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Article

Primary source of dietary protein is correlated with differences in the intestinal microbiome diversity

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SUMMARY

In spite of the recent popularization of the vegan diet, there is currently a deficit of studies that compare the impact and associated health benefits of dietary protein source, one of the main differentiators between vegan and non-vegan diets, on the intestinal microbiome. The objective of this study was therefore to correlate observed changes in the intestinal microbiome to changes in dietary protein sources. We hypothesized that changes in dietary protein source are associated with changes in the composition of the intestinal microbiome. The participant changed their diet over four separate one-week periods to include plant proteins only, plant proteins and dairy proteins, animal proteins only, and finally animal proteins and dairy proteins. The participant followed their respective dietary treatments for one week. Bacterial 16S dual-index sequencing and bioinformatic analysis identified seven genera and two families of bacteria. Of these, *Bifidobacterium*, *Bacteroides*, *Lactobacillum*, and *Clostridium* exhibited population trends correlated with changes in diet. Moreover, a comparison of α-diversity indicated a statistically significant change over the course of the experiment. Although the results of this experiment cannot be generalized due to the sample size, the hypothesis of this experiment was validated: changes in dietary protein source are correlated with changes in the diversity of the intestinal microbiome. However, we recommend further research into the impacts of variance in dietary protein source on the microbiome, especially by implementing longer-term dietary treatments.

INTRODUCTION

The recent popularization of the vegan diet as being a healthier, more environmentally sustainable alternative to modern non-vegan diets has mandated critical evaluations of both, including their impacts on the physiological health of an individual (1, 2). Given the extent to which the intestinal microbiome is implicated in maintaining homeostasis, changes to it as a result of the aforementioned diets have been a frequent subject of research (3). Interestingly, key differences between both diets have been observed to have significant modulatory impacts on the microbiome. The most apparent difference between the two is animal-product consumption: the vegan diet is defined by an abstinence from animal-based products like meat and dairy, while non-vegan diets often include some form of animal products (4, 5). Furthermore, the vegan diet generally has higher fiber content than most popular non-vegan diets (6).

While fiber has been extensively researched in relation to gut flora, macronutrients like proteins have been relatively overlooked (7). Dietary protein source varies significantly between the vegan and non-vegan diets: plant proteins and animal proteins respectively (5). However, comparatively little is known about changes in the microbiome associated with such changes in dietary protein source. Furthermore, there is currently a deficit in comparative studies that consider the merits of vegan and non-vegan diets in relation to one another. Most research done into the impacts of both diets only consider the diets in isolation (8). While numerous studies claim that vegan diets promote a stable microbial gut composition, more studies comparing vegan and non-vegan diets are needed to validate these assumptions.

To that end, our study compared the impacts of a "lean-protein" based diet—which includes chicken and fish—with the impacts of a vegan diet, on the intestinal microbiome. Hence, the hypothesis of this study is that changes in dietary protein source are associated with changes in the diversity of the intestinal microbiome. In order to test this hypothesis, the participant followed four dietary treatments that limited their intake of protein to either plant protein or animal protein and took stool samples at three points during each treatment to evaluate the changes in their microbiome. While this study validated this hypothesis, we specifically observed increases in the diversity of the microbiome during the animal protein dietary treatment phases, which contrasts with the findings of previous studies on the impacts of the vegan diet on the microbiome. Hence, our findings underscore the need for further research into the differential impacts of differing dietary protein sources on gut flora, as well as increased participant diversity in microbiome studies.

RESULTS

The sole participant followed four different dietary treatments for one-week periods: plant protein only, plant and dairy protein, animal protein only, and animal and dairy protein. The components of the participant's normal diet outside of this experimental setting, and the dietary treatments followed for this experiment, have been tabulated (**Table 1**). The participant collected three fecal samples during each dietary treatment period, leaving two days between each sample collection date, and we sequenced the samples using 16S dual-index sequencing to identify the genera and abundance of bacteria present in each sample.

