Dietary Protein Quality, Muscle Mass,

and Strength in Vegetarian Athletes

by

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ABSTRACT

Vegetarian diets can provide an abundance of nutrients when planned with care. However, research suggests that vegetarian diets may have lower protein quality than omnivore diets. Current protein recommendations assume that vegetarians obtain a majority of their protein from animal products, like dairy and eggs. Studies have shown that this assumption may not be valid. The recommended dietary allowance (RDA) may not be adequate in vegetarian populations with high protein requirements. The purpose of this study is to analyze dietary protein quality using the DIAAS (Digestible Indispensable Amino Acid Score) method in both vegetarian and omnivore endurance athletes. 38 omnivores and 22 vegetarians submitted 7-day food records which were assessed using nutrition analysis software (Food Processor, ESHA Research, Salem, OR, USA). Dietary intake data was used to calculate DIAAS and determine the amount of available dietary protein in subject diets. Dietary data was compared with the subjects' lean body mass (obtained using DEXA scan technology), and strength (quantified using peak torque of leg extension and flexion using an isokinetic dynamometer). Statistical analyses revealed significantly higher available protein intake in the omnivore athletes ($p<.001$). There were significant correlations between available protein intake and strength ($p=.016$) and available protein intake and lean body mass ($p<.001$). Omnivore subjects had higher lean body mass than vegetarian subjects ($p=.011$). These results suggest that vegetarian athletes may benefit from higher overall protein intakes to make up for lower dietary protein quality.

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INTRODUCTION

Overview

In the United States, approximately 3% of people follow a vegetarian diet (1). Many different forms of vegetarianism exist, and some diets may be much stricter than others. Pescatarians avoid all meat besides fish and other seafood; lacto-ovo vegetarians avoid all flesh products; vegans avoid all animal products including all forms of flesh, dairy and eggs. People may choose to leave meat off their plates for ethical reasons, concern for the environment, religious beliefs, or for the potential health benefits.

Vegetarian diets can often be more healthful and nutrient rich than omnivorous diets. These diets may be abundant in fiber and antioxidants, but they also may lack appropriate amounts of iron and other minerals if not planned correctly. The only RDA (recommended dietary allowance) that is different for vegetarians is iron. This recommendation is crucial because vegetarian diets are not only lower in total iron intake, but vegetarian sources of iron are much more difficult to absorb. Non-heme iron can have an absorption rate as low as 2% (2). Other nutrients may also be cause for concern when avoiding animal foods; experts recommend supplementation of iron, calcium, and B-12 for strict vegetarians (3, 4). Since vegetarians often have such different diets from omnivores, it would be beneficial to explore different recommendations for these nutrients as well.

Protein is another nutrient concern when planning a vegetarian diet. The current DRIs (dietary reference intakes) recommend the same amount of protein for both omnivores and vegetarians, which is 0.8 g of protein per kg of body weight (5). However, DRI assumes that most vegetarians are getting half of their protein from animal sources, which may not be the case for stricter vegetarians and vegans. Research suggests that vegetarians may only be getting about 21% of their protein from animal sources (6). Vegetarian diets tend to be higher in carbohydrates and lower in saturated fats and protein than omnivorous diets (7). Not only is protein quantity a concern for vegetarian diets, but protein quality is crucially important as well. While some vegetarians may take in plenty of animal protein in the form of eggs and dairy, others may rely on beans and nuts as their primary protein sources. Plant proteins may have a lower digestibility than animal proteins, and this can be due to various anti-nutritional factors or an imbalance of different amino acids. Inadequate intake of specific amino acids may limit protein synthesis in the body (8, 9, 10). Amino acids that may be limiting in plant protein include lysine, tryptophan, threonine, and sulfur-containing amino acids (11, 12).

Protein quality and content is an important concern for athletes in particular. Experts suggest that protein intake for athletes should be higher than for the general population. Endurance athletes should consume approximately 1.2 to 1.4 grams of protein per kilogram of body weight, and some strength training athletes may require as much as 1.7 grams per kilogram of body weight according to the American College of Sports Medicine and the Academy of Nutrition and Dietetics (13). This level of protein intake can be met without supplemental protein or amino acids. Many athletes tailor their diets to fit their training regimen. Vegetarian athletes need to pay additional attention to where they are getting their protein because plant sources may not be as effective as animal sources when it comes to building and maintaining lean mass due to their lower digestibility and the effect of limiting amino acids. According to the Institute of Medicine, a separate protein recommendation for vegetarians is not necessary if adequate animal protein (from dairy and eggs) is consumed (5). The Institute does recognize, however, that this may not be true for vegans, and that protein quality becomes a genuine concern when little to no animal protein is consumed. If vegetarians are getting only 21% of their protein from animal sources, it may be appropriate to re-examine the current recommendations (6). Research into this area is critical for athletes who want to leave meat off their plates and still optimize their performance.

Purpose

The purpose of this cross-sectional study is to analyze dietary protein quality and its relation to muscle mass and strength in vegetarian and omnivore endurance athletes using secondary data.

Research Aim and Hypotheses

- H1: Protein quality (intake as measured using Digestible Indispensable Amino Acid Score (DIAAS)) will directly correlate to lean body mass in vegetarian athletes.
- H2: Protein quality (intake as measured using DIAAS) will directly correlate to strength in vegetarian athletes.

- H3: Protein quality (intake as measured using DIAAS) will be higher for omnivore athletes compared to vegetarian athletes.

Definition of Terms

- Protein quality: Quality of protein is determined by indispensable amino acid content in food and its ability to meet dietary requirements.
- Indispensable Amino Acid (IAA): These 9 amino acids cannot be synthesized by the body and must be obtained from food. The 9 IAA are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These are also referred to as “essential amino acids” (EAA).
- Vegetarian diet: A diet that excludes all meat, poultry, and seafood. Stricter versions of this diet may also eliminate dairy, eggs, and other animal by-products.
- Omnivore diet: A diet that does not exclude meat and other animal foods.
- DIAAS: Digestible Indispensable Amino Acid Score. This is a system used to evaluate dietary protein quality based on amino acid content and true ileal digestibility. This method was suggested by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 2013.
- PDCAAS: Protein Digestibility Corrected Amino Acid score. This is a system used to evaluate dietary protein quality based on amino acid content and fecal digestibility. This has been the preferred method of protein quality evaluation since 1993; this was developed by FAO/WHO.

- DRI: Dietary Reference Intake. DRIs are sets of nutrient recommendations developed by the Institute of Medicine of the National Academies. These include daily recommended dietary allowances, estimated average requirements, and tolerable upper intake levels.
- RDA: Recommended Dietary Allowance. This value is part of the DRI system. This number represents the amount of a nutrient that is likely to meet the needs of 97.5% of the healthy general population.
- Limiting amino acids: These amino acids may inhibit protein synthesis if they are not consumed in adequate amounts. Limiting amino acids include lysine, tryptophan, threonine, cysteine, and methionine.
- DEXA: Dual-energy X-ray Absorptiometry is used to measure the composition of a human body, typically by analyzing bone mass, lean mass, and body fat.
- Isokinetic dynamometer: a device used to measure force or power applied during performance of a physical exercise.

Chapter 2

REVIEW OF LITERATURE

Vegetarianism

Motivations Behind Reduction of Meat Consumption

Vegetarian diets have been present in societies for thousands of years. In recent decades, as nutritional science has become more advanced, we are beginning to see many benefits from a reduction of meat intake. Several studies have shown that vegetarians may experience less problems with weight and may also live longer and healthier lives (14). As the rate of obesity continues to rise in the United States, it has become necessary to explore many different routes and methods of long-term weight maintenance and overall health improvement. Recent research has revealed that at least 2% of Americans identify as vegetarian, while 10% of Americans report having tried a vegetarian diet in the past (15).

Another very important reason to cut back on meat intake is the impact of animal agriculture on the environment. Concern for the state of the environment has grown rapidly in recent years due to the expanding amount of evidence regarding climate change. Many Americans are choosing to do their part by adopting “green” practices, for example, recycling, utilizing reusable shopping bags and water bottles, or switching to electrically powered cars. One main contributor to pollution and climate change has become the elephant in the room. Livestock and animal agriculture are responsible for 18% of greenhouse gas emissions, while exhaust from all forms of transport combined (cars, buses, trains, planes, and ships) are responsible for 13% (16).

Adoption of a vegetarian diet may improve the lives of Americans as well as the health and stability of our planet in the long-term. Avoiding all meat may seem extreme to some, so a simple reduction in meat intake may be a more sustainable route for many people.

While there are many different motivators that may drive a person to begin a vegetarian diet, these motivations are not necessarily always stable (17). For some, a completely new set of ideas encourage them to continue with their meat-free ways. For example, someone may adopt the diet to lower their cholesterol. As they learn more about the diet and the realities of factory farming, their primary motivator may move toward animal welfare instead.

Animal welfare is a primary concern and motivator for many vegetarians. There is a wide spectrum of what is considered acceptable treatment of animals when it comes to the vegetarian community. The concept of "humane meat" is very controversial. If an animal has lived a comfortable life with no mistreatment, and their death is quick and painless, is it humane? Many vegetarians and vegans will argue simply that killing is inhumane, and therefore all meat is inhumane regardless of the circumstances. Others identify factory farming as the main reason to avoid meat. The conditions in which factory farm animals live are not "comfortable", the animals are usually confined to very small spaces, and are treated as a commodity rather than as a living creature. For many ethical vegetarians, a combination of both concepts motivates them to avoid meat. Ethical vegans argue that using cows for milk and chickens for eggs is a form of exploitation; therefore, they avoid dairy, eggs, and other animal products like gelatin and leather.

Somewhere past the end of this spectrum of ethical vegetarianism are "conscientious omnivores". Conscientious omnivores do not abstain from meat, instead they consume meat from conditions that they deem humane (for example, free-range, grass-fed cattle from small family farms). Arguments against a conscientious omnivore diet note that even the strongest proponents of the diet do not follow it 100% of the time, and often consume factory-farmed meat rather regularly (18), and also that a conscientious omnivore diet is far more difficult to maintain than a vegetarian diet (free-range meat is generally far more expensive and far less available and accessible).

One study revealed that among younger vegetarians (40 or younger), ethical and environmental reasoning is their primary motivator, while older vegetarians (41-60) identify health as their main motivator (19). A growing number of vegetarians cite environmental concerns as an important factor in their choice to go meat-free (20).

Nutritional Implications of Vegetarian Diets

Some people choose to reduce their meat intake to get their health under control or to prevent health issues like obesity, cardiovascular disease, and type 2 diabetes. Numbers of vegetarians adopt the diet in an effort to lose weight, though the prevalence of weight-loss oriented vegetarians is not known. Adoption of a vegetarian diet can have beneficial health effects due to the lower fat and cholesterol intake and higher intake of fiber and many beneficial micronutrients, specifically antioxidants like vitamin C and E, as well as health-promoting phytochemicals, such as lycopene. A higher intake of healthy fats and antioxidants can have an anti-inflammatory effect that may have benefits in treating and preventing heart disease (21, 3). Well-planned vegetarian diets have been shown to reduce the risk of cardiovascular disease and can even reverse atherosclerosis. (22, 23).

Vegetarian diets are often associated with lower blood pressure (3, 24); this is likely a result of many different aspects of a healthful vegetarian lifestyle (whole foods, healthy body mass index, and physical activity). These diets also tend to be lower in sodium, and higher in potassium (24). A 2014 meta-analysis of 7 controlled interventions and 32 observational studies confirmed the association between vegetarian diets and lower blood pressure. Systolic pressure was 4.8 mm Hg lower in the intervention studies, and 6.9 mm Hg lower in the observation studies. Diastolic pressure was 2.2 mm Hg lower in trials, and 4.7 mm Hg lower in the cross-sectional observations. The effect sizes are roughly half of those seen with pharmacological treatment. However, even small improvements in blood pressure can help prevent further health complications down the line (3, 24).

Antioxidants are compounds found in food that have the ability to stabilize free radicals and prevent cell damage. Common antioxidants include vitamins C, E, and beta-carotene. Other nutrients can act as coenzymes or other components in antioxidant reactions; this includes most B vitamins, and minerals like copper, zinc, selenium, and manganese (25). Vegetarian diets with high intakes of fruits and vegetables provide an abundance of antioxidants. A meta-analysis of antioxidant studies revealed that vegetarians have higher serum levels of vitamins C, E, and beta-carotene, but often have lower serum levels of zinc, copper, and selenium when compared with omnivores (25).

