proteins reported to beneficially influence athletes are milk proteins (whey and casein), soy, and egg protein (7).

Amino Acid Metabolism

Amino acids are catabolized in varying degrees of tissues, but occur mostly in the liver in order to produce or store energy (5). Glucogenic amino acids, such as glycine, arginine, and valine, can be catabolized for energy in gluconeogenesis, and can also be converted to glycogen for energy storage. Ketogenic amino acids, such as lysine and leucine, are catabolized in the Krebs Cycle and can be converted to ketone bodies or fatty acids. The primary step in the metabolism of amino acids involves the removal of the amine group through the process of either transamination and/or oxidative deamination. In a transamination reaction, an amine group is transferred from an amino acid to an α–keto acid (17). The α–keto acid now becomes an amino acid, and the amino acid that originally lost its amine group now becomes the α–keto acid. Enzymes known as aminotransferases couple with vitamin B6 to catalyze these transaminase reactions, which generate either glutamate or aspartate. The other catabolic reaction amino acids undergo is known as oxidative deamination. This reaction involves the removal of an amine group from an amino acid, but unlike transamination, it does not transfer to another compound (5,17). During this reaction, both the amine group and the hydrogens located on the amino acid are removed in order to form the non-toxic products ammonia and hydrogen peroxide, which are disposed of through the urinary cycle. In addition to these chemical reactions, dietary intake and the gastrointestinal tract can also aid in the synthesis of ammonia. Once ammonia is formed, it is converted into urea so that it may be excreted through the urea cycle. The urea cycle consists of five reactions that convert ammonia to urea or uric acid, because these two products are much less toxic to the body (5).

Protein Influence On Skeletal Muscle

One of the most common methods used to assess protein metabolism and requirements is known as a nitrogen balance (9, 23). A nitrogen balance curve is drawn through calculating the measure of nitrogen input minus nitrogen output (18). Positive nitrogen balance indicates there is more nitrogen in the body than what is being excreted, and is commonly associated with skeletal muscle synthesis in response to the body reaching an anabolic (muscle building) state (18). In other words, this occurs when protein intake surpasses protein excretion, meaning the body is retaining the needed amounts of protein (23). Negative nitrogen balance is achieved when more nitrogen is being excreted from the body than is being used, and is often seen in times of malnutrition due to an increase in muscle protein breakdown. In order to achieve skeletal muscle hypertrophy, muscle protein synthesis (MPS) must exceed muscle protein breakdown (MPB). Physical activity paired with an increase in dietary protein leads to muscle hypertrophy due to the achievement of a positive protein balance (9). Resistance training, similar to dancing calisthenics, allows the body's metabolic pathway to reach an anabolic state where protein accretion and muscle fiber hypertrophy may arise (14). Significant contraction of muscles, as seen in resistance training exercises, in addition to protein consumption leads to this anabolic state (23). Essential amino acids obtained through exogenous food sources serve as regulators in MPS (14). The three BCAAs have a profound effect on skeletal MPS as well. Leucine, in particular, stimulates MPS by initiating signaling pathways to enhance protein synthesis upon exercise completion (6,7). Leucine also donates nitrogen to glutamine and alanine, which both serve crucial roles in muscle reformation (7). MPS is a highly energetic consuming process that the body must undergo to reform muscles. If an individual is not consuming enough dietary protein, a restriction in energy will result, and the balance between MPS and MPB will shift from a neutral to a negative balance (6). A negative protein balance promotes the breakdown of muscle tissue, resulting in a loss of lean body mass. The effects of skeletal muscle mass loss can pose critical risks for athletes, such as an increase in susceptibility to injury (14).

Protein Influence On Adipose Tissue

An increase in muscle protein synthesis not only leads to muscle growth, but it also plays a vital role in the enhancement of fat loss. The beneficial effects that account for an increase in fat loss with higher protein intake are due to an increase in energy expenditure (diet-induced thermogenesis), and an increase in satiety (4). Thermic effect of protein can be defined as the energy that is needed by the body to process, absorb, and excrete the nutrients ingested through diet (16). Protein has the largest thermic effect percentage in comparison to both carbohydrates and fats, therefore resulting in the highest energy expenditure. Research reveals that the larger the quantity of protein consumed, the higher the thermal effect (2). A diet lower in carbohydrate and higher in protein consumption is also shown to have advantageous effects on body weight homeostasis. The synergistic relationship between pairing increased protein intake along with exercise will therefore lead to a preservation of muscle mass and in increase in fat loss (24). Other contributions to the regulation of fat mass is through satiety related hormones leptin, and cholecystokinin (CCK). Leptin, a hormone that functions in appetite and weight control, experiences enhanced effects on the body when combined with protein intake (15). Insulin, another hormone associated with weight control, functions synergistically with leptin to signal the central nervous system to decrease appetite (15). Insulin may act by suppressing hunger itself, or by indirectly modulating the secretion of leptin (15). Leptin circulates in the hypothalamus in search of cells expressing corresponding leptin receptors. Once bound to the receptor, specific neurons are then able to produce a satiety feeling by suppressing appetite (20).

Both mechanisms account for a loss in fat mass with an increase in protein intake. A limited protein diet has opposite effects on the preservation of fat mass. If a negative protein balance is achieved, the cell is forced to prioritize energy expenditure. Muscle synthesis is an energetically costly mechanism, meaning the body will be required to store the excess energy as fat if it experiences a lower energy constraint (6). A low protein diet has been shown to lead to approximately 90% of extra energy in the body being stored as fat (11). Therefore, a reduction in energy availability due to diminished nutrient absorption may cause an increase in fat mass preservation.