We identified seven genera of bacteria: *Bifidobacterium*, *Coriobacterium*, *Bacteroides*, *Lactobacillum*, *Clostridium*, *Selenomonas*, and *Mollicutes* (**Figure 1**). We also

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	Carbohydrates	Proteins	Fiber	Dairy	Vitamins/Minerals
Normal Diet	Bread, Rice, Pasta, Cereal	Chicken Fish Legumes Nuts	Zucchini Broccoli Green Beans Black Beans Cabbage Celery Spinach Oats	Cow's Milk Yogurt Cheese Ice Cream	Zucchini Broccoli Capsicum Lettuce Tomato Green Beans Black Beans Cabbage Carrots Celery Spinach Peas Cucumber Onion Eggplant Kidney Beans Black-eyed Peas Gourds Cauliflower Corn Potato
Plant Proteins + No Dairy		Legumes Nuts		Nut Milk Yogurt (Nut-milk-based)	
Plant Proteins + Dairy				Cow's Milk Yogurt Cheese Ice Cream	
Animal Proteins + No Dairy		Chicken Fish	Zucchini Broccoli Cabbage Celery Spinach	Nut Milk Yogurt (Nut-milk-based)	Zucchini Broccoli Capsicum Lettuce Tomato Cabbage Carrots Celery Spinach Cucumber Onion Eggplant Gourds Cauliflower Corn Potato
Animal Proteins + Dairy			Zucchini Broccoli Cabbage Celery Spinach	Cow's Milk Yogurt Cheese Ice Cream	

Table 1. Components of the participant's normal diet and each of the dietary treatments. Components of the participant's normal diet and each of the dietary treatments followed during the experiment, stratified by the corresponding nutrient.

identified bacteria within the families *Erysipelotrichales* and *Betaproteobacteria*, but in insufficient abundances to identify specific genera (**Figure 1**). Subsequently, we calculated Shannon Diversity Indices, an α-diversity metric that quantifies the diversity of species within a singular sample, for each of the obtained samples (**Figure 2**). There was a net increase in the derived Indices values, from 1.45 to 1.60, indicating that the recorded diversity of the participant's microbiome had increased over the course of the experiment. We observed a statistically significant difference in α-diversity between all four dietary treatment groups (Friedman test, p = 0.0421). Although we also compared the composition of samples taken at corresponding times during each dietary

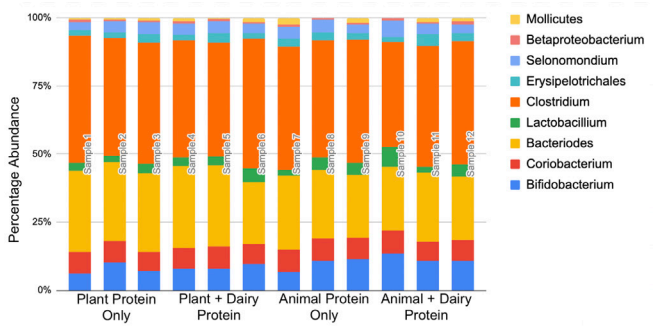

Figure 1. Percent abundances of identified bacteria genera does not significantly change with protein source. Percent abundances of the identified genera of bacteria present in each of the samples. The samples are stratified by dietary treatment. The protein source of protein was changed for the single participant over four separate weeks, and 16S sequencing was per-formed on 3 samples taken throughout each diet change (for a total of 12 samples).

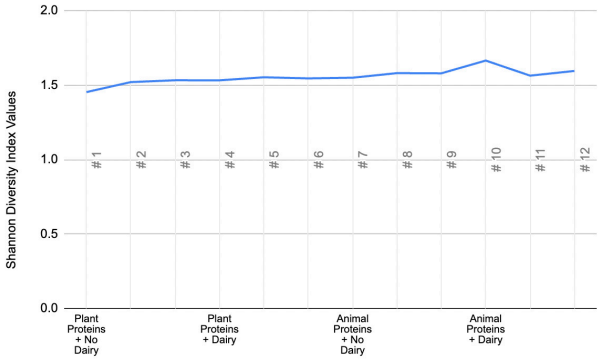

Figure 2. α-diversity increases significantly with animal protein consumption. Trend in the calculated α-diversity values for each of the samples taken over the course of the experiment. The samples are serially numbered on the graph from #1 to #14. Source of protein was changed for the single participant over four separate weeks, and 16S sequencing was per-formed on 3 samples taken throughout each diet change (for a total of 12 samples).