Saturated fats are most commonly found in animal products. Meats, high-fat dairy, and egg yolks are all high in saturated fat. Plant sources include palm oil and coconut oil. The USDA's Dietary Guidelines for Americans are very clear in stating that Americans should reduce their intake of saturated fats. The scientific evidence, however, is much more ambiguous (26). A meta-analysis from 2010 concluded that there is not enough evidence to support the idea that saturated fat intake is associated with cardiovascular disease risk (27). Not all saturated fats are created equal. Research has shown that saturated fat intake from whole milk and butter may increase both LDL and HDL, leading to higher total serum cholesterol (26). Vegetarian diets may be lower in saturated fat depending on the amount of dairy intake. Vegetarian diets are also often lower in dietary cholesterol than omnivore diets, while vegan diets contain no cholesterol at all. Dietary cholesterol recommendations underwent a recent overhaul, as more research reveals dietary cholesterol intake has less of an effect on blood cholesterol levels than previously thought. A specific limit is no longer included in the 2015-2020 USDA dietary guidelines, but it is still recommended to reduce cholesterol intake as much as possible (28).

Omega-3 fatty acids have become a hot topic in recent years, touted for their anti-inflammatory effects. Omega-6 fatty acids, however, are more prevalent in the typical American diet and warrant discussion. Vegetarian diets tend to be low in the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, vegetarians consume similar levels of the omega-3 fatty acid alpha-linolenic acid (ALA) when compared to meat eaters (29). Linoleic acid (LA) is an omega-6 fatty acid;

vegetarians tend to have higher LA intake than omnivores. ALA can be converted within the body to EPA and DHA, though this process is highly variable and rather inefficient. Excessive intake of LA may interfere with ALA conversion because they compete for the same enzymes (29).

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid found in meat products, eggs, and milk. Vegetarian diets are typically low in AA, and vegan diets contain no AA at all. Arachidonic acid can act as both an inflammatory agent and an anti-inflammatory agent. Increased AA intake in healthy individuals is unlikely to cause inflammation. Individuals with a medical history of inflammatory conditions may benefit from a reduction of arachidonic acid. Studies that have implemented arachidonic acid supplementation have produced mixed results. Supplementation of AA while undergoing strength training may have an anti-inflammatory effect. While AA is not harmful in a healthy population, an increased intake of AA may negate the effects of omega-3 supplementation (30, 31).

The overall ratio of omega-6 to omega-3 fatty acids is extremely important when trying to reduce cardiovascular disease risk (32). On average, Americans may be consuming a ratio of approximately 15:1 omega-6 to omega-3 (33, 34). Experts recommend a ratio of 4:1 or lower (33, 35). While vegetarians may consume lower amounts of arachidonic acid, linoleic acid intake is higher, leading to a less than ideal ratio (especially when omega-3 intakes are low). In order to improve this ratio, it is beneficial to replace consumption of omega-6 containing foods with omega-3 containing foods. Common sources of linoleic acid include safflower, sunflower, sesame, and corn oils. Foods high in ALA include, chia seeds, flaxseeds and flax oil, hempseeds, and canola oil. Walnuts, soybeans, and wheatgerm are high in both ALA and LA. EPA and DHA are primarily found in fish, seafood, and eggs but microalgae have been identified as a plant source (29). Special attention to fat sources may be beneficial to both vegetarians and omnivores.

Analysis of Vegetarian Diet Quality

A 2012 cross-sectional study surveyed subjects who consume various diets with different levels of animal product restriction (7). 1,475 subjects participated in this online survey, which included vegans (n=104), vegetarians (n=573), semi-vegetarians (n=498), pescatarians (n=145), and omnivores (n=155). Subjects were at least 20 years old and living in Belgium. Researchers used a 52-item food frequency questionnaire (FFQ) which was adapted from a previously validated 50-item FFQ. Additional items commonly consumed by vegetarians were added to the questionnaire, such as soy products, imitation meats, and hummus. The survey asked about consumption of foods within the past year and provided nine different options for frequency, which ranged from “never” to “more than 3 times per day”. Calorie, fat, carbohydrate, fiber, protein, sodium, calcium, and iron intake were calculated by using a standard set of nutrition data for each item included in the questionnaire. Subjects self-identified their diet type, but some were reclassified due to answers given in the FFQ. Subjects also provided their height, weight, age, gender, and education level.

Healthy Eating Index 2010 (HEI-2010) and the Mediterranean Diet Score (MDS) were used as measures of diet quality. Possible scores for the HEI-2010 range from 0 to 100, while possible scores for the MDS range from 0 to 9. In both cases, higher scores represent better adherence to dietary guidelines (Food Guide Pyramid for HEI-2010, Mediterranean Diet recommendations for MDS).

Analysis revealed significantly lower calorie intake among the vegan participants (2,383 ± 804) when compared to the other diets. Calorie intake did not differ between vegetarian (2,722 ± 875), semi-vegetarian (2,849 ± 858), or pescatarian subjects (2,744 ± 797) (7). Intake of polyunsaturated fats, fiber, and iron correlated with level of dietary restriction; vegans had the highest intake of these nutrients. It is unexpected that vegans had the highest intake of iron, but absorption of the iron is likely lower due to plant sources containing only non-heme iron. Vegans had significantly lower intakes of protein, sodium, total fat, saturated fat, monounsaturated fat, and cholesterol when compared to the omnivore group. Again, the differences for those nutrients between the other three groups were not significant. Omnivores had significantly lower calcium intakes than the vegetarian and semi-vegetarian group, possibly related to a higher intake of dairy products and fortified soy products. Total carbohydrate intake did not differ between groups, but the percent of energy from carbohydrates was related to restrictiveness of the diet.

Overall dietary quality according to HEI-2010 was greatest among vegans (65.4 ± 8.3). Second highest HEI-2010 score belonged to the semi-vegetarian group (59.4 ± 7.4), followed by vegetarians (58.7 ± 8.9) and pescatarians (58.7 ±7.9). Omnivores received the lowest HEI score (54.2 ± 9.0). The results of the MDS analysis also ranked the vegan diet at the top (5.8 ± 1.3), followed by pescatarians (5.5 ± 1.4), semi-vegetarians (5.2 ± 1.5), vegetarians (4.6 ± 1.5), while the omnivore diet again took last place (4.1 ± 1.6) (7). This study has a number of limitations, notably the convenient sample which does not represent the general population. 75% of survey participants were female, and 47% of participants were between the ages of 20 and 29. Height and weight were self-reported which, if inaccurate information was submitted, may lead to an under or overestimation of BMI averages. The addition of popular vegetarian foods may also have skewed results; soymilk and all other soy products were included under the plant protein category, resulting in scores of zero in the dairy category for the vegan group. A long recall period may also reduce the accuracy of the FFQ results.

Though diet quality is highly variable from person to person, research suggests diets lower in meat may be of higher quality. Vegetarian and pescatarian diets likely have lower intake of saturated fats and a lower omega-6 to omega-3 fatty acid ratio. In diets with fewer animal foods, it can logically be assumed that intake of plant foods may be higher. Not all plant foods are health-promoting, especially when highly processed, but meals rich in fresh vegetables, fruits, legumes, nuts, seeds, and whole grains can provide an excellent base for a healthful diet (36).

Vegetarian Diet and Inflammation

A study by Adam et al. (2003) implemented dietary changes in patients with rheumatoid arthritis (RA) and observed changes in their symptoms (37). 68 RA patients were divided into two groups. One group was instructed to consume their standard Western diet, and the other group was placed on an anti-inflammatory diet (AID). Patients in both diet groups were randomly given either fish-oil capsules or placebo capsules for three months. The fish-oil capsules contained 245.3 mg of eicosapentaenoic acid (EPA), which is an omega-3 fatty acid. After a two-month wash-out period, the participants received the opposite capsule for the last three months. Data and measurements taken each month during the study include the patient's pain (self-assessed on a scale of 0-10, 10 being excruciating pain), global assessment of disease activity, grip strength, and morning stiffness. Joint swelling and tenderness were evaluated by a physician using ACR criteria. Routine blood analysis occurred at each monthly meeting. Before and after each three-month period, blood samples were taken for eicosanoid, cytokine, and erythrocyte lipid analysis, and 24-hour urine was collected.

Participants in the anti-inflammatory group followed a modified vegetarian diet. They were instructed to avoid egg yolks, and high fat dairy. The only oils they could use during this period had to be plant-based. Meat was limited to less than 2 servings per week (to keep arachidonic acid to less than 90 mg per day) (37).

60 participants completed this trial. The study's results show that the diet low in arachidonic acid increased the benefits of the fish-oil supplement. The AID group showed 14% improvement in symptoms with diet alone. 17% symptom improvement was seen in the patients with fish-oil supplementation and the Western diet. When AID and fish-oil were combined, symptom improvement went up to 31%. When on the AID and fish-oil treatment, participants were able to decrease their intake of NSAIDs as

recommended by a doctor. A significant positive correlation was seen between disease activity and arachidonic acid intake ($p<0.001$). The investigators believe that the higher EPA:AA erythrocyte lipid ratio may account for the therapeutic effects of this treatment (37).

A small study by Donaldson et al. (2001) examined the effects of a primarily raw vegetarian diet in subjects with fibromyalgia (38). 20 subjects completed this strict dietary intervention. Participants were instructed to avoid all meat, dairy, eggs, alcohol, caffeine, refined flours, refined sugar and corn syrup, and refined oils. Dietary instruction encouraged consumption of fresh fruits and vegetables, carrot juice, nuts and seeds, root vegetables, whole grains, flaxseed oil, and extra virgin olive oil. Participants were given barley grass juice powder, a laxative blend of herbs and psyllium, and were given juicers and instructed on how to juice carrots. This intervention lasted for 7 months.

Before and after the intervention, physical performance was assessed by a physical therapist based on 12 measurements that looked at pain, range of motion, and flexibility (39).Three questionnaires were self-administered at months 0, 2, 4, and 7: Fibromyalgia Impact Questionnaire (FIQ), the SF-36 (“short form”, 36 questions) health survey, and a validated Quality of Life (QoL) survey. A food frequency questionnaire designed by the researchers was also given at months 2, 4, and 7 to monitor adherence to the diet.

At the end of this trial, 18 participants returned for physical testing. Significant improvements were seen in physical performance: in shoulder pain and range of motion, the sit-and-reach flexibility test, the chair test (which measures how many times the subject can stand up and sit down in one minute), and a six-minute walk. 20 patients returned surveys consistently throughout the study. Most patients followed the dietary instructions fairly well; 11 admitted to drinking soda at times, two did not consume barley grass juice, and average carrot juice consumption decreased as time went on. FIQ results showed significant improvement ($p<0.05$) of 45% at seven months. Composite QoL scores also improved significantly, from an average of 3.9 to 4.9 after seven months (on a scale of 0-7). SF-36 scores also improved in seven out of eight areas (38). Significant improvements were not seen in the “bodily pain” area, but “general health”, “physical functioning”, “role physical”, “role emotional”, “vitality”, “social functioning”, and “mental health” all saw marked beneficial changes. The investigators believe the improvements were a result of many factors in this diet. This study does have its flaws (no control group, self-reported measures and food frequencies, small sample size) but the results are still intriguing and the subject deserves further research.

This research suggests that a whole-food, mostly plant-based diet can aid in prevention of inflammation, particularly when animal-sourced saturated fats and omega-6 fatty acids are avoided. Further benefits may be seen if omega-3 fatty acids (especially EPA and DHA) are supplemented either through foods or fish oil capsules (37, 38). Ethical vegetarians may be likely to avoid fish oil supplements, but new advancements are being made regarding microalgal supplements (40) which may be able to make up for the lack of EPA and DHA in vegan and strict vegetarian diets. Supplemented vegetarian diets may prove to provide ideal nutrition for individuals suffering from chronic inflammatory conditions.

Nutrients of Concern Among Vegetarian Populations

A 2002 *Journal of Nutrition* article (Venti and Johnston) (4) highlighted specific nutrients of concern for vegetarians and introduced a vegetarian food guide pyramid to illustrate a healthful diet that will meet macronutrient and micronutrient requirements. Protein is a nutrient of concern for vegetarian diets due to lower overall protein quality in plant foods. The 2016 Academy of Nutrition and Dietetics position paper on vegetarian diets reports that vegetarians typically meet protein recommendations when overall calorie intake is adequate (41). However, it does not provide any detail regarding protein digestibility. Research at the time of Venti and Johnston's article suggested that vegetarians may digest only 90% of protein intake; while vegans may digest only 76%. The authors recommend raising protein recommendations by 20% for vegetarians to make up for lower digestibility (1.0g/kg/day; current recommendations are 0.8g/kg/day regardless of diet type). More recent research also supports this idea (6) which will be discussed in greater detail in subsequent sections of this review. Further research is needed to examine true digestibility of various diets and support increasing daily protein recommendations, especially when considering populations with higher requirements.