Protein Influence On Bone

Bone Mineral Density (BMD) reveals possible risks for developing stress factures and osteoporosis later in life (12,13). Female dancers often have below average BMD due to a lack of nutrients caused by an inadequate diet and calorie intake (13). Negative risk factors for BMD include: malnutrition, hormonal instability, and prolonged strenuous exercise. Positive risk factors include: exercising, lean mass, and dietary calcium consumption (12). Dancers can have both negative and positive risk factors contributing to low BMD and injuries. Although protein greatly affects BMD, calcium intake serves a large function in the formation of bones. An insufficient consumption of calcium along with protein does not present as many beneficial effects to bone mineral density (13). Other minerals such as phosphorous and magnesium also affect bone health by providing structure during the formation of bone (13). Physical activity positively contributes to bone strength, and is a positive risk factor in dancers. However, if an athlete is malnourished and is experiencing a restriction in energy availability, a decrease in overall bone mass will occur (13).

Protein Requirements

Protein is considered a key nutritional component to athletes due to its ability to stimulate the re-growth of skeletal muscles (7). Studies show that an RDA value of 0.8 g/kg of protein per body weight for a typical athlete is not great enough to reach a balanced nitrogen curve (21, 24). Increasing protein intake in addition to performing physical exercise will cause muscular benefits by allowing the body to reach a state of positive nitrogen balance that is needed for damaged muscle fibers to repair (21, 27). Several controversies exists between what an optimal protein requirement in an athletic population should be. Tarnopolsky et al. revealed that an increase in protein is needed in an athletic population due to an increase in amino acid oxidation during exercise. Amino acid oxidation is increased during strenuous activity because of the shift from carbohydrate to amino acids as the primary source of energy (28). However, this hypothesis is shown to be more relevant to endurance training athletes. Resistance training athletes typically undergo more muscle damage, and will therefore require more protein to aid in the synthesis of new muscle (24). Both forms of exercise demonstrate a difference between what is an optimal protein intake value, but still concurrently states that an increase from the RDA value is required for physically active individuals. Therefore, a protein diet concurrent with resistance and/or endurance training athletes undergo should lead to low fat mass and high lean body mass.

Body Composition Ideology in Dance

The desire to achieve weight loss is a customary goal amongst dancers due to the aesthetic nature of the sport. Many dancers increase the load of training and decrease total food consumption in order to achieve optimal fat loss (6). Protein is an essential macronutrient in an athlete's diet because of its extensive role in muscle protein synthesis, repair and thermogenesis. Female aesthetic athletes are often not in a positive energy balance because the environmental pressures of maintaining a lean physique overpower the concern for consuming an adequate diet

(7). Research has demonstrated that under-consuming protein can have adverse effects such as an increase in fat mass and a decrease in lean mass, and a higher dietary protein intake will attenuate the loss of fat free mass (19).

Protein is shown to have a positive association with body composition, energy availability, thermogenesis, and satiety levels (19). Athletes aiming to reduce fat mass while maintaining lean muscle mass should then consume higher quantities of protein than the RDA values advise. Therefore, the purpose of this study was to investigate the association between protein and body composition (fat mass and lean muscle mass), in female collegiate dancers. It is hypothesized that a diet high in protein is associated with lower fat mass and higher lean muscle mass compared to diets lower in protein.

Methods

Participants

Thirty collegiate female dancers (n=30) between the ages of 18 and 28 years were recruited to participate in this study. The participants were informed of the study protocol and the potential risks and benefits associated with the study. Prior to testing, all procedures were approved by the Florida State University Institutional Review Board and informed consent was obtained by all participants. Participants were recruited from the Florida State University Dance Department as well as local Tallahassee dance studios. For participants to be eligible in the study, they must have met the following criteria: A minimum of 8 consecutive years of dance training as well as dancing a minimum of two times per week. Non-smoking, and have had no significant musculoskeletal injuries or other medical conditions over the past 6 months. No contraindications to exercise based on the American College of Sports Medicine (ACSM) and

American Heart Association (AHA). Free of uncontrolled hypertension, may not be currently taking blood pressure medications, or have been diagnosed with cardiovascular disease, stroke, diabetes, thyroid, or kidney dysfunction. No risk factors for cardiovascular disease as determined by ACSM guidelines.

Testing Procedure

The initial visit consisted of an informational session, which involved a thorough explanation of the purpose of the study as well as the risks and benefits associated. All participants signed a written informed consent, and a three-day dietary food log was then distributed. The food logs were completed over three-days and included two weekdays and a one-weekend day. Participants were asked to maintain normal eating patterns and to record all dietary intake as precisely as possible. The remainder of the information session was used to provide the participants with time to ask any questions or express any concerns regarding the study. Participants were informed that the second visit would include completing a Dual Energy X-Ray Absorptiometry (DXA; Hologic, Marlborough, MA) scan where body composition would be analyzed.

Once the food log had been completed, participants were asked to schedule a time to complete a second visit. All testing was conducted in the Institute of Sports Sciences and Medicine (ISSM) at Florida State University. Upon arrival of the second visit, all food logs were collected and screened and the corresponding data was analyzed using the Food Processor dietary analysis software (McGraw-Hill 10.13.1, New York, NY). Participants were asked to wear a sports bra and spandex shorts in preparation for the DXA scan. Participants were also asked to complete a medical history form and a secondary informed consent to further explain the risks associated with the DXA scan. Anthropometric measurements (height and weight) were recorded using a wall-mounted stadiometer and a digital scale (SECA 216, Hamburg, Germany). Once these measurements were obtained, participants then lied supine on the scanning bed where a certified DXA technician placed both hands and feet in a secure position to ensure no unwanted movements occurred during the scan.