treatment using Manhattan Distances (a ß-diversity metric) and tabulated the resulting values, there were no observed significant differences between the corresponding samples (Mann-Whitney-Wilcoxon Test, p = 0.18684) (**Table 2**). Similarly, we could not identify any statistically significant changes in the percent abundances of specific genera, including *Lactobacillum* and *Bacteroides* (**Figures 3, 4**).

DISCUSSION

This experiment supported our hypothesis; changes in dietary protein source are correlated with changes in the diversity of the intestinal microbiome. The net increase in microbial diversity over the course of the experiment was of particular note, as existing literature indicates that plant-based diets are associated with higher gut flora diversity than the Western diet. Moreover, the magnitude of increase in diversity between each sample was relatively higher during the animal protein dietary treatment phase than during the plant protein dietary treatment phase.

The marked increase in microbial diversity observed as the dietary treatments transitioned from solely plant protein (i.e., the vegan diet) to animal and dairy protein (i.e., the Western diet) contrasted the findings of existing literature (**Figure 2**). Specifically, it opposed the conclusion that decreased diversity in gut flora is a significant consequence of the Western diet (9). In addition, the lack of discernible trends in the ß-diversity of the obtained samples reflected a key potential limitation of this study: the long-term stability and resilience of the participant's intestinal microbiome is unknown. Higher stability and resilience are associated with higher microbial diversity, but establishing a baseline level of microbial diversity against which the diversity of the participant's intestinal microbiome could be compared was evidently unfeasible. The aforementioned lack of significant trends in ß-diversity could be attributed to the resilience of the participant's microbiome to changes in composition in response to short-term dietary changes. The composition of the participant's microbiome potentially did not change significantly over each dietary treatment, but the diversity did

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	Samples Compared	Manhattan Distance
Plant Protein Only vs. Animal Protein Only	# 1 - # 7	0.0988
	# 2 - # 8	0.0937
	# 3 - # 9	0.1694
Plant + Dairy Protein vs. Animal + Dairy Protein	# 4 - # 10	0.2447
	# 5 - # 11	0.1529
	# 6 - # 12	0.0648

Table 2. Manhattan distances for corresponding samples across dietary treatments. Calculated Manhattan Distances between the corresponding samples taken during each dietary treatment. The samples were serially numbered from #1 to #14. Manhattan Distance is a ß-diversity metric used to compare the compositional similarity of two samples.

change significantly over the entire sample collection period (**Figure 2**).

While short-term dietary changes have been demonstrated to reproducibly induce significant changes in gut microbial composition, the studies that demonstrated those changes implemented dietary shifts of relatively greater magnitude than the dietary shifts implemented here (10). The primary reason for the difference in magnitude is that this study intended to isolate one specific component of diet, dietary protein source, leaving the others consistent and thereby limiting the magnitude of the dietary shift. Future study of the correlation between dietary protein source and gut microbial composition, therefore, should consider lengthening the time that the subjects follow each dietary treatment to a great extent. If feasible, moreover, measures to ensure that the other components of the subjects' diets are kept constant across each treatment should be taken. For instance, logging the grams of fiber consumed each day would allow for fiber content to be controlled across the different diets.

Another consideration for future study would be identifying with greater certainty the specific taxonomic groups that displayed changes in abundance correlated with changes in dietary protein source. In this study, for instance, there was an observed net decline in the abundance of *Bacteroides* during the animal protein diet phases of the experiment (**Figure 3**). However, previous experiments indicate that the abundance of *Bacteroides* typically increases in subjects following a primarily animal-based diet. This contrast could be due to differing abundances of protein; in animal-based diets, the relative amount of protein consumed daily would likely exceed that of the amount consumed daily during the animal-protein dietary phases of the experiment. As *Bacteroides* are known to be involved in both amino-acid fermentation and carbohydrate degradation, the observed decline during the dietary treatments that were likely lower in carbohydrates and protein overall is logical (11).

