



Abstract

Diet variations have been prevalent among societies around the globe, be it by choice or inherited from culture. According to the CDC, in recent years it was found that 17.1% of those 20 years and older in the United States were on a special diet, most commonly for weight loss¹. The purpose of this study is to determine what, if any health and wellbeing markers can be identified from a significant change in diet accompanied by gut microbiome changes. The participant, a healthy individual in her early twenties, originally omnivorous, followed a strict vegan diet for a six-week period. Urine, blood, and fecal samples were collected before and during the study. Metadata included measured vital signs such as blood pressure, heart rate, weight, BMI and subjective reports as well as information regarding bowel movements and urinations. The participant recorded these various measures in a daily log. Fecal samples were sent to an outside sequencer for 16S rRNA sequencing in order to determine bacterial composition in the gut. Using QIIME2 and R programs, the metadata collected along with the resulting sequences were analyzed. Results indicate presence of a positive association between the microbiota present and overall health and wellbeing markers. Although correlations were found, there remain ways to further study the effect of the gut microbiome on overall wellness. Future research may include investigations into whether these fluctuations in the microbiota causative of the recorded impacts on wellbeing markers are.

Rationale

The approach of individualizing medicine is a fairly newly accepted practice. Organizations such as they Mayo Clinic are known and have been for years for their use of this approach which as led to the ill all over the world seeking their assistance. Why would you want a generalized approach to your health when you can have one tailored to your own body? This study utilizes an individualized approach to study the participant's health and well-being changes during and after a significant change in diet. The Vegan diet was chosen due to its increasing popularity and its extreme differences from the diet of the participant before the study.

Methods

Study Design

Dietary intervention methods incorporating both discovery and hypothesis-based scientific exploration.

Participation

The participant of this study is a 22-year-old white, female student from the United States with no pre-existing physical or psychological medical conditions.

Dietary Intervention

The subject will follow the Vegan diet for the six weeks of the study. The Vegan diet is void of all animal products including, meat, eggs, dairy, and honey.

Sample Collection

Fecal samples were collected weekly for analysis of the gut microbiome.

Blood samples were collected biweekly for metabolic, lipid, and vitamin analysis.

Urine samples were collected daily for analysis of color, concentration, and content.

A variety of metadata was also collected multiple times during each day of the study including vitals, weight, energy levels, and other descriptors.