Analysis

Once the scans were complete, weight (kg), fat total (kg), fat percentage (%), lean total (kg), lean percentage (%), appendicular skeletal muscle mass (ASM), and appendicular skeletal muscle mass index (ASMI) were analyzed. Once the food logs were entered into the database (Food Processor, McGraw-Hill 10.13.1, New York, NY), total calories, calories from fat, calories from carbohydrates, calories from protein, total fat (g), total carbohydrates (g), total protein (g), fat percent (%), carbohydrate percent (%), protein percent (%), protein compared to body weight (g/kg), Cholesterol (mg), Vitamin D (IU), Calcium (mg), and Phosphorous (mg) were analyzed.

Statistics

Protein was expressed as g/kg/d and divided into tertiles (Tertile 1: low protein-LP, n=9; Tertile 2: moderate protein-MP, n=10; Tertile 3: high protein-MP, n=10). LP consumed $\leq$ 1.18 g/kg, MP consumed between 1.19-1.55 g/kg, and HP consumed $\geq$ 1.56 g/kg of protein. An initial power analysis testing for a 2.3 kg difference in fat mass between the LP and HP groups suggested that 30 participants in each treatment group would provide 80% power to reject the null hypothesis (30). All data was analyzed using one-way analysis of variance (ANOVA) to compare group means and further Tukey Post Hoc testing was used determine group differences

(SPSS v.21.0 SAS, Cary, NC.). Significance was set at $P < 0.05$ and all data is reported as means ± SD.

Results

A retrospective power analysis with the 30 total participants who completed the study provided a power of 0.44. Data for all participants are included in analysis except for one participant who consumed greater than 2SD's (3.3 g/kg/dy) than the mean protein intake for the entire population (1.6 g/kg/dy). There were no significant differences in participant characteristics (**Table 1**).

Average dietary intake is shown in **Table 2**. There were no significant differences between LP and MP or MP and HP for total daily caloric intake. However, there was a significant difference in total daily caloric intake between LP and HP (1,876.60 ± 471.46 vs. 2,465.25 ± 334.85, P = 0.011) (**Table 2**). Significant differences were also seen when comparing total protein intake between all groups. The comparison between protein tertiles and lean mass % are presented in **Figure 1**. No differences were observed between fat and carbohydrate compositions of the diet in any of the tertiles. Total cholesterol intake was significantly greater in MP than LP (P = 0.018). Calcium was also significantly greater in HP than LP (+377 mg; P = 0.017). All other minerals analyzed in this study showed no significant differences between groups.

There were no significant differences between any body composition measurements of any group. No differences in body weight, lean mass, fat mass, weight, ASM or ASMI were observed between the groups (**Table 3**). The association between protein tertiles and body weight (kg) are presented in **Figure 2.** Although not significantly different, the HP group had lower body weight when compared to LP and MP groups (55.11 ± 9.22 kg, 60.38 ± 4.58 kg, respectively). Fat mass % was lower in HP and MP compared to LP. Protein tertiles in comparison to fat mass % are shown in **Figure 3.** There were no significant differences among groups for BMD (**Table 3**). **Table 4** shows the protein spread % between all groups and reveals how closely related each classified group is to each other. There was no difference in the protein spread % between groups (**Table 4**).

Table 1
Descriptive characteristics by tertile of total protein intake

	LP	MP	HP
Total (*n*)	9	10	10
Demographics			
Age	22.11 ± 2.76	20.70 ± 1.57	19.8 ± 1.23
YOD	16.55 ± 3.68	14.00 ± 3.90	15.80 ± 1.23
Anthropometrics			
Height (m)	1.67 ± 0.08	1.68 ± 0.07	1.64 ± 0.07
Weight (kg)	60.95 ± 8.18	60.38 ± 4.58	55.11 ± 9.22
BMI (kg/m^2)	21.79 ± 2.22	21.52 ± 1.68	20.39 ± 2.71

[1] Mean ± SD (all such values).
[2] T1 (low protein tertile, •1.18 g/kg); T2 (moderate protein tertile, 1.19-1.55 g/kg); T3 (high protein tertile, •1.56 g/kg); YOD, years of dance; BMI, Body Mass Index

Table 2
Dietary Intake

	LP (P = LP:MP)	MP (P = MP:HP)	HP (P = LP:HP)
Total (*n*)	9	10	10
Total (kcal/d)	1,876.60 ± 471.46 (*P* = 0.298)	2,154.22 ± 375.09 (*P* = 0.223)	2,465.25 ± 334.85 $^{+}$ (*P* = 0.011)
Fat (kcal/d)	620.32 ± 223.47 (*P* = 0.242)	786.69 ± 180.77 (*P* = 0.942)	819.71 ± 250.93 (*P* = 0.150)
Fat (g)	98.92 ± 24.83 (*P* = 0.241)	87.44 ± 20.11 (*P* = 0.940)	91.18 ± 27.91 (*P* = 0.148)
Fat (%)	32.34 ± 4.41 (*P* = 0.416)	36.70 ± 7.15 (*P* = 0.562)	33.21 ± 9.50 (*P* = 0.968)