Additional observations of trends that apparently contrast existing literature include the divergence in abundances of bacteria of the phyla Firmicutes and Bacteroidetes from the expected trend in their abundances. Existing literature holds that increased consumption of plant-based nutrients is associated both with an increased abundance of *Firmicutes*, and increased production of Short-Chain Fatty Acids like butyrate by bacteria in this phylum (12). This could explain the slight increase in *Lactobacillum*, for instance, observed over the course of the plant-protein diets, due to the relatively higher rate of plant-based nutrients during those diets relative to the animal-protein diets (**Figure 4**). However, the reasons behind the rapid fluctuation in *Lactobacillum* abundance observed during the animal-protein diets are less evident. Further complications arise with the marked decrease in *Bacteroidetes* bacteria (*Bacteroides* specifically) over the course of the animal-protein diets, which sharply contrasts with a previous observation that *Bacteroides* abundance is positively associated with diets rich in animal protein (**Figure 3**) (2). However, Tomova et al. noted that this observation was made in individuals who followed a long-term diet rich in animal proteins and saturated fats (2). Given the short-term nature of the animal-protein dietary treatments, it is possible that the observed decrease in *Bacteroides* during those treatments was anomalous and would have been reversed if the duration of those treatments had been extended.

This particular trend also reflects an aforementioned limitation of this experimental design: the inability to conduct comparative analysis. The participant's normal diet diverges from the average diet of an individual living in a Western country, the U.S. specifically, as meat was not a major component of the participant's normal diet, or of the participant's immediate family's normal diet (13). Though intended to replicate the Western diet, it is likely that the animal protein treatments (both with and without dairy) likely contained less protein overall, and more closely resembled a generalized omnivorous diet. Moreover, as the implemented sources of plant protein, such as beans and legumes, were rich in both fiber and protein, it is possible that the plant protein dietary treatments had greater fiber content than the animal protein dietary treatments. Similarly, it is highly likely that the plants consumed during the animal protein dietary treatments had plant protein as well, albeit smaller amounts than typical

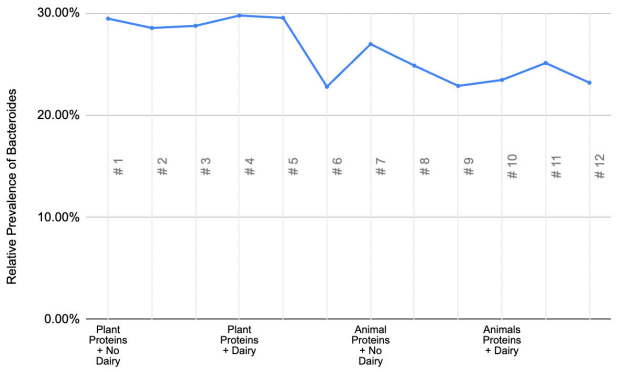

Figure 3. Abundance of bacteria of the genus *Bacteroides* declined overall but did not significantly change with protein source. Abundance of bacteria of the genus *Bacteroides* in each of the samples taken over the course of the experiment. The samples are serially num-bered on the graph from #1 to #14. Source of protein was changed for the single participant over four separate weeks, and 16S sequencing was performed on 3 samples taken throughout each diet change (for a total of 12 samples).