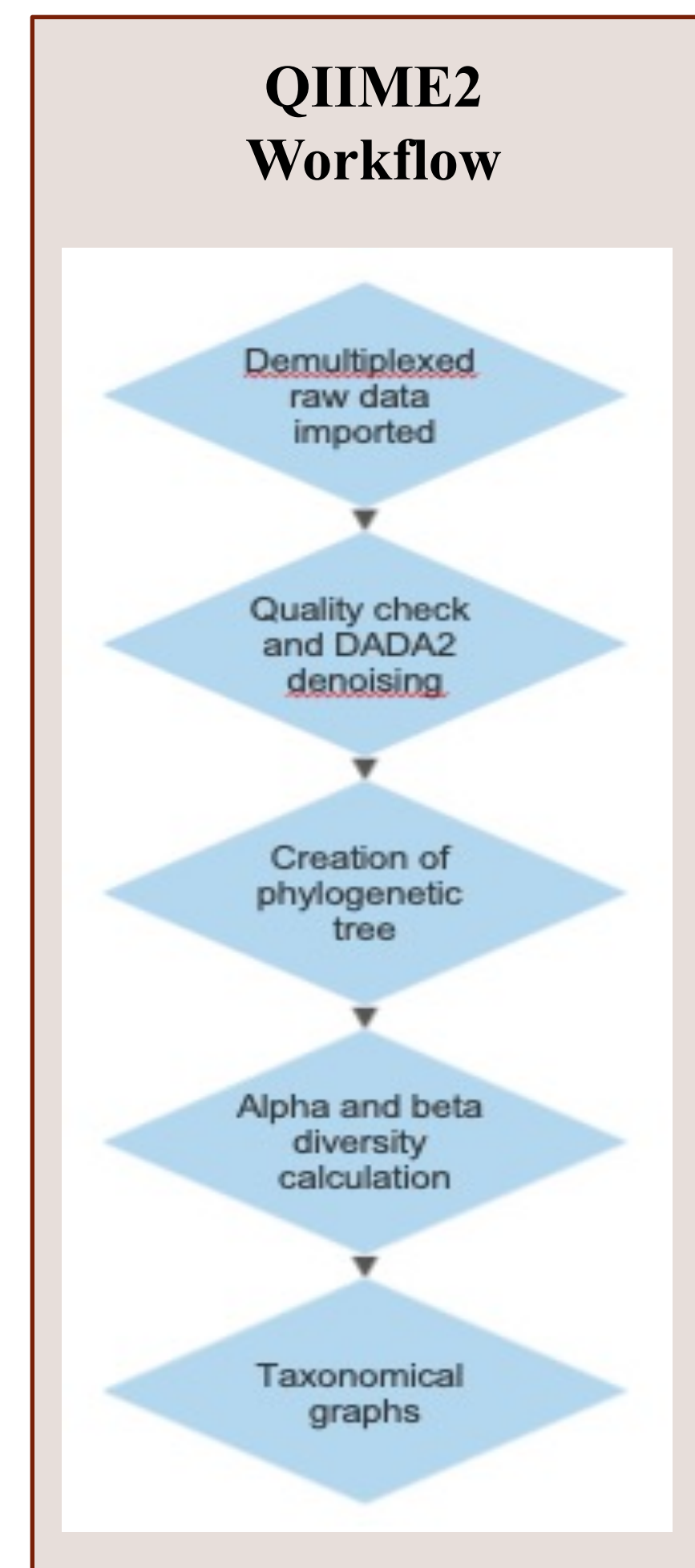


Table 1 shows metadata values with statistically significant changes over the course of the study and the direction in which the change occurred.