Carbohydrate (kcal/d)	1,005.78 ± 296.48 (*P* = 0.888)	1,072.10 ± 298.29 (*P* = 0.399)	1,259.50 ± 333.85 (*P* = 0.211)
Carbohydrate (g)	251.45 ± 74.12 (*P* = 0.888)	268.03 ± 74.57 (*P* = 0.399)	314.87 ± 83.46 (*P* = 0.211)
Carbohydrate (%)	54.03 ± 9.84 (*P* = 0.566)	49.29 ± 8.43 (*P* = 0.919)	51.10 ± 11.72 (*P* = 0.810)
Protein (kcal/d)	215.81 ± 29.14 * (*P* = 0.008)	326.98 ± 44.85 $^{\#}$ (*P* = 0.014)	428.85 ± 116.83 $^{+}$ (*P* = 0.000)
Protein (g)	53.95 ± 7.28 * (*P* = 0.008)	81.50 ± 11.21 $^{\#}$ (*P* = 0.014)	107.21 ± 29.21 $^{+}$ (*P* = 0.000)
Protein (%)	11.96 ± 2.42 (*P* = 0.117)	15.37 ± 2.27 (*P* = 0.381)	17.60 ± 5.35 $^{+}$ (*P* = 0.007)
Protein (g/kg)	0.89 ± 0.14 * (*P* = 0.000)	1.35 ± 0.11 $^{\#}$ (*P* = 0.000)	1.93 ± 0.33 $^{+}$ (*P* = 0.000)
Cholesterol (mg)	109.34 ± 76.69 * (*P* = 0.018)	316.79 ± 219.22 (*P* = 0.834)	276.14 ± 116.58 (*P* = 0.073)
Vitamin D (IU)	30.76 ± 25.40 (*P* = 0.700)	88.29 ± 81.48 (*P* = 0.812)	132.15 ± 257.73 (*P* = 0.360)
Calcium (mg)	432.69 ± 182.26 (*P* = 0.156)	669.05 ± 276.45 (*P* = 0.500)	809.86 ± 327.35 $^{+}$ (*P* = 0.017)
Phosphorus (mg)	404.18 ± 306.69 (*P* = 0.560)	560.77 ± 337.42 (*P* = 0.992)	578.71 ± 338.45 (*P* = 0.506)

[1] Mean ± SD (all such values).
[2] LP (low protein tertile, •1.18 g/kg); MP (moderate protein tertile, 1.19-1.55 g/kg); HP (high protein tertile, •1.56 g/kg); * indicates significant differences (*P* • 0.05) between LP and MP; $^{\#}$ indicates significant difference (*P* • 0.05) between MP and HP; $^{+}$ indicates significant differences (*P* • 0.05) between LP and HP.

Table 3
Body Composition Analysis

	LP (P = LP:MP)	MP (P = MP:HP)	HP (P = LP:HP)
Total (*n*)	9	10	10
Weight (kg)	60.95 ± 8.18 (*P* = 0.985)	60.38 ± 4.58 (*P* = 0.282)	55.11 ± 9.22 (*P* = 0.231)
Fat Mass (%)	27.70 ± 4.24 (*P* = 0.838)	26.35 ± 3.15 (*P* = 0.745)	24.71 ± 5.74 (*P* = 0.414)
Lean Mass (kg)	40.23 ± 5.51 (*P* = 0.925)	41.12 ± 2.43 (*P* = 0.481)	38.52 ± 5.16 (*P* = 0.739)
Lean Mass (%)	68.59 ± 4.12 (*P* = 0.747)	70.34 ± 3.71 (*P* = 0.896)	71.34 ± 5.54 (*P* = 0.475)
ASM (kg)	18.94 ± 2.81 (*P* = 0.986)	19.15 ± 1.69 (*P* = 0.646)	18.04 ± 2.91 (*P* = 0.762)
ASMI (kg/m^2)	6.71 ± 0.83 (*P* = 0.978)	6.64 ± 0.43 (*P* = 0.533)	6.28 ± 0.73 (*P* = 0.436)
BMD	1.11 ± 0.08 (*P* = 0.901)	1.13 ± 0.07 (*P* = 0.899)	1.11 ± 0.09 (*P* = 1.000)

[1] Mean ± SD (all such values).
[2] LP (low protein tertile, •1.18 g/kg); MP (moderate protein tertile, 1.19-1.55 g/kg); HP (high protein tertile, •1.56 g/kg); * indicates significant differences (*P* • 0.05) between LP and MP; $^{\#}$ indicates significant difference (*P* • 0.05) between MP and HP; $^{+}$ indicates significant differences (*P* • 0.05) between LP and HP. ASM, Appendicular Skeletal Muscle; ASMI, Appendicular Skeletal Muscle Index; BMD, Bone Mineral Density.

Table 4
Protein Spread: % Difference Between Tertiles

Tertile Comparison	(%)	(g/kg)
LP:MP	33.0	0.66
MP:HP	35.0	0.65
LP:HP	57.0	0.43

[1] LP (low protein tertile, •1.18 g/kg); MP (moderate protein tertile, 1.19-1.55 g/kg); HP (high protein tertile, •1.56 g/kg)