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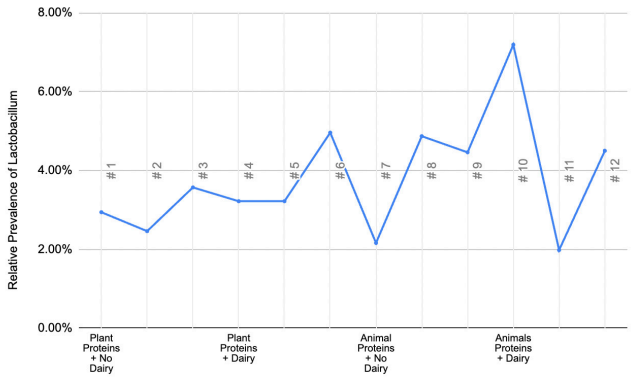

Figure 4. Abundance of bacteria of the genus *Lactobacillum* increased overall but did not significantly change with protein source. Abundance of bacteria of the genus *Lactobacillum* in each of the samples taken over the course of the experiment. The samples are serially numbered on the graph from #1 to #14. Source of protein was changed for the single participant over four separate weeks, and 16S sequencing was performed on 3 samples taken throughout each diet change (for a total of 12 samples).

plant protein sources. Evidently, completely isolating dietary protein source was not feasible. However, the difference in fiber content was likely minimized by the relative abundance of vegetables and plant products over animal products in the participant's diet, even during the animal-protein phase of the experiment (**Figure 1**). Perhaps the most limiting factor of this study was that there was only one participant due to resource limitations, which made comparative analysis unfeasible and precluded generalizing the findings of this study.

The intestinal microbiome remains a significant frontier in bio-centric research due to the extent of the microbiome's involvement in various aspects of the human body, including the health and functionality of the immune and cardiovascular systems, as well as the integrity of the intestinal barrier (14 - 16). As diet significantly modulates the composition of this microbiome, further research into how dietary interventions, like eliminating plant or animal protein sources for two-week-long periods, could help optimize therapeutic interventions that modify the patient's diet to positively modify their microbiome.

While protein source is a crucial distinguishing factor between the vegan and Western diets, there is less information on the specific therapeutic potential of protein source and intake. The primary difference in animal and plant proteins is in their composite amino acids: plant proteins have been widely observed to contain comparatively lower quantities of methionine, tryptophan and lysine (17). Proteolytic bacteria use these amino acids—albeit as peptides—to produce bioactive molecules implicated in numerous facets of gut health (18). For instance, metabolites produced from tryptophan uptake are associated with improved gut barrier function, while other amino acid-produced metabolites have been noted to increase tissue permeability and colitis severity (18). This reinforces the need for further research on the subject; a better understanding of how various dietary components correlate to changes in the composition of the microbiome would allow for therapeutic advancements. This improved understanding could further resolve the findings of this study that contrasted existing literature, such as the increase in microbial diversity during the animal protein dietary treatments, as opposed to the plant protein dietary treatment. In addition, this contrast underscores the need for gut flora studies to be conducted with participants from a wider range of ethnicities, due to the significant differences in dietary patterns observed between ethnic groups, which could lead to significant differences in gut flora (19). As the participant in this study was of South Asian descent, their habitual dietary patterns prior to the experiment, which significantly influenced their baseline microbiome, likely diverged widely from members of other ethnic communities. As such, future gut microbial studies should prioritize sample diversity, to better guarantee the generalizability of their obtained results.

MATERIALS AND METHODS

Participant

The sole participant was a 16 year old male of South Asian descent, who gave informed consent to participate. Informed consent was also provided by the parents of the participant.

12 OMNIgene GUT liquid buffer kits and OM-AC1 toilet accessories, provided by DNA Genotek, were used to store the collected fecal samples.

Methodology

The participant's diet was changed to include various dietary protein sources: plant proteins, animal proteins and dairy proteins (isolated due to being a potentially confounding factor) (20). During week 1, plant proteins were the only protein source. In week 2, the participant ate both plant and dairy protein sources. For week 3, only animal protein was eaten, and in week 4 both animal and dairy protein sources were eaten. Given that the microbiome has been observed to change significantly over the timeframe of a week, the sample-collection period was divided into 4 one-week intervals (10). Three samples were taken during each interval, using OM-AC1 toilet accessories, and stored in OMNIgene GUT liquid buffer kits (DNA Genotek, cat. # OMR-200) (21).