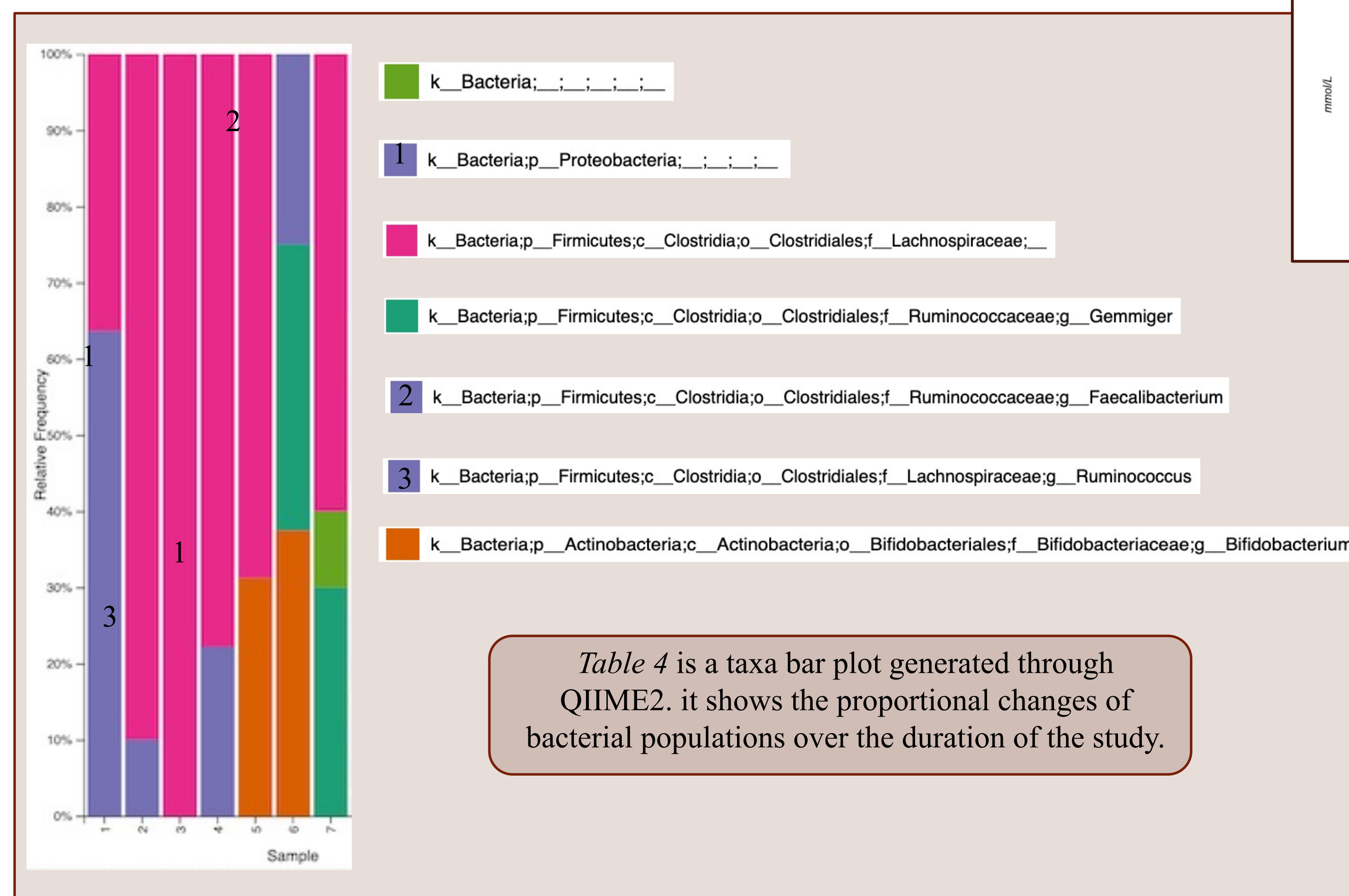
Significant Changes Noted

LDL Cholesterol	Decreased with introduction of the diet
Alertness	Increased over the course of the diet
Systolic Blood Pressure	Decreased over the course of the diet
Weight	Decreased with introduction of the diet before leveling out over time
Vitamin B₁₂	Decreased between the two samples obtained with supplementation

Table 2 provides a simplified outline of the QIIME2 workflow conducted during data analysis stages.

Table 3 provides Phred scores representing the quality of nucleobases.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean Quality Score	31.64	31.35	31.3	31.23	31.48	31.42	31.33



Results

Samples Used for Analysis

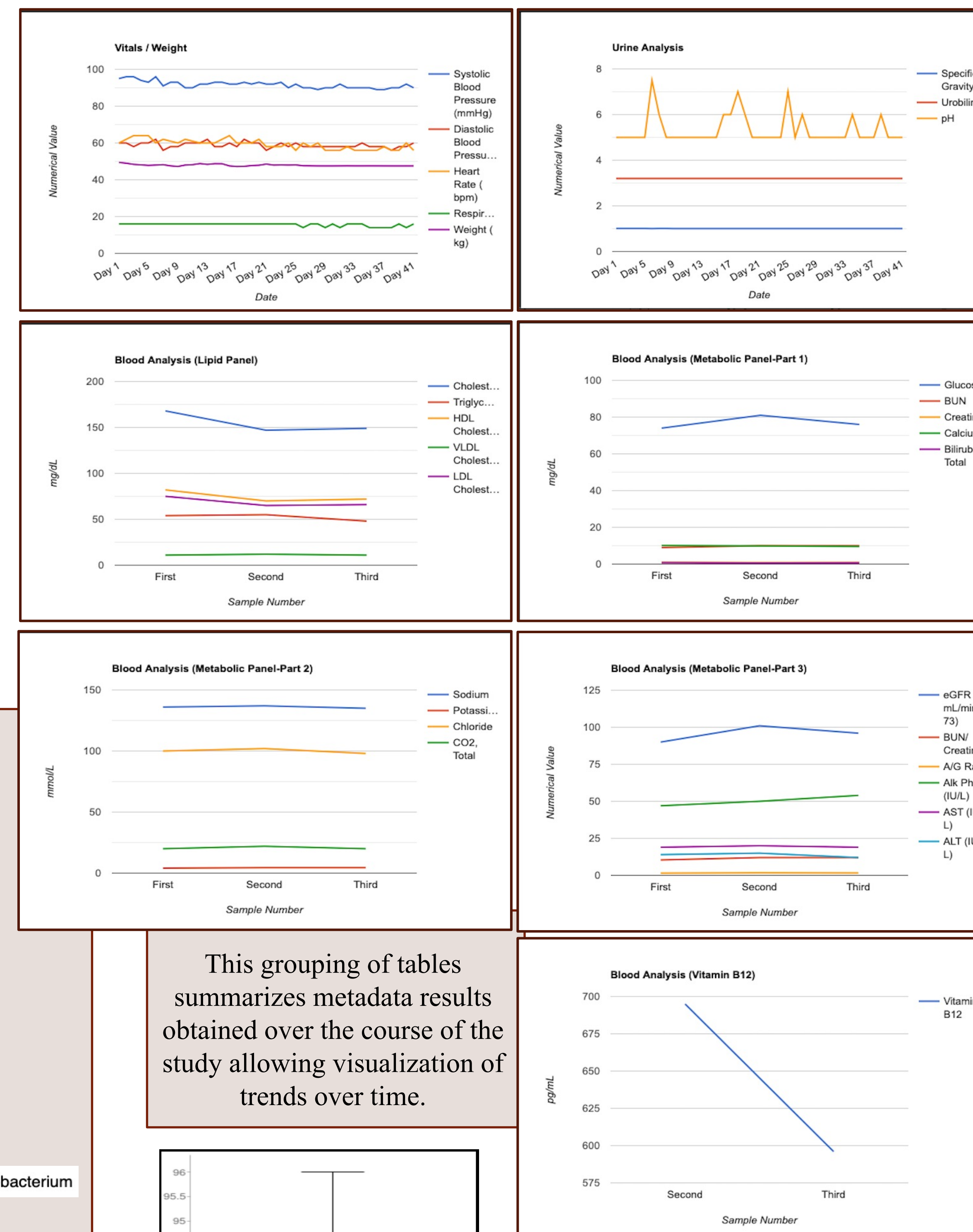
- A variety of different bodily samples were collected from the participant throughout the study. Fecal (n=7), urine (n=43), and blood (n=3) samples were obtained from the participant for use in the determination of the significance of the vegan diet on both the gut microbiome and human health.