Vitamin B-12 is primarily found in animal foods, meaning that a vegan diet may be completely devoid of B-12 without addition of fortified foods. In developed countries, the general meat-eating population exceeds recommended B-12 intake (2.4 µg) (42). Lacto-ovo-vegetarians and vegans often have low intakes of B-12; as many as 78% of vegans may be deficient (4). B-12 is stored in the liver, meaning it may take up to 5 years of inadequate intake to see clinical signs of deficiency. This puts long-term strict vegetarians at greater risk of deficiency. “A common mistake is to think that the presence of dairy products and eggs in the diet … can still ensure a proper intake of [B-12], despite excluding animal flesh” (42). One egg and one cup of milk per day would contribute just two-thirds of the RDA for B-12 (41). Inclusion of fortified foods or a B-12 supplement may be recommended to counteract low intake of animal foods, but further research is needed to identify proper supplementation amounts.

Nutrients that contribute to bone health are of unique concern to vegans and vegetarians with limited dairy intake. There is evidence to suggest that vegetarians are at greater risk for osteoporosis than the general population (43). Fortified dairy products are the main dietary contributors for calcium and vitamin D in omnivore diets. Calcium absorption is significantly lower in vegetarian diets, so higher overall calcium intake may be required for vegetarians and vegans (4). Although leafy greens and legumes contain calcium, plant-sourced calcium is less bioavailable. Experts recommend inclusion of dairy for vegetarians and calcium-fortified soy products and other dairy alternatives for vegans (43). Calcium and vitamin D work together within the body to promote bone health; the benefits of high calcium intake are essentially negated when vitamin D is not available (44). Vitamin D is found naturally in a limited number animal foods (cod liver oil, egg yolks, etc.), and is commonly found in fortified dairy. This puts vegetarians and vegans at considerably higher risk of deficiency due to exclusion of these foods. An 18-year study involving post-menopausal omnivore women revealed that intakes of >12.5 µg are associated with a 37% reduction in hip fracture risk when compared to intakes <3.5

μg (45). Though vitamin D may be synthesized within the body, many people are unable to get sufficient sun exposure to meet minimum requirements, particularly during the winter, in upper latitude regions, and with the use of sunscreen. Both omnivores and vegetarians should include fortified foods (dairy, soy products, breakfast cereals, and orange juice) in order to maintain adequate vitamin D status. Supplementation may be considered in high-risk populations (such as the elderly or people living in Northern regions) or when intakes are especially low (43).

A 2003 article in the *American Journal of Clinical Nutrition* described bioavailability of minerals in vegetarian diets, specifically iron, zinc, and trace minerals like copper (2). While plant foods can provide an abundance of iron and trace minerals, most dietary intake of zinc and highly-bioavailable heme iron comes from animal foods. "The total iron content of a diet … provides little information about its content of bioavailable iron, which is considerably influenced by the foods in the diet and can vary 10-fold from different meals of similar iron content" (2). Plant foods provide only non-heme iron. Heme iron has much higher rates of absorption (~15-40%) than non-heme iron (1-15%). The body's ability to absorb non-heme iron is highly variable; absorption is up-regulated in individuals with low iron stores, and those with high iron stores may absorb very little non-heme iron. Evidence suggests that iron deficiency anemia is not more common among vegetarians; the body's ability to up-regulate absorption may be the cause of this (46). Though vegetarians often have lower iron stores, below average iron stores in the absence of iron deficiency anemia have not been shown to have a negative effect on function (2).

A cross-over study (Hunt JR, 1999) revealed nearly 6 times higher iron absorption when consuming a non-vegetarian diet. The study included 21 healthy adult women who were given two controlled diets as planned by registered dietitians for 8-week periods (47). All foods were prepared and weighed by the researchers; energy intakes were planned to meet weight maintenance needs of each individual. Both diets contained similar amounts of total iron (~18 mg). The vegetarian diet contained 2.5 times the amount of fiber and 3 times the amount of phytic acid in the non-vegetarian diet; the vegetarian diet also contained 21% more ascorbic acid. At week 4 of each diet period, non-heme iron absorption was measured by way of a radioisotopic tracer injected into the primary non-heme iron containing foods for a full day. Whole-body scintillation counting technology was used to estimate iron absorption. Fasting blood samples were taken at weeks 7 and 8 of each diet period; the two results were averaged. Total feces collection took place at the final 14 days of each diet period. Results revealed total iron absorption of 0.14 mg/d and 0.89 mg/d for the vegetarian and non-vegetarian diets respectively. 0.48 mg/d of iron absorbed during the non-vegetarian diet was non-heme. Non-heme absorption rates were 1.1% from vegetarian diets and 3.8% from non-vegetarian diets. Though absorption rates were significantly different, no difference was found in blood iron markers (including serum ferritin) from each diet period. Fecal analyses revealed six times higher iron excretion during the non-vegetarian diet. Fecal ferritin was not correlated with iron absorption, but was correlated with serum ferritin. Serum ferritin was inversely correlated with iron absorption. This research suggests that serum ferritin is not easily altered by a change in dietary intake; it may take several years of adherence to a specific diet to see a significant difference in serum ferritin. This may mean that long term adherence to a vegetarian diet can put individuals at risk for iron deficiency. A separate set of RDA values for vegetarians has been developed: 14 mg/d for men, 33 mg/d for premenopausal women. Recommendations for non-vegetarians are 8 mg/d for men and 18 mg/d for women. Foods commonly consumed in a vegetarian diet may limit iron absorption: phytic acid is found in grains, nuts, and legumes; polyphenols are found in tea, coffee, wines, and some plant foods; soy protein and eggs may also inhibit bioavailability (2). Ascorbic acid can also boost non-heme iron bioavailability, though it may not always be enough to reach absorption rates as high as heme-iron. Vegetarians can maintain healthful iron status through careful diet planning, increasing overall iron intake, and pairing vitamin C with iron-containing foods.

The majority of zinc in American diets comes from animal sources. A 2013 meta-analysis of 26 studies found significantly lower serum zinc concentrations and zinc intake in long-term vegetarians (48). When separating subjects by gender, vegetarian females fared worse with lower zinc intake and serum zinc than vegetarian males. The current adult RDA for zinc is 11 mg for males and 8 mg for females (49). Though zinc deficiency is rare in developed countries, vegetarians and vegans should still aim to increase their dietary zinc as a precaution. Zinc can be obtained from plant foods, like nuts, seeds, legumes, and whole grains, but zinc bioavailability is much lower in these due to their high phytate content. Phytates bind zinc and inhibit its absorption. High intake of calcium-fortified foods may also inhibit zinc absorption. Because of these factors, vegetarians may need 50% higher zinc intakes than non-vegetarians to account for poor absorption (2, 50, 51).

When it comes to macronutrients, protein is the main source of concern in vegetarian diets. Vegetarian diets typically provide sufficient quantities of fat and carbohydrates, but protein adequacy is debatable when protein quality and digestibility is brought into question.

Evaluation of Protein Quality

Methods of Evaluation

In 2013, the FAO published a report titled “Dietary Protein Quality Evaluation in Human Nutrition”, which outlined current methods of protein quality assessment and suggested an improved form of evaluation (52). In 1989, the Protein Digestibility Corrected Amino Acid Score (PDCAAS) method was recommended by the FAO/WHO Expert Consultation on Protein Quality Evaluation. While this scoring system has proven to be useful in many different applications, there are also limitations that must be considered (53). PDCAAS is calculated by multiplying the limiting amino acid in a food by the percent of true fecal digestibility of the crude protein content of food. Evidence has shown that true ileal digestibility (determined at the end of the small intestine) may be a better representation of amino acid absorption. Taking samples from the large intestine does not account for the leftover proteins that were not absorbed by the small intestine. Another limitation of PDCAAS is the truncation of high quality proteins; all proteins with an excess of essential amino acids are truncated to a maximum score of 1.0. The protein reference pattern used in PDCAAS represents the minimum recommended intake and may not be useful for calculation of optimal intake. "The questions about the validity of the amino acid scoring pattern and the application of the true fecal rather than the true ileal digestibility correction, as well as the truncation of PDCAAS values warrant a critical evaluation of PDCAAS in its current form as a measure of protein quality in human diets." (10). PDCAAS also does not account for other factors that may influence protein digestibility. Anti-nutritional factors such as trypsin inhibitors, tannins, and phytates may increase protein losses during the digestion process. These are not included in the protein digestibility percent values in the PDCAAS calculations, and thus, digestibility may be overestimated (9).

The Digestible Indispensable Amino Acid Score (DIAAS) was developed to address these limitations. DIAAS uses true ileal digestibility of individual amino acids rather than crude protein fecal digestibility. More research is needed to build a larger database of ileal digestibility of human foods; these values would ideally be determined using human subjects but growing pigs and rats suffice when human data is unavailable. In practice, fecal digestibility may be used when no ileal digestibility percent has been determined for that food. In addition, the DIAAS system does not truncate the values of individual foods, therefore, high quality protein sources can have a value higher than 1.0. Using PDCAAS, milk protein and soy both have a score of 1.0; using DIAAS, milk protein has a higher score. When using DIAAS, values over 100% should never be truncated, the only exception being total scores for mixed diets or sole source foods, such as breast milk or infant formula. Two basic main amino acid patterns (this refers to the amino acid content of the reference protein) are recommended for calculating DIAAS. One uses the amino acid pattern of human breastmilk (this is used for infants and young children), and the other uses a reference protein developed by researchers which represents a hypothetical ideal protein source.

"DIAAS is defined as: DIAAS % = 100 x [(mg of digestible dietary indispensable amino acid in 1g of the dietary protein) / (mg of the same dietary indispensable amino acid in 1g of the reference protein)]" (52).

The FAO report identifies three practical uses for the DIAAS method:

- Calculation of DIAAS in mixed diets to assess dietary adequacy.
- Identification of high quality protein sources and the benefits of their addition to diets containing lower-quality proteins.
- Monitoring of protein content and quality in consumer food products.

Table 1. DIAAS CALCULATION TABLE FOR MIXED DIET

	Weight (g)	Protein (g/100g)	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp	Protein content (g)	Lys	SAA	Thr	Trp
	A	B	C	D	E	F	G	H	I	J	AxB	(AxB) X CxG	(AxB) X DxH	(AxB) X ExI	(AxB) X FxJ
Wheat	400	11	28	38	29	12	.82	.895	.86	.91	44	1010	1488	1097	480
Pea	100	21	71	25	37	9	.79	.69	.73	.66	21	1178	362	567	125
Milk Powder	35	28	78	35	44	13	.95	.94	.9	.9	10	726	322	388	115
Total	535										75	2914	2172	2052	720
Amino Acids mg/g (total for each AA/total protein)												38.9	29.0	27.4	9.6

	Reference Pattern (mg/g)				Digestible IAA Reference Ratio				DIAAS for mixture (%)
	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp	
Infants (0 to 6 months)	69	33	44	17	**.56**	.88	.62	.56	56 (Lys)
Children (6 months to 3 years)	57	27	31	8.5	**.68**	1.08	.88	1.13	68 (Lys)
Older Children/Adults	48	23	25	6.6	**.82**	1.26	1.10	1.45	82 (Lys)

The above table is adapted from: “Dietary protein quality evaluation in human nutrition.” FAO/WHO 2013.

The table above is adapted from the FAO report; its purpose is to identify limiting amino acids and DIAAS percentages for mixed diets (Table 1). This table includes the most commonly limiting amino acids: lysine, methionine and cysteine, threonine, and tryptophan. Other essential amino acids may also be included in this calculation table for a more thorough analysis. Limiting amino acids are given their name because protein synthesis is limited by the inadequate content of that essential amino acid in a particular food or mixed diet. The table below illustrates the adult reference protein pattern for DIAAS calculation (Table 2).

Table 2. DIAAS Reference Protein Pattern for Adults

Amino Acid	Reference Protein Pattern (mg/g)
Histidine	15
Isoleucine	30
Leucine	59
Lysine	45
SAA* (Methionine + Cysteine)	22
AAA** (Phenylalanine + Tyrosine)	38
Threonine	23
Tryptophan	6
Valine	39

*SAA: Sulfur Amino Acids
**AAA: Aromatic Amino Acids

The above table is adapted from: “Dietary protein quality evaluation in human nutrition.” FAO/WHO 2013.