In order to keep the protein sources constant across the first week of sample collection, all dairy and meat products were removed from the participant's diet. Moreover, one week before the beginning of this experiment, all sources of dairy were removed from the participant's diet to ensure that there would be no residual dairy proteins that could contaminate the first dietary treatment. As substitutes, plant milks (specifically almond milk and oat milk) and plant milk-based products (Silk Almondmilk Yogurt Alternative) were used due to their lack of dairy proteins. Similarly, all meat-based products were substituted for plant-based alternatives (Beyond Meat and Don Lee Farms Chipotle Black Bean Burgers Don Lee Farms). For the latter half of the sample collection period, however, which involved animal proteins and dairy proteins, all sources of plant protein were stricken from the participant's diet. This included the aforementioned plant-based meat alternatives, as well as beans, nuts and other protein-rich legumes. For the animal protein phase of sample collection, these were replaced with chicken and fish-based foodstuffs.

Fecal samples were taken every three days, amounting to three samples per dietary treatment, and stored in liquid buffer kits due to refrigeration constraints. After the sample collection period elapsed, the collected samples were sent to the University of Minnesota's Genomics Center for 16S dual-index sequencing (similar to 16S amplicon sequencing)

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and preliminary bioinformatic analysis (22). Specifically, sequencing was conducted by amplifying marker genes of interest with adapter-tailed primers, and the resulting data was analyzed via the Qiime2 pipeline. Diversity analysis was then conducted, also by the University of Minnesota's Genomics Center, using the Qiime2 'diversity core-metrics-phylogenetic' command. Further analysis, including alpha and beta diversity metric calculation, was performed using Google Sheets. This software was used to graph the relative abundance of the identified genera of bacteria over the course of the experiment, which allowed possible correlations between dietary protein source and the abundance of certain genera of bacteria to be identified.

As we conducted this experiment with four different dietary treatments, during which we took one samples every three days, there were three α-diversity values calculated for each treatment using Google Sheets. Thus, the Friedman test was conducted on VassarStats.com with the number of "samples" set to four, for each dietary treatment, and each "measure" within the "sample" set to three, for each calculated α-diversity value. The Shannon Diversity Index was chosen over other metrics as the Shannon Index is an information statistic that does not differentially weight the abundances of the taxonomic groups of interest in calculation. The non-parametric Friedman test, similarly, was chosen because all of the obtained samples were taken from the same individual, hence independence between the samples could not be assumed.