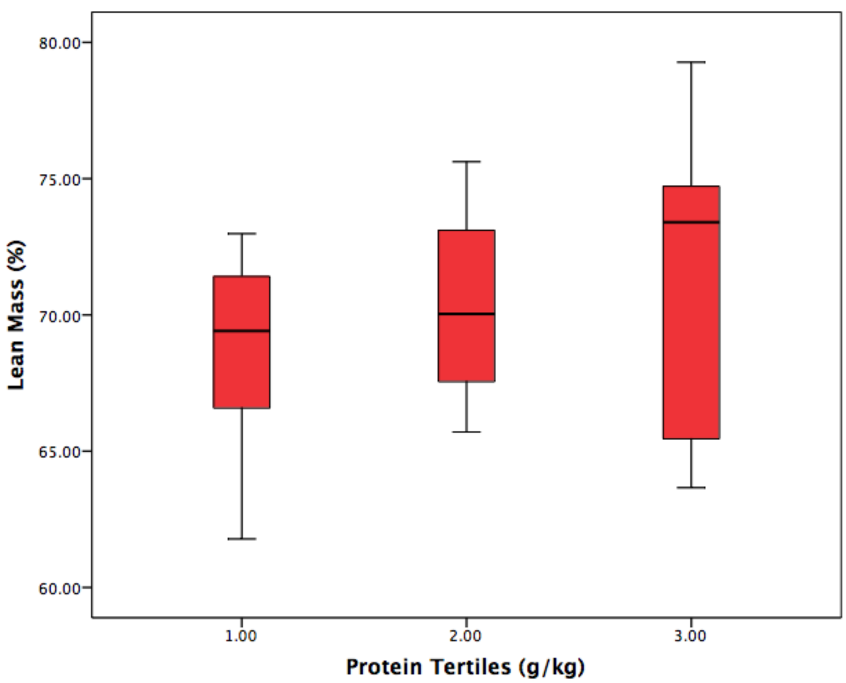

Figure 1. Total lean mass percentage compared to each tertile group of protein intake in g/kg. 1.00 = low protein (≤1.18 g/kg), 2.00 = moderate protein (1.19-1.55 g/kg), 3.00 = high protein (≥1.56 g/kg).

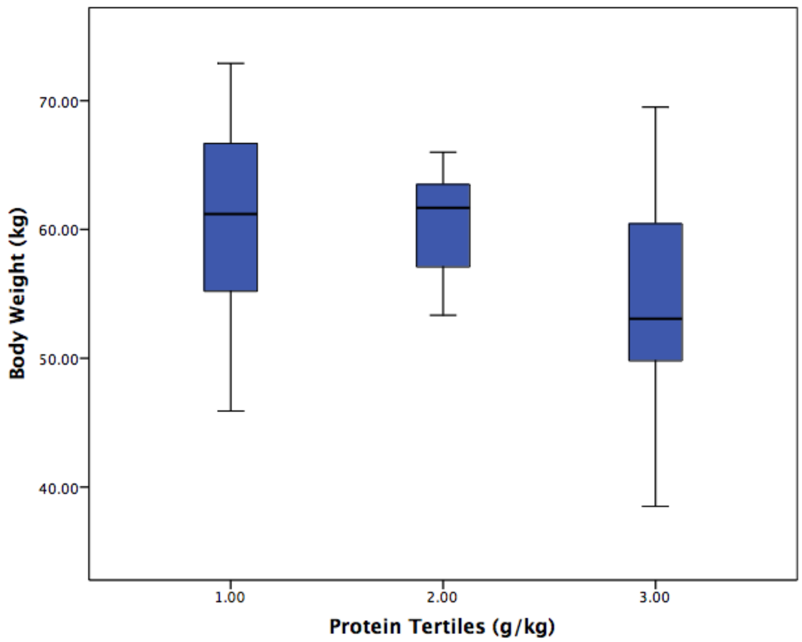

Figure 2. Total body weight in kg compared to each tertile group of protein intake in g/kg. 1.00 = low protein (≤1.18 g/kg), 2.00 = moderate protein (1.19-1.55 g/kg), 3.00 = high protein (≥1.56 g/kg).

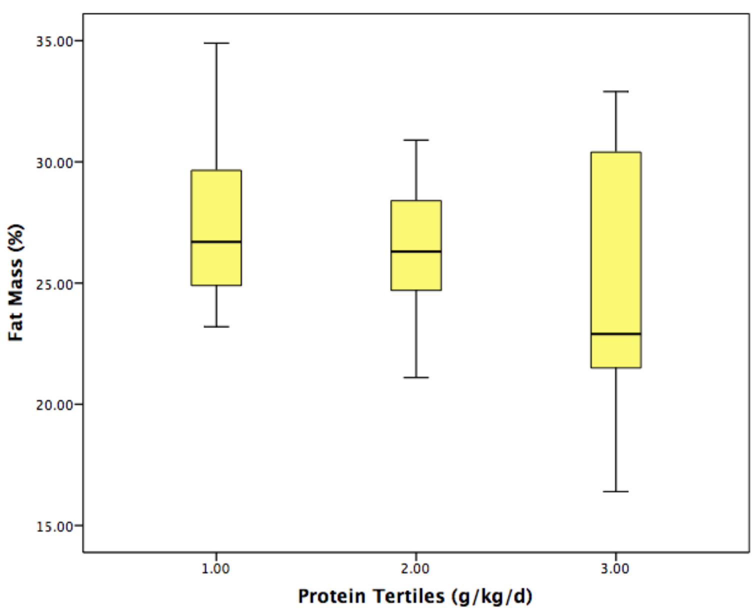

Figure 3: Total fat mass percentage compared to each group of protein intake in g/kg. 1.00 = low protein (≤1.18 g/kg), 2.00 = moderate protein (1.19-1.55 g/kg), 3.00 = high protein (≥1.56 g/kg).