Estimation of Protein Needs

Several techniques may be used to calculate an individual's protein needs. The nitrogen balance method is one of the most common ways to estimate protein needs despite its limitations. A 2003 meta-analysis (54) sought to analyze nitrogen balance studies in order to recommend new daily protein requirements, which had been previously set by FAO/WHO in 1985. 19 "primary estimation" studies (235 total subjects) were included in analysis. These studies presented data for each subject from a minimum of 3 different time periods. Participants were given specific diets ("test intakes") for 10-14 days, and urinary and fecal samples were collected for 5-day periods to calculate nitrogen excretion. Diets used in these studies were classified as "animal", "vegetable", or "mixed". Animal and vegetable diets contained >90% of protein from their respective sources. For the purpose of the review, protein intake was considered sufficient at whatever amount brought nitrogen balance to zero (equilibrium). Not enough evidence was available to set an ideal number for positive nitrogen balance at the time of this analysis; researchers were unable to justify use of any number beyond equilibrium. Intakes were estimated to be near levels that would reach nitrogen equilibrium; end results were calculated using the protein intake amounts in order to find the specific value that would produce exact nitrogen balance for that individual. Estimated average requirements (EAR) were calculated from the results using two methods. Estimates represented the median, meaning these values would be sufficient for 50% of the healthy adult population. Median was used because the results were not normally distributed. First, the median of all included subjects was calculated. Then, all subjects included in the analyses were divided into substudies (if their particular study examined >2 different groups of people or >2 different diet types). Medians were calculated for each substudy, and then the overall median was calculated using the substudy median values. All analyses were performed using SPSS.

Results revealed high variability from subject to subject, as well as variability over time for individual subjects. The median for older subjects revealed a need for more than 27 additional mg N/kg/d when compared to younger groups, though these results were not statistically significant. Males had significantly higher nitrogen requirements than females (an additional 20 mg N/kg/d). However, when comparing substudy medians, this difference was no longer significant (54).

Authors of this analysis estimate the EAR “of the healthy adult population as 105 mg N x kg1 x d1 (0.65 g good-quality protein x kg1 x d1)”. The estimated recommended dietary allowance (RDA), “that would be expected to meet the requirements of most (97.5%) of the healthy adult population [is] 132 mg N x kg1 x d1 (0.83 g good-quality protein x kg1 x d1)” (54). These numbers apply to the general population of healthy adults; analysis of separate groups (age, sex) did not produce enough convincing data to recommend different requirements. Analysis of animal, vegetable, and mixed diet groups did not reveal a significant difference in nitrogen requirements. The “vegetable” diets included in analysis contained several “complementary” proteins, meaning that several different plant-based protein sources were included in these diets. High quality plant proteins like soy showed similar digestibility to meat. However, diets made primarily of wheat and other cereals had much lower protein digestibility than more varied diets. The authors recommend inclusion of animal proteins, or several different plant proteins to ward off lysine inadequacy as lysine is the most common limiting amino acid in wheat and grains (common staples of a plant-based diet).

This meta-analysis did not control for physical activity in these subjects, so no conclusions can be drawn from the data to create recommendations for trained versus untrained individuals.

Dietary Protein Quality Among Vegetarians

A previous study from ASU (Kniskern and Johnston 2010) analyzed the dietary protein quality of vegetarian women (6). Twenty-two self-declared vegetarian women (ages 19-40) were recruited using advertisements near Arizona State University campus. The subjects were instructed to complete 4-day food records, including one weekend day, and were asked not to change their eating habits during the 4-day time period. Participants were instructed to be as specific as possible regarding food brands, categories, and serving sizes. Subjects were also asked to include all condiments and beverages in the food records. All food record data was entered into Food Processor 10.3 (ESHA Research, Salem, OR, USA); a standardized list of foods and serving sizes was used when adequate detail was not available. Daily protein contribution from several different food groups was calculated, and DRI reference values were used to estimate protein digestibility (85% for cereals, 80% for legumes, 95% for animal protein sources, 70% for nuts and seeds, and 60% for fruits and vegetables) (5). DRI reference values did not include nuts and seeds or fruits and vegetables, so percentages recommended by other sources were used. Lysine and sulfur amino acid (SAA) scores were calculated by multiplying the average amino acid content of each category by the corresponding digestibility percentage. Lysine (mg per g protein) values used were: cereals = 31, legumes = 67, nuts/seeds = 33, animal sources = 78, and fruits/vegetables = 43. SAA (mg per g protein) values used were: cereals = 38, legumes = 26, nuts/seeds = 29, animal sources = 30, and fruits/vegetables = 10. PDCAAS values for lysine and SAA were calculated for each 4-day food log using the following equation:

(limiting amino acid [mg] in 1 g of test protein)/ (same amino acid [mg] in 1 g of reference protein) x true digestibility percentage

Reference values used were 51 mg/g for lysine, and 25 mg/g for SAA. Of the 22 subjects, one was removed from all analyses due to meat consumption on multiple days. Two participants reported seafood consumption on one day; these days were omitted from the food records (3-day records were used for these subjects). The lowest of the two PDCAAS values for each day were used to calculate the 4-day average digestibility score.

The average total protein digestibility score (82 ± 1%) for the 21 vegetarians differed significantly ($p < .001$) from the 88% DRI reference score. Average 4-day PDCAAS (80 ± 2%) was also significantly lower ($p < .001$) than the DRI reference value (100%). These results suggest that vegetarians may be consuming lower quality protein sources than previously assumed. Current protein recommendations may not be adequate for some vegetarians who consume fewer animal products. For this study, a convenient sample of young, healthy women was used, and therefore results cannot be generalized to the entire population. Further research is warranted to investigate adequacy of protein quality in other populations (55).

A 2011 study analyzed essential amino acid (EAA) intake and total protein intake and its relationship to muscle mass in healthy, adult women (56). The 63 women were divided into three groups: omnivore, vegetarian, and vegan. Levels of physical activity ranged from "sedentary" to "moderately active" (as defined by the researchers as less than 3 hours per week of moderate intensity exercise or less than 5 hours per week of low intensity exercise). The subjects were required to have adhered to their respective diets for a minimum of two years. The subjects were monitored for two three-day periods approximately six months apart. 72-hour urine samples and daily fasting blood samples were taken from all subjects, in addition to three-day food records and anthropometric measurements. Lean body mass was estimated using urinary creatinine concentrations. Past studies have shown correlations between creatinine content within the body and creatinine excretion in urine (57, 58). Two separate formulas were used to calculate muscle mass content; meat eaters often have higher urinary creatinine levels. The formulas were developed in 1976 by researchers Forbes & Bruining and have been validated (59). Skeletal muscle mass estimations that resulted from these calculations were then divided by height in meters, squared (as is done in BMI calculations) to create a muscle mass index value.

- Vegan & Vegetarian Groups:
    - "SM1" (skeletal muscle mass, kg) = 11.8 x creatinine (g/24 hr) + 10.1

- Omnivore Group:
    - "SM2" (skeletal muscle mass, kg) = 14.4 x creatinine (g/24 hr) + 3.6

The three-day food records were evaluated using the Nutrient Database at University of Massachusetts at Amherst.

The groups varied significantly in body weight, as the vegan group (53 ± 6 kg) was lighter on average than the two other groups (both 57 ± 6 kg). Muscle mass did not differ between groups. The omnivore group subjects consumed diets that were significantly higher in protein (total grams per day) and had a higher value for g protein/kg of body weight per day. The omnivore diets were also significantly lower in carbohydrates and fiber than the vegetarian and vegan diets. Despite the difference in quantity of protein intake, no significant difference was found for intakes of essential amino acids (isoleucine, leucine, lysine) or in the ratio of EAA to total protein intake. Results of this study suggest that vegetarian and omnivore diets may result in similar muscle mass in adult women when adequate amounts of essential amino acids are consumed. Limitations of this study include: the use of food records, which may not be an accurate representation of dietary intake, and the use of urinary creatinine to calculate muscle mass (DEXA or MRI scans are proven to be more accurate methods). Results cannot be generalized to the entire population as only healthy Caucasian women below the age of 65 were included in this study (56).

A 2009 article published in the *British Journal of Nutrition* discussed an investigation into possible relationships between intake of protein from animal sources and muscle mass (60). Researchers recruited 21 omnivores and 19 vegetarians in Helsinki, Finland; all were healthy adult women, and were sedentary or moderately active (evaluated using a frequency questionnaire). Vegetarian subjects were required to have maintained a meat-free diet for a minimum of two years. Subjects were studied on four separate occasions for five consecutive days at a time. At each visit, 72-hour urine collection, 3-day blood samples, and 5-day food records were obtained. Lean body mass was estimated using urinary creatinine concentrations derived from the 72-hour samples. The article states "Janssen and Heymsfield et al. demonstrated that this indirect method is valid for measuring fat-free mass and skeletal muscle mass in human subjects, but required certain specific conditions:

1. The consumption of the same diet as normal during data collection.
2. To minimize emotional stress and physical activity during data collection.
3. The absence of severe renal insufficiency.
4. The collection of urine during 3 consecutive days" (60, 61).

Subjects were asked to begin recording dietary intake two days before the start of each 72-hour urine collection and to continue for the duration of the collection period. The goal of this was to control for the fact that subjects may alter their eating habits during the 3-day period. Investigators used the following equation to calculate muscle mass.

- ((Means of quantity of urine/100) x (means of quantity of creatinine in mg/dl) x 21.8)/1000

Muscle mass index was also calculated by dividing the result of the equation above by height of the subject in m^2.

Subjects were asked to maintain their typical diets during the five-day food record process. Dietary analysis was performed using a 1983 coding system developed by the nutrition department at the University of Helsinki. Macronutrient values were recorded and protein values were separated by source: animal or plant protein. Protein intakes were given as g/day and g protein/kg body weight.

Statistical analysis revealed that while the two groups did not differ in age, body weight, BMI, or activity level, they did differ significantly in muscle mass and muscle mass index; both values were higher in the omnivore group. Analysis of dietary data revealed significantly greater plant protein intake among vegetarians and significantly greater animal protein intake among the omnivores. Total protein intake and total energy intake did not differ between groups. Even after controlling for plant protein intake, results still revealed the same significant differences between groups. Researchers performed Pearson correlations and found that animal protein intake (both g/day and g/kg body weight) correlated with muscle mass index. Ratio of animal protein to plant protein also correlated with muscle mass index. These results suggest that intake of animal protein may promote a greater lean body mass. It is important to note that these results cannot be generalized to the public as only healthy, Caucasian women were used in this study. (60)

A 2016 review sought to gather information comparing athletic performance of vegetarians with that of meat eaters (62). Three theories were suggested as reasons why a vegetarian diet may be beneficial for physical performance: 1. Higher carbohydrate intake may lead to increased glycogen stores; 2. Increased intake of antioxidants and phytochemicals may reduce oxidative stress related to exercise and improve immune response; 3. Intramuscular acidity may decrease performance of high-intensity activity, and vegetarian diets may hypothetically increase alkalinity of the body's acid-base balance. Eight studies were identified according to the researchers' search criteria. Three of the identified studies analyzed effects of resistance training between omnivore and vegetarian groups in older men. No significant difference in strength was found in the three studies, apart from one (63) in which the only significantly greater strength gain among vegetarians was in performance of leg extensions. Four of the articles included in the review compared aerobic and anaerobic performance of omnivores and vegetarians, three of which discovered no significant differences in aerobic or anaerobic capacity. Hietavala et al. (2012) found no difference between length of time to exhaustion, but found that oxygen consumption was significantly higher among vegetarians (64). The last study included in the review reported no difference in measures of immune system response between vegetarians and omnivores after exercise (65). Overall conclusions state that evidence is limited and more research is warranted to draw conclusions as to whether vegetarian diets may have positive or negative effects on athletic performance (62).

A 2015 cross-sectional study analyzed cardiorespiratory fitness and strength among omnivore and vegetarian athlete subjects (66). 70 endurance athletes were recruited for this study: 27 vegetarians and 43 omnivores. 7-day food logs were collected and analyzed using Food Processor software (ESHA Research, version 10.11.10, Salem, OR, USA). Anthropometric measurements and DEXA scans were used to determine BMI and body composition of these subjects. Cardiorespiratory fitness was assessed by measuring maximal oxygen uptake while subjects ran on a treadmill. Participants were encouraged to run for as long as possible. Strength was analyzed by measuring peak torque; this was recorded using an isokinetic dynamometer during performance of leg extensions and flexions.

Results revealed lower body weight and lower lean body mass among vegetarians. Maximal oxygen uptake was significantly higher among vegetarian subjects. Peak torque did not differ between groups. This suggests that a vegetarian diet does not influence strength in endurance athletes, but may increase aerobic capacity, particularly in subjects with lower body weight (66). This may be related to the significantly higher carbohydrate content found in the diets of vegetarian participants. Further research is needed to examine differences among athletes whose sports are more dependent on anaerobic capacity and strength. Subject data from this study was used as secondary data for the study detailed in following sections of this document.