Sample Quality

- As per the report, samples ranged from roughly 200,000 to 300,000 reads and 65-94 Mbases. All samples had greater than 71% of bases with a quality score of greater than 30 with all samples having a mean quality score of 31.23 to 31.64.

Filtering

- Due to the limited number of fecal samples collected for analysis, and their high Phred scores, all sequenced samples were kept for further analysis.
- A standard 10 bp were removed from the 5' end in processing.



This grouping of tables summarizes metadata results obtained over the course of the study allowing visualization of trends over time.

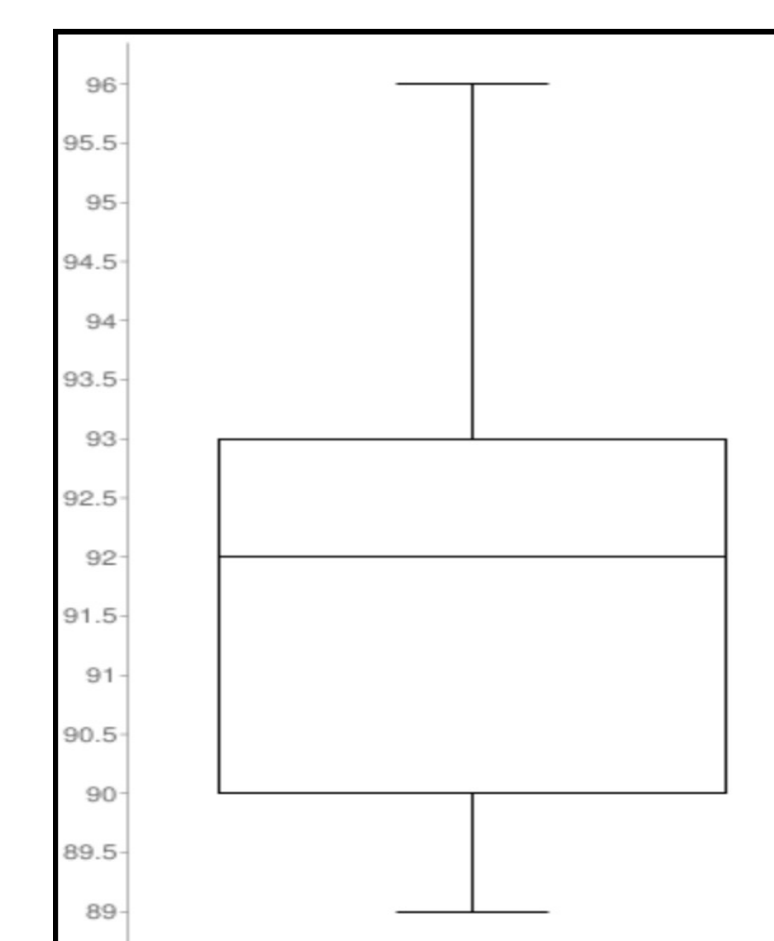


Table 5 shows a box plot generated for viewing the distribution of supine systolic blood pressure values obtained throughout the duration of the study.

Microbe ID (Genus)	Morphology	Gram Reaction	Metabolic Role in Gut	Associated Diseases
Gemmiger	Appearance of pairs or chains of spherical or tear drop shaped cells, generally a small and large body ⁶	Negative / Variable ⁶	Carbohydrate fermentation, formic and butyric acid production ⁶	IMIDs (Crohn's, Ulcerative Colitis, MS, and Rheumatoid Arthritis) ⁶
Faecalibacterium	Non motile and rod shaped ⁷	Positive ⁷	Butyric acid production ⁷	IBD, Colorectal Cancer, Obesity, Celiac ⁷
Ruminococcus	Cocci form pairs or chains ⁸	Positive / Variable ⁸	Degradation of complex polysaccharides into nutrients ⁸	IBD, Parkinson's, Celiac, Arthritis
Bifidobacterium	Clubbed or branching rods with some species exhibiting swollen coccoid forms ⁹	Positive / Variable ⁹	Digest dietary fiber and produce vitamins ¹⁰	Ulcerative colitis, IBD, Diarrhea, Other Infections of the Intestine ¹⁰

Table 6 summarized the bacterial genus' present over the course of the study. See table 4 for their estimated percentage of presence during collection of each of the samples.

Conclusions

- Although there is more than one factor that contribute to microbiome changes, this study focuses on the changes produced by diet variation, specifically, those associated with the vegan diet.
- Just as the metadata may impact the microbiome, the microbiome may impact the metadata as well. For this reason, correlation is all that can be observed accurately with this method.
- Results indicate presence of a positive association between the microbiota present and overall health and wellbeing markers. Although correlations were found, there remain ways to further study the effect of the gut microbiome on overall wellness.
- The ability to perform more accurate testing, and analysis based solely off an individual's own characteristics and create an individualized treatment plan is a great asset to the medical community.

References

- Products - DATA Briefs - number 389 - NOVEMBER 2020. (2020, November 03). Retrieved April 18, 2021, from <https://www.cdc.gov/nchs/products/databriefs/db389.htm>
- Buy Quality Medical Products and Equipment. (n.d.). Retrieved December 05, 2020, from https://www.medical-and-lab-supplies.com/?utm_campaign=English+Speaking
- Mariotti, F. (2017). Plant Protein, Animal Protein, and Protein Quality. Vegetarian and Plant-Based Diets in Health and Disease Prevention. 621-642. doi:10.1016/b978-0-12-803968-7.00035-6
- Bioinformatics. (n.d.). Retrieved December 05, 2020, from <https://www.genome.gov/genetics-glossary/Bioinformatics>
- Genome center home genome center. (n.d.). Retrieved April 18, 2021, from <https://systemsbiology.columbia.edu/genome-sequencing-defining-youreperiment#:~:text=Paired%2Dend%20reading%20improves%20the,the%20assembly%20of%20repetitive%20regions.>
- Forbes, J., Chen, C., Knox, N., Marrie, R., El-Gabalawy, H., Kievit, T., Domselaar, G. (2018, December 13). A comparative study of the gut microbiota in immune-mediated inflammatory diseases: does a common dysbiosis exist? Retrieved April 19, 2021, from <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-018-0603-4>
- Martin, R., Miquel, S., Benevides, L., Bridonneau, C., Robert, V., Hudault, S., ... Langella, P. (2017, June 30). Functional characterization of Novel *FAECALIBACTERIUM prausnitzii* strains isolated from HEALTHY Volunteers: A step forward in the use of *F. prausnitzii* as a NEXT-GENERATION PROBIOTIC. Retrieved April 19, 2021, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5492426/>
- Ruminococcus. (n.d.). Retrieved April 19, 2021, from <http://web2.uwindsor.ca/courses/biology/fackrell/Microbes/17400.htm>
- BIFIDOBACTERIUM. (n.d.). Retrieved April 19, 2021, from https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/Bifidobacterium.htm

Acknowledgements

I would like to thank the Science Department at Dominican College for funding this study, as well as Dr. Bernadette Connors and Dr. Emma Sarro for their mentorship. We also thank Siobhan O'Sullivan for laboratory assistance.