Discussion

The purpose of this study was to investigate the association between protein and body composition (fat mass and lean muscle mass), in female collegiate dancers. The primary findings from this study are that HP results in lower body weight than LP diets. However, no other statistically significant associations were shown in this study for fat mass or lean muscle mass. Interestingly, although not significant, HP appeared to be associated with improved total fat mass percentage, lean muscle mass, and overall body weight compared to LP. Although previous studies suggest that a higher protein intake leads to positive alterations in body composition, this present study did not support this hypothesis. Arciero et al., revealed lower body weight and body fat in HP groups as compared to LP groups (31). Similarly, Weigle et al. (15) showed that participants consuming an isocaloric high protein diet had a decrease in energy intake, body weight, and fat mass. These studies both successfully demonstrate an association between a high protein diet and positive modifications in body composition.

Bosse et al. hypothesized a theory that may help explain the discrepancies between the results of this study and others. This theory, “protein spread”, states that there must be a significant percent difference (>58.4%) in g/kg/day of protein intake between all groups observed (27). LP compared to MP reveals a 33% difference between groups; MP compared to HP reveals a 35% difference between both groups, while as LP in comparison to HP reveals a 57% difference. This theory may account for the absence of significant differences observed in body composition between the populations. Nevertheless, the magnitude of difference between groups was apparent, albeit not statistically significant, and indicated a benefit to consuming more protein in the diet.

Several other factors such as the dietary food log and type of dancing may have also affected the results of this study. The dietary food logs may present inaccurate information through incorrect portion sizes or failing to document all items consumed. However, researchers verified all food intakes and personally questioned each participant to mitigate this issue. Although the results demonstrate that the amount of protein consumed in a typical diet of female collegiate dancers was not positively associated with body composition, HP was associated with lower body weight compared to LP intake. The body composition data agrees with Hoffman et al., that report protein intakes above the RDA in strength/power athletes had no positive alterations in body composition (21). Strength gains were seen throughout all 3 groups measured, but the data revealed no significant differences between the groups. Antonio et al also showed no changes in body weight and lean body mass in resistance-trained individuals consuming a high intake of protein (2). However, generally, the higher protein consuming groups in the present study were trending towards a lower overall fat mass percentage. This reveals that a protein intake above the RDA may actually positively influence body composition in dancers. Arciero et al., reported that increasing the intake of protein more frequently and consuming more meals a day led to a decrease in fat mass and increase in lean muscle mass (31). Similarly, Baer et al., revealed a decrease in fat mass after 6 months of protein supplementation (30).

The difference between our findings and that of others may be the type of subjects that were utilized in this study. The type of dancing (ballet, jazz, contemporary, etc) that was ritually practiced by the subjects was not consistent amongst our population. Many of the other studies used either resistance-trained or strength trained athletes, but dancing may be a combination of both strength and endurance training. Another difference is that our population was not only limited to collegiate level dancers, but the study was also restricted to a female population only. Other studies also performed their experimentation over a longer period of time in comparison to our study, which was analyzed over only a 3-day time frame. Our study also analyzed body composition using the DXA method, while as other studies used different methods such as the Bod Pod.

Conclusion

Results of the present study suggest that consuming a diet high in protein in combination with physical exercise presents no statistically significant positive modifications in body composition. However, there were general non-significant trends for improved fat mass, lean muscle mass, and overall body weight with higher versus lower protein intake. Figures 1, 2, and 3 show a general trend towards lower body weight and fat mass percentage in individuals that consumed higher quantities of protein. The HP group also had greater lean muscle mass when compared to MP and LP groups, although not significantly. In addition, several other researchers have shown that protein can help attenuate fat loss and increase muscle mass (30, 31). Future studies will include executing this present study over a larger and more diverse population of female dancers. Also, assessing the association between protein intake and overall performance will be tested as well as intervention studies to glean the impact of adding protein to the diet of dancers over time.

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Appendix

A.

The Effect of Dietary Protein Consumption on Performance Capabilities in Female Collegiate Ballet Dancers.

Informed Consent Form

Title of Project: The Effect of Dietary Protein Consumption on Performance Capabilities in Female Collegiate Ballet Dancers.

Principal Investigator: Dr. Michael Ormsbee

Other Investigators: Ann Frost (co-PI), Dr. Tom Walsh, Dr. Carla Prado, and Dr. Robert Moffatt

Participant's Printed Name:____

1. Voluntary Consent

I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled "The Effect of Dietary Protein Consumption on Performance Capabilities in Female Collegiate Ballet Dancers." This study is being conducted by Ms. Ann Frost and Dr. Michael Ormsbee, of the Department of Nutrition, Food, & Exercise Sciences at Florida State University.

2. Purpose of the Research

The primary purpose of this study is to examine the extent to which protein intake in female ballet trained dancers effects physiological characteristics and performance capabilities among college-aged dancers.

Sixty highly trained ($\geq$ eight years of ballet training) female dancers (18-26 years of age) from Florida State University and the local Tallahassee area will be recruited for this study.

3. Procedures

Participants in the study will be required to perform laboratory testing at the School of Dance and the Department of Nutrition, Food and Exercise Sciences (Human Performance Laboratory; HPL) at Florida State University. All measurements and assessments to be completed are described in detail below.

I must meet the following criteria to be included in the study: (1) minimum of 8 consecutive years of ballet training, (2) have no contraindications to exercise based on the American College of Sports Medicine and American Heart Association (ACSM/AHA) risk stratification criteria including uncontrolled hypertension, currently taking blood pressure medications, or have been diagnosed with cardiovascular disease, stroke, diabetes, thyroid, or kidney dysfunction, (3) have no risk factors for cardiovascular disease as determined by ACSM guidelines, and (4) have no significant musculoskeletal injuries or other medical conditions over the past 6 months.

