COMPARISON OF ANIMAL VERSUS PLANT-BASED PROTEIN SUPPLEMENTATION TO NITROGEN BALANCE IN FEMALE COLLEGE STUDENTS

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ABSTRACT

Comparison of Animal Versus Plant-Based Protein Supplementation to Nitrogen Balance in Female College Students

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Plant-based diets have become popular in the past 10 years, with approximately 11% of Americans self-identifying as vegan or vegetarian, and many others trying to reduce meat consumption. Due to this increasing interest, the plant-based food market has exploded with several novel innovative products serving as alternatives to animal-based products. One such example is almond protein powder, a fairly new protein supplement created as an alternative to whey protein. A number of studies have compared animal-based protein supplementation, such as whey to plant-based supplementation, such as soy, on muscle protein synthesis and skeletal muscle preservation. Due to the novelty of almond protein, little is known regarding how well the protein supplement performs in the body. The effects of both almond and whey-based protein beverage on nitrogen balance, body composition, and hydration in the body were investigated in the work presented herein. Twenty female students aged 20-25 were randomly assigned to consume either an almond or whey-based protein drink twice daily for 7 days. A 24-hour urine collection was performed at baseline and endpoint of the 7-day treatment period, and nitrogen balance was assessed. The effects of supplementation on nitrogen balance in almond and whey protein were equally capable of increasing significantly the N balance from 8.58g to 11.66g (p =0.05), indicating that almond protein powder may be a functional plant-based replacement to whey protein powder, and may be of interest in future research regarding muscle mass and body composition changes.
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1. INTRODUCTION

It is estimated that 30-40% of all food produced in the United States goes to waste, corresponding to 133 billion pounds and $161 billion worth of food lost per year (USDA, 2017). Mitigating food waste is of interest among all points along the farm to fork continuum and it is of particular interest in the agricultural sector due to lost profit and inefficient flow of food to those who need improved access to it. The United States Environmental Protection Agency (EPA) has established the Food Recovery Hierarchy which illustrates the recommended flow of food in order to decrease this waste (Figure 1), including source reduction and feeding food insecure individuals. However, dietary choices are not considered in this figure. It is postulated that adjustment of dietary habits may decrease food waste. This may lead to increased food access for food insecure individuals, significantly more than by simply adjusting how food is currently consumed.

Figure 1: Food waste recovery hierarchy illustrating most to least optimal use of food. (Adapted from EPA Food Recovery Hierarchy. US EPA, 2020)
Current Western diets consist of considerable amounts of highly processed foods, red meats, and high-fat dairy products, with a significant portion of dietary protein from animal based protein sources (Angela & Laguipo, 2020). It is noteworthy that meat production requires considerable land use, with 30 million square kilometers worldwide, accounting for 60% of the world’s agricultural land use (Global Agriculture, 2016). Beef production results in a fossil fuel to energy ratio of 40:1, and all animal-based proteins demonstrate an average ratio of 25:1. This ratio is 11 times greater than that of grains, illustrating the considerable impact of animal-based protein consumption on the environment and possibly climate change (Pimentel & Pimentel, 2003). Greenhouse gas emissions vary across protein source as well, with beef producing 60 kg CO\textsubscript{2} equivalents/kg, whereas soy produces less than 1 kg CO\textsubscript{2} equivalents (figure 2).

![GHG Emissions of Protein Sources (kg CO2 equivalents/kg product)](image)

**Figure 2:** Illustration of popular protein sources and their greenhouse gas (GHG) emissions. Fish represents both wild and farmed and does not include prawns. Adapted from Harvard School of Public Health, 2018 and Neufeld, 2020.
While food waste is traditionally thought of as a visible issue, i.e., food scraps or leftovers in the garbage, the issue extends far beyond the consumers’ household waste production and management. To prevent food waste, one must consider opportunity loss. More specifically, the largest driver behind food waste prevention via plant-based protein is such a case, since opportunity food loss considers food loss associated with consuming resource intensive animal-based products in place of plant-based alternatives (Shepon et al., 2018). Plant-based diets are estimated to produce a 20-fold more nutritionally similar and twofold more nutritionally similar food per cropland than that of beef and eggs, respectively. This optimization of resources and food production output generated, could result in food surplus to sufficiently feed 350 million additional people, a most helpful approach when considering the rapidly growing population at a global scale (Shepon et al., 2018). The opportunity food loss concept illustrates the potential savings beyond conventionally defined food waste, and further emphasizes the need for plant-based alternatives for animal products given the resource optimization with minimized environmental negative impact that is achieved through plant-based food production systems (Shepon et al., 2018).

While plant-based food alternatives extend several environmental benefits, it is critical that nutritionally they function in a similar manner to animal-based protein sources, in order to encourage consumers to modify dietary habits without sacrificing nutritional benefits. Plant-based protein functionality has been compared to that which is animal-based on several occasions (Banaszek et al., 2019; Jenkins et al., 2012; Salter, 2013), with evidence suggesting decreased disease risk and numerous health benefits with plant-based options (Williams & Patel, 2017). Plant-based proteins have also
exhibited mixed effects on muscle protein synthesis (MPS) in human studies when compared to animal-based protein suggesting that for those who are prioritizing muscle building maximization, animal-based proteins may still constitute the best option (Banaszek et al., 2019; Candow et al., 2006).

Almond protein powder, a more recently commercialized protein powder, has not been thoroughly investigated in terms of its effects on MPS. There is evidence suggesting that soy and pea protein powders may not be as effective as whey protein (Banaszek et al., 2019; Candow et al., 2006; Tang et al. 2009), and the amino acid profile of almond protein powder differs from other vegan and vegetarian proteins, such as soy and pea. Almond protein powder has decreased essential amino acid presence than both soy and pea protein, in all essential amino acids (Gorissen et al., 2018; Banaszek et al., 2019). This may result in different effects on MPS. Investigating the functionality of almond protein powder compared to a well-established animal-based counterpart is important for developing nutritional recommendations for vegetarians/vegans interested in consuming more protein and ensuring adequate amount and quality of dietary protein intake.

In this context, the first objective of the study presented herein was to determine the body composition in relation to protein status of female college students, including lean body mass, percentage body fat, and visceral fat mass. It was hypothesized that female students will be marginal in terms of their protein intake or display lower levels of protein consumption as compared to nutritional guidelines (Schwitzer et al., 2010).

The second objective was to assess body composition and nitrogen balance during a protein supplementation period and compare effects from using either an animal or
plant-based protein source. It was hypothesized that both supplements would improve protein status as evaluated through nitrogen balance.

The third and final objective was to compare the effects of the protein source, either animal or plant-based, on nitrogen balance and protein status. It was hypothesized that there will be a similar positive effect on nitrogen balance, regardless of the supplementation source.
2. LITERATURE REVIEW

Consumer interest in plant-based diets has rapidly risen in the past decade while shifts towards such diets are increasingly becoming commonplace. Although these diets were once perceived as the purview of the odd few, riddled with stigma and representing a minority of the population, health and environmentally conscious individuals of all ages have consistently been shifting the needle toward an increasingly plant-based format, creating a new trend from both dietary and food production/product standpoints. In 2015, 3.4% of Americans self-identified as vegetarian, and 0.4% as vegan (The Vegetarian Resource Group, 2016). In 2018, the figures presented 8% of Americans as vegetarian, and 3% as vegan (Gallup, 2018), representing more than a 50% increase in both groups in the span of merely 3 years. These numbers have been significantly impacted by the onset of COVID-19 as well, with a recent International Food Information Council (International Food Information Council, 2019) survey stating that 24% of participants reported eating more plant-based protein in 2021 than before the pandemic (International Food Information Council, 2019). The elevated interest observed is linked to several factors, including the rise of the plant-based meat-like industry, an increased awareness of and support for the benefits of a plant-based diet to the consumer and the environment (Institute of Food Technologists, 2020; Good Food Institute, 2020).
A plant-based diet usually refers to a diet whereby plants and plant-based proteins are the only source of nutrition. There are certain subsets of the plant-based diets, including those that exclude processed plants and oils, but generally and for practical purposes, one can accept the original definition of a diet whose sole source of nutrition is from plant and plant-based proteins. The concept of a plant-based diet can be used to classify the eating patterns of vegans and vegetarians. Some researchers perceive the definition somewhat more broadly, utilizing it as an umbrella term to encompass diets significantly less heavy on animal protein than those of omnivores. Veganism is well defined, with its adherents following a strict no animal product diet. This diet also excludes eggs, dairy, and honey. Vegetarianism includes several subsets, such as lacto- and ovo-vegetarians, with individuals consuming dairy and eggs, respectively. Pescatarians limit animal-based protein intake to fish (figure 3). The desire to follow one of these reduced animal protein diets may be due to a variety of reasons, including cost, concern regarding animal treatment, lessened environmental impact, and overall health benefits of a plant-based diet (Robert & Shmerling, 2019).

![Plant-Based Dietary Spectrum](image)

**Figure 3:** Illustration of the plant-based dietary spectrum, ranging from omnivore to vegan (Robert & Shmerling, 2019)
While sustainability of plant-based diets is an important factor, consumer interest tends to be focused on nutritional effects and palatability. Consumer education regarding taste, sustainability, and nutrition, while present in the media and the literature, needs to be expanded to offset the powerful marketing campaigns of big agriculture and large food processors. Viewing a plant-based diet through the bi-focal lens of nutrition and environmental impact, and providing bifurcated education on these two topics, may result in a positive change in consumer behavior. The objectives of this review are to illustrate the environmental considerations and the health effects of a plant-based diet, providing an extensive reference for those considering a plant-based diet.

2.1 Sustainability and plant-based diets

It is estimated that the global population will grow to 10 billion people by 2050, and it is projected that global dietary patterns will shift toward a more western-style diet: more calories, more protein, and more animal-based foods (Sataloff et al., 2016). This shift in dietary patterns could be problematic for a variety of reasons, including the facts that it is environmentally taxing and may potentially threaten food security (Vågsholm et al., 2020). Agriculture was historically considered relatively regenerative, with animals and crops frequently found on the same plot, and land was not used exclusively for a single crop. Industrial agriculture has over time resulted in a visible shift where more focus is placed on economy of production (output scaling) rather than minimizing environmental impact. Modern farms are frequently large single use, producing only one crop. Production of these crops requires the use of significant resources, including nonrenewable energy from fossil fuels, environmentally impactful fertilizers and medications, and substantial amounts of irrigation water. The output, or food produced,
often does not contain enough energy to result in a net energy gain; interestingly, for every 10.3 quads of energy used to produce food, animal or plant-based, 1.4 quads of food energy is created. This creates a large imbalance rendering food production inefficient from a pure energy balance standpoint (Sabaté & Soret, 2014).

Specifically, meat production results in significant fossil fuel use, with beef and eggs representing the highest fossil fuel energy demand: protein energy ratios, at 40:1 and 39:1, respectively (Pimentel, D. & Pimentel, M., 2003). Overall, 11 times greater fossil fuel energy is required to produce an animal protein than a plant protein, which drives the point that the production of a plant-based protein is significantly more efficient requiring less energy use to produce, and therefore less burdening to the environment (Pimentel, D. & Pimentel, M., 2003). Despite the substantial amounts of land used and GHG emissions produced, only 18% of the global calorie supply comes from meat and dairy (Ritchie and Roser, 2020). This creates a considerable gap for increased efficiency along the food production chain, which may be ameliorated via a plant-based protein approach.

Food waste also plays a significant role in terms of both environmental issues and plant-based diets. It is estimated that between 30-40% of the food supply goes to waste in the United States, which corresponds to a $161 billion worth loss of food (FDA, 2020). Not included in these numbers are water, energy, and labor losses that could have been more effectively allocated elsewhere (FDA, 2020). Paradoxically, 10.5% of US households reported experiencing food insecurity at some point in 2019 (USDA, 2020), representing a disparity between food wasted and food needed. To combat this inefficiency, there are efforts being initiated to divert food waste and lengthen a food’s lifespan, with disposal to a landfill being a last resort. The Food Recovery Hierarchy,
developed by the Environmental Protection Agency (EPA), suggests planning food waste reduction in the following order:

1. Reduce the volume of surplus food generated
2. Feed hungry people
3. Feed animals
4. Use for industrial purposes
5. Composting
6. Landfill as a last resort (US EPA, 2020) (Figure 1)

Animal based diets play a significant role in food waste and food security. It is estimated that if all Americans switched to a plant-based diet, enough food could be produced to feed 350 million more people. This number far exceeds the food that is lost in the supply chain, and could effectively feed more people, decreasing food insecurity (Chang, 2019). This would also allow for more effective land use, as the surface of land used to produce 100 grams of plant-based protein can only produce 4 grams of beef protein. This results in an opportunity food loss (OFL) of 96% for plant-based protein production when the resources are used to produce animal protein instead of plant protein (Pimentel, D. & Pimentel, M., 2003).

The primary focus in terms of food waste and plant-based diets is the OFL, as the OFL of animal-based diets have been shown to exceed all food losses in plant-based diets. In a study by Shepon et al., OFL of animal-based consumption was compared to that of plant-based alternatives. It was found that the OFLs of beef, pork, dairy, poultry, and eggs were 96%, 90%, 75%, 50%, and 40%, respectively. However, OFLs of plant-based replacements were significantly lower. Plant-based alternatives have the potential
to produce significantly more food using much less land. In fact, plant-based foods can produce a 20-fold increased output of nutritionally similar food per cropland acre when compared to beef, and twofold more when compared to eggs. It was then estimated that in the US plant-based proteins could produce enough food to feed 350 million more people, significantly decrease OFLs, and significantly minimize supply chain waste (Shepon et al., 2018).

2.2 Produce Standards

Despite the shift in dietary habits towards plant-based diets observed recently, produce remains one of the highest food waste generating categories in the United States. A 60% yield for most crops is considered “good” (Huckaby, 2021), with loss occurring along all points on the farm to fork continuum. Interestingly, it is not taste that causes the disposal of produce, but appearance. Produce frequently gets rejected due to unsightly appearance, typically deemed “ugly produce”. Initiatives to encourage consumption of such “ugly” produce are increasing in grocery stores and food delivery programs in the United States, with the goal to change consumer standards regarding perfect produce, which is no different from a dietary quality or taste and texture standpoint compared to the “ugly” counterpart. While these programs are somewhat successful (Tesdall, 2016), it is difficult to convince consumers to rapidly shift views on produce standards after years of demand for perfect product. Therefore, the most effective way to utilize ugly produce is in the creation of new plant-based products to mask appearance and decrease waste. One such example is in carrot production at Grimmway Farms in Bakersfield, CA, where 40% of carrots were going to waste due to appearance. Innovation regarding utilization of the carrot, such as production of baby carrots, juices, chips, and precut salad mixes
decreased that number to 0.5-1% waste, which is purposed for animal feed (Huckaby, 2021). Similar processes are being utilized in beets, sweet potatoes, and pumpkin (Huckaby, 2021; Watson, 2018). These are not necessarily common practices, and conversations regarding improved efficiency to decrease waste and increase profits need to be had.

Shifts towards vegan and vegetarian diets require growth of more produce, and acceptability of more ugly produce. Creation of salsas, salads, and flours from ugly produce is done occasionally already, but investment in new products that utilize this produce effectively is key. Reframing produce standards with emphasis on taste and nutrition, rather than appearance, may help decrease waste on both the farm and consumer ends of food production.

2.3 Plant-based diets’ effects on health

The associated health benefits of plant-based diets are significant driving forces supporting the adoption of the relevant dietary patterns. In a 2006 study surveying consumer perception of a plant-based diet, it was found that the highest perceived benefit of a plant-based diet was decreased saturated fat intake and increased fiber intake (Pohjolainen et al., 2015) while eating a more natural diet and intaking more vitamins ranked second. However, 42% of respondents identified lack of information regarding these diets as their largest perceived barrier for adoption of such dietary practices. Since the study surveyed 415 Australian adults, it is more representative of an Australian population. Similar results have been found in British and American populations as well (Pohjolainen et al., 2015). In a survey conducted by the Institute of Food Technologists, Protein division, U.S. respondents reported the largest motivating factor behind
consuming a plant-based diet was “to be healthier” (56%), followed by “it tastes good” (42%). Moreover, 86% of respondents reported regularly consuming protein from nuts. Nuts are a readily available protein source with ease of consumption for many consumers (Institute of Food Technologists, 2020). Clarification of potential health benefits, such as contribution to decreased risk for Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease (CVD) onset, helps to garner consumer interest, as interestingly environmental impact did not rank highly in these studies (Pohjolainen et al., 2015, Institute of Food Technologists, 2020).

2.3.1 Plant-based diets and T2DM

It is estimated that 39% of the United States Population is obese, defined by a body mass index (BMI) >30.0. This is frequently due to hypercaloric, unhealthy diets, combined with sedentary lifestyles (Centers for Disease Control and Prevention, 2021). Obesity can lead to many adverse health consequences, including T2DM (Centers for Disease Control and Prevention, 2021). Approximately, 26 million people in the United States are estimated to be suffering from T2DM, and while pharmacological treatments exist to mitigate disease effects, good control of T2DM remains a challenge for the majority of diagnosed patients. The onset of T2DM is largely influenced by diet and lifestyle factors. Dietary changes are often prescribed for T2DM individuals to help with attaining better glycemic control. Plant-based diets typically lead to decreased calorie intake, less fat, less saturated fat, and less dietary cholesterol, and decrease in BMI. As a result, plant-based diets are of interest to investigators seeking to decrease T2DM risk and improve disease management (Trapp & Levin, 2012).
A 2004 study in Belgium investigated plant versus animal protein intake and the effect on obesity and weight. A group of 3,083 volunteers was surveyed regarding dietary habits, employing two non-consecutive 24-hour dietary recalls. Animal based protein included dairy products, meat and meat products, fish and shellfish, and eggs and egg products. Plant-based protein included potatoes and other tubers, vegetables, legumes, fruits, nuts and seeds, cereal products, and soy products. Thirty four percent of male participants were deemed overweight based on BMI, and 10.1% were classified obese. Twenty five percent of females were determined to be overweight, and 10.5% obese. Overall, animal protein intake provided the majority of the energy intake for the participants, with the mean energy intake being 0.795 mj/d, or 46 g/d of protein. Plant protein energy intake was much less, with an average of 25 g/d of protein. Both protein intakes were higher in males. Animal protein intake was also positively associated with BMI and waist circumference in males. Increased plant protein intake was inversely associated with BMI in both groups, revealing a potential link between decreased obesity risk and plant-based diets (Lin et al., 2011).

A plant-based diet may be beneficial for individuals with T2DM. Participants in the Adventist Health Study, a study initiated in 2002 that follows 97,000 Adventist church members in the US and Canada, were surveyed regarding their dietary habits. 22,434 men and 38,469 women participated, and participants were asked to self-identify as vegan, lacto-ovo vegetarian, pesco-vegetarian, semi-vegetarian, and non-vegetarian. Dietary habits were then connected to BMI and T2DM incidence. Mean BMI was lowest in vegans, at 23.6. BMI then increased as animal protein intake increased, with non-vegetarians mean BMI being 28.8. T2DM incidence increased similarly, with prevalence
being 2.9% in vegans to 7.6% in non-vegetarians. After adjustments for external/confounding factors, including age, sex, ethnicity, education, income, and physical activity, it was found that vegan and lacto-ovo vegetarians demonstrated a nearly one-half reduction of T2DM risk, and vegetarians of any type exhibited an overall decreased risk of T2DM, when compared to non-vegetarians. This may be because vegetarian diets are rich in fruits and vegetables, which may decrease chronic inflammation and oxidative stress, while also contribute to fiber intake increase. Vegetarian diets also contained significantly less saturated fat, which may increase insulin sensitivity (Tonstad et al., 2009).

A second study utilizing participants from the same Adventist Health Study investigated the association between dietary choice and T2DM onset. Participants were surveyed regarding their animal-based and plant-based protein intake and were determined to be non-diabetic at baseline (n=8,401). Within the 17-year follow-up, 543 cases of T2DM were reported. It was found that those who consumed meat weekly, were 29% more likely to develop T2DM, and individuals who consumed processed meats, including salted fish and hot dogs, were 38% more likely to develop T2DM. When compared to a long-term vegetarian diet, a long-term carnivorous diet was associated with a 74% increase in the incidence of T2DM. While these results may be affected by weight, after adjusting for weight and weight change, weekly meat intake was still found to be a significant risk factor for T2DM (Vang et al., 2008).

Increased fiber and magnesium intake are associated with a diet high in fruits and vegetables, and lower in red meat. In a study evaluating fiber and magnesium intake and T2DM onset, 9,702 men and 15,365 women aged 35-65 were assessed on their dietary
patterns and fiber and magnesium intake using a food-frequency questionnaire. They were also observed for T2DM onset between 1994 to 2005. 844 cases of T2DM were observed, and it was found that high cereal fiber intake was inversely associated with diabetes risk, but fruit fiber was found to have no significant association. Magnesium intake was not found to have an association either, although meta-analysis has shown inverse associations in the past. Cereals typically include wheat, rice, oat, and corn, and are characteristically found in a plant-based diet. The elevated fiber intake in plant-based diets may decrease T2DM risk (Schulze et al., 2007).

While cereal grains were shown to be associated with decreased T2DM risk in the previous study, there may be other sources of dietary fiber which also contribute in decreasing T2DM risk. A 2012 study evaluated an increased legume intake, compared to an increased whole wheat, in T2DM individuals to determine which regime generated better disease management. One hundred and twenty one participants were provided either a low glycemic index (GI) legume diet, or a diet rich in whole wheat for 3 months. Hemoglobin A1c (HbA1c) levels were assessed at baseline and at the end of the 3 month-period. It was found that the low GI legume diet, reduced HbA1c levels by 0.5%, whereas the wheat diet reduced levels by 0.3%. While these reduction differences may seem small, they were determined to be statistically significant and can provide a significant difference in physiological function. Risk of coronary heart disease (CHD) was also evaluated, and CHD risk reduction on the legume diet was 0.8%. This risk reduction was found to be coupled with decreased systolic blood pressure in the legume diet as well. These data align with those obtained by the previously discussed study, as increased wheat fiber intake did result in better T2DM management and decreased
disease risk, but it is evident that there may be other plant-based foods potentially better suited for T2DM management (Jenkins et al., 2012).

A 2014 study investigated the effect of protein substitution on overweight T2DM patients, to assess cardiometabolic risk factors. Thirty-one participants with T2DM were assigned to either a control, legume free diet, or a legume-based diet for 8 weeks. The legume-based diet replaced two servings of red meat with legumes, 3 days per week. After an 8-week washout, the groups then followed the alternate treatment. Blood pressure, BMI, waist circumference, and fasting plasma glucose and insulin were assessed. It was found that the legume-based diet significantly decreased fasting plasma glucose and insulin, without significant changes in BMI or waist circumference. Conclusively the authors stated that minor supplementation with a plant-based protein may result in better glycemic control in T2DM patients and may decrease cardiometabolic risk (Hosseinpour-Niazi et al., 2015).

There is convincing evidence that increased animal-protein intake is associated with elevated T2DM risk, and decreased meat intake may result in better disease management. However, T2DM patients are unique, in that they are responsible for as much as 95% of their own care. This is a much higher level than what is typically seen with other chronic diseases. Because of this significant requirement for high individual responsibility, it may prove challenging to self-adopt and implement a plant-based diet. This may be due, at least in part, to economic or environmental factors. Education toward a plant-based diet with T2DM individuals as a pathway to healthier eating, veering the conversation away from what one should include and should avoid eating, can be an effective means of teaching ownership. Education on how to consume a plant-based diet,
meal tips and ideas, and small dietary changes can exert a significant impact and may be proven easier for a person to follow (Sataloff, 2015). This approach also removes the concept of restriction, which typically results in an individual removing a food for a period of time, before relapsing and overconsuming that food (Lebow et al., 2015). Small and easily navigable dietary recommendations for a plant-based diet may be more achievable in terms of economic factors as well, such as swapping an animal protein for a plant-based alternative, for example tofu or legumes, at one meal three days a week (Sataloff et al., 2015).

2.3.2 Plant-Based diets and CVD

Cardiovascular disease (CVD) risk is significantly increased in T2DM patients, with mortality risk doubled in T2DM patients, when compared to non-T2DM individuals (Laakso, 2008). Due to the apparent link between omnivorous diet and T2DM, it is likely that a similar link is present in individuals with CVD. This is speculated to be due to the increased consumption of lipids, in the form of cholesterol ester, and saturated fatty acids (SFAs) commonly associated with an animal-based diet (Salter, 2013). There is evidence that plant-based diets decrease CVD risk due to low LDL cholesterol. There are evidentiary links between decreased risk of mortality due to CVD and plant-based diets (Fraser, 2009). 81,337 men and women selected from the Adventist Health Study were assessed for their dietary patterns using a food frequency questionnaire, and animal and plant-based protein consumption was determined based on response. Individuals were followed for up to 12 years, during which time 2,276 deaths due to CVD were reported. It was found that an 18g increase in animal protein intake was significantly associated with higher risk of CVD mortality. Hazard risk of CVD mortality was 61% higher in the meat
protein factor than for those in the first quintile, where the nuts and seeds protein factor had a 40% lower mortality risk. No associations were found for grains, processed foods, and fruits and vegetables. This 40% decrease can be attributed to the high levels of L-arginine in nuts, which functions as a precursor of nitric oxide, a neurotransmitter important for cardiovascular health (Tharrey et al., 2018).

CVD risk factors and their association to vegetarian diets were evaluated in 6,555 adults in four regions of India. Dietary habits were assessed, as well as tobacco and alcohol use, physical activity, and medical history, all of which constitute risk factors for CVD onset. 32.8% of the study population were vegetarian and did not differ from non-vegetarians in terms of tobacco use, BMI, and disease presence at baseline. However, they were found to have a higher standard of living, less likely to drink alcohol, and were less physically active. Vegetarians presented lower levels of total cholesterol, triglycerides, and diastolic blood pressure than non-vegetarians, suggesting that a vegetarian diet may have cardiovascular health benefits (Shridhar et al., 2014). It is important to note that these results may be affected by the higher standard of living and decreased alcohol consumption reported, but diet may be a larger factor.

While plant-based diets are perceived to be healthier, not all are equal. Some plant-based diets can be high in refined grains and sugar sweetened beverages, which are frequently associated with increased cardiometabolic risk. Assessing CHD onset in healthful and unhealthful plant-based diets is vital to assess true benefit of the diet. Individuals in the Nurses’ Health Study and Health Professionals Follow-Up study, without CHD at baseline, were asked about food intake via questionnaires every 2 to 4 years. Responses were then sorted into three plant-based diet indices (PDI): 1) PDI
(plant-based foods given positive scores, animal given reverse), 2) hPDI (positive scores to healthy plant foods, reverse given to less healthy plant foods, and 3) uPDI (positive scores given to less healthy plant foods, reverse given to healthy plant food and animal food groups). Additionally, CHD onset was also assessed. A considerable amount of participants developed CHD over the time-period considered (n=8,631), and PDI was found to be inversely associated with CHD incidence. hPDI and CHD onset also demonstrated an inverse relationship, and this was found to be much stronger than that of PDI. A positive association with uPDI and CHD onset was observed, but this relationship was not linear. Remarkably, higher intake of a healthier plant-based diet may be effective in reducing CHD risk, whereas a less-healthy yet still plant-based diet may increase CHD risk more than an animal-based diet. Ensuring consumption of complete and healthy protein is key to decreasing CHD risk. Plant-based diets must be evaluated carefully as they may not be as healthy as they are presumed to be (Satija et al., 2017).

CVD risk is greatly impacted by genetic susceptibility, as well as diet. A plant-based diet may not affect an individual with increased genetic risk for CVD. Qi et al., investigated healthy plant-based diets with incidence of CVD while considering genetic susceptibility. Individuals, free of CVD at baseline, were asked about dietary habits (n=156,148). Healthful-PDI’s were then created, with healthy plant-based foods receiving positive scores, and less healthy plant-based and animal-based foods receiving negative scores. Genetic risk scores were also calculated. 1,812 cases of CVD were observed within the 5 years of follow-up. Higher healthful-PDI scores were significantly associated with a lower CVD risk, regardless of genetic susceptibility. A healthy plant-based diet
may be effective at preventing CVD, even in genetically susceptible groups (Heianza et al., 2020).

2.3.3 Plant-based diets and the Microbiome

Another dietary trend seen currently includes “eating for your gut” as a way to increase beneficial bacteria in the gut and decrease the detrimental bacteria, effectively positively altering gut microbiome. There is increasing evidence that the health of the microbiome may have a significant role in onset or prevention of several diseases (Hadrich, 2018).

The human microbiome is comprised of two primary phyla: Bacteroidetes and Firmicutes. In a healthy individual, this ratio typically favors Bacteroidetes over Firmicutes. This effectively places the individual in a state of eubiosis. Eubiosis typically consists of a gut bacterial composition of 95% Bacteroidetes and 5% Firmicutes (B/F). In this state, the microbiome efficiently produces short chain fatty acids (SCFAs) and branched chain amino acids (BCAAs), affects positively lipid metabolism, and generates other key metabolites. However, this ratio has been seen to be altered, especially in obese individuals, favoring Firmicutes over Bacteroidetes instead, thus resulting in dysbiosis. This is believed to be due to the nature of Firmicutes, as they metabolize insoluble carbohydrates, resulting in an increased energy harvest (Sikalidis & Maykish, 2020; Jandhvala et al., 2015). Due to the fact that many plant-based protein sources are higher in carbohydrates, it may be possible to see a similar ratio in vegans and vegetarians.
Interest regarding microbial composition and dietary habit is not new as researchers have been investigating this connection since as early as the 1970s. Interestingly, colon cancer incidence varies geographically, with high incidence rates in Europe and North America, and low in South America, Africa, and Asia (Shadmani et al., 2017). Diet is believed to be a major driver behind colonic tumorigenesis, and Western, high fat diets are believed to be associated with elevated risk. Reddy et al., investigated this association, with individuals consuming a mixed Western, high meat, high fat diet, followed by a non-meat diet, consisting largely of fruit, beans, rice, and pasta. Skim milk was consumed in both groups. Omnivores had increased levels of anaerobic microflora, as well as increased counts of \textit{Bacteroides}, \textit{Bifidobacterium}, \textit{Peptococcus}, and \textit{Lactobacillus}. It was concluded that diet plays a role in modifying intestinal microflora and may be linked to increased colon cancer risk. While this study is dated, it relays...
valuable information regarding microbial composition and diet, and demonstrates that interest in this approach has been longstanding (Reddy et al., 1975).

In separate experiments with humans, male rugby players were assessed to determine dietary impacts on gut microbial diversity. The athletes were chosen as a group because they typically demonstrate an “extreme,” i.e., extreme diet and exercise. Athletes also typically consume higher amounts of protein, carbohydrates and fat, demonstrating a higher overall calorie intake. Athlete’s metabolic status, microbiome footprint, and inflammatory status was compared to both a low BMI healthy control group and a high BMI healthy control group. It was found that athlete gut microbiota was significantly more diverse than that of the control group, while lower inflammatory markers and improved metabolic markers were exhibited by the athlete group (Clarke et al., 2014).

More specifically, 22 phyla, 68 families, and 113 genera were detected in the athlete fecal sample, as opposed to 11 phyla, 33, families, and 65 genera were detected in the low BMI group. Moreover, 9 phyla, 33 families, and 61 genera were identified in the high BMI group. Bacteroidetes was significantly less abundant in athletes, and the largest changes were in the Firmicutes. These differences were attributed to protein intake, as athlete’s protein intake was significantly higher than either control group (22% in athletes vs. 16% and 15% in low and high BMI groups, respectively). Meat and meat products represented the most significant contributing source of protein intake. However, after review of dietary data, it was evident that athletes consumed significantly more fruits and vegetables, and plant-based proteins as well, suggesting that the discrepancies in microbiome status may also be affected by this characteristic (Clarke et al., 2014).
While high protein diets are frequently consumed by athletes, similar diets have become mainstream for health conscious, but less active individuals. Promotion of high-protein, low-carbohydrate (HPLC) diets, have resulted in dietary shifts, with emphasis on protein for weight loss, especially for obese individuals. These diets may have adverse consequences, including elevated risk of colon cancer (similar to what was discussed in Reddy et al.) and decreased fecal SCFA concentrations, including butyrate. Flint et al., investigated the effects of HPLC diets in obese males (n=17), as HPLCs are frequently recommended to obese individuals to promote weight loss and prevent T2DM onset, with dietary regimes being implemented for 4 weeks. The authors reported that total SCFA concentrations in fecal samples decreased significantly. Reduction of dietary carbohydrates resulted in decreased levels of *Roseburial Eubacterium rectale* group of butyrate producers, members of the *Lachnospiraceae* group. Interestingly, the proportion of *Bacteroides* decreased by 22% with the HPLC diet. Decreased levels of fecal cancer-protective metabolites were also observed (Russell et al., 2011). Due to the fact that *Bacteroidetes* levels decreased, high meat-based protein and disease risk may be due to decreased butyrate levels, and not the B/F ratio previously discussed.

There is evidence to support the concept that the microbiome demography changes rather quickly (Turnbaugh et al., 2007) through dietary change, and that appears to be the case for plant-based diets as well. David et al., investigated the effects of a plant-based diet on the microbiome, including the speed and reproducibility of these changes. Specifically, 11 individuals were placed into one of two groups: 1) plant-based, consisting of vegetables, rice, lentils, fruit, and cereal, or 2) animal-based, consisting of eggs, bacon, pork, beef, cured meats, and cheese. Individuals followed these diets for 5
days without caloric restriction. Fecal samples were collected, and microbial community patterns were assessed using 16S rRNA sequencing. Microbial diversity within each subject at a given point (alpha-diversity) and between each subject at baseline and diet-associated gut microbiota (beta-diversity) were evaluated. The animal-based diet appeared to produce a greater impact on the microbiome than the plant-based. No significant change in alpha-diversity was observed, but there was a significant increase in beta-diversity in the animal-based diet. After hierarchal clustering of bacteria, the abundance of 22 clusters changed significantly on the animal-based diet, compared to only 3 on the plant-based. Overall, the animal-based diet increased the abundance of bile-tolerant organisms, and decreased levels of *Firmicutes*, bacteria that metabolize dietary plant polysaccharides (David et al., 2014).

Because the microbiome changes rather quickly, many studies investigate short-term effects of dietary changes. Because of the relative ease of researching on a short-term basis, long-term effects of vegetarian and vegan diets on the microbiome are not well understood. However, Matijašić et al., aimed to study microbial differences in long-term omnivore versus vegan/lactovegetarian diets. Slovenian adults (n=60) were surveyed as per their dietary habits and separated into one of 3 groups: 1) lactovegetarian, 2) vegan, and 3) omnivore. Fecal samples were collected, and microflora was assessed using 16s rRNA sequencing. The vegetarian diet was associated with a higher ratio of *Bacteroides-Prevotella, Bacteroides thetaiotamicron, and Faecalibacterium prausnitzii*, but a lower ratio of *Clostridium*. While variance due to age, lifestyle, and sex was analyzed, diet represented up to 4% of variance in microbial community, the largest of
any dietary habit. It was concluded that vegetarian diets result in a significantly more varied microbiome than omnivorous diets (Matijašić et al., 2014).

In separate experiments, 98 individuals underwent microbial analysis using 16s rDNA sequencing to determine effects of diet on microbial status. Nutrients from fats versus plant products and fiber showed inverse associations with microbial taxa. Similar results were shown with protein versus carbohydrates and fat versus carbohydrates. This included increased presence of Bacteroidetes and Actinobacteria. Increased fiber intake was positively associated with increased presence of Firmicutes and Proteobacteria. Samples were then clustered into enterotypes, with high levels of *Bacteroides* and *Prevotella* identifying two clusters. Increased presence of the *Bacteroides* enterotype was positively associated with animal protein, whereas *Prevotella* was in turn associated with vegetarian and vegan diets. This analysis was then followed by a short-term feeding study to assess rapid changes in the microbiome. In these experiments, 10 individuals were randomly assigned to a high fat/low fiber or high fiber/low fat diet for 10 days and fecal samples were collected daily. Changes in microbial composition were detectable within 24 hours in all 10 individuals. All participants’ microflora fell in the *Bacteroides* enterotype, and while no stable switch to the *Prevotella* enterotype was observed, some crossover between the two was noted. It was therefore concluded that while the microbiome does change rapidly with dietary intervention, long term dietary adherence is more effective in creating a stable gut environment (Wu et al., 2011).

In a systematic review by Medawar et al., the short- to moderate-term effects (≤ 24 months) of plant-based diets on the microbiome, weight status, inflammation, and energy metabolism were investigated. In this analysis, 32 studies were determined to be
eligible, with 19 of these including obese individuals or individuals with T2DM. Vegan diets were found to be associated with a larger reduction of HbA1c values and decreased waist circumference when compared to omnivores. Reduced need for T2DM medication and increased glucose sensitivity was also observed. All results were observed with similar calorie intake among all groups. This was attributed to microbial changes within the gut (Medawar et al., 2019).

It is evident that the pattern between the gut microbiome and plant-based diets is not clear. While the consensus on the ideal B/F ratio has been proposed to be 95% Bacteroidetes, plant-based diets may induce changes favoring Firmicutes due to the increased carbohydrate and fiber intake. Despite this, plant-based diets are continuously associated with decreased weight, regardless of calorie intake. Conversely, elevated Firmicute levels are associated with increased energy harvest. However, data representing these changes are inconsistent. More research investigating this link between plant-based diets and microbiome status is necessary to provide dietary recommendations, including length an individual must follow a diet for in order to demonstrate consistent changes.

### 2.3.4 Plant and animal-based diets and muscle mass

There appears to be a misconception in the public that vegan and vegetarian diets result in decreased protein intake, resulting in muscle breakdown, weakness, and fatigue (Pezeshki et al., 2016). The recommended dietary intake for adults is 0.8g per kg of bodyweight, or 1.2g per kg for athletes. For a 75kg adult, 60g of protein would be adequate (Volpi, 2017). Several studies have shown that American adults tend to eat 100g of protein per day, far exceeding this recommendation, including those on a vegetarian or vegan diet (Egan, 2017). While protein intake may not differ between plant
and animal-based diets, one element that should be considered is protein quality, and its effect on muscle mass and growth.

When protein is consumed, the acidic environment of the stomach results in denaturation of the protein, making it more accessible for enzymes to break the polypeptide chain. Proteolytic enzymes such as pepsin target the protein chain, resulting in the generation of shorter amino acid chains, a degradation that continues further in the lumen of the intestine, where the pancreas releases more proteolytic enzymes. The process results in the generation of free amino acids and shorter di- and tri-peptides that can be absorbed and utilized (Berg et al., 2002). Out of the 20 common amino acids used for human protein translation, 9 are considered essential as these cannot be made by humans, so they must be obtained through diet. A food that contains these 9 essential amino acids is considered a “complete protein” (Hoffman & Falvo, 2004). Complete proteins are easily accessible for omnivorous individuals, as eggs, beef, and whey protein are considered high quality proteins. (Hoffman & Falvo, 2004). Therefore, it is not necessarily the amount of protein a vegetarian consumes that may result in inadequate protein intake, but also the quality of the protein. It is more difficult to find complete proteins that are not from animal sources. Legumes and soy-based proteins are frequently nearly complete, with lower levels of leucine, an amino acid essential for growth, in comparison to animal based proteins (Berrazaga, 2019). This means that plant based protein sources may not be as effective as animal based proteins in various areas of the body, and plant-based consumers may need larger portions to meet similar amino acid intake (Petre, 2016) (Table 1). Regular consumption of plant-based Due to this lack of essential amino acids,
it may be necessary to investigate the differences between animal and plant-based proteins on muscle mass and muscle protein synthesis (MPS).

Table 1: Amino acid composition of popular protein sources (g/100g) (American Egg Board, 2021, Ahmad et al., 2018)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Red Meat</th>
<th>Chicken Breast</th>
<th>Pork</th>
<th>Eggs</th>
<th>Lentils</th>
<th>Tofu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>0.6</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>0.7</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.9</td>
<td>2.9</td>
<td>2.7</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Valine</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.4</td>
<td>1.4</td>
<td>0.7</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.7</td>
<td>2.5</td>
<td>2.3</td>
<td>1.1</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Serine</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5</td>
<td>4.5</td>
<td>4.6</td>
<td>1.6</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Proline</td>
<td>1.4</td>
<td>1</td>
<td>1.2</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.9</td>
<td>1.8</td>
<td>1.7</td>
<td>0.7</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.1</td>
<td>1.1</td>
<td>1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.1</td>
<td>2.1</td>
<td>1.8</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>3</td>
<td>2.9</td>
<td>2.7</td>
<td>1.3</td>
<td>1</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Along those lines, 40 Caucasian women, both omnivorous and vegetarian, were evaluated for muscle mass index and protein intake, to determine the effects of their dietary habits on muscle mass. There were significant differences observed between the two groups for muscle mass index and body weight. Muscle mass index was found to be strongly associated with animal protein intake, but not with plant protein or total protein intake, with each group consuming similar amounts of protein. This may be because plant proteins contain lower levels of essential amino acids, such as leucine and methionine (Aubertin-Leheudre & Adlercreutz, 2009).

On a separate consideration, sarcopenia is defined as the gradual loss of muscle during aging, and can lead to increased risk of fall, fracture, and loss of strength. To investigate the effects of dietary habits on muscle mass during aging, 275 Polish women aged 32-60 were assessed. The participants were asked about their dietary patterns over the course of 14 days, and three major nutrient patterns were determined: Animal Protein-Vitamins, Fiber-Plant Protein-Minerals, and Fats. Skeletal muscle mass was also evaluated. It was found that strong adherence to an animal protein-vitamin diet was associated with increased muscle mass, and plant protein and fat were not found to be associated accordingly (Danielewicz et al., 2019).

Maximizing muscle mass is of particular interest in athletes and fitness-centric individuals, with the protein supplementation industry estimated to be worth $32.6 billion by 2027 (James, 2020). In a market once dominated by whey protein, elevated interest in plant-based diets has resulted in the development of plant-based protein supplements. Animal-based supplements currently account for 67.9% of the market ($21.7 billion), but it is believed that plant-based protein will reach $11.05 billion by 2027 (James, 2020). It
is therefore vital to determine if animal and plant-based protein powders function similarly in terms of muscle growth and synthesis. Phillips et al., evaluated the effects of whey, casein, and soy protein isolate on muscle protein synthesis both at rest and after exercise. Eighteen men who regularly engaged in whole body resistance training (2-3 days/week) were asked to consume one of the three drinks before exercise. Ingestion of whey protein stimulated muscle protein synthesis more than casein or soy protein, both at rest and after exercise. Soy, overall, performed better than casein. This is interesting as casein is animal based as well, but functions differently than whey in this case. This may be due to protein functionality, as whey and soy both function quicker than casein, meaning they are digested more rapidly. This results in an amino acid presence in the blood sooner, but for a shorter period of time (Tang et al., 2009).

Creatinine excretion assessment is frequently used to estimate total-body skeletal muscle mass, as creatinine is produced by non-enzymatic dehydration of intracellular creatine from muscle tissue. Creatinine excretion is affected by dietary protein intake, regardless of source. Muscle mass and its relation to protein source was assessed in 76,633 individuals, aged 18-91. Dietary habits and lifestyle factors were determined, and protein source was divided into animal protein, dairy protein, fish/meat/egg protein, and plant protein. When consumption of total protein, animal protein, and fish/meat/egg protein increased, creatinine excretion increased in both men and women across quartiles. This relationship was not observed in plant or dairy protein. Plant protein was significantly positively associated with creatinine excretion in the male 26-35 age group only, after adjusting for confounding factors. In order to preserve muscle mass, this
conclusion regarding dietary protein may be used to adjust dietary protein intake as the individual ages (Alexandroy et al., 2018).

Elderly individuals in Taiwan were recruited for a cross-sectional study assessing low muscle mass and dietary protein intake. Individuals were sorted into low muscle mass or normal groups, based on skeletal muscle mass measurements estimated by bioelectrical impedance analysis. The low muscle mass group consumed significantly less total protein and vegetable protein, while fat intake was slightly higher than the normal group. Those with the lowest total protein intake exhibited a threefold risk for low muscle mass compared with those with the highest protein intake. Low vegetable protein intake was also associated with a twofold risk in low muscle mass compared to those with high vegetable protein intake. It is important to note that individuals were not clustered into vegetarian, vegan, or omnivorous groups in this study. Animal-based protein may have therefore influenced muscle mass. It is plausible that animal-based protein sources provided enough nutrients to supplement for plant-based protein, representing the decreased risk for low muscle mass. (Huang et al., 2016).

While it is evident that plant-based diets provide beneficial health effects for T2DM and CVD prevention, evidence is not as clear for muscle mass growth and sarcopenia prevention. Animal based proteins and whey protein powder appear to function better in terms of MPS than plant-based proteins. It may therefore be necessary for bodybuilders, strength athletes, and elderly individuals to consume higher amounts of plant-based protein to achieve optimal MPS status.
2.4 Dietary Recommendations

United States dietary habits and trends are at an interesting crossroads. There is growing interest in plant-based diets for beneficial health effects, with simultaneous interest in increased protein intake for muscle mass and sarcopenia prevention. Current dietary recommendations consist of 0.36g of protein per pound of body weight, or roughly 56g/day for the average sedentary man, and 46g/day for the average sedentary woman (Pendick, 2015). This recommendation is well under the protein levels the typical American consumes, whether that be plant or animal-based protein (Physicians Committee for Responsible Medicine, 2021). There is evidence to suggest that plant-based diets result in decreased BMI, reduced T2DM and CVD risk, and that it may alter the microbiome in a beneficial way. However, there is also evidence to suggest that plant-based diets alone may not promote muscle mass, especially in sarcopenia prevention. Optimal protein intake for sarcopenia prevention is 1-1.2 g/kg body weight per day, but it is the quality of the protein that is vital (Yanai, 2015). Several studies suggest that the source of discrepancies between plant and animal-based protein and muscle mass, lay in the amino acid composition of protein. As previously mentioned, consumption of all the essential amino acids is the best way to ensure adequate protein intake. Leucine, one of the essential amino acids, is frequently implicated with MPS, as it extends signaling supporting protein synthesis and MPS. Leucine is responsible for activating the mammalian target of rapamycin (MTOR) pathway, a pathway responsible for growth. MTOR regulates skeletal muscle mass and anabolic and catabolic processes (Yoon, 2017). Leucine is also linked to the release of alanine from the muscle, whereby alanine functions as a gluconeogenic precursor, inducing gluconeogenesis (Maykish & Sikalidis,
Dietary sources of leucine include meat, tofu, cheese, and beans, but the leucine content varies significantly between sources. A chicken leg or 6oz of skirt steak will provide 180% of the RDI for leucine, whereas one cup of navy beans provides 61% of the RDI (USDA, 2019), making it significantly more challenging for vegans and vegetarians to consume adequate leucine.

Several studies have observed increased MPS through leucine supplementation. Katsanos et al., investigated this relationship in elderly individuals to decrease sarcopenia risk. Individuals in two elderly and two young groups were assessed before and after ingestion of 6.7g of an essential amino acid (EAA) formula. Formula was composed of either whey protein (26% leucine) or enriched in leucine (46%). In the elderly group consuming the enriched supplement, stimulation of postprandial MPS and accretion of muscle proteins occurred, whereas in the whey-only elderly group, this was not observed. This is especially of interest, as the enriched supplement contained 3g of leucine, providing evidence that even a small amount can provide protective effects (Katsanos et al., 2006).

Koopman et al., studied the effects of carbohydrate or carbohydrate plus protein and free leucine in eight elderly (75 ± 1 year) and eight young (20 ± 1 year) individuals. The aim was to evaluate change in MPS with age. Individuals consumed either carbohydrate or carbohydrate plus protein and free leucine after completion of 30 minutes of standard daily activities. In both groups, the leucine supplementation showed improved protein balance, when compared to the carbohydrate only. MPS rates were also increased in the supplement group. Leucine and EAA supplementation are frequently
studied in elderly individuals to prevent sarcopenia, however it is evident that supplementation is beneficial in young people as well (Koopman, 2006).

Ten adults in a randomized, double-blind study completed three energy deficient periods (-30%) lasting 5 days each. Diets were supplemented with EAA enriched whey, whey only, or a mixed-macronutrient meal after exercise. Protein synthesis was 15.8 and 19.4 greater in the EAA enriched supplement than the whey and meal, respectively. No significant difference was observed between whey and meal. Addition of an EAA enriched supplement resulted in whole-body protein balance in energy deficit, again illustrating the importance of complete protein (Gwin et al., 2021).

Leucine supplementation appears to be beneficial in active individuals. However, aging is typically accompanied with immobilization periods, which further increases sarcopenia risk due to decreased muscle use (Magne et al., 2012). Supplementation with protein during recovery is therefore of interest. Casein diets with either alanine (control), or leucine were provided to elderly rats after an 8-day period of immobilization. Muscle atrophied by 20%, and did not recover fully, even after 40 days post-immobilization. The leucine group demonstrated a greater protein anabolism, but muscle mass recovery was not observed. However, the high protein control diet was efficient in inducing muscle mass recovery. Therefore, while leucine supplementation does appear to be effective in preventing muscle loss prior to immobilization, consumption of high protein meals with complete protein may be more effective at preserving muscle mass after immobilization events (Magne et al., 2012).

Hydroxyl-methyl butyrate (HMB) is an active metabolic form of leucine and is believed to extend anticatabolic effects on skeletal muscle by minimizing muscle damage
and muscle proteolysis. May et al., 2002). This occurs through use of inhibitors of leucine transamination, generating HMB, and effectively suppressing the inhibition of protein degradation (Zanchi et al., 2006). Nissen et al., evaluated the effects of HMB on muscle metabolism during resistance exercise training in two studies. Study 1 provided three levels of HMB supplementation (0, 1.5, or 3g/day) with either a normal (117g/day) or high protein (175g/d) diet. Individuals then weight lifted for 1.5 hours for 3 days/week. HMB supplementation was found to significantly decrease the exercise-induced rise in muscle proteolysis. The amount of weight that was able to be lifted also increased over the course of three weeks with this group, in comparison to the control (Nissen et al., 1996).

Study 2 provided individuals either 0 or 3g/day of HMB and weight lifted for 2-3 hours for 6 days/week for 7 weeks. Fat-free mass was significantly increased in the HMB supplemented group, when compared to the control, and results were significant by week 2 (Nissen et al., 1996). Both studies demonstrated effects with small supplementation amounts, displaying beneficial effects at as low as 1.5g/day.

Hsieh et al., assessed the effect of HMB supplementation on body composition and protein metabolism in bedfast elderly during tube-feeding. Participants either consumed an HMB supplement (2g/day) or resumed normal dietary habits. Blood urea nitrogen (BUN) decreased significantly in the HMB group but remained unchanged in the control after 14 days. Urinary urea nitrogen (UUN) excretion decreased in the HMB group as well, whereas the control group demonstrated significant increases in UUN levels. Bodyweight and BMI increased slightly, but significantly, in the HMB group.
was therefore concluded that HMB supplementation may be beneficial in prevention of muscle degradation in elderly residents in an inpatient unit setting (Hsieh, 2010).

While these studies do not focus solely on animal or plant-based proteins, they illustrate the importance of consumption of complete protein for MPS. Due to concerns about protein intake and plant-based diets, it may be beneficial for individuals to consume EAA supplements, particularly those high in leucine content, along with a plant-based diet. While this may not be necessary for a younger person, it may be of interest to elderly individuals who have followed a long-term plant-based diet or a vegan or vegetarian diet who are interested in gaining muscle.

2.5 Conclusions

Plant-based diets are becoming increasingly popular for a variety of reasons, including the desire to lead a healthier lifestyle and to decrease negative impacts on the environment. Plant-based alternatives, instead of meat options, can reduce OFLs represented by current agricultural practices, and may contribute to the decrease of food waste as well. Plant-based diets have been linked to decreased T2DM and CVD risk but may not be effective at preserving muscle mass during aging. Identification of a plant-based product with similar protein and amino acid content that functions similarly to animal products is key for encouraging major positive shifts in the American diet.
3. MATERIALS AND METHODS

3.1 Participants

A group of 20 female college students, aged 20-25 were recruited for this study, all of which were enrolled full-time at California Polytechnic State University, San Luis Obispo. Participants were recruited from the College of Agriculture, Food, and Environmental Sciences (CAFES), as well as the Kinesiology department in the College of Science and Mathematics (COSM) and all participants had a basic knowledge of nutrition (introductory Nutrition general education class completed). The study was conducted between May 24 and June 11, 2021. All participants completed an online survey to determine eligibility (see Appendix A). Participants were eligible if they were within an age range of 20-25, lacked allergies to dairy or tree nuts, were able to consume both animal and plant protein, had no present chronic diseases, did not regularly consume protein supplements (3x or more per week), and did not take medication for hypertension, glycemic control, dyslipidemia, or antibiotics. Participants were informed on the purpose of the study, experimental procedures to be used, and any potential risks. All participants were not pregnant at the beginning of the study. Written consent via an informed consent form was obtained from all participants before beginning the study. Incentives in the form of a $10 gift card were provided at completion of the study. The experimental protocol and all procedures were approved by the Human Subjects Research Committee of California Polytechnic State University, San Luis Obispo, California (IRB 2021-091).

3.2 Experimental Protocol

The experimental protocol implemented was designed to examine the effect of receiving whey or almond protein supplementation on nitrogen balance, hydration, and fat mass. Randomized single blind design was used, in which study participants were
assigned to either the whey (n=10) or almond (n=10) supplementation group through randomization in Excel. In addition, individuals completed 3-day dietary records and exercise logs prior to supplementation to assess nutrient differences. At the initial and post-supplement test days, participants’ anthropometry was analyzed, including BMI, height (cm), weight (kg) (EB9380H Bodyweight Scale, Etekcity), percentage body fat and muscle mass, and percent body water. Body composition, bone density, fat mass, and muscle mass were assessed using dual-energy X-ray absorptiometry (DXA) at baseline. Secondary analysis of body fat, muscle mass, water, basal metabolic rate (BMR), and visceral fat was assessed using bioelectrical impedance (Tanita). Participants performed 24-hour urine collections at baseline and at conclusion of the study to assess changes in nitrogen levels due to supplementation. 24-hour urea nitrogen and urine specific gravity were analyzed to determine effects on nitrogen balance (NB) and hydration.

### 3.3 Supplementation

Supplementation was provided in the form of protein powder, with commercially available whey (animal source) and almond (plant source) protein powder. Powders were then to be added to almond milk and enriched chocolate flavoring to create a protein shake. Both shakes were identical in protein content, color, taste, and texture. Ingestion of the supplement was twice daily over the course of seven days and provided 30g of additional protein per day to each participant. Individuals were asked to maintain their typical dietary and exercise habits. The almond shake contents consisted of 34.5g of almond protein powder, 16.8g of vitamin enriched chocolate flavoring, and 295ml of almond milk per drink. Whey shakes consisted of 17g of whey protein powder, 16.8g of vitamin enriched chocolate flavoring, and 295ml of almond milk per drink. This totaled
15g of protein per shake (whey protein powder contains a more concentrated amount of protein than almond, requiring less powder). Almond milk was used in both the whey and almond protein shakes to ensure uniformity. The total amount of protein ingested per day was within safety guidelines as available by the American Society for Nutrition, with recommendations not to exceed 125g/day for a 140 lb. person (Pendick, 2015). Caloric content of the shakes included 285kcal/shake for the almond shake, and 205kcal/shake for the whey shake. Participants were provided ingredients on campus along with written instructions and were asked to make their own drinks in their place of residence due to concerns pertinent to COVID-19. Supplement and shake ingredients were provided to each participant in individually packaged containers and included clear containers with lids and a plastic scoop (table 2). Shaker bottles for drink production was also provided. Compliance with the supplementation protocol was monitored via distribution of a twice daily survey through e-mail (Appendix B).

Table 2: Ingredient breakdown of each shake. Each shake totals 15g of protein, or 30g of protein per day.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Almond Shake (plant)</th>
<th>Whey Shake (animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond Milk</td>
<td>10oz</td>
<td>10oz</td>
</tr>
<tr>
<td>Chocolate Flavoring</td>
<td>16.8g</td>
<td>16.8g</td>
</tr>
<tr>
<td>Protein Powder</td>
<td>34.5g</td>
<td>17g</td>
</tr>
</tbody>
</table>
3.4 Dietary Analyses and Consultations

Dietary intake was recorded in the 72 hours prior to supplementation. Standardized dietary assessment procedures were utilized throughout the study. Participants used standardized 3-day food and exercise records created by the Sports Nutrition Team at California Polytechnic State University, San Luis Obispo, to record what they ate three days prior to supplementation. All dietary records included two weekdays and one weekend. Time of day, portion size, food description (including brand), and preparation method were all recorded. Participants were given a portion size guide to estimate food amounts (Appendix C, Appendix D). Exercise logs consisted of date and time of day, duration, and exercise description (Appendix C). Records were then entered into ESHA Food Processor Nutrition Analysis software (ESHA Cloud Services, Version 1.5) to obtain values regarding nutritional intake of participants, including daily calorie and macronutrient intake, and their relation to recommended values. ESHA’s default recommended values are based on the participant profile, including age, sex, height, weight, and activity level. All participants were determined to be “lightly active”, unless no exercise was reported on the exercise log, and were thus determined to be “sedentary”. Additionally, participants were asked to consistently consume the same dietary intake as recorded in their three-day food records during the protein supplementation phase.

3.5 Body Composition

Body composition was assessed at the Nutrition and Health Assessment Laboratory in the Food Science and Nutrition department at California Polytechnic State University, San Luis Obispo. Measurements were performed using DXA on a Lunar
iDXA (GE Healthcare, Madison, WI, USA), and all procedures were completed according to GE Lunar specifications. Weight, height, and ages were recorded immediately before the DXA scans. Bone density, fat mass, and fat-free mass were assessed using DXA data at baseline. 12-hour fasting was required prior to scanning and all subjects were required to present a negative pregnancy test before the scan. Subjects were placed in a supine position, with arms slightly abducted from the trunk, and legs slightly separated. Total body scans were performed using standard mode and the same individual performed all DXA scans. Participants were given a copy of their results following the scan. Study ID numbers were used for all DXA files to ensure participant privacy.

Bioelectrical impedance analysis (BIA) was also performed via the Tanita MC-780U Plus P (Tanita Corp., Tokyo, Japan). BIA was conducted both at baseline and at conclusion of the study. Subjects stood barefoot on the metal sole plates, and a 2.2lb tare was entered as per manufacturer’s recommendation to account for clothing. All measurements were performed using standard prediction equations, as no participants were determined to require the athlete setting. BIA was utilized to determine total body fat, muscle mass, visceral fat, body water, and BMR.

3.6 Nitrogen Analysis

24-hour urine collection was performed at baseline and at the conclusion of the study. Participants consumed protein shakes for 7-8 days, including on the last day when they completed their urine collection. Participants delivered their own urine samples to Pacific Diagnostics Laboratories in San Luis Obispo, CA. Total nitrogen and specific gravity were measured and analyzed per standard approved established protocols (Laboratory
Participants were provided collection containers (Fisherbrand Low Form 24-hour Urine Collection Container, Fisher Scientific) for 24 hour total urine collection at the beginning of the study and were required to keep samples refrigerated until delivered for analysis.

NB was determined using the standard nitrogen balance equation below (Champan et al., 2020) with miscellaneous nitrogen excretion assumed to be 4g/day (Poortmans & Dellalieux, 2000).

\[ NB = \text{Nitrogen intake (g)} - \text{Urinary urea nitrogen(g)} + 4 \]

3.7 Statistics

JMP Pro (Version 14.0) (SAS Institute, Cary, NY) was used for all calculations for the study. Paired Sample T-tests were used to assess differences between pre- and post-protein supplementation for significant physiological change and urea nitrogen change, both overall and per supplementation group. Normality was assessed before conducting tests, and transformed using log10 if not normal. Independent t-tests were utilized to assess significant differences between the almond and whey groups. Descriptive statistics were utilized to quantify the mean and standard deviation of the height, weight, and age. Calculations regarding nutrient intake included mean calorie intake, protein, carbohydrate, and fat, as well as the percent intake of each macronutrient.
4. RESULTS AND DISCUSSION

4.1 Body Composition

An on-line screening questionnaire applying inclusion/exclusion criteria for study participation was completed by 192 individuals. Of these, 45 individuals were determined to be eligible for inclusion in the study, and 20 participants enrolled and successfully completed the study. Eligible participants were females aged 20-25 with no underlying health conditions who did not regularly consume protein supplements (3x or more/week), were not pregnant and did not receive any medication. Baseline characteristics of these individuals included: age of 21.9 ± 1.21 years, and body weight of 60.85 ± 7.45 kg. Average BMI was 22.29, placing all participants in the “normal” classification, or “standard” body composition for the age range. Body fat was measured via BIA to be 21.56 ± 5.89 %, whereas DXA indicated a total body fat percentage of 30.30 ± 5.05%. The disparity between the two methods of assessment recorded was determined to be statistically significant (p=0.0021). While there is no universally accepted official standard for assessing body fat, two commonly utilized references are the American Council on Exercise (ACE) and Beth Israel Winchester Hospital (Woods, 2016; Muth, 2009). ACE suggests a range of 21-24% as “fitness” for women, without considering age, whereas Beth Israel Winchester Hospital suggests the ideal range for women 20-29 years old to be 21-32%. Hence, regardless of scale, our study participants demonstrated low levels of body fat as assessed via BIA, but normal levels as assessed by DXA (Woods, 2016; Muth, 2009; Healthline, 2019). Our observations on the differences in body fat assessment between BIA and DXA closely align with previous research reports (Newton et al., 2005; Wattanapenpaiboon et al., 1998; Gupta et al., 2011) observing BIA to consistently produce a lower body fat percentage compared to DXA, particularly in
females, which as per sex is reasonable since females exhibit a higher body fat percentage under normal body composition compared to males. The differences observed between the two assessment methods (i.e.: BIA vs. DXA) may be attributed to the formulae utilized to calculate body fat by the built-in software of the equipment. Different equations used to predict/extrapolate body fat can result in large variation between DXA and BIA, ranging from 0.3-8.1% body fat (Wattanapenpainboon et al., 1998). Additionally, body water percentage was determined to be in range, indicating adequate hydration. Visceral fat was low, and BMR was determined to be average (table 3).

4.2 Nutritional Analysis

Three-day dietary records were collected from all 20 participants. Analysis of the records conducted via ESHA determined an average caloric intake of 1,672.7 \( \pm \) 492.36 kcal/day. Typical dietary recommendations include 2,000 kcal/day for females, irrespective of exercise levels (FDA, 2020b). In this context, our findings represent an underconsumption of calories by 328 calories/day in the observed population. However, adequate macronutrient consumption was observed for all three macronutrients (carbohydrate, lipid, protein), with 48% of daily calories from carbohydrates (201.66g), 36.2% from fat (68.41g), and 15.7% from protein (23.17g) (figure 5). These percentages for carbohydrates and protein are in accordance with acceptable macronutrient distribution ranges (AMDR) of 45-65% for carbohydrates and 10-35% for protein, according to the Institute of Medicine (Institute of Medicine of the National Academies, 2002).
The AMDR for lipid is 20-35%, hence our participants demonstrated a slight overconsumption for lipids in terms of daily energy contribution. This percentage represents total lipid, and does not consider source and/or type (i.e.: plant vs. animal and saturated, unsaturated/polyunsaturated/monounsaturated). In the case of our study, participants frequently consumed avocados and peanut-butter and rarely consumed sweets and red meat, according to dietary records. It is therefore plausible that the relatively high fat-derived energy intake percentage is primarily due to unsaturated fat consumed, as this has also observed by others (Cotton, 2018; De Souza et al., 2015).

While the considered macronutrient percent energy contribution ranges do not take age, sex, or physical activity into account, nutritional analysis indicates adequate intakes and reasonably aligned contribution of each macronutrient, revealing desirable dietary habits in this regard. It is important to note that all participants ensured basic nutrition knowledge (introductory nutrition College-level class had been taken), and the observed eating habits are likely not representative of those of the Campus’ general student population. Our observations align with research conducted by Hong et al. at San Diego State University, whereby BMI of nutrition majors was compared to that of non-nutrition majors. A total of 202 female students were assessed, concluding that nutrition majors exhibited a significantly lower BMI than that of non-nutrition majors (Hong et al., 2016). The biggest factor in this finding was deemed to be nutrition knowledge informing food choice; nutrition majors were more likely to choose a healthier meal option, whereas non-nutrition majors were more likely to choose a meal based on convenience (Hong et al., 2016). Due to the characteristics of our studied population, it is likely that a higher-than-average nutrition knowledge, coupled with possibly more interest and motivation towards
nutrition and health may at least partially explain the dietary habits of our study participants.

Additionally, participants performed cardio workouts more frequently than strength workouts, and for longer durations. The average time for a cardio workout was 33.1 +/- 36.4 minutes, whereas strength workouts were found to last 8.5 +/- 19.6 min, on average. Most cardio workouts consisted of low impact workouts, such as walks around town or to various buildings on campus. This indicates that participants are “lightly active”, on average, categorized as activity including daily living, plus 30-60 minutes per day of moderate activity, such as walking (ESHA Research, 2020).

4.3 Effects of Protein Supplementation on Body Composition

All body composition values are as indicated via BIA. Pre-supplementation values include all participants, and are not separated by assigned protein supplement. After completion of protein supplementation, participant weight increased by an average of 0.785kg (p=0.0047), representing a significant weight change during the supplementation period. No significant weight change was observed between the almond and whey protein supplemented groups (p=0.63). Interestingly, changes in body fat percentage were not determined to be significant overall (p=0.59), however changes in body fat between the whey and almond groups following supplementation were determined to be significant (p=0.033). For the entire study population, total muscle remained statistically unchanged, regardless of protein supplementation source. Thus, protein source does not seem to affect muscle for the period of time and at the level of supplementation we introduced (8 days and 30g/day of protein supplemented). The observed weight change may be attributed to the increased caloric intake due to the
protein shakes, increased water retention, or general typically observed natural fluctuations in overall weight. Body fat changes were greater in the whey group than in the almond, which is of interest considering the fact that the almond shake contains 79 more calories than the whey. This difference in body fat percentage could be due to differing exercise levels per group, or changes in dietary habits throughout the week, especially considering the end of the quarter and final examinations. No other significant changes were observed regarding body composition, and results are summarized in table 2. The small body composition changes were not a surprising finding, as significant body composition changes typically take 4 weeks at minimum to manifest and longer supplementation periods are likely necessary to confer change (Doylestown Hospital, 2014).

Table 3: Body composition characteristics of female college students, including pre- and post-supplementation. (*BIA). Statistical significance was determined via matched pairs t-test, and is on a per row basis. BMR: Basal Metabolic Rate calculated through BIA equipment software.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre- Supplementation (Baseline)</th>
<th>Post- Supplementation (Overall)</th>
<th>Post- Supplementation (Almond group)</th>
<th>Post- Supplementation (Whey group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>60.84 ± 7.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.63 ± 8.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.14 ± 9.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.11 ± 6.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body fat* (%)</td>
<td>21.5 ± 6.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.29 ± 5.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.42 ± 5.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.15 ± 5.56&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle mass (%)</td>
<td>33.75 ± 2.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.01 ± 2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.51 ± 2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.5 ± 2.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body Water (%)</td>
<td>51.97 ± 3.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.15 ± 4.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.58 ± 4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.71 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Visceral Fat</td>
<td>1.25 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMR</td>
<td>1446.65 ± 136.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1441 ± 134.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1434.40 ± 159.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1447.60 ± 113.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 5: Percentage of caloric intake contribution from macronutrients.

4.4 Protein Supplementation effects on Urine Specific Gravity

Urine-specific gravity (USG) was assessed by calculating the ratio of urine density to the density of water (Stuempfle & Drury, 2003). USG gravity was determined to be $1.01 \pm 0.0067$, placing participants within the normal/physiological range of 1.005 to 1.030 (Brennan, 2021). This finding indicates good levels of hydration, suggesting good fluid intake habits and adequate water intake. Following protein supplementation, USG increased slightly ($1.011 \pm 0.00655$), regardless of protein source. This change was not determined to be significant ($p=0.26$), and it can be concluded that increased protein intake did not impact hydration levels significantly. This is of interest due to current dietary trends, which include high protein diets. High protein diets are often recommended due to protein’s satiety effects and ability to increase lean muscle mass, when appropriately combined with exercise (Olsen, 2020). However, such practice, if not regulated, can lead to excess protein intake, and concern regarding dehydration and
potential renal damage. Elevated USG levels would indicate such a condition. Goodwin et al. investigated hydration status of five endurance athletes during consumption of low, moderate, and high protein diets, with each dietary regime four weeks, whereas fluid intake remained unchanged. Degree of hydration was inversely related to protein intake, and urea nitrogen levels were abnormal at high protein intakes, indicating kidney stress and dehydration due to high protein diets (Goodwin, 2002). Similarly, Martin et al. assessed increased protein intake and hydration indexes in males. Increased USG was correlated with both high and moderate protein diets, however fluid balance remained unchanged (Martin et al., 2006). While the observed increase in USG in our study was not significant, the overall increase does align with previous research observations. It is possible that a longer supplementation period or larger sample size would result in further increased USG.

4.5 Protein Supplementation effects on Urine Urea Nitrogen

In our study all 20 participants exhibited low urine urea nitrogen (UUN) prior to supplementation (5,892.7 ± 2,197.4 mg/24hr), placing them well below the acceptable range of 12,000-20,000 mg/24hr. None of the 20 participants were within this range prior to supplementation. On average, participants were determined to be in a very slight state of positive nitrogen balance overall (0.585g/24hr), representing a state of anabolism and excess nitrogen pool. However, almost half (n=9) of participants were in a negative nitrogen balance, indicative of a low protein intake (Dickerson, 2016). This correlates to the low UUN levels observed, as protein intake and UUN are closely linked. While the AMDR for protein was met overall, it may not be enough for this sample population of young females who exercise regularly. None of the participants were members of Cal
Poly athletic teams, and exercise did not impact protein intake. Protein consumption amounts may also have been overreported due to estimation of portion size. This is further illustrated by the UUN to dietary nitrogen ratio. Literature suggests an expected ratio of UUN to dietary nitrogen of $0.81 \pm 0.05$ as a valid estimate of dietary intake (Bingham, 2003). The ratio in our study was found to be $0.562 \pm 0.577$, on average. This is far below the expected ratio and indicates a lack of dietary protein. It is thus reasonable to speculate that protein intake was significantly overreported.

After supplementation, UUN levels increased significantly overall, to $7,802.1 \pm 1,909 \text{ mg/24h}$ ($p=0.0016$). No significant difference was found between protein source ($p=0.9494$), indicating that the amounts of both the almond and whey protein increased nitrogen in the body similarly.

Nitrogen balance (NB) was also increased significantly following supplementation, from $8.58\text{g nitrogen}$ to $11.66\text{g nitrogen}$ ($p<0.0001$). Protein source was not determined to be significant ($p=0.7760$). A nitrogen balance from within -4 to -5 g/day to + 4 or +5g/day is typically considered equilibrium, thus indicating that our participants were in a slightly anabolic state prior to supplementation. Protein supplementation further aided that positive nitrogen balance, regardless of protein source. Such increase is of interest in several areas of health and nutrition. Results are summarized in table 4. Additionally, table 4 indicates nitrogen intake (NI) and differs among almond and whey groups due to differing protein intake at baseline between the two groups, as assessed via dietary records.

When combined with resistance exercise, positive NB aids in muscle growth and MPS. This is due to the increased amino acid pool associated with increased nitrogen
intake. Consumption of EAAs immediately following exercise has been shown to significantly increase MPS 24h after resistance exercise, when compared to rest (Burd et al., 2009). Protein consumption immediately following exercise has been linked to increased MPS on several occasions (Phillips, 2013). This is due to the increased amino acid concentration in the blood, combined with the stimulation of muscle tissue following resistance exercise. However, popular plant-based protein supplements, such as soy and pea protein, have displayed decreased effect on MPS, in comparison to whey, however results appear inconsistent (Candow et al., 2006; Tang et al., 2009). Supplementation with whey and almond protein following resistance training would be of interest to assess protein source impact on MPS, in addition to NB, to further investigate the effect of plant-based proteins and muscle growth, also taking into consideration different protein profile and quality due to different plant source.

Functionality of almond protein, in comparison to whey, appears to be similar in terms of NB and USG, despite the differing amino acid content/profile (table 3). This approach of supplementation with almond-based protein may be beneficial for several reasons, including desire to increase protein in a vegan diet, desire to increase muscle mass without consuming whey protein (animal products), or desire to consume a higher protein diet. High protein diets have become popular due to their satiety effects which may aid in weight loss. Between 2013 and 2014 alone, there was a 49% increase in snacks making high protein claims (Van Allen, 2014), further illustrating this trend. Protein intake is believed to stimulate metabolic hormones that communicate energy status to the brain, in a process termed signaling fullness. Greater weight loss on high protein diets is attributed to this phenomenon, combined with a decreased carbohydrate
intake which subsequently depresses insulin. Increased protein intake may also be beneficial for bone health, as high-protein diets could positively affect bone homeostasis through calcium absorption. Increased calcium absorption may attenuate bone resorption and preserve bone health (Cuenca-Sánchez et al., 2015).

Table 4: Amino acid content of whey and almond protein powder (Banaszek et al., 2019).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Almond Protein (g/100g)</th>
<th>Whey Protein (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>0.539</td>
<td>1.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.751</td>
<td>4.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.473</td>
<td>8.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.568</td>
<td>7.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.157</td>
<td>1.6</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.132</td>
<td>2.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.601</td>
<td>4.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.211</td>
<td>1.3</td>
</tr>
<tr>
<td>Valine</td>
<td>0.855</td>
<td>4.4</td>
</tr>
</tbody>
</table>

While high protein diets may preserve bone mass, they can also be taxing on the kidneys, especially in individuals with impaired kidney function. Glomerular filtration rate (GFR) increases when protein consumption is increased, with long-term elevation in GFR leading to kidney damage and ultimately potential failure. Several studies have shown that increased GFR, when combined with a high protein diet, is detrimental to those with chronic kidney disease (CKD). However, effects of these high-protein diets are not well-established in healthy individuals. Long-term high protein diets may result in CKD onset as well, but this pathway is not clear in humans. Nevertheless, plant-based proteins may be beneficial in CKD maintenance. CKD patients have been reported to
consume 0.7-0.9 g/kg/day of plant-based protein with no negative health effects. Plant-based diets have also shown reductions in hypertension, hyperphosphatemia, and metabolic acidosis, which may favor CKD patients (Joshi, Shah, & Kalantar-Zadeh, 2019). Plant-based diets have also been linked to decreased mortality among CKD patients (He et al., 2021). Therefore, increased plant-based protein intake may be optimal to reduce disease risk and increase MPS. This may be of interest in our study’s observed population, because all UUN levels were below standard acceptable levels at baseline. While none of our 20 participants had CKD, low UUN levels, if chronic, may suggest increased underlying disease risk.

Table 5: Summary of nitrogen balance changes pre- and post- supplementation, including protein source. Pre-supplementation includes all participants, and is not separated by protein source. Statistical significance was determined using matched pairs t-test and are on a per row basis (see materials and methods for method of NB calculation/ formula) (+ indicates positive nitrogen balance) NI: nitrogen intake, UUN: Urinary urea nitrogen (nitrogen out).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre-Supplementation (Baseline)</th>
<th>Post-Supplementation (Overall)</th>
<th>Post-Supplementation (Almond)</th>
<th>Post-Supplementation (Whey)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>60.84 ± 7.67a</td>
<td>61.63 ± 8.16b</td>
<td>62.14 ± 9.65b</td>
<td>61.11 ± 6.85b</td>
</tr>
<tr>
<td>NI (g)</td>
<td>10.48 ± 3.80a</td>
<td>15.28 ± 3.80a</td>
<td>14.42 ± 3.99a</td>
<td>16.14 ± 3.60a</td>
</tr>
<tr>
<td>UUN (g)</td>
<td>8.90 ± 2.20a</td>
<td>7.62 ± 3.05b</td>
<td>8 ± 3.63b</td>
<td>7.35 ± 2.51b</td>
</tr>
<tr>
<td>N balance (g)</td>
<td>+ 8.58 ± 4.59a</td>
<td>+ 11.60 ± 5.09b</td>
<td>+ 10.54 ± 6.10b</td>
<td>+ 12.79 ± 3.83b</td>
</tr>
</tbody>
</table>

4.6 Limitations

A limitation to this study is the small sample size of 20 participants. Rationale for this sample size included specific eligibility criteria and timing in the academic year and school quarter. Of the 192 total survey respondents, 31.4% regularly consumed protein supplements (3x or more per week), making these individuals ineligible to participate. However, this exclusion was vital to ensure minimal interaction between the provided
protein supplement and regular protein dietary intake. Research was conducted near the end of the quarter, making it difficult for students to participate as well. A larger sample size may have resulted in less variability regarding protein source (± 2,401.42 for almond, ± 2,366.13 for whey), as well as elevated UUN levels at baseline.

A second limitation is the method used to assess dietary intake. Use of three-day food logs may result in under- or over-reporting or misreporting (Yang et al., 2010; Banna et al., 2015). It is likely that the observed population was overreporting food intake, particularly protein intake. Protein intake has been observed to be the most commonly over-reported food group (Macdiarmid & Blundell, 1998). This is illustrated in the observed population by the reported 15% protein intake, but low UUN levels measured.

Participants were recruited from a specific College on campus, with many of those recruited members of the Nutrition program. This may not be indicative of the entire Cal Poly campus, particularly in terms of dietary habits. Female College students, overall, tend to have decreased energy, nutrient, fruit, and vegetable intake, and increased high-fat, high-calorie food intake (Deshpande et al., 2009; Racette et al., 2005). Female college students also tend to be more body image conscious, and frequently skip meals (Fujiwara et al., 2020). These typical eating habits were not reflected with our study’s population.

4.7 Future Research

Due to the novelty of almond protein powder, there are considerable research opportunities to further explore the functionality of this protein powder. One interesting insight would be a longer supplementation period to observe changes on muscle mass and
body fat. Four to six weeks are regarded as the standard for muscle mass change. Adherence to the protein shake consumption protocol for this period of time would help to determine if, over time, almond protein affects muscle mass, in addition to UUN and NB levels.

Supplementation in a different age group would be of interest as well. For example, individuals in their 30s or 40s likely have a different body composition than those in their 20s, and it is known that bone mass starts changing at that point. Bone density loss starts occurring at about 30 years old, and bone thickness plays a significant role in the onset and progression of osteoporosis (Romito, 2020). Bone mineral density appears to be positively associated with dietary protein intake as well (Wallace, 2019). Investigating effects of almond protein powder on bone density and prevention of bone loss in individuals over 30 years of age, would be thus an interesting investigation.

Similarly, comparing almond protein effects between those who have and have not reached peak bone mass would be of interest as well.

Finally, high protein diets are known to be detrimental on individuals with CKD, and may result in dehydration (Joshi et al., 2019; He et al., 2021). Investigating the effects of an overabundance of plant-based protein in the diet would be of interest, particularly almond protein powder, to see if health detriments are still present.
5. CONCLUSIONS

The overall objective of this research was to assess functionality of almond protein on NB, in comparison to whey protein. It was hypothesized that the two proteins would increase NB significantly and similarly, due to the increased protein intake. No significant difference was observed between protein source on NB, and NB was increased overall, from 8.58g to 11.66g. This indicates that almond protein powder may be a good vegan substitute to whey protein powder. This is particularly important considering almond popularity in the United States, especially in the non-dairy milk alternative market.

The United States almond industry has grown over 30% in the past decade, with most of the country’s almonds grown in California. This demand far exceeds that of any other tree nut and is due to a variety of factors including image changes regarding the nut and desire for plant-based protein sources. Almonds and tree nuts were once regarded as a high fat snack and were thus avoided due to caloric concern. Almonds, however, are a good source of healthy fats. This realization helped to shift the image and acceptability of the nut, beginning its increase in popularity (Huckaby, 2021). Almonds have also been linked to improved heart health, weight management, and longer lifespans. This coincides with a shift from traditional protein sources, especially in the non-dairy milk industry. It is estimated that almond milk occupies 63% of the total plant-based milk alternative market, further illustrating its popularity (Huckaby, 2021). Investigating the benefits of almonds, such as in almond protein powder, may aid in dietary shifts towards plant-based diets and result in decreased OFLs.
It is important to note that almonds notoriously use a considerable amount of water, one almond requires 1.1 gallons of water to grow, and the land space to do so. This number already represents a 33% reduction in water usage from 1990 to 2010, particularly via implementation of micro-irrigation. The California Almond Board has established goals to reduce this number by another 20% by 2025, representing a significant decrease in water usage. However, red meat production still uses more water, 106 gallons of water is used for producing one ounce of beef, as opposed to 23 gallons for the production of one ounce of almonds (Holthaus, 2015).

Several other plant-based options exist that may be more sustainable than almonds. However, the growing popularity of almonds and almond milk place this particular nut in a position to possibly lead the shift in dietary habits and increase sustainable diets in the United States. The observed functionality of almond protein powder reduces the myth that plant-based proteins do not provide adequate protein, while it highlights a beneficial product that may encourage more consumers to follow a plant-based diet.
REFERENCES


Cohort Study. *American Journal of Kidney Diseases*, 0(0). https://doi.org/10.1053/j.ajkd.2021.03.023


Huckaby, Jeff. (2021, April 12). Personal interview.


population-based study. *Epidemiology and Health, 39*, e2017020. https://doi.org/10.4178/EPIH.E2017020


Tonstad, S., Butler, T., Yan, R. U., & Fraser, G. E. (2009). *Type of Vegetarian Diet, Body Weight, and Prevalence of Type 2 Diabetes*. https://doi.org/10.2337/dc08-1886

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Appendix A: Eligibility Survey

Eligibility Questionnaire

Thank you for your interest in this study! This questionnaire will take no more than 5 minutes to complete.

* Required

Which of the following best describes your gender? *

- Female
- Male
- Prefer not to say
- Other: __________

Next

Are you between the ages of 20-25? *

- Yes
- No

Back Next
Eligibility Questionnaire

Are you allergic to dairy or tree nuts? *

- Yes
- No

Eligibility Questionnaire

Are you comfortable consuming both an almond (plant-based) and whey (animal-based) protein source? *

- Yes
- No
Eligibility Questionnaire

* Required

Eligibility Questionnaire

Do you have a chronic disease? *

- Yes
- No

Eligibility Questionnaire

Do you regularly consume protein supplements? (3x per week or more) *

- Yes
- No
Eligibility Questionnaire

* Required

Eligibility Questionnaire

Do you use medications for stage 1 hypertension, glycemic control, dyslipidemia, or antibiotics? *

- Yes
- No

Eligibility Questionnaire

* Required

Eligibility Questionnaire

Participation in this study requires urinary analysis, which means you will be asked to collect your urine over the course of 24 hours. Specific instructions and equipment to do so will be provided. Are you comfortable doing this? *

- Yes
- No
Appendix B: Shake Survey

Protein Research Questionnaire

Please answer these questions honestly

Email *
Valid email

This form is collecting emails. Change settings

Have you consumed your protein shake this morning? *

☐ Yes
☐ No

Protein Research Questionnaire

Reporting

Are you currently experiencing any of the above?

☐ Nausea
☐ Elevated heart rate
☐ Hives
☐ Itching in throat
☐ Dizziness
☐ Rash

☐ Other (anything that may be related to shake consumption. Please do not check this box if you are feeling sick or unwell outside of shake consumption)

☐ None of the above
Protein Research Questionnaire

If you are experiencing any adverse reactions, do not finish this survey. Call at (805) 756-6126 immediately.

If not, please continue

Morning Screening

Is there anything else you would like to report?

Your answer
Appendix C: 3-Day Food and Exercise Record

Food Record Instructions

In order for your diet analysis to be accurate, you must do the best job possible of accurately writing down what you eat.

- **We also encourage you to take pictures of meals and labels** because this can help our accuracy. Just email us the photos with info regarding when it was eaten.
- **Questions – cpsportsnutrition@gmail.com or Dr. Reaves by text 805-215-9273**

Instructions for the Food Record:
1. You will be recording everything you eat for three days.

Tips for recording accurately:
1. Record each individual food item plus any supplements you take. For example, if you ate oatmeal, fruit and coffee for breakfast, the meal would be represented as shown below:

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Portion Size (amount)</th>
<th>Food Description including location/brand</th>
<th>Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00 am Breakfast (B)</td>
<td>½ cup dry</td>
<td>Quaker Oats, old fashioned, dry</td>
<td>As instructed</td>
</tr>
<tr>
<td>B</td>
<td>2 teaspoons</td>
<td>Smart Balance light buttery spread with flax</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1 large</td>
<td>egg</td>
<td>scrambled</td>
</tr>
<tr>
<td>B</td>
<td>1 cup</td>
<td>Lactaid milk, 1%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1 tablespoon</td>
<td>Brown sugar</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>½ cup</td>
<td>Blueberries, fresh</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1 cup</td>
<td>Brewed drip coffee</td>
<td>As instructed</td>
</tr>
<tr>
<td>B</td>
<td>1 tablespoon</td>
<td>Land O’Lakes Nonfat half and half</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1 tablet</td>
<td>Men’s One-A-Day vitamin and mineral supplement</td>
<td></td>
</tr>
</tbody>
</table>

Please notice all details about the oatmeal including added foods (buttery spread, milk, brown sugar), brand names if applicable (Quaker, Smart Balance, Land-o-Lakes), descriptions of the food products (“light,” “1%”) and specific amounts (“2 teaspoons,” “1/2 cup”) are provided. Please be as specific as possible (“fresh,” “frozen,” “blanched,” “fried,” “steamed”) so proper assessment of the quality of your diet can be evaluated.

2. If you don’t know the exact portion, revert to the Food Portions Guide as a tool to estimate your portion size. Some examples are provided in the table below:

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Portion Size (amount)</th>
<th>Food Description including location/brand</th>
<th>Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 pm Dinner (D)</td>
<td><strong>1 deck of cards = 3 oz</strong></td>
<td>Safeway Select Chicken breast, boneless skinless</td>
<td>Sautéed w/ canola oil and</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Portion Size (amount)</td>
<td>Food Description including location/brand</td>
<td>Preparation Method</td>
</tr>
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<td>-------------</td>
<td>-----------------------</td>
<td>-------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>D</td>
<td>1 ½ quarters = ½ tablespoon</td>
<td>Canola oil</td>
<td>salt then finished in the oven</td>
</tr>
<tr>
<td>D</td>
<td>¼ teaspoon</td>
<td>Salt</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1 fist full = 1 cup cooked</td>
<td>Brown rice, long-grain</td>
<td>As instructed</td>
</tr>
</tbody>
</table>

Remember to record the amount of salt you add when you cook. It is not necessary to add other spices and seasonings; we are more concerned with added salt, which contains the mineral sodium. Notice that preparation method details have also been included when appropriate.

/  

Name: _____________________     Weight:________   Height:________       Age:________

Date: ______________   Day: __________________

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<th>Time of Day</th>
<th>Portion Size (amount)</th>
<th>Food Description including location/brand</th>
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<th>Time of Day</th>
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<td>Date and Time of Day</td>
<td>Duration</td>
<td>Exercise Description</td>
<td>Additional Details</td>
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Appendix D: Portion Size Guide

Folding Instructions
- Fold in Half
- Fold in Half Again
- Fold Once
- Last Time
- Insert into Pocket

Visualization Key
- Computer Mouse
- Deck of Cards
- Baseball
- Tennis Ball
- Golf Ball
- Nire-Volt Square Battery
- Small Postage Stamp

Fruits
- 1 oz dried fruit
- 1 cup strawberries
- 1 cup blueberries

Veggies
- 1 cup broccoli
- 1 cup green beans
- 1 medium baked potato

Dairy
- 1 & ½ oz cheese
- 1 cup milk
- ½ cup frozen yogurt
- 3 oz cooked chicken
- 2 tbsp peanut butter
- ½ cup cooked beans

Fats and Oils
- 1 tsp butter
- 1 tsp vegetable or olive oil
- 1 tbsp salad dressing

Grains
- 1 cup cold cereal
- ½ cup cooked rice
- 1 whole grain muffin

Meat, Nuts and Beans
- 1 medium cooked chicken