Influences of Vegan Status on Protein Intake, Strength, and Bone Mineral Density

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2023 by
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ARIZONA STATE UNIVERSITY

May 2023

ABSTRACT

Plant-based eaters are known to reap nutritional benefits due to their diet choice, but it is important to evaluate dietary differences that may put them at a disadvantage compared to omnivores. Stark differences exist in daily intakes of protein between vegans and omnivores, which may lead to several risks including decreased strength and bone density. The purpose of this study was to analyze the differences in protein intake, lean mass, strength, and bone density in vegans versus omnivores in order to support the argument for an increased recommended daily allowance (RDA) for protein for plant-based eaters. Participants in this study were assigned to groups based on omnivorous (n = 25) or vegan (n = 19) dietary pattern. Nineteen matched pairs were created based on age and BMI. Data was collected at a single lab visit and included health history and physical activity readiness questionnaires, 24-hr food recall, and anthropometric measures. Bone mineral density (BMD) was measured using DEXA and strength was assessed using hand and Biodex dynamometers. Statistical analyses were conducted using independent samples t-tests and Pearson's correlation tests to evaluate differences in body composition, bone density, strength, and dietary intake between the two groups with significance set at $p \leq .05$. Differences were seen in daily calorie ($p=.007$), protein ($p<.001$), fat ($p<.001$), and fiber ($p=.009$) intake. Lean mass ($p=.282$) and bone density ($p=.651$) were not different between groups, but lower body strength was different ($p=.008$). There was a correlation between lower body strength and protein intake ($p<.001$), and lean mass was correlated with lower body strength ($p<.001$), grip strength ($p<.001$), and bone density ($p<.001$), but not LBM ($p=0.158$). Correlations were also

observed between BMD and lower body strength ($p=.004$). These data suggest that there is a significant difference between protein intake in vegans versus omnivores, which appears to have a positive association with strength. BMD also has a positive association with strength as well as lean mass. Cumulatively, the results suggest that it may be beneficial for vegans to increase daily protein intake.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my wonderful thesis advisor, Dr. Carol Johnston, for her unwavering support and guidance throughout this process. She took me under her wing without question when original plans were not working out and has made this process easier than I ever thought it would be. To my friends and family, thank you for your constant support and patience with me throughout this journey. Thank you for listening to me rant about my excitements and frustrations over the last several years and for always helping me stay grounded (and sane). You all keep me going and it is only with your help that I have been able to make it to this point. I am forever indebted and grateful for you all.

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CHAPTER 1

INTRODUCTION

Over the last several years, plant-based diets have seen a rise in popularity. Currently, about 5% of people in the United States self-identify as vegetarian[1]. The increase in popularity of eating a plant-based diet can be, in part, contributed to an increase in plant-based product development and sales. In fact, from 2018 to 2019 there was an 11% increase in plant-based food sales[2]. Some individuals follow a plant-based diet for the associated health benefits. Vegetarian and vegan diets have been found to have some positive effects on health such as up to a 12% decrease in incidence rate for all cancers, decrease in type 2 diabetes incident by 50% over omnivorous counterparts, plus a 40% reduction of the risk of dying from heart disease[3]. However, plant-based diets can lead to deficiencies in essential nutrients including, but not limited to vitamin B12, zinc, and iron[4].

The current study, however, focuses on dietary protein. It is well-documented that plant-based sources of protein are not as digestible and bioavailable as protein from animal sources[5]. Moreover, studies have shown that total daily protein intake is 2-5% lower in plant-based versus meat eaters[6]. Considering adequate protein intake is necessary for muscle anabolism, there are reductions in muscle synthesis and lean body mass (LBM) over time in plant-based eaters versus their omnivorous counterparts[5]. In fact, research has demonstrated that simply increasing daily protein intake in sedentary vegans and vegetarians increases LBM over an 8-week period[7].

What is not as well-established as the relationship between adherence to a plant-based diet and LBM, is the effect that a plant-based diet has on bone mineral density

(BMD). Much of the research that has been done until this point on BMD in vegans and vegetarians shows that omnivores tend to have higher BMD[8,9]. Adults from an NHANES survey who identified as vegan or vegetarian were found to have significantly lower BMD compared to nonvegetarians[10]. Other results demonstrate that plant-based eaters had lower BMD over omnivores at the lumbar spine, femoral neck, and whole body[11]. Yet the research is not conclusive. One study showed that BMD was 4%-5% lower in vegans and vegetarians, but this result lacked statistical significance and Z scores did not differ between groups[12].

It is generally agreed that diet and physical activity are key factors influencing BMD[13]. Individuals who follow a plant-based diet are potentially at a greater risk for developing osteopenia or osteoporosis because they often have deficiencies in nutrients essential for bone health such as calcium and vitamin D[14,15]. These nutrients are crucial for bone health as vitamin D stimulates calcium absorption from the gut[16]. If vitamin D or calcium intake is low, the body will take calcium from the skeleton in order to maintain blood levels[17]. As previously mentioned, plant-based eaters also commonly do not consume adequate protein, though sufficient protein intake is important for bone accrual and maintenance in addition to lowering the incidence of fracture[18]. Studies have shown that results from strength measures like the grip test are positively associated with BMD[19].

Amount and type of physical activity is also known to influence bone health. Due to the forces generated by physical activity, small amounts of deformations in the bone tissue are caused and perceived by the bone cells as unusual[20]. This initiates an adaptive response that leads to production of new bone tissue by osteoblasts[20]. Exercises most commonly noted as being best for bone are weight-bearing exercises including, but not limited to jumping, aerobics, and resistance training[20]. Bone strength improvements have been cited in adolescents engaging in weight-bearing activities, and similar exercise patterns can slow the progression of bone loss[21-23]. In other words, repeated impact exercises have shown positive results across all age groups.

Despite the knowledge presented by the literature related to the relationship between protein intake, strength, and BMD in plant-based eaters, the current recommended dietary allowance for plant-based eaters and omnivores remains the same (0.8g/kg bodyweight per day)[24]. However, the inference that can be made from the research is that it may be wise to increase the protein RDA for individuals that do not consume most of their protein from animal sources.

The purpose of this study is two-fold: 1) to examine the differences in BMD between plant-based eaters and omnivores and link BMD to protein intake and 2) to observe the correlation between BMD and strength within participants between sedentary matched omnivorous and plant-based eaters in the Phoenix metropolitan area. We hypothesize that lower average whole-body BMD will be observed in vegan and vegetarian participants versus omnivorous individuals. Furthermore, we postulate that stronger individuals will have higher BMD and that dietary protein will be linked to BMD.

CHAPTER 2

REVIEW OF LITERATURE

Defining Plant-Based and Omnivorous Diets

Over the last several years, plant-based diets including vegetarian, vegan, pescatarian, lacto-ovo-vegetarian, and flexitarian, have seen a rise in popularity. The most common and widely followed in the United States is vegetarian. In 2018, about 5% of adults in the United States self-identified as vegetarian while about 3% identified as vegan.[25] However, recent evidence suggests that vegetarians and vegans make up a combined 10% of the adult population in the United States.[26] The increase in popularity of eating a plant-based diet can be, in part, contributed to an increase in plant-based product development and sales. In fact, from 2018 to 2019 there was an 11% increase in plant-based food sales. However, while adherence to and interest in plant-based diets has increased over the last several years, an omnivorous diet, characterized as a diet pattern that includes daily consumption of animal proteins, is still widely followed by most individuals living in the country. A recent poll of over 1,000 people showed that just under 90% of individuals living in the U.S. include meat in their diet, but over half of respondents expressed interest in eating more plant-based foods.[27]

The term “plant-based” is broad and encompasses many styles of eating. While many people think of plant-based as exclusion of meat and potentially even all animal products, the term is simply defined as a diet that is made up primarily of plant-based foods like vegetables, legumes, and grains, but in some cases can contain cheeses, fish, and even meat sparingly. Perhaps the most recognizable ‘plant-based’ eating styles are vegetarianism, referring to individuals who do not consume meat but will consume animal products like cheese and honey, or veganism, which is the exclusion of all animal products from one's diet. Other less restrictive forms of plant-based eating include consumption of fish but no other meat (pescatarian), eggs and dairy but no other animal products (lacto-ovo vegetarian), and inclusion of meat occasionally with a heavy concentration on legumes, grains, and produce (Mediterranean). For the purposes of this paper, we will be discussing all plant-based diets, with a major focus on vegetarianism and veganism specifically.

Health Outcomes

There is a growing body of evidence suggesting that plant-based diets, including vegetarian, vegan, lacto-ovo-vegetarian, pescatarian, and Mediterranean, can offer numerous health benefits when compared to omnivorous ones. Studies have shown that plant-based diets, specifically vegan ones, are associated with lower risks of chronic diseases such as cardiovascular disease (CVD), type 2 diabetes, and certain types of cancer. For example, a meta-analysis published in 2021 found in a pooled analysis of data from seven studies whose participants adhered to diets ranging from omnivorous to vegan that greater adherence to plant-based diets was accompanied by significantly lower risks of CVD.[28] Yet another study examining vegans, lacto-ovo-vegetarians, pescatarians, semi-vegetarians, and omnivores found an almost 5% difference in prevalence of type 2 diabetes between vegans and meat-eaters.[29] Interestingly, as diet groups became increasingly more liberal with meat consumption, prevalence of diabetes increased.[29] Furthermore, studies have found significantly lower incidences of prostate, breast, and cancers of the gastrointestinal tract, and overall cancer risk in non-meat-eaters versus meat-eaters,[30,31] with the lowest incidences reported in vegans.[30]

As a result of increased consumption of nutrient-dense foods like fruits and vegetables, whole grains, and legumes, plant-based diets also tend to be higher in important vitamins and minerals, antioxidants, and fiber, the combination of which is responsible for many of the positive health outcomes seen in plant-based eaters. Epidemiological research has produced evidence that strongly suggests that a diet high in plant polyphenols as well as a high intake of fiber can provide protection against development of diseases like cardiovascular disease (CVD), cancers, diabetes, and more.[32-34] The literature shows that polyphenols are found to prevent platelet aggregation[35] as well as improve endothelial dysfunction,[36] thereby aiding in limiting the incidence of coronary artery disease, myocardial infarction, and other heart-related diseases.[37-39] Fiber has a similar effect on heart disease due to its ability to prevent absorption of some fat and cholesterol, which aids in reduction of CVD risk by lowering total serum triglyceride and cholesterol.[34] In fact, an umbrella meta-analysis published in 2017 looked at several studies conducted over almost three decades and found significant reductions in incidence and mortality from CVD in individuals with higher daily intakes of fiber.[40] Additionally, increased antioxidant consumption is known to reduce growth and number of tumors,[41] the effects of which have been observed in cancers of the mouth, stomach, duodenum, colon, liver, lungs, breasts, and skin.[41] Fiber provides further protection against colon cancer by expelling carcinogens in the intestines, as well as allowing for higher short-chain fatty acid production which reduces the ability of intestinal cells to become cancerous.[42] Polyphenols and fiber also play a role in inhibition of glucose absorption[43] and blood sugar control,[34] respectively, which contribute to prevention and management of diabetes. These benefits, among others, are a primary reason why many health professionals recommend plant-based diets, and many individuals adhere to them. However, consumption of meatless diets is not without drawbacks. For example, iron deficiency is more common in individuals that do not eat meat due to the lower availability of iron in plant foods.[44] Additionally, vitamin B12, a vitamin only found in animal products, as well as fatty acids like omega-3s are often consumed in smaller quantities in plant-based eaters.[44] Most symptoms associated with deficiencies in any of these vitamins are mild and include weakness, fatigue, dry skin, or brittle nails.[45-47] However, there are some more serious potential symptoms like difficulty breathing in the case of low iron[48] intake or increased heart rate caused by vitamin B12 deficiency.[49]

For the purposes of this paper, the most important nutrient difference to underscore is protein. There is growing evidence that protein intake is inadequate in plant-based eaters compared to omnivores, and the discrepancy increases with restrictiveness of diet. Considering the importance of protein for many biological functions, this difference in protein intake may pose some risks to plant-based eaters. However, before we assess the potential risks associated with low protein intake, we must first understand the function of protein in the body.

The Biological Role of Protein

There are many roles that protein plays in our bodies, including but not limited to aiding in satiety, helping repair and build body tissues, catalyzing chemical reactions, and coordinating cell signaling pathways. One of the more common associations people make when thinking about protein, however, is its effects on muscle, lean body mass (LBM), and strength. It is known that protein is heavily involved in maintenance and growth of muscle50. Research has found that protein ingestion stimulates muscle protein synthesis (MPS)[51] and, at the same time, are part of the structural components of muscle hypertrophy.[52] This process is largely controlled by the quality of protein that we consume as well as the daily shift in negative and positive protein balance in our bodies that results from periods of fasting and feeding, respectively. However, regarding increasing strength and muscle mass, it is important to understand how quantity and quality of protein can provide an impact.

Many researchers have sought to determine the precise role of dietary protein on LBM in varying populations, and though not all results present the same impact, there are many that clearly show positive impact of greater protein intake on LBM. For example, one study examining the effects of ~30g additional protein per day for 12 weeks in adults >69 years showed that added protein contributed to a larger relative increase in LBM as well as a decrease in fat mass in comparison to a control group not receiving additional protein.[53] However, there was not a significant difference in muscle strength between the two groups pre or post intervention.[53] These results indicate that in relatively active elderly adults, the loss of muscle mass due to age can be attenuated with an increase in daily protein intake. In other randomized controlled trials observing aspiring female physique athletes, it was found that those in the high-protein group (≥2.4g protein/kg/d) found a significant increase in fat free mass in the high-protein versus low-protein (0.9g/kg/d) group.[54] Additionally, in the high-protein condition, greater increases in LBM and decreases in fat mass were observed.[54]

While the association of increased protein intake with lean body mass is well-established, not as much is known of how animal versus plant protein sources impact

LBM and strength. Bioavailability and protein density of plant protein sources are not as abundant as animal sources; plant protein is, on average, 80% digestible while animal proteins are about 93% digestible.[55] Although this information may lead to the assumption that those who consume plant-based proteins cannot achieve the same muscle mass as those who consume animal proteins, the research is not as clear. A meta-analysis by Messina et. al. compared the differences in muscle mass gains and strength when individuals supplemented with soy protein versus animal protein. While there have been some acute studies conducted showing whey protein having a more significant impact on LBM than plant sources, the results of this meta-analyses showed that neither whey nor soy alone affected LBM significantly.[56] When coupled with resistance training, however, both proteins produced a significant effect on strength.[56] These results show that potentially similar outcomes can be achieved on vegetarian or plant-based diets when compared to animal protein-rich diets.

Protein Quality

While consuming adequate amounts of protein in the diet is essential to well-being and proper body functions, it is important to note that not all proteins are the same in terms of quality. Proteins are made up of amino acids (AA), all of which vary in quality and digestibility.[57] The quality of dietary protein is primarily characterized by their indispensable amino acid content.[58] Indispensable amino acids are those that cannot be synthesized by the human body and must therefore be obtained from the diet.[58] These AAs include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

Measures of Protein Quality

DIAAS Scores

The current method for determining protein quality and AA digestibility is the Digestible Indispensable Amino Acid Score or DIAAS. This score assesses the digestibility of individual AAs that have been processed in different ways (e.g. heated). Considering AA absorption takes place in entirety in the small intestine, the DIAAS method measures protein digestibility at the end of the small intestine. Currently, the protein sources that are among the most digestible are primarily animal proteins, namely pork meat, casein, egg, and whey.[58] This suggests that animal proteins are a better choice of protein if one is attempting to optimize digestibility, with whey protein often being cited as the highest quality with a DIAAS of 100.[59] However, potato and soy proteins also have high DIAAS scores and are therefore promising choices for being able to support a plant-based diet[58,60]. For example, average DIASS for soy products including soy protein isolate, concentrate, soymilk, etc., is around 85.[61] Furthermore, a new study recently found that 30g of additional protein per day in the form of potato protein strongly increased muscle protein synthesis in men both at rest and during the recovery period after exercise.[62]

Biological Value

There are a few different rating scales for protein that help to determine a protein's overall quality. One other important aspect of protein or amino acid quality is bioavailability. This is defined as the proportion of amino acids that enter systemic circulation to become part of body protein synthesis.[55] Proteins that are easy to digest, absorb, and become new proteins in the body are considered to be of high bioavailability.

Protein bioavailability is quantified in a value known as the biological value. To calculate this value, nitrogen utilized for tissue formation is divided by nitrogen absorbed from food, then multiplied by 100 and expressed as a percentage of nitrogen utilized.[63] The higher the higher a protein source's biological value is, the higher supply of AAs that protein contains. The research has shown many times that protein sources with the highest biological value like whey, milk, and egg all come from animal sources while plant-based proteins like wheat gluten are significantly lower.[63]

Net Protein Utilization

This technique is very similar to biological value, but it varies in one distinct way. While both tests measure the same factor of nitrogen preservation, protein utilization is calculated from nitrogen ingestion versus nitrogen absorption as we see with biological value.[63] Considering its similarities with biological value, it is no surprise that protein utilization values follow a similar pattern to biological value, with animal proteins like egg and whey ranking among the highest and plant proteins like wheat gluten and black beans in the lowest.[63]

Protein Efficiency Ratio

Though it is not the most accurate way to measure protein quality, the protein efficiency ratio is determined by measuring the growth of an animal. Determining this value requires feeding rats different types of test proteins and measuring their weight gain per gram of protein consumed. The standard value of casein, 2.7, is used as the comparison. This means that any protein with an efficiency ratio greater than 2.7 is considered an excellent source of protein. The primary limiting factor to this method, though, is that it does not provide a strong correlation to the growth needs of human, rather only rats.[63,64] Still, as with the other measurement techniques, protein efficiency ratios are higher in animal sources than plant sources, with egg, whey, and beef proteins with scores of 3.9, 3.2, and 2.9, respectively while the closest plant-based competitor is soy protein with a score of 2.2.[63]

Protein Type	DIAAS	Biological Value	Net Protein Utilization	Protein Efficiency Ratio
Pork	117	74	--	--
Casein	117	77	76	2.5
Whey	85	104	92	3.2
Egg	101	100	94	3.9
Soy Protein	91	74	61	2.2
Wheat	48	64	67	64
Potato	100	95	--	--

Differences in Plant vs. Animal Proteins

Despite some similarities in DIAAS scores, protein efficiency and utilization, and biological value between plant and animal-based proteins, plant-based sources overall appear to fall short on stimulating muscle protein synthesis as effectively. Recent research has found that plant-based proteins do not have as significant of an effect on muscle protein synthesis when compared to animal-based proteins. Studies have shown that beef protein, when compared with soy-based beef product, is superior in muscle protein synthesis stimulation.[64] Similarly, soy protein isolates and dairy proteins have demonstrated differences in their ability to stimulate muscle protein synthesis in resting and post-exercise conditions.[66-68] The reduced effect of plant-based protein on muscle anabolism is likely related to the digestibility of plant proteins, as measured by the DIAAS score, which is not as high as that of animal sources.[69] Additionally, some studies have found that AAs derived from certain animal proteins like those from dairy are less likely to convert to urea than plant sources,[70] which further contributes to a lower potential for plant-based protein sources to stimulate anabolic effects on skeletal muscle. Moreover, plant-based protein sources tend to have a lower content of leucine,[69] the amino acid most commonly linked with triggering muscle anabolism.[71]

Not surprisingly, this difference in ability of vegetarian vs omnivorous sources of protein to aid in the anabolism of muscle leads to a difference in strength and lean body mass as well. A recent study used serum creatinine as an indicator of lean body mass in omnivores and vegetarians and found significantly higher levels of serum creatinine and therefore greater LBM in omnivorous versus vegetarian participants.[72] Furthermore, strength assessments determined that omnivores were significantly stronger than vegetarians.[7] These data show that, regardless of diet, it appears that serum creatinine levels do correlate with LBM and strength in adults. Another study determined omnivorous and vegetarian participant's seven-day food record DIAAS scores along with available protein, LBM, and strength, and found that for all measures, there was a significant difference between the two groups. Namely, DIAAS score, LBM and available protein was higher in omnivores than vegetarians, and there was a significant correlation between strength and available protein.[5] Based upon available protein, it was determined that vegetarian participants in this study would need to increase their daily protein consumption by about 10g per day in order to reach the recommended intake. These results present a strong case for increasing the daily recommended intake of protein for plant-based eaters.

Despite the evidence that plant sources of protein are not as effective as animal proteins performing the same functions in our body, there is evidence to support that an increase in daily protein intake from non-animal sources can produce improvements in LBM and strength in vegetarians and vegans. One study examined the effect of supplementing 18g of mung bean protein per day for eight weeks on sedentary vegetarians and was able to identify a significant difference in percent change for grip, flexor, and extensor strength between the test and control groups.[7] This strongly indicates that, in the absence of exercise, consumption of additional daily vegetarian protein can positively affect strength in vegetarians and vegans. Another study examined how protein-matched diets (1.6g/kg/d) in vegetarians and omnivores affected strength in male athletes. In this study, daily protein intake came from participant's regular diet plus an additional supplement of soy protein isolate for vegetarians and whey protein supplementation for omnivores for participants to meet the desired amount for their bodyweight. The results found that men in both groups had an increase in lean mass throughout the body, yet there was no difference between groups for any of the measured outcomes.[73] This indicates that supplemental plant-based proteins may not be inferior to animal sources when attempting to increase muscle mass. These results also demonstrate the efficacy of protein quality measures as we know that soy protein isolate is comparable to animal protein sources in DIAAS, biological value, and protein efficiency ratio.[63]

Aside from LBM, muscle protein synthesis, and strength, total level of protein intake as well as protein type is also associated with other health indices. For example, one meta-analysis found several studies that have reported that total protein intake has a positive correlation with all-cause mortality.[74] These results point to animal proteins having a harmful effect on cardiovascular disease mortality.[74] The same meta-analysis found that participants of studies who has lower daily consumptions of protein also had lower daily intakes of vitamins and minerals like vitamin B12, choline, and magnesium. Furthermore, participants with lower protein intakes also had higher incidences of functional limitations. Combined, these results suggest the double-edge sword of protein in health.[75]

Vegetarian vs. Omnivore Protein Intake

As has already been established, the overall quality of vegetarian sources of protein is inferior to that of animal sources which can lead to lower protein assimilation and utilization and a subsequent negative effect on several health parameters. To further the difference in discrepancies between daily protein intake in plant-based versus omnivorous eaters, vegetarians and vegans are likely at risk of consuming less than the recommended amount of daily protein due to the fact that most plant proteins sources are not as protein dense as animal sources. For example, three ounces of chicken contains 23 grams of protein while the same amount of tofu or chickpeas contains only seven or 16 grams, respectively. Taking that into consideration, one may assume that individuals who consume more plant-based protein sources consume less daily total protein. The EPIC-Oxford study identified average daily protein intake in meat-eaters, fish-eaters, lacto-ovo-vegetarians, and vegans. For meat-eater, average intake was 90 grams, while all other diet types were 12-29% lower.[76] These data demonstrate that even the exclusion of protein from meat and poultry sources can dramatically decrease total daily protein intake and complete exclusion of all animal proteins produces the most significant effect on total daily protein with almost 30% less daily protein based on this study. Other studies have demonstrated similar effects. Data from a study conducted by Alles et al showed that meat-eaters consumed an average of 84 grams of protein per day while those who identified as neither meat-eaters nor vegans consumes ~64 grams and vegans showing the lowest daily consumption at 60 grams per day.[77] These results as well suggest that individuals who adhere to a largely plant-based diets are likely not consuming adequate amounts of protein daily while also not reaping the same benefits of animal proteins due to quality differences.

Bone Health

In addition to protein intake and the concerns at hand regarding lower consumption of protein in vegetarians and vegans, bone health is of growing interest as numbers of plant-based eaters rise. One major aspect of bone health is bone mineral density (BMD) which refers to the amount of minerals contained in a certain volume of bone. This measurement is used to determine risk for or diagnose osteopenia or osteoporosis, where osteopenia refers to one's BMD being lower than average for their age, and osteoporosis being bone loss that leads to weakening and makes bones more susceptible to fracture. Data from the 2005-2010 NHANES report estimates that over 10 million adult Americans over the age of 50 years old have osteoporosis and an additional 43.4 million had low bone density. Furthermore, based on this data, it was estimated that there would be a 19% increase in adult osteoporosis and low bone mass from 2010-2020 and a 32% increase from 2020-2030.[78] While a plant-based diet contributes to known improvements in many heath conditions, results from meta-analyses point toward bone mineral density (BMD) being negatively affected by adherence to vegetarian and vegan diets.[79] However, not all the evidence supports these conclusions as strongly. To understand the potential effects that a plant-based diet could have on BMD, we must first talk about some of the common nutrients related to bone health that are often consumed in smaller quantities in vegetarian and vegans versus omnivores.

Calcium in Vegetarians vs. Omnivores

Calcium is the most abundant mineral in the body and is the primary component of bone. In fact, around 70% of bone is calcium in the form of hydroxyapatite and 99% of the body's calcium is stored in bones. Perhaps the most notable source of calcium is dairy products like milk and cheese, but many plant sources like leafy greens, beans, and tofu are also good sources of calcium. Still, many plant-based eaters consume less than the recommended amount of calcium, though vegan diets take the biggest hit.[79,80] One study compared calcium intake among vegans, vegetarians, pescatarians, and omnivores and determined that the participants with the lowest calcium intake were vegans while vegetarian calcium intake was on par with pescatarians and meat-eaters.[81]

Vitamin D in Vegetarians vs. Omnivores

Vitamin D is not found in many food sources, but the foods most known for containing vitamin D include fatty fish and fish liver oils as well as certain varieties of mushrooms.[82] However, the best way for one to get vitamin D is through sun exposure. In fact, depending on skin tone, only 15 minutes of sun exposure without sunscreen produces enough vitamin D to reach the recommended daily intake of about 600 IU.[83] However, darker skin tones may require up to an hour of sun exposure, and other factors affecting vitamin D production via the sunlight include geographical location and how much skin is being exposed.[83] Considering vitamin D is difficult to get from food and many people are not out in the sun for even 30 minutes a day, vitamin D deficiency is a common issue worldwide. Across the world, over 12% of the population is deficient in vitamin D. However, in the United States, the incidence of vitamin D deficiency is around 35%.[84] Seeing as the main food sources of vitamin D primarily come from animals, it is no surprise that vegetarians and vegans may have lower intakes of the vitamin. A review done by Naufingerl et. al. found that across all studies that assessed vitamin D intake from food only, average intake was lowest among vegetarians and vegans versus pescatarians and meat-eaters.[85] Additionally, studies that have analyzed serum vitamin D status have had similar findings. The same review found that average serum vitamin D across all studies was 22.8μg/L and 21.9μg/L in vegetarians and vegans, respectively, whereas levels in pescatarians were averaging 28.9μg/L and meat-eaters at 26.2μg/L.[85] These results show that, while plant-based diets have many health positive health effects, it is also not unlikely that plant-based eaters may struggle to meet RDAs for certain micronutrients not found as abundantly in plant-based foods.

The Biological Role of Calcium

While the main function of calcium is maintaining healthy bones and teeth, it is also necessary for blood clotting, muscle contraction, and regulating normal heart rhythms.[86] With around only around 1% of the body's calcium being free calcium to circulate in the blood, muscles, and other tissues, it is important that our serum calcium is tightly regulated and maintained. If there is too much calcium circulating in the blood (hypercalcemia), this can result in bone weakening, kidney stones, and interferences in heart and brain function.[87] Interestingly, the symptoms of hypocalcemia, or too little calcium in circulation, can lead to very similar signs and symptoms like weakening bones and alterations in the brain, in addition to cataracts and changes in dentition.[88] Additionally, regardless of if an individual has hypercalcemia or hypocalcemia, it can most likely be attributed to abnormalities in the parathyroid gland, leading to abnormal levels of circulating parathyroid hormone (PTH). Parathyroid hormone, released from the parathyroid gland, is responsible for keeping serum calcium under tight regulation. When blood calcium falls too low, PTH is released which triggers calcium from the bone to be released into circulation.[89] To further help maintain blood calcium, PTH will also help the intestines absorb calcium from food more efficiently and allow the kidneys to hold on to calcium and return it to the blood as opposed to excreting in the urine.[89] Conversely, if blood calcium is high, PTH will not be released in high quantities. In some cases, however, there are issues in the way that they parathyroid gland functions which can lead to inappropriate release of PTH, and therefore unstable blood calcium levels. In the case of hyperparathyroidism, the most common treatment is surgery of one or more of the parathyroid glands. However, there are also medications that can be taken to control high blood calcium and improve bone density.[89] Hypoparathyroidism is a situation in which the parathyroid glands do not produce enough PTH. This is typically a result of damage to the parathyroid glands during thyroid or neck surgery.[90] It may however also be caused by an autoimmune attack on the parathyroid glands, low blood magnesium levels, or very rarely, radioactive iodine treatment for hyperthyroidism.[90]

The Biological Role of Vitamin D

Vitamin D plays an almost equally important role in bone health as does calcium. Some potential health risks associated with vitamin D insufficiency include incorrect growth patterns in children and muscle weakness and mood changes in both children and adults.[84] The biggest health risk associated with vitamin D deficiency, however, is bone pain and weakness. This is because the primary effect of vitamin D in the body is enhanced calcium absorption for maintenance of bone health.[91] When the body recognizes low blood calcium levels, increased PTH secretion signals the kidneys to produce vitamin D_3, the most readily available form of vitamin D.[91] Once this has happened, the vitamin D binds to calcium channels in the small intestinal cells to promote calcium uptake.[91] What this means is that when the body has insufficient vitamin D, it cannot optimally absorb calcium which can lead to lower bone density and increased fracture risk, both of which have been shown through research.

Protein and Bone Mineral Density

Protein also has a function in BMD as it relates to calcium metabolism. Interestingly, both too much protein and too little protein can have negative impacts on overall bone health. Many studies have found that high protein intake induces urinary calcium loss, negatively affects calcium balance, increases bone turnover, and is possibly associated with a higher fracture risk.[92]

Similarly, too little protein can be harmful for bones as well. As a diet that is high in protein can lead to suppressed levels of PTH, one that is too low in protein can elevate PTH. When this happens, blood calcium may rise too high, which, as discussed previously, can lead to decreases in bone density and kidney stones (primary hyperparathyroidism). Additionally, protein intake equal to or below 0.8g/kg may cause secondary hyperparathyroidism due to reductions in intestinal calcium absorption.[92] Several studies have shown the effects that lower protein intake can have on BMD. A study conducted by Devine et. al. measured BMD in over 1,000 elderly women. Protein intake was measured via food frequency questionnaire and bone mineral density was measured once at baseline and again one year later. When participants were split into tertile of protein intake (<66g/d, 68-87g/d, >87g/d), subjects in the low-protein group had significantly lower heel bone mass and hip BMD than those in the high protein group.[93] Similarly, when the moderate and high-protein groups were combined, average BMD was significantly higher than that of the low-protein group alone.[93] Another study analyzing almost 110,000 participants found that, after adjusting for age, a 10g increase in total daily protein intake resulted in a 10% lower chance of sustaining a hip fracture in women.[94] However, when further adjustments were made for BMI and lifestyle factors, this significance was attenuated.[94] The results of these studies, among others, are of interest and warrant further research to determine optimal quantities of protein intake to gain more insight into the mechanisms by which protein affects the bones.

Protein and BMD in Vegetarians vs. Omnivores

As discussed above, we know that it is not uncommon for individuals who follow a plant-based diet to consume less protein than those who consume animal protein regularly. As may be expected, the effects of a plant-based diet on BMD reflect those of a high-protein diet on BMD. For example, one study looking at data from NHANES cycles 2007-2010 found that in statistical models adjusted for age, sex, race/ethnicity, menopausal status, and education level, BMD was significantly lower in vegetarians than omnivores.[95] However, after adjusting for lifestyle factors, BMI, and waist circumference, the differences were attenuated and no longer held significance.[95] It is important to note, though, that these results mean that lower BMD in vegetarians may not be a result of differences in diet composition. Rather, it may be more significantly attributed to higher BMI, which past studies have found is associated with greater BMD from extra weight-bearing.[96,97]

Another study found that BMD was reduced 4-5% in individuals adhering to meatless diets as opposed to omnivores, but this was not significant.[12] They also determined that Z scores, though not statistically different, placed BMD between the 75th and 80th percentiles for meat-eaters and between the 60th and 70th percentiles for vegetarians. Furthermore, those following a vegetarian diet consumed ~30% less protein daily as compared to meat-eaters.[12] In many cases, the nutrient profile of a vegan or vegetarian diet versus an omnivorous diet vary significantly. For example, one study found that vegans had significantly lower calcium intake compared with omnivores.[98] Yet another saw significantly higher intake of magnesium, folate, and vitamin K in vegans versus omnivores.[12] Many of these differences could potentially impact bone metabolism, some positively and some negatively. So, the results of this study indicate that the bone-enhancing properties of a well-balanced meatless diet may negate the unfavorable properties that affect bone mineralization.

Overall, considering the evidence that is available, it would appear as though plant-based populations and specifically vegetarians and vegans may require higher daily protein intakes than omnivores. Unfortunately, restricting animal protein in the diet creates a barrier to consumption of higher quality proteins and therefore imparts some risks related to lean mass, strength, and bone density in those who follow a meatless diet. For this reason, the most feasible way for plant-based eaters to meet a higher recommendation would likely be through increasing quantity of plant-based proteins consumed. Further research is necessary in this area and should examine how much additional protein would be necessary in order to match results of strength tests and bone scans in plant-based versus meat-eaters.

CHAPTER 3

METHODS

Study Design and Sample

This study is a follow-up trial of a study examining the effect of a daily protein supplement on lean body mass in healthy, vegetarian adults. The study will consist of 23 omnivores and 23 vegetarians, matched for age and BMI stratification. Other research utilizing knee flexion and extension measures was used for the sample size calculation.[99] The p-value for the outcomes of this study was set at 0.05 and the beta error level at 0.2 with an estimated sample size of 34-92 participants. Vegetarian participants were recruited from the parent study[100] and matched omnivores were recruited from Arizona State University's campus and around the metropolitan Phoenix area via posted fliers, listservs, and word-of-mouth. To be eligible for the trial, participants needed to be healthy female adults between the ages of 18-50 years and be of similar age and BMI to already qualified vegetarian women. Pregnant and unhealthy women as well as those who were injured or reporting a chronic illness were excluded from participation. An online survey was used to screen potential subjects and ensure inclusion criteria was met and that subjects were willing to follow the study protocol. Individuals that cleared the initial screening survey were contacted via email to schedule a lab visit to confirm eligibility and collect data. The present study protocol was approved by the Institutional Review Board of Arizona State University (ASU) (STUDY00005383) and written informed consent was obtained from all participants.

This follow-up trial was designed to test the difference in quantity and quality of protein intake in vegetarians versus omnivores and relationships with bone density and muscle strength. Participants were instructed to arrive to the testing site fasted (12hr no food or drink) on the morning of their visit. Participants completed a COVID-19 screening before being taken into the lab to sign an informed consent form. Once in the lab, subjects received and completed a data collection packet consisting of a health history questionnaire, 24hr dietary recall, and results release form. Following completion of paperwork, participants' anthropometrics (height, weight, waist/hip circumference, BIA) were taken along with a DXA scan. Subjects were then asked to fill out a physical activity readiness questionnaire (par-Q), had resting blood pressure taken, and completed the Biodex and grip strength dynamometer assessments.

Biodex Multi-Joint System Pro

The Biodex Multi-Joint System Pro (Biodex) is an advanced technology designed to test and rehabilitate human's musculoskeletal system in the knees, ankles, hips, elbows, forearms, and wrists. Biodex methods of operation involve isokinetic, passive, isometric, isotonic, and reactive eccentric. For the purposed of this trial, only isokinetic and isometric modes were used. Along with the Biodex machine is a Windows-based Biodex Advantage Software that connects to the system and allows for easy set-up, protocol selection, and data recording.

In the present study, participants right leg torque and power was assessed at their lab visit. Tests were conducted by individuals trained in managing the Biodex system in accordance with the knee flexion/extension protocol laid out in the Biodex setup/operation manual. Prior to subjects performing the tests, calibration of the Biodex system was completed and proper accessory attached to the machine. Once calibration was complete, assessments began.

At the start of each assessment, participants sat on the chair attached to the Biodex and the operator entered subject information (participant ID, weight, leg dominance). Next, the knee attachment was secured to the dynamometer and the participant was stabilized with the thigh strap over their right leg, waist, and chest straps. They were then moved into position such that the point of flexion on their right knee was positioned in line with the center of the knee attachment. Following positioning, participant's right ankle was strapped into place one to two inches above the point of flexion in their ankle and range of motion (ROM) stops were set. To set ROM stops, participants were asked to perform a single knee flexion and extension, that is flexing their knee toward them as far as possible and extending their knee away as far as possible. These are the toward and away limits, respectively. Then, the Biodex operator identified the anatomical 90-degrees in the subject's knee using a goniometer and set that angle as well. Once these steps were completed, assessments began.

Each participant in the present study completed a total of five knee extension/flexion tests via the Biodex. These tests consisted of isokinetic flexion and extension at speeds of 150, 120, and 90 as well as isometric flexion and extension at a 60-degree angle. For isokinetic assessments, participants were instructed to kick as hard as they could forward and contract as hard as they could backward a total of three times. This was one round. Participants repeated two rounds of isokinetic extension and flexion at all three speeds. For isometric assessments, the Biodex arm was set and locked at a 60-degree angle and participants were asked to kick forward (extension) or contact backward (flexion) as hard as they could and hold for five seconds. They completed this three times for each condition.

Hand Dynamometer

Subjects also performed hand dynamometer tests to assess grip strength. Participants were asked to sit with shoulders back and neutrally rotated with the elbow held at a 90-degree angle against the body. The hand dynamometer was placed in the participant's dominant hand with the researcher supporting the base of the instrument to prevent dropping. The indicator needle was set to zero at the start of each assessment. A practice round was performed by each participant at sub-maximal strength to ensure clarity of instruction. Once participants understood how to perform the test, they were asked to squeeze the instrument with full strength and release. Each hand was tested twice, and the best effort was documented.

Diet Analyses

Dietary data were collected using the multiple-pass 24-h recall method. Participants were asked by researchers to recall all foods and beverages consumed the day prior, including condiments, spices, and fats used for cooking. Questions such as "upon waking, what was the first thing you consumed?" and "what, if anything, did you consume between lunch and dinner?" were asked to help participants thoroughly recall intake. Each question was asked a total of two times to increase accuracy of data and to allow participants to recall missing information. Dietary data were analyzed using Food Processor SQL nutrition and Fitness Software by ESHA Research, Inc. (version xx, Salem, OR). Dietary data was inputted by writer. If the exact food or beverage code was unable to be determined from the dietary record, a default list of > 450 food and beverage codes were used to identify the item. Micronutrient and macronutrient data were tabulated for comparisons between diet groups. A specific aim was to relate protein intake to BMD and muscle strength in participants.

Statistical Analysis

Data are presented as mean±SD and normality was assessed using the Shapiro- Wilk test. Outliers and confounders will be identified and controlled for. Pearson's correlation coefficient was used to assess relationships and data was considered significant at $p \leq 0.05$. IBM SPSS statistical software will be used for analysis.

CHAPTER 4

RESULTS

Of the 44 total participants in this study, 25 were omnivores and 19 were vegans. Omnivores and vegans were matched for age and BMI and a total of 19 matched pairs were created. As a result of the matching process, there were no significant differences between participants' age, height, weight, BMI, and waist circumference (WC) (Table 1). There was a significant difference in METS between groups (p=0.038), which did not affect the significant outcomes between groups reported below.

Table 2. Descriptive Statistics by Diet Group

Variable	Diet	Mean ± SD	p-value*
Age	OMNI	28.3 ± 8.2	.338
	Vegan	30.9 ± 9.5	
Height (cm)	OMNI	164.2 ± 7.5	.480
	Vegan	162.5 ± 6.3	
Weight (kg)	OMNI	63.4 ± 9.9	.628
	Vegan	61.7 ± 13.1	
BMI	OMNI	23.5 ± 3.3	.872
	Vegan	23.3 ± 4.3	
WC (cm)	OMNI	74.7 ± 8.7	.674
	Vegan	73.2 ± 11.6	
METS	OMNI	48.1 ± 34.4	***.038***
	Vegan	29.0 ± 20.2	

*Significance is set at p < .05
**p-value represents group differences analyzed by independent samples t-test

Nutrient intake data was gathered from 24-hr food recalls entered into Food Processor. Upon analyzing nutrient data using an independent samples t-test, there were no significant differences in carbohydrate intake between the two groups (Table 2). However, omnivores consumed significantly more calories, protein, and fat than their vegan counterparts but had significantly lower intakes of fiber. Additionally, vegans had a significantly lower intake of protein per kg bodyweight than did omnivores (p=<.001).

Table 3. Nutrient Intake by Diet Group.

Nutrient (Daily Avg. Intakes)	Diet	Mean ± SD	p-value*
Kcals	OMNI Vegan	1913.8 ± 523.8 1487.1 ± 456.7	***.007***
Pro (g)	OMNI Vegan	73.9 ± 22.9 43.0 ± 15.5	***<.001***
Pro (g/kg)	OMNI Vegan	1.2 ± 0.4 0.7 ± 0.3	***<.001***
CHO	OMNI Vegan	228.3 ± 85.3 216.9 ± 70.3	.629
Fat	OMNI Vegan	76.7 ± 22.9 49.7 ± 21.8	***<.001***
Fiber	OMNI Vegan	20.6 ± 8.2 27.8 ± 10.6	***.009***

*Significance is set at $p < .05$
**p-value represents group differences analyzed by independent samples t-test

Evaluation of mean fat mass, percent body fat, visceral fat, lean mass, and BMD did not differ significantly between the two groups (Table 3).

Table 4. Body Composition Measures by Diet Group

Variable	Diet	Mean ± SD	p-value*
Fat Mass (g)	OMNI Vegan	21284.7 ± 7098.1 21684 ± 7904.4	.861
% Body Fat	OMNI Vegan	34.7 ± 6.9 35.9 ± 5.9	.553
Visceral Fat (g)	OMNI Vegan	337.9 ± 414.7 345.5 ± 369.1	.949
Lean Mass (g)	OMNI Vegan	39291.6 ± 4602.5 37494.0 ± 6152.3	.282
BMD (g/cm^2)	OMNI Vegan	1.1 ± 0.1 1.1 ± 0.1	.651

*Significance is set at $p < .05$
**p-value represents group differences analyzed by independent samples t-test

Peak torque adjusted by body weight (BW) was measured and analyzed at three different speeds to obtain data for flexion and extension strength (Table 4). There was no significant difference in flexion or isometric strength of omnivores versus vegans at any speed. When analyzing maximum extension torque, omnivores were significantly stronger than vegans at 90 EXT (p=.009). However, that significance was not maintained at 120 EXT (p=.063) and 150 EXT (p=.120). Overall trends with extension showed increased significance as difficulty of exercise increased.

Table 5. Peak Torque BW % by Diet Group

Variable	Diet	Mean ± SD	p-value*
90 FLEX	OMNI Vegan	120.0 ± 28.3 110.8 ± 20.6	.219
90 EXT	OMNI Vegan	178.8 ± 36.3 151.9 ± 28.4	***.009***
120 FLEX	OMNI Vegan	106.8 ± 27.3 99.4 ± 17.3	.283
120 EXT	OMNI Vegan	158.8 ± 41.4 139.5 ± 24.4	***.063***
150 FLEX	OMNI Vegan	92.0 ± 26.3 83.5 ± 17.5	.213
150 EXT	OMNI Vegan	135.5 ± 45.2 118.5 ± 23.1	***.120***
ISO	OMNI Vegan	216.0 ± 58.2 196.1 ± 38.1	.184

*Significance is set at p < .05
**p-value represents group differences analyzed by independent samples t-test

Peak torque independent of BW was measured and showed similar trends to peak torque/BW (Table 5). There were no significant differences between peak flexion torque at any speed nor peak isometric force. Vegans had significantly less strength at extension speeds of 90 (p=.008) as well as 120 (p=.047), but significance was lost at 150 (.063). As with torque/BW, differences in extension peak torque became less significant as speeds became more difficult.

Table 6. Peak Torque Newton-Meters (N-M) by Diet Group

Variable	Diet	Mean ± SD	p-value*
90 FLEX	OMNI Vegan	74.8 ± 16.7 67.9 ± 17.1	.184
90 EXT	OMNI Vegan	111.6 ± 20.9 93 ± 23.2	***.008***
120 FLEX	OMNI Vegan	66.6 ± 16.0 60.7 ± 14.3	.210
120 EXT	OMNI Vegan	99.2 ± 24.5 85.3 ± 19.9	***.047***
150 FLEX	OMNI Vegan	57.4 ± 14.1 50.8 ± 12.4	.105
150 EXT	OMNI Vegan	84.5 ± 25.2 72.2 ± 17.0	***.063***
ISO	OMNI Vegan	140.0 ± 27.6 123.0 ± 31.5	.065

*Significance is set at p < .05
**p-value represents group differences analyzed by independent samples t-test

Average and peak grip strength as well as systolic and diastolic blood pressure was not different between groups.

Table 7. Grip Strength and Blood Pressure by Diet Group

Variable	Diet	Mean ± SD	p-value*
Avg Grip (kg)	OMNI Vegan	25.5 ± 4.4 25.2 ± 4.5	.836
Peak Grip (kg)	OMNI Vegan	27.1 ± 4.3 26.7 ± 4.7	.769
Systolic	OMNI Vegan	114.2 ± 10.5 113.1 ± 10.1	.707
Diastolic	OMNI	73.9 ± 8.6	.390

Vegan	71.7 ± 7.4

*Significance is set at p < .05
**p-value represents group differences analyzed by independent samples t-test

Strength as measured by 90 EXT (N-M) (p<.001) as well as peak grip strength (p<.001) were correlated to lean mass, while strength as measured by 90 EXT (%BW) and lean mass were not correlated. However, 90 EXT (%BW) was correlated with 90 EXT (N-M) (<.001) as well as peak grip strength (p = .017). Both 90 EXT (%BW) (p = .003) and 90 EXT (N-M) (p<.001) were correlated to daily protein (g) while peak grip strength was not. Daily calorie intake was correlated with 90 EXT (%BW) (p = .029) as well as 90 EXT (N-M) (p = .003), but not peak grip strength. Protein intake (g/kg) was correlated with 90 EXT(%BW) (p<.001) but no other strength measures. Bone mineral density was correlated with 90 EXT (N-M) (p = .004) and peak grip strength (p = .004).

Table 8. Strength Correlations

		Lean Mass (g)	**90 EXT (%BW)**	**90 EXT (N-M)**	**PRO (g)**	**PRO (g/kg)**	**BMD (g/cm²)**	**Peak Grip Strength (kg)**	**Kcals**
90 EXT (%BW)	Pearson Correlation	.149	1	.697**	.442**	.531**	.220	.358*	.333
	P-value	.335		***<.001***	***.003***	***<.001***	.150	***.017***	***.029***
	N	44	44	44	44	44	44	44	43
90 EXT (N-M)	Pearson Correlation	.705**	.697**	1	.507**	.293	.422	.450**	.446**
	P-value	***<.001***	***<.001***		***<.001***	.053	***.004***	***.002***	***.003***
	N	44	44	44	44	44	44	44	43
Peak Grip (kg)	Pearson Correlation	.593**	.358	.450**	.000	-.051	.425**	1	.135
	P-value	***<.001***	***.017***	***.002***	.999	.740	***.004***		.389
	N	44	44	44	44	44	44	44	43

Lean mass and BMD were correlated with one another ($p<.001$). Daily protein intake in grams was correlated with protein intake (g/kg) ($p<.001$) as well as daily calorie intake ($p<.001$).

Table 9. Lean Mass, Bone Density, and Dietary Correlations

		Lean Mass (g)	**PRO (g)**	**Pro (g/kg)**	**BMD (g/cm²)**	**Kcals**
Lean Mass (g)	Pearson Correlation	1	.217**	-.078	.527**	.282
	P-value		.158	.614	***<.001***	.067
	N	44	44	44	44	43
BMD (g/cm²)	Pearson Correlation	.527**	.169	.078	1	.124
	P-value	***<.001***	.272	.614		.429
	N	44	44	44	44	43
PRO (g)	Pearson Correlation	.217	1	.914**	.169	.723**
	P-value	.158		***<.001***	.272	***<.001***
	N	44	44	44	44	43
PRO (g/kg)	Pearson Correlation	-.078	.914**	1	.078	.617**
	P-value	.614	***<.001***		.614	***<.001***
	N	44	44	44	44	43
Kcals	Pearson Correlation	.282	.723**	.617**	.124	1
	P-value	.067	***<.001***	***<.001***	.429	
	N	43	43	43	43	43

CHAPTER 5

DISCUSSION

The present study was designed to determine the differences between protein intake, BMD, and strength in matched omnivore versus vegan subjects. We hypothesized that lower average whole-body BMD would be observed in vegan participants and that stronger individuals would have higher bone mineral density. Furthermore, we postulated that higher dietary protein intakes would be linked to BMD and strength. We were also seeking to confirm that there is a difference in daily protein intake between vegans and omnivores, in order to help establish a case for increasing the protein RDA for vegans and vegetarians. While the results did not support all our predictions, significance was seen in several key areas and positive correlations were maintained throughout.

There were no significant differences between the BMD of omnivorous and vegan participants, despite literature showing lower bone densities in vegetarians and vegans than omnivores.[78] However, not all the research shows clear differences between dietary groups.[12] Lower dietary intakes of several nutrients including vitamin D, calcium, and protein[98] often observed in vegetarians and vegans are regularly cited as potential causes of lower BMD in plant-based eaters versus omnivores due to the important role that these nutrients play in bone health.[91,101,102] Conversely, it has been documented that higher dietary animal to plant protein ratios are associated with an increase in bone resorption.[98,103,104] In the present study, omnivores consumed a significantly higher ($p<.001$) amount of protein per day (1.2g/kg) compared to vegans (0.7g/kg), which may be contributing to the similarity in bone density between the two groups. Matching participants by age may have played a role in bone density similarities as well. We know that age, especially in women, is an important factor in bone health.[105] Bearing in mind that subjects of this study were closely age matched, this may lead to a similar pattern in bone densities between the two groups.

There was a weak but positive correlation between bone mineral density and daily protein intake in grams and g/kg with respective Pearson correlation values of .169 and .078. Despite an insignificant correlation in BMD and protein intake, BMD was significantly correlated to peak grip strength ($p = .004$) as well as 90 EXT N-M ($p = .004$). Considering dietary protein intake is directly correlated to strength,[106] it is interesting that there is no correlation between BMD and daily protein intake even though protein intakes between the two groups is different and BMD is correlated with strength.

Furthermore, there were substantial correlations seen in relation to protein intake, LBM, and strength. Lower body strength measures had significant positive correlations with protein intake. When compared to 90 EXT (%BW), protein intake measured as grams per day had a p-value of .003 and was correlated even further when compared to 90 EXT (N-M) ($p<.001$); protein intake (g/kg) also had a significant correlation with 90 EXT (%BW) ($p<.001$), but significance was not maintained when measured against 90 EXT (N-M) ($p = 0.53$). To date, no other research has been done to examine strength differences in vegans and omnivores using this type of strength measure. One of the supposed reasons for the difference in strength between the two groups is due to their significant differences in daily protein intake, as we know that higher protein intakes are associated with greater strength and muscle mass.[50] Interestingly, despite the correlations between strength and protein intake in lower body assessments, comparison of peak grip with protein intake (g) showed a Pearson correlation coefficient of .000 which was exacerbated to -.051 when compared with protein intake (g/kg). This suggests that as protein intake increases, grip strength decreases.

Finally, while LBM was not different between the two groups, it was significantly correlated to strength measures, as previous literature has indicated.[100] This outcome is expected due to the ability of greater muscle mass to produce greater force.[107] What is noteworthy is that, while the only lower body strength tests where significant differences were observed between omnivores and vegans were 90 EXT (%BW) and 90 EXT (N-M), leg extension tests at the three different speeds (90, 120, 150) demonstrated decreased significance as the exercises became easier. In other words, the more difficult an exercise was, the more similarly subjects performed. Although METS were significantly different between groups ($p = .038$), significance of both 90 EXT measures as well as trends between speeds were maintained.

This study helps to fill the literature gap that exists regarding BMD, LBM, strength, and protein intake in omnivores versus vegans. Overall, these results support the need for an increased protein RDA for vegans due to significantly lower protein intake and strength in vegan participants versus omnivores. However, further investigation, particularly as it relates to bone density between the two groups is warranted, as many other studies have demonstrated varying outcomes related to this measure.

Gold standards of assessment including DEXA, hand dynamometer, and Biodex dynamometer, were used throughout the present study. However, limitations and delimitations were present as well. One of the major limiting factors is that this study was not a randomized controlled trial, hence causality cannot be assessed. Furthermore, the sample size was quite small which may affect significance of results. Additional limitations exist as a result of dietary data collection using a 24-hr food recall. Success of a 24-hr recall is dependent on participant's memory, cooperation, and effective communication of portion sizes. Additionally, these types of diet assessments do not account for day-to-day variability of intake. Delimitations include the study population being limited to the Phoenix Metropolitan area, lending itself to smaller generalizability of results. Finally, lower body strength assessments were conducted with participants' right leg only which may affect results in left-leg dominant participants.

CONCLUSIONS

Based on the outcomes of this study, the data suggests that there are significant differences in daily protein intakes between vegans and omnivores, in addition to the known differences in quality of plant versus animal proteins. This is possibly a factor in the differences in strength that were observed between the two groups as well as the correlation seen between protein consumption and lean body mass. Furthermore, protein intake is correlated with bone density of subjects, suggesting that lower daily intake of protein may contribute to lower BMD. All these results are consistent with the current body of literature and provide reasonable evidence to consider adjusting the protein RDA for plant-based eaters, specifically vegans. However, there was also a lack of difference seen between LBM and BMD of vegans and omnivores, which may be due to the matching process. Future research is necessary to determine how much daily plant protein would be required for vegans to meet similar intakes to omnivores. Additional research examining bone density differences between the two groups over longer periods of time is also needed to determine long-term effects of a vegan diet.

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APPENDIX A

IRB APPROVAL FROM FEBRUARY 2022

APPROVAL: MODIFICATION

Carol Johnston
CHS: Health Solutions, College of 602/496-2539 CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:
On 2/8/2022 the ASU IRB reviewed the following protocol:

Type of Review: Modification / Update	
Title:	Examination of the Effect of Supplementary Dietary Protein (21g per day) on Lean Mass and Strength in Sedentary, Adult Vegetarians
Investigator: Carol Johnston	
IRB ID: STUDY00005383	
Funding: None	
Grant Title: None	
Grant ID: None	
Documents Reviewed: None	

The IRB approved the modification.

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).

REMINDER - Effective January 12, 2022, in-person interactions with human subjects require adherence to all current policies for ASU faculty, staff, students and visitors. Up-to-date information regarding ASU's COVID-19 Management Strategy can be found here. IRB approval is related to the research activity involving human subjects, all other protocols related to COVID-19 management including face coverings, health checks, facility access, etc. are governed by current ASU policy.

Sincerely, IRB administrators