First Visit – upon arrival to the HPL, the written informed consent will be signed and the 3-day food log will be distributed and explained by the researchers. The food log is to be completed before returning to the laboratory for the second visit. Anthropometrics (see below) will be measured followed by body composition measurements using dual energy X-Ray absorptiometry (DXA). The first visit will require an hour of my time that will be scheduled with the investigator.

Dietary Analysis

Dietary intake will be measured using a 3-day food log where I will be asked to maintain my normal eating patterns and habits. Foodwise dietary analysis software (McGraw-Hill, New York, NY) will be used for analysis. It is important to note that I will be asked to replicate eating patterns prior to both visits to the best of my ability.

Anthropometrics

My height and weight will be measured via the use of a wall-mounted SECA 216 stadiometer and a digital scale (SECA, Hamburg, Germany), respectively. All my measurements will be taken without shoes wearing minimal clothing (e.g. dance clothing). Measured anthropometric values will then be used to calculate body mass index (BMI). My waist, thigh, hip, and upper arm circumference measurements (cm) will also be obtained using a Gulick tape measure.

Body Composition

After the aforementioned circumferences are obtained, my body composition will then be assessed via DXA scan. My height and weight will be measured again and used for analysis. I will be asked to change into clothing that is free of metal and/or hard plastic (buttons, zippers, snaps, etc.) and asked to remove all metal from the body (jewelry, eyeglasses, hair accessories, etc). The body composition of my total body will be measured noninvasively via the use of the Lunar DXA® Scanner (GE Lunar iDXA; General Electric Company, Fairfield, Connecticut), with one scan; anteroposterior (AP) view of the total body lying supine. Very low doses of radiation are used; however, this test is non-invasive. Testing will be completed according to the manufacturer's instructions and specifications by a certified X-ray technician. My hands and feet will be secured in place in order to avoid unwanted movements during the body scan. The scan will take approximately 10 minutes to complete. From the scan, my lean mass (kg), fat free mass (kg), percent fat, and bone density will be determined. If I have not had a menstrual cycle in the past 28 days or am not using a reliable form of birth control a pregnancy test will be highly recommended before undergoing DXA scan.

Second Visit - HPL and the School of Dance for all performance measurements. The second visit will require two hours of my time that will be scheduled with the investigator.

Warmup

Upon arriving at the Nutrition Food and Exercise Science Laboratory, I will begin with a five-minute warm-up on a cycle ergometer set at 2kp resistance.

Muscle Performance Testing

After completing the 5 minute cycle, I will have my lower body muscle performance measured by my results from the vertical jump test, Wingate anaerobic power test, the flexibility test (sit and reach), and strength determined using the Biodex System 3 (Biodex Medical Systems, Shirley, New York) exercise dynamometer performing an isokinetic and isometric strength test. Lower body range of motion measurements will be taken prior to lower body muscle performance testing using a wall sit (with knees flexed to 90 degrees and hips maximally rotated externally) for maximal time.

Testing will then move on to the dance studios at Montgomery Hall to perform a Dance Specific Aerobic Fitness Test (Wyon et al., 2003). The testing will begin with a 6-minute warm-up including stretching of my choice. The test will include five stages of dance choreography, which increase in intensity with each stage. Heart rate will be monitored (Polar HR monitor strap and watch) throughout the test, blood lactate (finger prick method) and maximal oxygen uptake (VO_2; Indirect calorimetry) will be taken at the end of each stage. Prior to beginning exercise, the testing protocol will be described to me in detail. My maximal oxygen uptake (VO_{2max}) will be measured via indirect calorimetry using a portable metabolic measurement system (Parvomedics Truemax® 2400, Consentius Technologies, Sandy, UT, USA). During the test I will wear headgear with a mouthpiece attached, noseclip, and a heart rate monitor around my chest. This will be done to measure my aerobic fitness.

4. Discomforts and Risks

I understand there is a minimal level of risk involved if I agree to participate in this study. I may experience some muscle soreness from the exercise testing sessions. The risks associated with exercise testing are minimal and the selected protocols have been previously used in other studies. There is the possibility of muscle fatigue or soreness related with exercise training or testing. The risk will be minimized by using qualified investigators to supervise testing and ensure proper procedures. The risk of a cardiovascular event during testing will be minimized by careful review of my medical history and monitoring of my exercise sessions. All mouth pieces, breathing hoses and nose clips will be sterilized and cleaned with disinfecting solutions. Utilized equipment will be soaked in disinfecting solution for 20 minutes, rinsed with water, and air-dried.

Body composition will be evaluated by Dual-Energy X-ray Absorptiometry (DXA). This involves low exposure to radiation less than 5 mREMs per DXA scan. Doses received from DXA examinations are small in comparison to other common radiation sources and are believed to represent no significant health risk. No risk of adverse health conditions have been established for lower exposures of 5000 mREM or less. By comparison, natural background radiation is about 300 mREM/year, an x-ray of the spine is 70 mREM, a mammogram is 45 mREM, and a round trip transcontinental plane flight is 6 mREM. The measurement of body composition using DXA is non-invasive.

5. Possible Benefits

Benefits of participating in the study will include gaining knowledge of my current fitness level, my body mass index, my body composition, and my bone mineral density (BMD). The benefit to society relates to a better understanding of the dietary effect on dance performance, body composition, and overall health. It will also be beneficial to understand the stresses placed on dancers to achieve a functional and aesthetic physique while maintaining strength for performance without injury.