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REFERENCES

1. Sentient Media. "Increase in Veganism: Why Is Veganism on the Rise in 2021?" *Sentient Media*, 26 Oct. 2021, sentientmedia.org/increase-in-veganism/.
2. Tomova, Aleksandra, et al. "The Effects of Vegetarian and Vegan Diets on Gut Microbiota." *Frontiers in Nutrition*, vol. 6, no. 47, Apr 2019, https://doi.org/10.3389/fnut.2019.00047.
3. El Aidy, Sahar, et al. "Temporal and Spatial Interplay of Microbiota and Intestinal Mucosa Drive Establishment of Immune Homeostasis in Conventionalized Mice." *Mucosal Immunology*, vol. 5, no. 5, 2012, pp. 567–579., https://doi.org/10.1038/mi.2012.32.
4. Ostfeld, Robert J. "Definition of a Plant-Based Diet and Overview of This Special Issue." *Journal of Geriatric Cardiology*, 2017, https://doi.org/10.11909/j.issn.1671-5411.2017.05.008.
5. Shi, Zumin. "Gut Microbiota: An Important Link between Western Diet and Chronic Diseases." *Nutrients*, vol. 11, no. 10, 2019, p. 2287., https://doi.org/10.3390/nu11102287.
6. Craig, Winston J., et al. "The Safe and Effective Use of Plant-Based Diets with Guidelines for Health Professionals." *Nutrients*, vol. 13,11 4144. 19 Nov. 2021, https://doi:10.3390/nu13114144.
7. Calatayud, Marta, et al. "Comparative Effect of 22 Dietary Sources of Fiber on Gut Microbiota of Healthy Humans in Vitro." *Frontiers in Nutrition*, vol. 8, 2021, https://doi:10.3389/fnut.2021.700571.
8. Losno, Emily A., et al. "Vegan Diet and the Gut Microbiota Composition in Healthy Adults." *Nutrients*, vol. 13, no. 7, 2021, p. 2402., https://doi.org/10.3390/nu13072402.
9. Zinöcker, Marit, and Inge Lindseth. "The Western Diet–Microbiome-Host Interaction and Its Role in Metabolic Disease." *Nutrients*, vol. 10, no. 3, 2018, p. 365, https://doi:10.3390/nu10030365.
10. David, Lawrence A., et al. "Diet Rapidly and Reproducibly Alters the Human Gut Microbiome." *Nature*, vol. 505, no. 7484, 2013, pp. 559–563, https://doi:10.1038/nature12820.
11. Flint, H.J., and S.H. Duncan. "*Bacteroides* and *Prevotella*." Encyclopedia of Food Microbiology, 2014, pp. 203–208., https://doi.org/10.1016/b978-0-12-384730-0.00031-8.
12. Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, 308(5728), pp. 1635–1638. https://doi.org/10.1126/science.1110591.
13. Cordain, Loren, et al. "Origins and Evolution of the Western Diet: Health Implications for the 21st Century." *The American Journal of Clinical Nutrition*, vol. 81, no. 2, 2005, pp. 341–354, https://doi:10.1093/ajcn.81.2.341.
14. Zheng, Danping, et al. "Interaction between Microbiota and Immunity in Health and Disease." *Cell Research*, vol. 30, no. 6, 2020, pp. 492–506., https://doi.org/10.1038/s41422-020-0332-7.
15. Kazemian, Negin, et al. "Gut Microbiota and Cardiovascular Disease: Opportunities and Challenges." *Microbiome*, vol. 8, no. 1, 2020, https://doi.org/10.1186/s40168-020-00821-0.
16. Takiishi, Tatiana, et al. "Intestinal Barrier and Gut Microbiota: Shaping Our Immune Responses throughout Life." *Tissue Barriers*, vol. 5, no. 4, 2017, https://doi:10.1080/21688370.2017.1373208.
17. Nogal, Ana, et al. "The Role of Short-Chain Fatty Acids in the Interplay between Gut Microbiota and Diet in Cardio-Metabolic Health." *Gut Microbes*, vol. 13, no. 1, 2021, https://doi:10.1080/19490976.2021.1897212.
18. Eisenstein, Michael. "The Hunt for a Healthy Microbiome." *Nature*, vol. 577, no. 7792, 2020, https://doi.org/10.1038/d41586-020-00193-3.
19. Bennett, Grace, et al. "A Comparison of Dietary Patterns and Factors Influencing Food Choice among Ethnic Groups Living in One Locality: A Systematic Review." *Nutrients*, vol. 14, no. 5, 2022, p. 941, https://doi:10.3390/nu14050941.
20. Berrazaga, Insaf, et al. "The Role of the Anabolic

DOI: https://doi.org/10.59720/23-056

Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review." *Nutrients*, vol. 11, no. 8, 2019, p. 1825., https://doi.org/10.3390/nu11081825.
21. Zhao, Jianfei, et al. "Dietary Protein and Gut Microbiota Composition and Function." *Current Protein & Peptide Science*, vol. 20, no. 2, 2018, pp. 145–154., https://doi.org/10.2174/1389203719666180514145437.
22. Rettedal, Elizabeth A., et al. "The Effects of Unfermented and Fermented Cow and Sheep Milk on the Gut Microbiota." *Frontiers in Microbiology*, vol. 10, 2019, https://doi.org/10.3389/fmicb.2019.00458.
23. Fadrosh, Douglas W, et al. "An Improved Dual-Indexing Approach for Multiplexed 16S Rrna Gene Sequencing on the Illumina MiSeq Platform." *Microbiome*, vol. 2, no. 1, 2014, https://doi.org/10.1186/2049-2618-2-6.

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