Animal Versus Plant Protein Supplementation

Many studies have aimed to clarify the effects that animal and plant-based protein supplementation may have on muscle mass and athletic performance. Whey is a commonly used supplement, often considered the gold standard due to its high leucine content. However, more athletes are turning toward alternative protein sources such as soy protein, pea protein, rice protein, and hemp protein.

An article published in Nutrition & Metabolism (Phillips, 2016) gathered information about protein supplementation quality and muscle hypertrophy (67). Researchers examined dozens of articles and aimed to compare the effect of high and low-quality proteins on muscle gain when paired with resistance exercise. Phillips discusses the process of muscle hypertrophy and theories surrounding it. Essential amino acids are required for muscle protein synthesis, one of the most notable being leucine, which signals to stimulate protein synthesis. The “leucine threshold” is an idea that suggests ingestion of high quality protein will cause rapid leucinemia, which is followed by an increase in intracellular leucine concentrations. These high leucine concentrations will then trigger a surge in muscle protein synthesis. This theory suggests that identifying available leucine content may be just as important as overall essential amino acid content of a protein. This literature review took the leucine content of various proteins into account by calculating the “leucine amino acid reference ratio”. This compares the leucine content of a specific food to the leucine content of the reference protein used in DIAAS calculations.

Whey is notable for its high leucine content, which suggests that it is more rapidly absorbed, leading to faster protein synthesis. The author discussed a fairly recent review (68) which included 14 whey protein studies. Investigators compiled results of the reviewed articles and found that whey significantly increased lean body mass when paired with a resistance exercise routine. When compared to other proteins, whey supplementation did not lead to significantly greater muscle gain. Another study compared whey and soy supplementation paired with strength training for a 9-month period (69). Untrained men and women were randomly assigned one of three isoenergetic supplements: whey protein, soy protein, and carbohydrate. Subjects were given resistance training routines to perform and were supervised throughout the 9-month period (96 workout sessions). Body composition was analyzed at baseline and at months 3, 6, and 9. Blood leucine concentrations were analyzed at baseline and at month 9. Blood samples were taken in a fasting state, as well as after supplement ingestion (post-workout). Gains in muscle mass were significantly higher in the whey group than in the soy and carbohydrate groups. Muscle gain did not differ between the soy and carbohydrate groups. Fat loss did not differ between groups. Fasting blood leucine concentrations were significantly higher than at baseline in the whey group. The authors found that fasting blood leucine content is positively correlated with gains in lean body mass.

Another study compared whey and rice protein supplementation over an 8-week resistance training period in 24 college-age male subjects (70). This study aimed to overcome the leucine threshold by supplementing large amounts of these proteins. 48 g of each protein would provide 5.5 g and 3.8 g of leucine from whey and rice protein, respectively. This amount of leucine fully triggers the response to start muscle protein synthesis. Both groups saw significant increases in muscle mass and decreases in fat mass, but results did not differ between groups. This supports the idea that lower-quality proteins may produce similar results as higher quality proteins when consumed in larger amounts. In a real-world setting, it is unlikely that such large quantities would be consumed. If one were to half the portions of each protein, whey would still fully saturate the protein synthesis response, while the rice protein would not (67, 70).

A systematic review published in 2014 from the US Army Research Institute sought to analyze the evidence surrounding supplemental protein and its effect on muscle mass, strength, and fitness (71). The investigators used PubMed and Google Scholar to identify useful peer-reviewed articles published prior to the fall of 2013. Several combinations of keywords were used as search terms, including the keywords ‘protein’, ‘supplements’, ‘exercise’, and ‘muscle’, as well as many others. Searches were limited to studies with participants between the ages of 18 and 50, with no significant health issues, and with a typical daily protein intake near or above the 0.8 g/kg recommended dietary allowance. Studies that tested the effects of protein supplements containing other ingredients like vitamins, minerals, or herbs were excluded from the study. Single amino acid supplements were not included in the review. 32 protein supplementation articles were found that analyzed strength and muscle mass changes as a result of resistance training. A number of articles reviewed by the investigators compared the effects of protein supplementation and a non-energetic placebo. Results were varied in these studies when using non-athlete participants. Studies comparing the effects of different types of proteins, like whey or soy, were also included in this review. A notable study mentioned in this review found that whey and soy produced a near equal increase muscle mass and strength after six weeks of resistance training and supplementation (72). Overall protein intake during this study was very high, likely counteracting the differences caused by lower protein quality in the soy group. This review article also points out that studies in well-trained athletes may provide a clearer picture of the effects of protein supplementation. Untrained participants add a confounding variable – their bodies are adapting to new types of physical activity and becoming more efficient with each training session. This makes it less evident whether effects are from the supplementation or from the exercise routine itself (62).

A controlled intervention study from 2007 analyzed the effects of different protein sources by comparing muscle mass, strength, and muscle fiber size after 12 weeks of resistance training and post-workout supplementation of either dairy, soy, or carbohydrate (73). Researchers recruited young men between the ages of 18 and 30 who were either sedentary or lightly active (less than 2 to 3 hours of leisurely activity per week). This intervention required the subjects to perform resistance exercise training 5 days per week for 12 weeks. The exercise routine was broken into three separate categories of exercise: pushing exercises (bench press, triceps extension, etc.), pulling exercises (biceps curl, seated lateral pull down, abdominal exercises, etc.), and leg exercises (leg extension, calf raise, hamstring curl, etc.). Training sessions were supervised to ensure good form and routine compliance. All weighted exercises were completed using guided motion machinery as opposed to using hand-held free weights. The 5-day routine would contain two pushing exercise days, two pulling exercise days, and one leg exercise day.

Subjects were split into three groups: milk, soy, and carbohydrate (control group). After each training session, subjects were given 500 mL of their designated fluid supplement. The milk group (n = 18) received fat-free milk (17.5 g protein, 25.7 g carbohydrate, 0.4 g fat). The soy group received a soy protein drink that was nutritionally equal to the fat-free milk, containing the same calories and macronutrient ratio. The soy drink also was equal in nitrogen, free of isoflavones, and did not contain any fiber. The carbohydrate group received a drink with the same calorie content comprised only of carbohydrate. All drinks were vanilla flavored and served in opaque containers, and subjects were asked to refrain from discussing the beverages. All participants consumed one portion of their designated beverage immediately after exercise, and another portion one hour afterwards. No food or beverage with any macronutrient value was to be consumed within 2 hours prior to each training session. Subjects were not to consume any additional supplements throughout the course of the study. Prior to the study and at weeks 6 and 12, subjects completed 3-day food records. Daily macronutrient intake was calculated using Nutritionist V software (First Data Bank, San Bruno, CA). DEXA scans were used to analyze lean body mass before and after the 12-week training period.

Data analysis revealed significant increases in weight and muscle mass in all 3 groups. The milk group had the greatest increase in muscle mass. Fat loss was greater in the milk group in comparison to the soy and control groups. The 3-day diet records showed an increase in protein intake for all groups when comparing pre-study and post-study diet records. The control group had significantly higher intake of carbohydrates; protein intake did not differ between groups. All food records included post-workout beverages. Changes in strength were determined by comparing pre- and post-study one-rep maximum weight values. All groups displayed significant gains in strength. “A trend toward greater strength gains was observed for the leg press in the milk and soy groups than in the control group (P=0.075). A trend for greater strength gains was also observed in the milk group than in the soy and control groups for knee extension (P=0.077) and hamstring curls (P=0.082)" (73). No significant difference was found for all other measurable exercises. Researchers reported no bias; this study was funded in part by the US National Dairy Council.

Current Recommendations for Athletes

A 2016 joint statement from the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine outlines nutritional recommendations for optimal athletic performance. Dietary protein intake, when combined with exercise, promotes the "synthesis of contractile and metabolic proteins as well as enhancing structural changes in non-muscle tissues such as tendons and bones" (13). Research suggests protein synthesis mechanisms are stimulated by a rise of leucine within the body paired with enough dietary protein to fuel muscle mass gain. Protein intake immediately after and throughout the day after exercise may be beneficial due to the fact that protein synthesis and sensitivity to dietary protein intake are higher for 24 hours after a workout. While protein recommendations for the general public range from 0.8 g/kg to 1.0 g/kg, athletes may require anywhere from 1.2 g/kg to 2.0 g/kg depending on activity type, frequency, and intensity. General ranges are given, but the statement recommends that protein intake should be personalized to the athlete's goals and training routines. Excessive protein intake does not pose a threat; the idea that excess protein may cause a decline in kidney function is not supported by evidence. (74).

A 2003 review article discussed several different points concerning the effect dietary differences may have on athletic performance (75). The standard recommendations for protein intake may not be sufficient for elite athletes according to multiple nitrogen balance studies. Strength athletes may require up to 1.8 g/kg/day to reach a positive nitrogen balance, while endurance athletes may require 1.4 - 1.5 g/kg/day (60, 76). High needs may prompt some athletes to add supplementary protein to their diet, however, this may not be necessary in mixed diets. Studies show that on average, omnivore athletes consume 1.5 g/kg/day without supplementation.

High quality proteins are proven to be useful in the maintenance and accretion of muscle mass. Dairy protein has been shown in several studies to be superior to other protein sources (77), but further research is needed to compare effects of other high-quality proteins, such as egg, meat, and soy. A vegetarian diet may be nutritionally adequate for athletes, but proper planning is required to ensure appropriate intake of macro and micronutrients (11, 78). Different strategies can be used to overcome low protein quality in a restrictive vegetarian diet. Van Vliet (2015) suggests fortifying plant-based proteins with their limiting amino acids or modifying plant foods during breeding to improve amino acid content (79). These solutions would require years of further research and are not practical from an individual consumer's standpoint. The final two suggestions are much more achievable in the short-term: increase the quantity of plant protein sources consumed, and increase the variety of dietary plant protein sources in order to reach an optimal amino acid intake.

Restriction of meat and other animal foods can promote good health when planned appropriately. Regardless of physical activity level, vegetarian diets require special attention to quantity and quality of protein intake. Even elite athletes can thrive on meat-free diets, though more research is warranted to pinpoint optimal protein recommendations for vegetarians.

Summary

Although vegetarian diets can promote overall health and well-being, several nutrients require additional planning and consideration when eliminating meat and other animal products. Vegetarian diets are abundant in many antioxidants, like vitamins A and C and polyphenols. Adequate fiber intake can be easy to accomplish when eating a varied, minimally processed, plant-based diet. Vegetarian and vegan diets can improve intakes of healthy dietary fat sources and can reduce intake of more potentially harmful trans fats, saturated fats, dietary cholesterol, and arachidonic acid. Restriction of meat intake and increased intake of plant foods can have a multitude of health benefits, but restriction of animal foods may have health consequences when not appropriately planned. Vegetarians need to be aware of which foods contain vitamins B-12 and D, calcium, iron, and zinc so that they are able to consume a well-balanced diet and meet their micronutrient needs.

However, before micronutrients can be considered, one must look at the bigger picture. Macronutrient adequacy forms the basis upon which a healthful, micronutrient-rich diet is built. Protein deficiency is extremely rare in developed countries, as both omnivores and vegetarians tend to consume quantities of protein above the RDA. The RDA for protein is currently 0.8 g/kg for healthy adults. Many populations may have higher protein needs, including the elderly who are commonly affected by sarcopenia. Athletes also require additional protein to promote muscle synthesis and fuel their strength and athletic performance. Even if protein quantity is adequate, protein quality must be considered. Populations with higher protein needs may need to consume high-quality proteins to get the most benefit from their protein intake. This is a simple task for meat-eaters, but when animal foods are taken away, the risk of overall lower-quality dietary protein intake increases. Further research is necessary in this area; future research should examine effects of dietary protein quality in populations with varying levels of protein requirements.

Chapter 3

METHODS

Participants and Study Design

This cross-sectional study used previously collected data from a group of 35 vegetarian athletes and 35 omnivore athletes. Answers to questions regarding diet led to eight participants being recategorized as omnivore due to occasional meat consumption. Of the subjects recruited, only 60 submitted food records, bringing the total number of vegetarians and omnivores to 22 and 38, respectively. Data from participants without food records was excluded from analysis. Subjects were recruited from the Phoenix area via Stevebay.org (a popular website for endurance athletes), Facebook, and word of mouth. Inclusion criteria required participants to be healthy men and women who were active athletes over the age of 18. Subjects were required to be part of a National Collegiate Athletic Association (NCAA) Division 1 university team or training for a competitive endurance race (marathon, triathlon, etc.). Exclusion criteria included pregnancy or lactation, and current injury or rehabilitation. All subjects were given thorough verbal explanation of the study protocol and all provided written consent. The study from which this secondary data was obtained was approved by the Institutional Review Board at Arizona State University, number HS1211008557. Recruitment and all data collection occurred between August and November of 2015.

Lean Body Mass and Strength Assessment

Anthropometric measurements were obtained, which include weight, height, and waist circumference. Body fat percentage, lean body mass, and bone mineral density data were gathered using Dual-energy X-ray Absorptiometry (DEXA) (Lunar iDXA, General Electric Company, East Cleveland, OH, USA). DEXA analysis was conducted by a trained and certified radiology technologist.

Strength was measured by assessing peak torque on an isokinetic dynamometer (Computer Sports Medicine, Inc., Stoughton, MA, USA) for both leg extension and flexion. Torque can be described as the amount of force applied when rotating an object. Extension and flexion of both legs were performed at 3 different speeds: 60, 180, and 240 degrees per second. Three maximal effort repetitions were performed for each speed.

Participants were given thorough instructions and practiced at each speed to become familiar with the proper protocol. Participants rested for 30 seconds between each set of repetitions. Peak torque as identified by the isokinetic dynamometer was recorded for the self-reported dominant leg. Aerobic fitness was also assessed by measuring VO2max while the subjects ran on a treadmill, but this data was not included in analysis for the present study.

Dietary Analysis

Seven-day food records were collected and dietary analysis will be used to identify dietary protein sources. Subjects were instructed to record all foods, drinks, and supplements for seven consecutive days. Logs were intended to be as detailed as possible; participants were encouraged to include brand names and portion sizes when applicable.

Subjects were asked to maintain their typical diet during this time. All recorded food items were entered and analyzed using Food Processor 10.11.0 (ESHA Research, Salem, OR, USA). A default food list was used during analysis when detail was lacking in the food records or when given items were not available in the Food Processor database.

Data from Food Processor was entered into a Digestible Indispensable Amino Acid Score calculation spreadsheet adapted from FAO/WHO. A limited list of foods was used for the DIAAS spreadsheet. Foods were sorted into the most applicable category using a standard protocol. The DIAAS spreadsheet included several forms of dairy, eggs, meat, grains, beans, legumes, nuts, and seeds. Two types of protein powder were also included: whey protein concentrate and soy protein isolate. Fruits, vegetables, sugars, and oils were not included due to their minimal protein contribution. All seven days of food were entered into the DIAAS spreadsheet. Four subjects were missing at least one day from their 7-day food logs; these food records were still included in analysis.

Table 3. Protein information for all foods used in DIAAS calculation spreadsheet.

Food Product	Protein	IAA Composition* (g/100g)				True Ileal IAA Digestibility**			
	g/100g	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp
Milk	3.4	0.312	0.136	0.177	0.055	0.91	0.93	0.92	0.93
Egg	12.14	0.82	0.681	0.596	0.194	0.909	0.909	0.909	0.909
Bread	10.5	0.1464	0.2034	0.1536	0.0821	0.92	0.9	0.91	0.9
Cereal (corn)	7.5	0.07	0.24	0.2	0.04	0.66	0.76	0.69	0.42
Cereal (wheat)	11.2	0.3208	0.4126	0.3083	0.1417	0.85	0.79	0.67	0.75
Oats, dry	16.85	0.7012	0.7198	0.575	0.234	0.76	0.85	0.7	0.77
Oats, cooked	2.52	0.135	0.1432	0.0962	0.0402	0.76	0.85	0.7	0.77
Cheese	24.9	2.072	0.777	0.886	0.32	0.91	0.9	0.88	0.85
Cheese, cottage	12.39	1.002	0.49	0.55	0.138	0.91	0.9	0.88	0.85
Corn (flour)	8.5	0.263	0.364	0.351	0.0658	0.76	0.86	0.76	0.78
Tortilla, corn	5.8	0.1625	0.225	0.217	0.0417	0.76	0.86	0.76	0.78
Popcorn	12.86	0.339	0.472	0.454	0.0857	0.76	0.86	0.76	0.78
Beans, cooked	8.21	0.564	0.213	0.346	0.097	0.94	0.77	0.72	0.77
Peas, cooked	8.34	0.602	0.212	0.296	0.093	0.9	0.74	0.91	0.89
Peanuts, dry-roasted	23.68	0.85	0.595	0.811	0.23	0.94	0.96	0.89	0.84
Potato, baked	2.61	0.101	0.0436	0.0601	0.0178	0.52	0.52	0.48	0.47
Sweet potato, baked	2.02	0.0842	0.0648	0.107	0.0404	0.53	0.55	0.51	0.47
Rice, cooked	2.7	0.0968	0.1178	0.0962	0.0312	0.92	0.91	0.82	0.89
Soybean, boiled	16.64	1.108	0.492	0.723	0.242	0.8	0.72	0.81	0.68
Soybean, roasted	35.22	2.344	1.042	1.53	0.512	0.8	0.72	0.81	0.68
Soy milk	3.27	0.131	0.027	0.1082	0.038	0.8	0.72	0.81	0.68
Soy protein isolate	82.14	5.327	2.176	3.137	1.116	0.99	0.98	0.98	0.95
Sunflower seeds	22.78	0.937	0.945	0.928	0.348	0.77	0.81	0.77	0.8
Wheat (flour)	10.3	0.228	0.4024	0.2808	0.1272	0.8	0.88	0.83	0.88
Wheat pasta, cooked	5.7	0.1329	0.1778	0.2057	0.0829	0.8	0.88	0.83	0.88
Whey protein concentrate	83.3	7.321	13.278	5.723	1.441	0.91	0.9	0.89	0.87
Meat (all animal flesh)	25.01	2.125	1.013	1.056	0.292	1	1	1	1

*Total protein and amino acid composition information obtained from Bowes & Church's Food Values of Portions Commonly Used (80).
**True ileal digestibility values obtained from Gilani S (2011) (81), Evenepoel, et al (1998) (82).

Four indispensable amino acids were included in the calculation spreadsheet adapted from the 2013 FAO/WHO paper regarding protein digestibility. Lysine, sulfur amino acids (SAA = methionine + cysteine), threonine, and tryptophan are the most common limiting amino acids among plant foods. All animal flesh foods (meat, poultry, and seafood) were assumed to have 100% digestibility.

Food Item Categorization

Categorization was kept as standard as possible for mixed food items and foods not included in the DIAAS spreadsheet. Weight of all foods (g) was entered as reported by Food Processor unless food was a mixed item (sandwiches, soups, energy bars, etc.).

- All milk types, yogurt (plain, flavored, Greek, etc.), and any fluid dairy products were categorized under "milk".
- All animal flesh foods (beef, pork, poultry, fish, shellfish, etc.) were categorized under "meat".
- All non-dairy milk alternatives (soy milk, almond milk, cashew milk, etc.) were categorized under "soy milk".
- Quinoa, buckwheat, and other specialty grains were categorized under "wheat pasta, cooked".
- Crackers, cookies, muffins, and other baked products were categorized under "bread".
- All nuts and nut butters were categorized under "peanuts, dry roasted".

- All potato products (baked potatoes, mashed potatoes, French fries, and chips) were categorized under “potato, baked”.
- Hard granola bars were categorized under “oats, dry”; chewy granola bars were categorized under “oats, cooked”.
- Soy-based veggie burgers were categorized under “soybean, boiled”; bean-based patties (black bean burgers, falafel, etc.) were categorized under “beans, cooked”.
- All plant-based protein powders were categorized under “soy protein isolate”.
- Protein bars were categorized by their main protein-containing ingredient; weight (g) was adjusted to approximately match total protein content of the food item.
- Mixed food items were categorized by each of their protein-containing ingredients; weight (g) per ingredient was adjusted so that the sum of the ingredients approximately matched the protein content as reported by Food Processor.

DIAAS Calculation

All calculations were performed using Microsoft Excel (2016). See the following page for calculation examples.

Table 4. DIAAS CALCULATION TABLE FOR MIXED DIET: EXAMPLE

	Wt	Pro	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp	Protein content	Lys	SAA	Thr	Trp
	(g)	(g/ 100g)	(g/100g)				Ileal Digestibility				(g)	Digestible mg IAA			
	A	B	C	D	E	F	G	H	I	J	AxB/ 100	(AxB) X CxG	(AxB) X DxH	(AxB) X ExI	(AxB) X FxJ
Milk	100	3.4	0.312	0.136	0.177	0.055	0.91	0.93	0.92	0.93	3.4	283.92	126.48	162.84	51.15
Cereal (corn)	50	7.5	0.07	0.24	0.2	0.04	0.66	0.76	0.69	0.42	3.75	23.1	91.2	69	8.4
Total	150										7.15	307	217.7	231.8	59.6
Amino Acids mg/g (total for each AA/total protein)												42.9	30.4	32.4	8.3

	Reference Pattern (mg/g)				Digestible IAA Reference Ratio				DIAAS for mixture
	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp	
Older Children/Adults	48	23	25	6.6	**.89**	1.32	1.3	1.26	**89% (Lys)**

The above table is adapted from: "Dietary protein quality evaluation in human nutrition." FAO/WHO 2013.

The table above shows the basic DIAAS spreadsheet calculation process using two food items as an example: 100g of milk and 50g of corn flake cereal. Steps for manually calculating protein digestibility are as follows:

1. (weight of food consumed x protein content in 100g of food)/100 = g protein in food portion
2. (weight of food consumed x IAA content in 100g of food)/100 = g of IAA in food portion
3. (g of IAA in food portion * true ileal digestibility) *(1000) = Digestible mg IAA. Repeat for all amino acids.
4. Repeat for each food item.
5. Add all food item values (digestible mg IAA) to find total digestible dietary intake of each amino acid.
6. (Total digestible mg IAA / total protein intake) / reference pattern mg = Digestible IAA Reference Ratio. Repeat for each amino acid.
7. The lowest result for step 6 is the overall dietary protein digestibility (DIAAS). This also identifies the limiting amino acid.

The DIAAS spreadsheet performs each of these steps automatically after manually entering weight of all foods consumed.

Available protein was calculated by multiplying the overall dietary DIAAS by average daily protein as reported by Food Processor. This process was repeated for each subject.

Statistical Analyses

Data is reported as mean ± the standard error. SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Data was tested for normal distribution; abnormally distributed data were log-transformed to achieve normal distribution. No outliers were identified. Significance will be $p \leq 0.05$. Pearson correlations were used to evaluate relationships between digestible protein intake, lean body mass, and strength. Analysis of variance was also used to evaluate differences between groups.

Chapter 4

RESULTS

Group Descriptive Statistics

Table 5. Omnivore and Vegetarian Group Statistics

Variable		n	Mean ± Std. Deviation	p Value*
Age	Omn.	38	37.9 ± 9.4	0.474
	Veg.	22	36.1 ± 9.1	
Height (in)	Omn.	38	68.4 ± 3.2	0.060
	Veg.	22	66.8 ± 3.2	
Weight (lb.)	Omn.	38	164.0 ± 26.9	0.001
	Veg.	22	140.1 ± 22.4	
BMI	Omn.	38	24.6 ± 3.1	0.011
	Veg.	22	22.5 ± 2.9	
Sex		Male %(N)	Female %(N)	
	Omn.	60.5% (23)	39.5% (15)	0.313
	Veg.	50.0% (11)	50.0% (11)	

*Significance is set at p<.05

**p value represents group difference analyzed by independent samples t-test

Of the 70 participants recruited for this study, 60 returned food records. included 38 “omnivore” athletes and 22 “vegetarian” athletes. Note that although participants self-declared their diet status, some self-declared vegetarians (n=8) were moved to the omnivore group due to recorded meat consumption. The two groups showed no significant difference in age or height. When weight and BMI were analyzed, it was observed that the vegetarian subjects were significantly lighter in weight and had lower BMI values (Table 5). When controlling for gender, weight still differed significantly between groups (p=0.001). All subjects were endurance athletes; a majority were triathletes (n=27), and the rest were runners (n=20) or cyclists (n=10). 2 subjects played competitive lacrosse (n=2), and one was a long-distance hiker. These three subjects were grouped into the running category for the purpose of evaluation due to the nature of their sports. There were no significant differences in strength and lean body mass when comparing the three athlete groups.

A Shapiro-Wilkes normality test revealed that data for total protein and available protein were not normally distributed. These data were log transformed to achieve normality. No outliers were found in the data.

Table 6. Nutrient Intakes Across Omnivore and Vegetarian Groups

Nutrient (Daily Averages)		**n**	**Mean ± SD**	**p Value***
Total kcal	Omn.	38	2349.9 ± 636.8	0.447
	Veg.	22	2472.5 ± 520.7	
kcal from Fat	Omn.	38	315.7 ± 51.2	0.637
	Veg.	22	228.6 ± 48.7	
kcal from Saturated Fat	Omn.	38	99.0 ± 16.1	0.162
	Veg.	22	99.9 ± 21.2	
Carbohydrates (g)	Omn.	38	280.8 ± 79.7	0.015
	Veg.	22	332.4 ± 70.9	
Total Protein (g)	Omn.	38	101.6 ± 31.2	0.002
	Veg.	22	78.5 ± 17.7	
Protein Digestibility (%)	Omn.	38	99.9 ± .8	<0.001
	Veg.	22	89.9 ± 10.5	
Available Protein (g)	Omn.	38	101.5 ± 31.2	<0.001
	Veg.	22	71.0 ± 19.6	

*Significance is set at p<.05

**p value represents group difference analyzed by independent samples t-test

Seven-day food records were entered in Food Processor for nutrient analysis. Protein digestibility was calculated for daily diets using DIAAS formulas for common food items, and 7-day DIAAS average was calculated. Available protein values were calculated by applying digestibility percent to the averaged total protein intake from Food Processor. Upon analysis using independent samples t-tests, select macronutrients differed significantly between groups. Total calorie intake and calories from fat and saturated fat did not differ between groups. Total protein intake, digestibility percent, and available protein were significantly higher among omnivore participants. Carbohydrate intake was significantly higher for vegetarian participants (Table 6).

Table 7. Protein intake as a function of body mass.

Variable		**n**	**Mean ± SD**	**p Value**
Total Protein (g/kg)	Omn.	38	1.39 ± .47	0.258
	Veg.	22	1.24 ± .25	
Available Protein (g/kg)	Omn.	38	1.39 ± .47	0.014
	Veg.	22	1.12 ± .30	

*Significance is set at p<.05
**Data was log transformed to achieve normal distribution.

Evaluation of protein intake per kilogram of bodyweight revealed significantly higher available protein g/kg in the omnivore athletes. Total protein g/kg did not differ between groups (Table 7).

Table 8. Lean Body Mass and Strength Across Omnivore and Vegetarian Groups

Variable		n	Mean ± SD	p Value*
Lean Body Mass (kg)	Omn.	38	55.1 ± 9.6	0.011
	Veg.	22	48.5 ± 8.9	
Strength (torque foot-pounds)	Omn.	38	105.0 ± 33.0	0.074
	Veg.	21	88.5 ± 33.6	

*Significance is set at p<.05

**p value represents group difference analyzed by independent samples t-test

Total grams of lean body mass (as calculated using DEXA) differed significantly between the omnivore and vegetarian groups (p=0.011). Strength (recorded using HumacNorm isokinetic dynamometer to measure leg extension and flexion) did not differ significantly between groups (Table 8). When controlling for gender, lean body mass differed more significantly (p=.004); strength did not (p=0.106). One participant did not complete strength testing and was not included in correlation analyses.

Correlations

Table 9. Correlation between strength and lean body mass and protein intake measures for all subjects.

Correlations

		Strength	**Lean Body Mass**	**Total Protein**	**Available Protein**
Strength	Pearson Corr.	1	.758**	.372**	.314*
	p Value		.000	.004	.016
	n	59	59	59	59
Lean Body Mass	Pearson Corr.	.758**	1	.575**	.541**
	p Value	.000		.000	.000
	n	59	59	59	59
Total Protein	Pearson Corr.	.372**	.575**	1	.967**
	p Value	.004	.000		.000
	n	59	59	59	59
Available Protein	Pearson Corr.	.314*	.541**	.967**	1
	p Value	.016	.000	.000	
	n	59	59	59	59

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Strength and lean body mass were directly correlated ($p<0.001$). Available protein intake was significantly correlated to both lean body mass ($p<0.001$) and strength ($p=0.016$) (Table 9). Calculation of effect sizes using univariate ANOVA between the omnivore and vegetarian groups revealed a large effect size for log-transformed available protein ($\eta^2_p = 0.261$), and medium effect sizes for lean body mass and strength ($\eta^2_p=0.107$ and 0.055, respectively).

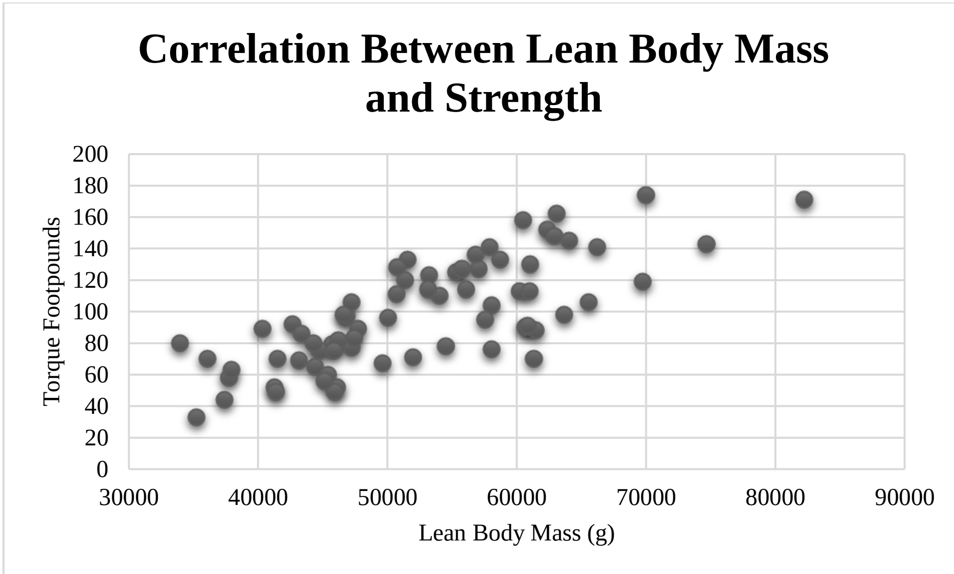

Figure 1. Significant positive correlation between lean body mass and strength (p=0.000)

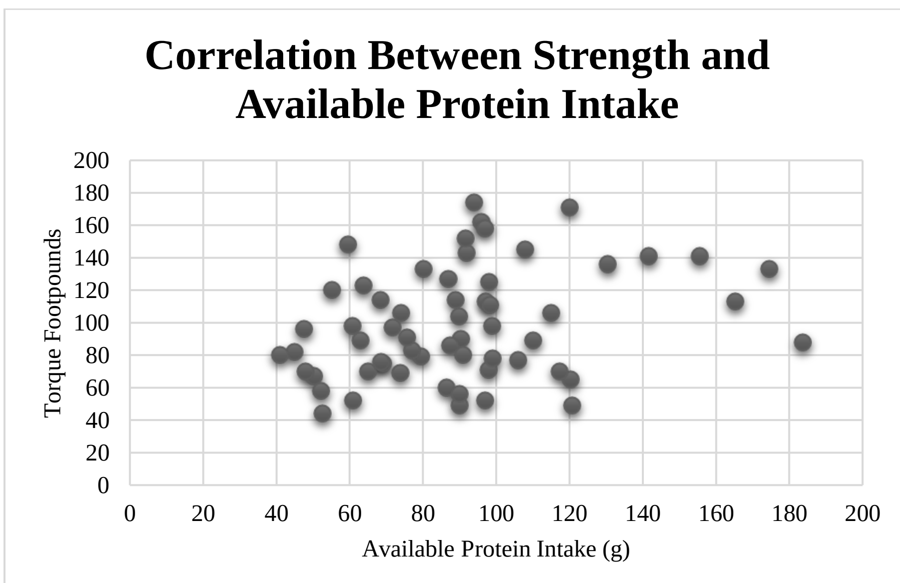

Figure 2. Significant positive correlation between strength and available protein intake (g) (p=0.016).

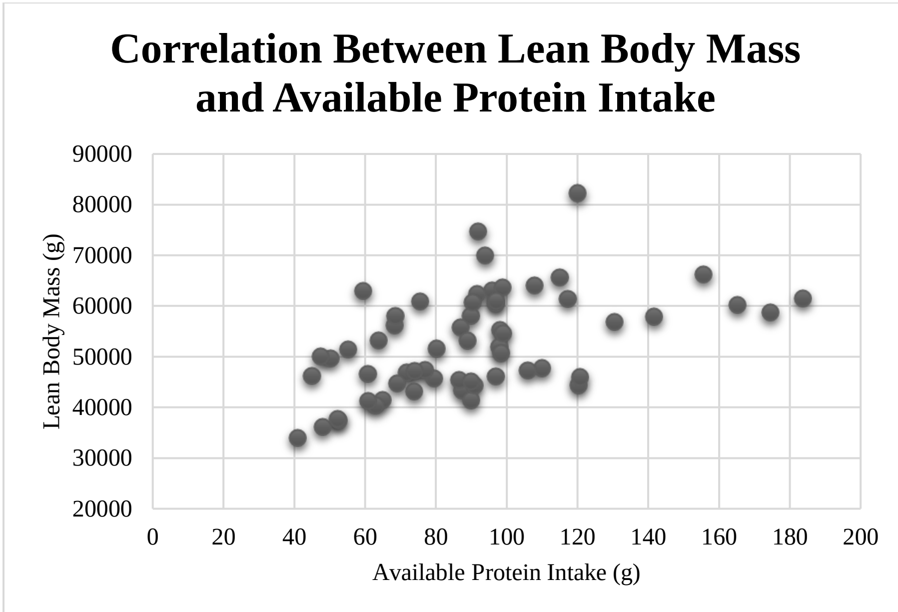

Figure 3. Significant positive correlation between lean body mass and available protein intake (g) (p=0.000).

Correlations Within Groups

Table 10. Correlations between strength and lean body mass and protein intake measures for vegetarian subjects (n=21).

Correlations

		Strength	Lean Body Mass	Total Protein	Available Protein
Strength	Pearson Corr.	1	.803**	.388	.156
	p Value		.000	.082	.501
	n	21	21	21	21
Lean Body Mass	Pearson Corr.	.803**	1	.620**	.446*
	p Value	.000		.003	.043
	n	21	21	21	21
Total Protein	Pearson Corr.	.388	.620**	1	.913**
	p Value	.082	.003		.000
	n	21	21	21	21
Available Protein	Pearson Corr.	.156	.446*	.913**	1
	p Value	.501	.043	.000	
	n	21	21	21	21

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 11. Correlations between strength and lean body mass and protein intake measures for omnivore subjects.

Correlations

		Strength	Lean Body Mass	Total Protein	Available Protein
Strength	Pearson Corr.	1	.710**	.281	.272
	p Value		.000	.088	.098
	n	38	38	38	38
Lean Body Mass	Pearson Corr.	.710**	1	.470**	.466**
	p Value	.000		.003	.003
	n	38	38	38	38
Total Protein	Pearson Corr.	.281	.470**	1	1.000**
	p Value	.088	.003		.000
	n	38	38	38	38
Available Protein	Pearson Corr.	.272	.466**	1.000**	1
	p Value	.098	.003	.000	
	n	38	38	38	38

**. Correlation is significant at the 0.01 level (2-tailed).

When looking at Pearson correlations for the two diet groups separately, the relationship between available protein and lean body mass retains its significance. The correlation between available protein and strength loses its significance when the two diets are analyzed separately (Table 10, Table 11).

Overall results reveal that higher intake of available protein correlates with higher lean body mass composition and physical strength in an athlete population.

Chapter 5

DISCUSSION

The present study was designed to evaluate and compare dietary protein quality in both vegetarian and omnivore athlete diets. We hypothesized that protein quality (available protein) would correlate with both lean body mass and strength. We also hypothesized that the omnivore group would have higher dietary protein quality (as expressed through quantity of available protein).

These hypotheses were proven true as available protein intake was significantly higher in omnivore subjects ($p<.001$). Available protein intake was 101.5 ± 31.2 g/day for omnivores and 71.0 ± 19.6 g/day for vegetarians. Overall digestibility percent (mixed-diet DIAAS) was significantly higher for omnivores (99.9 ± .8) than vegetarians (89.9 ± 10.5) ($p<.001$). Values for digestibility percent were not normally distributed and were not included in Pearson correlational analyses. The percentage of digestible protein is only useful in the context of total protein intake, as it is possible to meet all dietary needs with any DIAAS when a high enough quantity of protein is consumed. This supports the need for an increase of the protein DRI for vegetarians. The vegetarian athletes in this study had lower overall protein intake (78.5 ± 17.7 g/day) than the omnivore athletes (101.6 ± 31.2 g/day) ($p=.002$). When digestibility was taken into account, the difference between the groups' protein intakes grew by approximately 7 g. If the vegetarians had a higher overall protein intake, they may have been able to overcome the lower digestibility.

The significant association of available protein intake and lean body mass and strength is especially meaningful in the athlete population, as their performance may be affected by lower muscle mass and strength. The athletes in this study were endurance athletes; their sports depend more on their cardiovascular abilities than their strength. Endurance athletes tend to be very lean regardless of omnivore or vegetarian status. The vegetarians in this study were significantly lower in weight and lean body mass. In the context of their sports, this may not be a disadvantage. However, had these been athletes whose sports require high levels of strength and power (weightlifting, football, wrestling), it is possible that a long-term vegetarian diet would hinder their performance.

The main limitations in this study come from the use of the 7-day food records and methods of dietary analysis. Food records are extremely useful when they contain truthful and detailed information. The subjects were instructed to consume their normal diets during this 7-day period and to be as detailed and accurate as possible. However, there is the possibility of error when it comes to recording true portion sizes and reporting all foods, beverages, and condiments eaten. Many of the food logs were lacking in detail and required the use of a standardized food item portion list.

The Food Processor software also had its limitations, as not all food items in the subjects' records were available in the food database. Many participants consumed specialty foods like protein bars and protein powders, often from lesser-known brands. This required selection of alternative items in the food database. Nutritional content and ingredients were identified for the specialty foods, and items were selected from Food Processor that approximately matched the content of the recorded food items.

The list of food item categories from the DIAAS calculation spreadsheet provides additional challenges, as not all food items fit perfectly in each category. The categories were limited by availability of true ileal digestibility values for many foods. Quinoa, for example, is not in the DIAAS spreadsheet, so every instance of quinoa in the food records was categorized under “wheat pasta”. The rationale for this action is the higher protein content of wheat when compared to other grains on the spreadsheet. Several other food items required a similar method of categorization. This may mean that amino acid content of the original foods could have been lost in translation. Another thing to note is the exclusion of fruits and vegetables in the DIAAS spreadsheet. These were not included due to the low protein content of these foods and their extremely low protein digestibility. It is reasonable to assume that these foods would not contribute large quantities of amino acids to the DIAAS calculation. Although these were excluded from the DIAAS spreadsheet, they were still recorded in Food Processor and contributed to the total protein intake and thus, the available protein intake.

Since only endurance athletes were included in this study, these results are not representative of the general population. Results may reveal even greater differences in populations with different physical activity levels; further research is needed to compare effects of protein quality on muscle mass in sedentary individuals. Additional research using more strength-dependent athletes can also provide a better picture of the effect of protein quality on physical performance. Another thing to note is that many of this study’s participants regularly consume protein supplements in the form of powders, drinks, and bars. The general population may not be as likely to use these products;

possibly resulting in vastly different dietary protein quality and quantity, especially among vegetarians.

This study helps fill the literature gap that exists regarding application of the DIAAS protein quality evaluation method in mixed diets. To our knowledge, no other studies have used DIAAS to assess multiple-day food records. Many studies have used PDCAAS, but since the introduction of DIAAS in 2013, little has been done to apply the method to mixed diets as suggested by FAO/WHO. This may open the door to further refinement of dietary analysis using DIAAS. In addition, true ileal digestibility values are available for a limited number of foods. As research regarding ileal digestibility grows, more accurate DIAAS values can be obtained.

Conclusion

This study found significant correlations between available protein intake and lean body mass and strength. The significant difference in protein quality for vegetarian diets supports recommendations to increase the protein DRI from 0.8 g/kg to 1.0 g/kg. The DRI assumes that vegetarians are getting a vast majority of their protein from animal products and that they have levels of protein digestibility similar to those of omnivores (6). As more people turn away from animal products and toward the growing industry of plant-based alternatives, this assumption becomes less valid. This also does not account for those who follow strict vegan diets, who likely have even lower dietary protein digestibility. To make up for the lower quality of plant proteins, a higher quantity of protein intake is required. Vegetarian diets are an excellent, sustainable choice that can be planned to meet all dietary requirements; additional attention to protein is recommended to reach adequate intake of indispensable amino acids.

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APPENDIX A

IRB APPROVAL

CONSENT FORM

APPROVAL:CONTINUATION

Carol Johnston
SNHP: Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 5/16/2017 the ASU IRB reviewed the following protocol:

Type of Review:	Continuing Review
Title:	Body Composition, Strength, and Protein Source: A Comparison between Vegetarian and Omnivore Division I Collegiate Athletes
Investigator:	Carol Johnston
IRB ID:	STUDY00002775
Category of review:	(8)(c) Data analysis
Funding:	Name: Graduate College (GRAD)
Grant Title:	None
Grant ID:	None
Documents Reviewed:	

The IRB approved the protocol from 5/16/2017 to 6/14/2018 inclusive. Three weeks before 6/14/2018 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 6/14/2018 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
Karen Angelica Gomes Vilela Antunes
Heidi Lynch
Pamela Swan

Body Composition, Strength, and Protein Source: A Comparison between Lacto-Ovo Vegetarian and Omnivore Division I Collegiate

INTRODUCTON

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS

Drs. Carol Johnston and Pamela Swan, Nutrition and Exercise & Wellness professors, and Heidi Lynch, a doctoral student, at Arizona State University Downtown, Phoenix, have requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to evaluate associations between diet and health parameters in elite competitive athletes. Your coaches have stated their approval of this research; however, they will not be involved in any aspect of the research.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are generally healthy, competing for a major athletic competition, and have followed your current diet for more than 6 months. If female, you do not have a history of pregnancy or lactation within the past 6 months. Also, you are not currently injured or in rehab for a recent injury. This study will involve the completion of brief demographic and health history questionnaire to demonstrate the absence of conditions that may contraindicate participation.

This research entails that you visit our test facilities on one occasion on ASU's Downtown Phoenix campus. At your visit, you will be asked to complete a survey about your general health and views on food. Your height, bodyweight, and waist circumference will be measured. In addition, body composition (relative amounts of fat and lean tissue) will be determined by using a FDA-approved bone density measurement machine. The procedure is called Dual-energy X-ray Absorptiometry (DXA). You will be asked to lie face up on an open, padded table for 7 minutes while the scanner arm of the DXA machine passes over the entire body. You can wear regular clothing but any metal must be removed. You will be exposed to a small amount of radiation (1-4 microSieverts) that is within an acceptable range per the FDA. For comparison, you would be exposed to approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. (For test accuracy, you will be asked about test procedures using barium/isotopes in the recent past and be scheduled for your visit with an adequate lapse of time.) Next, you will complete a maximal oxygen volume (VO2 max) test under supervision; your fitness as evaluated by this test is measured in terms of milliliters of oxygen used in one minute. It can also be expressed in terms relative to one's body weight (milliliters of oxygen used in one minute per kilogram of body weight). This test will consist of you first walking and then running on a treadmill at progressively faster speeds and higher inclines while wearing a mask that collects the air you exhale. It is then analyzed by a computer for oxygen and carbon dioxide composition. A person is considered to have reached his/her VO2 max when the oxygen consumption is not changing, even though the workload increases. Finally, you will have your lower body power assessed using a dynamometer machine. For this test you will be seated on a machine and extend and bend your lower leg at a constant speed. The power and torque (force) you generate will be measured.

RISKS

Anytime you are exposed to radiation there is potential risk. If there is ANY chance of being pregnant then you should not undergo the DXA scanning. Females will take a urine pregnancy test prior to the DXA scan. A certified X-ray technician will complete all DXA scans. The mask used for the breathe assessment during the VO2 max test may feel tight and uncomfortable over your nose and mouth. You are asked to tell investigators to stop any testing at any time if desired. Any time someone participates in weight lifting there is risk of injury including a pulled muscle or broken bone. Close supervision by your coaches/trainers will be provided at all times during the lifting exercise.

BENEFITS

You will not benefit from this study, but you will be provided with all your health marker test results if desired including bone mineral density, body fat composition, VO2 max results, and maximal power generated on the dynamometer.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE
You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect you any manner.

COSTS AND PAYMENTS
You will receive a $20 Target gift card at the completion of data collection.

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 500 N. 3rd St., Phoenix, AZ 85004. [602-827-2265]

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

__________________________ ___ ____________________ __________
Subject's Signature Printed Name Date

__________________________ ___ ______________________________
Contact phone number Email

INVESTIGATOR'S STATEMENT
"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator______________________________ Date__________

APPENDIX B

RECRUITMENT FLYER

HEALTH HISTORY QUESTIONNAIRE

FOOD RECORD DOCUMENT

ASU Nutrition Study:

Diet and Performance

THE NUTRITION PROGRAM AT ASU IS RECRUITING HEALTHY VOLUNTEERS TO STUDY HOW DIET CHOICES IMPACT HEALTH BIOMARKERS AND FITNESS.

WE ARE RECRUITING BOTH **MEAT EATERS** AND **VEGETARIANS**.

Participation will include:

- You must be an athlete training for a major competition
- One visit to the Nutrition Bldg. (ABC1) at the downtown ASU campus (near 5th Street and Van Buren)
- Participation includes a DXA scan (measures body composition), VO2 max test on a treadmill, and performing a maximal power test on a dynamometer.
- All testing results will be provided to participants on request including VO2 max, body composition assessment, and bone quality.

You will also receive a $20Target card for your participation.

INTERESTED?? Please visit our recruitment site:

www.surveymonkey.com/r/ASUAthleteDietStudy

HEALTH HISTORY QUESTIONNAIRE ID#__________________

1. Gender: M F

2. Age: __________

3. Have you lost or gained more than 10 lbs in the last 12 months? Yes No
If yes, how much lost or gained? _________ How long ago? ___________

4. Ethnicity: (please circle one) Native American African-American Caucasian Hispanic Asian Other

5. Education (please circle) High school diploma AA/vocational degree College degree MS degree PhD degree

6. Do you smoke? No, never ________
Yes _______ # Cigarettes per day = ________
I used to, but I quit _______ months/years (circle) ago

6. Have you ever been pregnant? __________________
If yes, date of last pregnancy? ___________

7. What were the dates of your last menstrual cycle? ______________________

8. Do you take any medications regularly? Yes No *If yes, list type and frequency:*

Medication	Dosage	Frequency

9. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No *If yes, list type and frequency:*

Supplement	Dosage	Frequency

10. Have you ever been hospitalized? ______ If yes, for what? ______________________________________

11. Please ANSWER (YES/NO) if **you currently have** or if **you have ever** been clinically diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			Chest Pain		
High Blood Pressure			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorder			Depression		

Please elaborate on any condition listed previously.____
____

12. How would you rate your lifestyle?

Not active __________ Active __________

Somewhat active __________ Very Active __________

13. Please circle the total time you spend <u>in each category</u> for an average **week.**

Light activities such as:
Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as:
Mod. Moderate walking, cycling, singles tennis, moderate swimming, weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as:
Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

14. Have you ever trained for and competed in any of these endurance events?

	NO	YES	#	Date ?
5k run				
10k run				
½ marathon				
Marathon				
Triathlon				
Cycling race				
Distance swimming				

Other? Explain: ____

15. How much alcohol do you drink? (average #drinks per day) __________

16. Do you have any food allergies? Yes No If yes, explain: ______________________________

17. How long have you been following your current diet? ____________________

18. How many times per week do you consume meat, fish, or poultry? ____________________

19. How many times per week do you consume dairy products (milk, cheese, butter, yogurt)? ______________

20. How many eggs do you consume per week? ______________

FRIDAY

24 Hour Dietary Recall Guide

Subject #____________

Upon waking, what food and beverages did you consume?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

What was the next thing you ate or drank?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

What did you have to eat and drink for lunch?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

Did you have any snacks or beverages next?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

What did you have to eat and drink for dinner?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

Did you eat or drink anything else throughout the day or night?

Food/Beverage	Quantity/Portion (in slices, ounces, cups, tablespoons, teaspoons, etc.)

Now I would like to go back and add more detail on how much of each food and beverage you consumed as well as how the food was prepared, starting with the first item.

Is there any condiment, topping, seasoning or food you may have missed, such as: sugar, butter, ketchup, salt, cream cheese, etc.?

Think for a minute. Was there any food, beverage or anything else you may have missed that you consumed yesterday?

All right, now I would like to go through the foods and beverages we have listed and make sure they are listed correctly. Did you list brand or restaurant names, where applicable? Did you specify type of product (such as skim milk, cheddar cheese, whole wheat bread, cooked beans, etc)?

Was this a typical day in terms of dietary choices and eating patterns? What differs?