6. Statement of Confidentiality

The Effect of Dietary Protein Consumption on Performance Capabilities in Female Collegiate Ballet Dancers.

The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with my name and code number will be kept by the principal investigator, Dr. Michael J Ormsbee, in a locked drawer in his office. Data will be kept for 10 years and then destroyed.

7. Notice of Potential Injury

In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense.

8. Contact Information for Questions or Concerns

Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Ann Frost at (425) 894-1446 (af12h@my.fsu.edu) or Dr. Mike Ormsbee at (850) 644-4793 (mormsbee@fsu.edu) for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

If I have questions about my rights as a subject/I in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@magnet.fsu.edu).

10. Signature and Consent to Participate in Research

The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

____ ____________________

Participant (Print Name) Date

____

Participant Signature

Initials__________

FSU Human Subjects Committee Approved on 1/09/2014. Void after 12/10/2014. HSC#2013.11730

B.

ID #: ___________________

Dietary Food Record

Name:___________________________ Date:_________

Time	Food Items and Method of Preparation	Amount Eaten

Did you consume your protein? (Y/N) _____________________

Was this a typical day's intake? (Y/N, if No please explain).

____

____

Was this a typical day's worth of exercise? (Y/N, If No please explain).

____

____

C.

ID #: ________________________

Visit #: ________________________

DXA:

X-Ray Consent for Women of Childbearing Age

Onset of last menstrual period, date: ________________________

Date today: ___________________________

I am pregnant   Yes: ___________________   No: ____________________

A pregnancy test is highly recommended if I have not had a menstrual cycle in the past 28 days or if I am not using a reliable form of birth control. I recognize that if I am pregnant and have a radiation to the abdomen, there is a possibility of injury to the fetus. However, I understand that the likelihood of such injury is slight. I, therefore, wish to have this x-ray examination performed now.

____

Name of Patient

____

Signature of Patient

____

Signature of Witness

D.

Human Performance Laboratory
Florida State University
Nutrition, Food, and Exercise Sciences

HEALTH AND FITNESS HISTORY QUESTIONNAIRE

The following questions are designed to obtain a thorough preliminary medical history. The information you provide will help us to make the best determination about your eligibility for a particular study or other studies. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide will be kept confidential and will not be shared with any unauthorized person or organization unless you specifically request us to do so.

Name: ____

Street Address: ____________________________________

City, State, Zip code: _______________________________

Telephone Number: H ( ) ______________ W ( ) ______________

Email address: ____________________________________

Date of Birth: ____________ (mm/dd/yy) Age: ______

Sex: M ___ F ___

Personal Physician's Name: ______________ Phone: ( ) ______________

Address: ____________________________________

____

Height ______ in. ________ cm

Weight ______ lb. ________ kg

Signature: ______________________________

Date: ________   ID #: ________

Occupation

____

Race ____________

Personal Health History

Have you ever been hospitalized or had surgery? Yes____ No____
Please list all hospitalizations and surgeries to the best of your recollection.

Hospitalized for Disease/Operation	Duration	Age when hospitalized

List any disease or illness you have had not listed above (e.g., pneumonia, broken bones, etc.)

Are you allergic, sensitive or intolerant of any foods or medications?
Yes____ No____

If yes, please describe:

Food____

Medication____

Other____

Are you currently taking any form of birth control?
Yes______ No______

If yes, what kind?

2

Date: ________   ID #: ________

Do you still have a monthly menstrual period?
Yes______ No______

Is your menstrual cycle regular?
Yes______ No______

If no, please explain:

What was the first day of your last menstrual cycle?

Are you currently seeing a doctor or other health care provider for any reason?

Yes______ No______

If yes, please explain:

1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

Y N High Blood Pressure
Please indicate last known reading:
Blood pressure: _____/_____

Y N High Cholesterol or High Triglycerides
Please indicate last known reading:
Cholesterol: _____
Triglycerides: _____

Y N Diabetes (Circle: Type 1 or Type 2)
Note: Type 1 diabetes is insulin-dependent diabetes mellitus. It is typically diagnosed at an early age and requires insulin shots or an insulin pump immediately upon diagnosis. Type 2 diabetes is often diagnosed at an older age (past age 20) and is usually initially treated with changes in diet and/or medication (pills).

Y N Hypoglycemia (low blood sugar)

Y N Asthma

Date: ________ ID #: ________

2. Have you ever had a glucose tolerance test? Y N
If yes, what were the results?

3. Have you ever had a fasting blood sugar test? Y N
If yes, what were the results?

4. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

6. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain:

7. Do you smoke or use smokeless tobacco? Y N
If yes, how many cigarettes per day? ______

8. Do you drink coffee or other caffeinated beverages? Y N
What kind, how much and how often?

9. Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. How long have you been taking them and how frequently?

Are you willing to stop taking all nutritional supplements you are currently on for the duration of this research study? (Y/N) ____________________

Date: ________ ID #: ________

10. Do you have any food allergies or intolerances (e.g., allergic to dairy or lactose intolerance)? Please describe:

11. How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe:

12. What changes have you made in your diet in the last 6 months?

13. Do you exercise regularly outside of dance? Y N

How often do you have dance classes? Please be detailed in a description of your average week of training.

Please list the 3 most current performances that you have participated in and when they occurred:

14. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

15. Have you had a physical exam in the past 2 years? Y N
Please describe your assessment of your overall health: