Associations Between Usual Plant & Animal Protein Intake and Select Serum Biomarkers of Chronic Disease Risk

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Abstract

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Background

The prevalence of lifestyle-related chronic diseases has increased significantly in the U.S. over the last two decades, and nutrition interventions may help reduce disease risk. However, studies linking diet with chronic disease risk factors have yet to confirm optimal proportions and sources of certain dietary components such as protein. The purpose of this analysis was to identify associations between usual intake of plant and animal protein and standard clinical chemistries relevant to chronic disease risk.

Methods

This analysis of baseline screening data from an ongoing study within the Seattle Dietary Biomarker Development Consortium included a subset of adult participants screened for the first trial of the study. Screening data included usual diet and fasting blood measures of common clinical chemistries. Using linear regression models, animal- and plant-based protein intake were assessed relative to serum concentrations of select biomarkers, including LDL cholesterol, triglycerides, hematocrit, and albumin.

Additionally, mean biomarker concentrations were compared between tertiles of intake of select protein foods using ANOVA and post-hoc Tukey models.

Results

Data associated with a total of 33 participants were analyzed. The linear regression revealed a significant inverse relationship between plant-based protein intake as a percent of total calories and hematocrit (p=0.02). A significant positive relationship between animal-based protein intake as a percent of total calories and hematocrit (p=0.004) was also identified after adjustments. ANOVA tests revealed a significant difference in mean triglyceride concentrations between protein intake tertiles in terms of servings per week of poultry (p=0.04) as well as a significant difference in mean hematocrit in terms of servings per week of green beans and peas (p=0.007).

Conclusion

These results suggest that the proportions of plant and animal-based protein in the diet may meaningfully influence hematocrit levels and may be important considerations in addressing iron-deficiency anemia or mitigating CVD risk. However, large, randomized controlled trials are needed to confirm these findings.

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I. Introduction

The steady rise in the incidence and mortality of diet-related chronic diseases in the U.S. over the last 20 years demonstrates the importance of research related to preventative measures, including nutrition interventions.[1] According to the CDC, five of the 10 leading causes of death in the U.S. are associated with preventable chronic diseases, and lifestyle factors such as diet play an important role in their development and management.[1] Among the leading causes of death are cardiovascular disease, chronic liver disease, and kidney disease.[2] These conditions create a significant financial burden, costing nearly $400B combined in the U.S. annually.[3–5] Also, many people are now living with more than one chronic disease, multiplying the individual burden.[1] Research focused on dietary components that may contribute to or protect against the development of these diseases is critical to inform lifestyle interventions that may create cost savings and improve patient quality of life. This analysis is intended to add to the body of research on this topic.

Protein consumption in the U.S. has increased over the last two decades with the majority of intake coming from animal-based sources like meat.[6,7] Most Americans exceed the recommended dietary allowance for protein, and meat consumption in the U.S. is significantly higher than the global average, with approximately 340 grams of meat consumed per day per capita in the U.S. versus 118 grams per capita per day globally in 2021.[8,9] High intake of red and processed meat in particular has been associated with increased risk of several chronic conditions as evidenced by a growing body of research.[10] Conversely, increased consumption of plant-based foods, including plant protein sources like legumes, and pulses in particular, has been shown to be beneficial in the prevention and treatment of many chronic diseases.[11] Given these apparent associations, this analysis focused on animal and plant protein and how their consumption may be related to select serum biomarkers of disease risk.

Clinical biomarkers are objective measures that reflect normal or abnormal biological processes.[12] Certain serum biomarkers are important in characterizing disease risk by reflecting genetic predispositions and indicating early signs of disease development or progression.[12] For instance, higher serum concentrations of low-density lipoprotein (LDL) cholesterol and triglycerides are associated with increased risk of cardiovascular disease (CVD).[13] This association is well documented and widely referenced in clinical practice.[13] Hematocrit is a measure of the proportion of red blood cells in the blood, and low hematocrit can indicate iron-deficiency anemia, a condition that affects 30% of the global population and can be caused by inadequate dietary iron intake.[14,15] Iron-deficiency anemia has immediate effects including fatigue and shortness of breath that can significantly impair quality of life, and, if left untreated, it may have long-term impacts including increased heart failure mortality.[15,16] Alternatively, the results of some studies indicate that high hematocrit may be associated with CVD risk factors, but the research is limited.[17,18] Albumin, as the most abundant circulatory protein in the body and a carrier of various compounds, is an important measure of nutritional status, however, it can also indicate the presence of inflammation even in well-nourished individuals, so it is no longer used to diagnose malnutrition.[19,20] Albumin is still a useful marker however, as low concentrations may reflect infection and can also be indicative of the presence or increased risk of liver disease and kidney disease.[19] Biomarkers included in this analysis are all standard clinical chemistries and include LDL cholesterol, triglycerides, hematocrit, and albumin.

Current research reflects the potential benefits of increased plant food consumption and decreased animal product consumption on the development and management of some chronic conditions.[11] However, few studies have sought to identify optimal proportions of plant and animal-based protein in the diet or specific sources and serving amounts. For example, according to a 2024 scoping review of pulse consumption and health outcomes, pulse intake has been shown to have beneficial effects on blood lipid concentrations, but the authors note that more research is needed to better understand pulse types and optimal intake quantities to achieve desired health effects.[21] Also, given the high prevalence of iron-deficiency anemia, ample research exists on the relationship between diet and iron status, but more research may be needed to better understand the ideal balance of plant and animal-based foods, including proteins, in the diet to achieve sufficient concentrations of iron and other nutrients. A 2021 systematic review of nutrient status in plant-based eaters compared to meat-eaters found that dietary inadequacies existed in all groups, with those following plant-based diets having increased risk of inadequate iron and vitamin B12 and meat-eaters having increased risk of inadequate fiber and vitamin E, among others.[22] This demonstrates that while animal-based foods provide vital nutrients such as iron, plant-based foods can provide compounds such as fiber that may be protective against certain diseases, and research to determine the optimal balance of each is critical.

Additionally, while many studies have confirmed the influence of diet on circulating LDL cholesterol and triglyceride concentrations as well as iron status,[22,23] few studies have assessed the possible influence of dietary factors on albumin concentrations, and existing studies show conflicting results. For example, one study found a weak positive association between animal protein and serum albumin concentrations, while a second study found no association between protein intake and serum albumin concentrations.[24,25] Albumin concentrations may provide important indications of disease risk, and further research on dietary associations may provide additional points of leverage in chronic disease management.

This analysis was conducted using baseline participant screening data from an ongoing controlled feeding study. Focusing on the four biomarkers noted above, the primary aim of the investigation was to answer the following research questions:

1. Among the study sample, do concentrations of select serum biomarkers (LDL cholesterol, triglycerides, hematocrit, albumin) decrease with increasing proportions of plant-based protein intake relative to total daily energy intake?
2. Conversely, do concentrations of these biomarkers increase with increasing proportions of animal-based protein intake relative to total daily energy intake?

Secondary aims of this project were to answer the following exploratory research questions:

1. Is there a clinically significant difference in concentrations of select serum biomarkers demonstrated between varieties of plant-based protein foods or animal-based protein foods consumed, such as legumes and meat? Is there a threshold amount (servings/week) of animal or plant-based protein foods that may be needed to demonstrate a clinically meaningful relationship with concentrations of select serum biomarkers?
2. More specifically, is there a clinically significant difference in concentrations of select serum biomarkers demonstrated with increased weekly servings of soy and processed soy products relative to other legume varieties? Is there a clinically significant difference in select serum

biomarker concentrations demonstrated with increased weekly servings of red and processed meat relative to other meat varieties?

Based on an initial literature review, the following primary hypotheses were established, which assume the same relationship in terms of directionality of the association and reflect the level of confidence in the association based on current research.

3. *High Confidence*: LDL cholesterol and triglyceride concentrations are positively associated with animal-based protein intake due to increased saturated fat and cholesterol content. They are inversely associated with plant-based protein intake due to lower saturated fat and cholesterol content and higher fiber content.
4. *Moderate Confidence*: Hematocrit levels are positively associated with animal-based protein intake due to increased heme iron content. They are inversely associated with plant-based protein intake due to plant foods containing no heme iron.
5. *Low Confidence*: Albumin concentrations are positively associated with animal-based protein intake due to increased protein content relative to plant protein sources. They are inversely associated with plant-based protein intake due to reduced protein content compared to animal protein sources.

This research project was intended to address certain gaps in the literature related to dietary components and amounts that may be associated with chronic disease risk factors. As a future Registered Dietitian, the research and results of this analysis process were also intended to help expand my understanding of select biomarkers and inform my future assessments and recommendations.

II. Methods

Study Setting and Data Source

This project involved a secondary analysis of data collected at the start of an ongoing set of controlled feeding trials (Phase 1 of the primary study) nested within the Seattle Dietary Biomarker Development Consortium (SDBDC). The primary study is a three-phase project aimed to build upon prior efforts involving the identification and validation of dietary biomarkers that improve upon dietary self-report. Phase 1 is currently being conducted and includes two three-period crossover feeding trials, with each testing period lasting seven days. Each of the two trials contrasts one animal and one plant protein food and includes both pharmacokinetic and dose-response testing for biomarker discovery. The primary study was designed to be well suited for generally healthy participants. The study was approved by the Fred Hutch Cancer Center Institutional Review Board, and all participants provided written informed consent prior to eligibility confirmation.

Eligibility and Recruitment

For Phase 1 of the primary study, participants were recruited throughout the city of Seattle using community, workplace and university advertisements and social media postings. Interested participants completed an initial online screening questionnaire. Inclusion criteria included healthy adults, aged 18 years or older, with a body mass index (BMI) of 18.5 to 39.9 kg/m2. Those who were determined to potentially meet these criteria were contacted to schedule an orientation visit, and during the visit, detailed information about the study was provided before individuals signed the consent form. Height, weight, and blood pressure were measured for those who provided consent, and participants then scheduled an eligibility visit. Prior to the eligibility visit, participants completed an online food frequency questionnaire (FFQ), and during the visit, a baseline fasting blood screen of standard clinical chemistries was conducted. The blood samples were sent to Quest Diagnostics, a Clinical Laboratory Improvement Amendments (CLIA)-certified lab, for analysis to confirm eligibility. Only those with lab values within acceptable ranges were eligible to participate in the study (see Table 1 for clinical chemistries relevant to this analysis and Appendix 1 for all chemistries screened). Of note, some individuals who completed FFQs and baseline blood screening but were ultimately ineligible for the study based on blood test results were still included in the current analysis. Additional exclusion criteria for the primary study included history of certain diseases, use of certain medications, and other clinical and lifestyle factors (Appendix 1).

Data Collection

Baseline data collected for Phase 1 of the primary study, including FFQ output and fasting blood test results for 33 participants, were used for this analysis. Additional demographic and lifestyle data such as age, sex, education status, and race/ethnicity were collected at baseline with a participant-completed questionnaire.

Table 1. Relevant fasting clinical chemistries at screening - normal and acceptable values.

	Description	Normal Values	Acceptable Values
Comprehensive Metabolic Panel			
Albumin	Serum Protein	3.7-5.4 g/dl	3.5-5.9 g/dl
LDL cholesterol		<130 mg/dl	<160 mg/dl
Triglycerides		<150 mg/dl	<500 mg/dl
Complete Blood Count			
HCT (women)	Hematocrit	36-48%	35-48%
HCT (men)	Hematocrit	38-49%	37.5-49%

Data Management and Cleaning

The original datasets obtained for this analysis included 43 participant identifiers (IDs), however, six participants were screened more than once for both trials of the primary study, creating multiple IDs for each. Thus, only the data associated with the first screening were used for these participants, which narrowed the list of IDs to 36. Additionally, three participants completed blood tests but did not complete FFQ forms, so they were excluded. This resulted in a final total of 33 participants, for which associated data were used for this analysis.

FFQ Data

The Nutrition Assessment Shared Resource (NASR) FFQ created by Fred Hutchinson Cancer Center was used in the primary study as part of baseline participant screening. The NASR FFQ asks participants to report consumption frequency and portion size of approximately 125 line items, each of which is defined by a series of foods or beverages, over a specified time period (e.g., the last month).[26] It includes additional questions on food type and preparation methods, which allow for further refinement of nutrient calculations by analysis software.[26] The database for the NASR FFQ is based on the nutrient strings in the Nutrition Data System for Research developed by the Nutrition Coordinating Center at the University of Minnesota.[26]

The FFQ forms for each participant underwent batch processing to generate several datasets. The processing system performed error checking and made corresponding adjustments to the data. It then aggregated data and performed calculations to produce datasets including, but not limited to, daily nutrient intake and annual servings per participant. The daily nutrient intake dataset contained several variables that were used for the analysis including total daily energy (kcal), total daily protein (g), total daily animal protein (g), and total daily vegetable protein (g). To address primary research questions, the proportion of daily energy from animal and plant protein was calculated. Animal protein includes protein from all animal sources (meat, eggs, dairy) and plant protein includes protein from all plant sources. The data were then organized by participant into a spreadsheet that served as the input for primary statistical tests, with fields including participant ID, percent of daily energy from total protein, percent of daily energy from animal protein, and percent of daily energy from plant protein.

To address the secondary research questions related to protein food varieties and amounts, focus was placed specifically on consumption of legumes as a plant protein source and on meat (flesh foods) as an animal protein source. The following categories of foods were included in the analysis:

- Plant protein foods:
    - Legumes (Total)
    - Green beans and peas
    - Soy
    - All other legumes
- Animal flesh foods (excludes eggs and dairy):
    - Meat (Total)
    - Poultry
    - Fish and seafood
    - Red and processed meat

The majority of dietary consumption data were extracted from the daily nutrient intake dataset, and daily intake was converted to weekly servings based on standard serving size assumptions used to produce the annual servings dataset. However, fresh green beans and peas were not included in the daily nutrient intake dataset, and thus, weekly servings were calculated using annual servings data and dividing by 52 weeks. The resulting green bean and pea servings, as well as the soy servings, were added to the total legumes amounts as the original total only included dry beans. These data were organized into a spreadsheet and served as the input for secondary statistical tests.

Clinical Chemistries

Blood chemistries selected for this analysis include LDL cholesterol, triglycerides, hematocrit, and albumin, which are often referenced by medical professionals, including dietitians, in the clinical setting. The blood chemistry data were first inspected for errors or missing values and then incorporated by participant into the aforementioned spreadsheets with a field for each of the four biomarkers.

Analysis

The analysis was conducted exclusively using R statistical software (version 2024.04.2). Due to the small sample size, the distribution about the mean was assessed for all dietary and clinical chemistries data and a log transformation was performed on all numerical data to improve normality (Appendix 2).

Hypothesis Testing: Primary Analysis

Simple and multivariable linear regression models were conducted to address primary research questions. Exposure variables (x) included the percent of energy from plant-based protein and the percent of energy from animal-based protein. Outcome variables (y) included the four selected blood chemistries.

Total protein intake differed significantly among participants, so it was added as an adjustment variable in subsequent multivariable regression models to assess with greater precision the impact of differences in plant and animal protein proportions on the outcomes. Additionally, sex was added as an adjustment variable due to notable differences in protein intake and lab concentrations between male and female participants.

Overall, four regression models were conducted for each exposure relative to each outcome. The first model included no adjustment variables, the second model was adjusted only for total protein intake, the third model was adjusted only for sex, and the fourth model was adjusted for both total protein intake and sex.

Hypothesis Testing: Secondary Analysis

Log transformation was performed on the data prior to running tests, however, the input dataset used for secondary analysis tests included some zero values, so 1 was added to all values before taking the natural log to prevent errors in testing due to the log of zero being mathematically undefined.

A one-way ANOVA test was conducted for each legume and meat variety noted above to assess the mean differences in biomarker concentrations between tertiles in terms of servings per week. A separate test was performed for each food variety and each biomarker (32 tests total). The results of the ANOVA tests indicate whether there is a statistically significant difference in biomarker concentrations between servings tertiles, however they do not indicate which of the three tertiles significantly differ and whether the mean difference is positive or negative. To avoid Type I error, post-hoc analysis was performed using Tukey tests, the output of which provided the difference in means, confidence levels, and adjusted p-values for all possible pairs.

III. Results

Study Sample Characteristics

The majority of the participants were female (63.6%), young adults aged 20-40 (69.7%), non-Hispanic white (60.6%), with a BMI <30 kg/m^2 (75.7%), and well-educated with a 4-year college degree or higher (91%) (Table 2).

Table 2. Summary of Participant Demographics.

	Overall (N=33)
Sex	
Male	12 (36.4%)
Female	21 (63.6%)
Age in Years*	
20-30	10 (30.3%)
30-40	13 (39.4%)
40-50	5 (15.2%)
50+	5 (15.2%)
Race / Ethnicity	
Hispanic Multiracial	1 (3.0%)
Hispanic Prefer not to answer	1 (3.0%)
Hispanic Unknown	1 (3.0%)
Non-Hispanic Asian	4 (12.1%)
Non-Hispanic Black	1 (3.0%)
Non-Hispanic Multiracial	4 (12.1%)
Non-Hispanic Prefer not to answer	1 (3.0%)
Non-Hispanic White	20 (60.6%)
Highest Education Level Completed	
1-3 Years College	3 (9.1%)
4-Year College Degree	15 (45.5%)
Post-Graduate Degree	15 (45.5%)
BMI (kg/m^2)	
20-25	14 (42.4%)
25-30	11 (33.3%)
30-35	8 (24.2%)

n (%)

*Participant ages ranged from 23 to 75 years, with one participant over the age of 55.

Individuals with lab values outside of clinically acceptable ranges cited in Table 1 above were excluded from the primary study but were still included in this analysis. Thus, concentrations of LDL cholesterol for several participants were above both the clinically normal range and the acceptable range for the primary study (Table 3). Triglyceride concentrations were all within the acceptable range but were above the clinically normal range for some participants. Albumin concentrations and hematocrit for all participants were within clinically normal and acceptable ranges. On average, male participants had higher concentrations of all four clinical measures of interest than female participants.

Table 3. Summary of Participant Clinical Chemistries.

	Male (N=12)	Female (N=21)	Overall (N=33)
LDL (mg/dL)			
Mean (SD)	108 (22)	99 (31)	102 (28)
Median [Min, Max]	101 [86, 165]	93 [47, 164]	97 [47, 165]
Triglycerides (mg/dL)			
Mean (SD)	112 (46)	87 (49)	96 (49)
Median [Min, Max]	100 [54, 221]	71 [40, 244]	89 [40, 244]
Hematocrit (%)			
Mean (SD)	44.9 (2.5)	39.4 (2.2)	41.4 (3.5)
Median [Min, Max]	45.4 [39.0, 47.8]	38.8 [36.4, 44.3]	40.7 [36.4, 47.8]
Albumin (g/dL)			
Mean (SD)	4.5 (0.2)	4.3 (0.2)	4.4 (0.2)
Median [Min, Max]	4.5 [4.2, 4.8]	4.3 [3.9, 4.7]	4.4 [3.9, 4.8]

As shown in Table 4, among the overall sample, average daily animal-based protein intake as a percent of daily calories (11%) was higher than average daily plant-based protein intake as a percent of daily calories (6%). Total daily protein intake as a percent of daily calories was, on average, slightly higher among male participants than female participants. Animal-based protein intake was also slightly higher, on average, among males. Overall, total daily protein intake as a percent of daily calories ranged from 13% to 32% and was 18% on average.

Table 4. Summary of Participant Daily Protein Intake (% of kcal).

	Male (N=12)	Female (N=21)	Overall (N=33)
Total Protein Intake (% of daily kcal)			
Mean (SD)	19 (5)	17 (3)	18 (4)
Median [Min, Max]	17 [13, 32]	17 [13, 21]	17 [13, 32]
Animal-Based Protein Intake* (% of daily kcal)			
Mean (SD)	13 (7)	11 (3)	11 (5)
Median [Min, Max]	11 [5, 29]	10 [6, 17]	10 [5, 29]
Plant-Based Protein Intake (% of daily kcal)			
Mean (SD)	6 (3)	6 (2)	6 (2)
Median [Min, Max]	5 [3, 13]	6 [3, 14]	6 [3, 14]

*Includes protein from all animal sources (meat, eggs, dairy).

Results

Primary Research Questions

Four separate linear regression models were used to address primary hypotheses. The first included no adjustment variables, the second was adjusted for total protein intake, the third was adjusted for sex, and the fourth was adjusted for both total protein intake and sex.

Each model yielded at least one statistically significant ($p<0.05$) test result, all of which involved hematocrit. In all four models, hematocrit was found to be significantly inversely associated with plant-based protein intake. However, in the third and fourth models, which included sex as an adjustment variable, hematocrit was also found to be significantly associated with sex ($p<0.001$), with females having lower concentrations, on average, than males. According to model 4, adjusted for both total protein intake and sex, for every 1% increase in plant-based protein intake, hematocrit decreases by [log] 0.07% (95% CI: -0.13, -0.01; p=0.02) (Table 5).

Hematocrit was significantly and positively associated with animal-based protein intake in the adjusted models. However, total protein intake and sex (female) were also significantly associated with hematocrit, with each demonstrating an inverse association. According to the fully adjusted model 4, for every 1% increase in animal-based protein intake as a percent of daily kcal, hematocrit increases by [log] 0.11% (95% CI: 0.04, 0.19; p=0.004) (Table 5).

All other clinical chemistries included in the regression models (LDL cholesterol, triglycerides, albumin) were not significantly associated with either animal-based or plant-based protein intake. According to regression line trends, as animal-based protein intake increases, regression lines for LDL cholesterol, triglycerides, and hematocrit trend upward, and as plant-based protein intake increases, these regression lines trend downward (Appendix 3). Alternatively, the regression line associated with albumin concentration trends downward as animal-based protein intake increases and upward as plant-based protein intake increases.

Table 5. Associations Between Plant-Based vs. Animal-Based Protein Intake as a Percent of Total Calories and Log Values of Select Clinical Chemistries Among a Sample of 33 Healthy Adults.

	LDL ([log] mg/dL)			**Triglycerides ([log] mg/dL)**			**Hematocrit ([log] %)**			**Albumin ([log] g/dL)**		
Predictors	*Estimates*	*95% CI*	*p*	*Estimates*	*95% CI*	*p*	*Estimates*	*95% CI*	*p*	*Estimates*	*95% CI*	*p*
Model 1												
(Intercept)	4.2	3.35, 5.04	**<0.001**	4.01	2.66, 5.36	**<0.001**	3.45	3.21, 3.69	**<0.001**	1.51	1.36, 1.66	**<0.001**
Plant-Based Protein Intake	-0.14	-0.44, 0.16	0.348	-0.16	-0.63, 0.31	0.495	-0.1	-0.18, -0.01	**0.025**	0.01	-0.04, 0.06	0.657
Model 2												
(Intercept)	4.38	2.95, 5.8	**<0.001**	5.44	3.27, 7.62	**<0.001**	3.55	3.15, 3.94	**<0.001**	1.56	1.31, 1.81	**<0.001**
Plant-Based Protein Intake	-0.13	-0.44, 0.18	0.403	-0.08	-0.55, 0.39	0.739	-0.09	-0.17, -0	**0.04**	0.01	-0.04, 0.07	0.594
Total Protein Intake	0.09	-0.45, 0.63	0.743	0.68	-0.14, 1.5	0.102	0.05	-0.1, 0.19	0.537	0.02	-0.07, 0.12	0.621
Model 3												
(Intercept)	4.33	3.45, 5.21	**<0.001**	4.34	2.97, 5.71	**<0.001**	3.6	3.44, 3.76	**<0.001**	1.56	1.41, 1.7	**<0.001**
Plant-Based Protein Intake	-0.12	-0.42, 0.18	0.433	-0.11	-0.57, 0.36	0.646	-0.07	-0.13, -0.02	**0.012**	0.02	-0.03, 0.07	0.426
Sex: Female	-0.11	-0.32, 0.1	0.286	-0.28	-0.6, 0.05	0.094	-0.12	-0.16, -0.08	**<0.001**	-0.04	-0.08, -0.01	**0.024**
Model 4												
(Intercept)	4.43	3, 5.85	**<0.001**	5.55	3.41, 7.68	**<0.001**	3.6	3.34, 3.86	**<0.001**	1.58	1.34, 1.81	**<0.001**
Plant-Based Protein Intake	-0.11	-0.42, 0.2	0.47	-0.04	-0.5, 0.42	0.861	-0.07	-0.13, -0.01	**0.016**	0.02	-0.03, 0.07	0.42
Total Protein Intake	0.05	-0.50, 0.59	0.858	0.59	-0.22, 1.41	0.148	0	-0.1, 0.1	0.974	0.01	-0.08, 0.1	0.842
Sex: Female	-0.11	-0.33, 0.11	0.311	-0.24	-0.57, 0.08	0.135	-0.12	-0.16, -0.08	**<0.001**	-0.04	-0.08, -0	**0.03**
Model 1												
(Intercept)	4.81	4.21, 5.41	**<0.001**	5.08	4.14, 6.02	**<0.001**	3.88	3.71, 4.05	**<0.001**	1.45	1.34, 1.55	**<0.001**
Animal-Based Protein Intake	0.1	-0.17, 0.36	0.452	0.27	-0.14, 0.68	0.184	0.07	-0, 0.15	0.064	-0.01	-0.06, 0.03	0.542
Model 2												
(Intercept)	4.78	3.82, 5.73	**<0.001**	5.72	4.26, 7.17	**<0.001**	3.82	3.55, 4.09	**<0.001**	1.53	1.37, 1.69	**<0.001**

Animal-Based Protein Intake	0.11	-0.31, 0.54	0.586	-0.01	-0.65, 0.63	0.974	0.1	-0.02, 0.22	0.103	-0.05	-0.12, 0.02	0.148
Total Protein Intake	-0.04	-0.88, 0.8	0.923	0.72	-0.55, 2	0.254	-0.07	-0.31, 0.16	0.541	0.1	-0.04, 0.24	0.17
Model 3												
(Intercept)	4.85	4.25, 5.46	**<0.001**	5.18	4.26, 6.1	**<0.001**	3.92	3.81, 4.04	**<0.001**	1.46	1.36, 1.56	**<0.001**
Animal-Based Protein Intake	0.08	-0.18, 0.35	0.518	0.24	-0.16, 0.64	0.228	0.06	0.01, 0.1	**0.028**	-0.02	-0.06, 0.02	0.383
Sex: Female	-0.12	-0.33, 0.09	0.265	-0.27	-0.59, 0.05	0.095	-0.13	-0.16, -0.09	**<0.001**	-0.04	-0.07, -0.01	**0.025**
Model 4												
(Intercept)	4.76	3.8, 5.71	**<0.001**	5.68	4.26, 7.1	**<0.001**	3.8	3.63, 3.96	**<0.001**	1.53	1.37, 1.68	**<0.001**
Animal-Based Protein Intake	0.13	-0.3, 0.55	0.544	0.02	-0.61, 0.65	0.96	0.11	0.04, 0.19	**0.004**	-0.05	-0.12, 0.02	0.157
Total Protein Intake	-0.11	-0.95, 0.73	0.792	0.58	-0.68, 1.84	0.352	-0.15	-0.29, -0	**0.049**	0.08	-0.06, 0.21	0.259
Sex: Female	-0.12	-0.34, 0.1	0.261	-0.25	-0.57, 0.08	0.129	-0.13	-0.17, -0.09	**<0.001**	-0.04	-0.07, -0	**0.037**

Note:
Model 1 includes no adjustment variables.
Model 2 adjusted only for total protein intake as a percent of daily kcal.
Model 3 adjusted only for sex.
Model 4 adjusted for both total protein intake as a percent of daily kcal and sex.

Secondary Research Questions

For each protein food variety noted previously, participants were grouped into tertiles by ranges of servings per week (Tables 6.1 and 6.2) using the quantile function in R software, which creates quantiles corresponding to given probabilities with the smallest observation corresponding to a probability of 0 and the largest to a probability of 1.

Table 6.1. Legume Varieties - Servings per Week by Tertile.

	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Total Legumes				
Mean [SD]	0.65 [0.2]	1.5 [0.31]	6.2 [6.7]	2.8 [4.5]
Median [Min, Max]	0.63 [0.3, 0.96]	1.5 [0.99, 1.9]	3.7 [2.1, 24]	1.5 [0.3, 24]
	Tertile 1 (N= 12)	Tertile 2 (N= 10)	Tertile 3 (N= 11)	Overall (N= 33)
Green Beans and Peas				
Mean [SD]	0.087 [0.11]	0.64 [0.17]	3.4 [2.9]	1.3 [2.2]
Median [Min, Max]	0 [0, 0.24]	0.65 [0.42, 0.87]	2.4 [0.94, 9.4]	0.63 [0, 9.4]
	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Soy				
Mean [SD]	0.033 [0.035]	0.26 [0.11]	2.6 [4.1]	0.95 [2.6]
Median [Min, Max]	0.02 [0, 0.1]	0.27 [0.13, 0.47]	1.2 [0.49, 15]	0.27 [0, 15]
	Tertile 1 (N= 12)	Tertile 2 (N= 10)	Tertile 3 (N= 11)	Overall (N= 33)
All Other Legumes				
Mean [SD]	0.16 [0.11]	0.44 [0.085]	0.85 [0.4]	0.48 [0.38]
Median [Min, Max]	0.12 [0.02, 0.32]	0.44 [0.34, 0.57]	0.72 [0.58, 2]	0.43 [0.02, 2]

Table 6.2. Meat Varieties - Servings per Week by Tertile.

	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Total Meat				
Mean [SD]	4.5 [1.4]	9.2 [1.8]	23 [12]	12 [11]
Median [Min, Max]	4.5 [1.5, 6.3]	9.4 [6.7, 12]	17 [13, 49]	9.4 [1.5, 49]

	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Poultry				
Mean [SD]	1.2 [0.58]	3 [0.59]	9.9 [5]	4.7 [4.7]
Median [Min, Max]	1.3 [0.06, 1.9]	3 [2.3, 4.2]	7.7 [4.4, 19]	3 [0.06, 19]

	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Fish and Seafood				
Mean [SD]	0.31 [0.3]	1.5 [0.3]	3.1 [1]	1.6 [1.3]
Median [Min, Max]	0.25 [0, 0.71]	1.4 [0.87, 2]	2.9 [2, 4.7]	1.4 [0, 4.7]

	Tertile 1 (N= 11)	Tertile 2 (N= 11)	Tertile 3 (N= 11)	Overall (N= 33)
Red and Processed Meat				
Mean [SD]	1.7 [0.74]	3.5 [0.68]	12 [7.8]	5.9 [6.5]
Median [Min, Max]	1.9 [0.16, 2.5]	3.5 [2.5, 4.4]	8.8 [6.1, 29]	3.5 [0.16, 29]

Separate ANOVA tests were conducted for each protein food to identify if significant differences in clinical chemistry values exist between tertiles, which were designated by servings per week consumed. Of the 32 tests performed, two yielded statistically significant results (p<0.05).

Mean triglyceride concentrations differed significantly across the poultry intake tertiles (p=0.04). Post-hoc analysis revealed the primary difference occurred between tertiles 2 and 3, with the mean triglyceride concentration of tertile 3 being significantly higher than that of tertile 2 (Figure 1). Participants in tertile 3 consumed greater than 4.4 servings (10 servings on average) per week of poultry versus participants in tertile 2 who consumed between 2.3 and 4.2 servings (3 servings on average) per week.

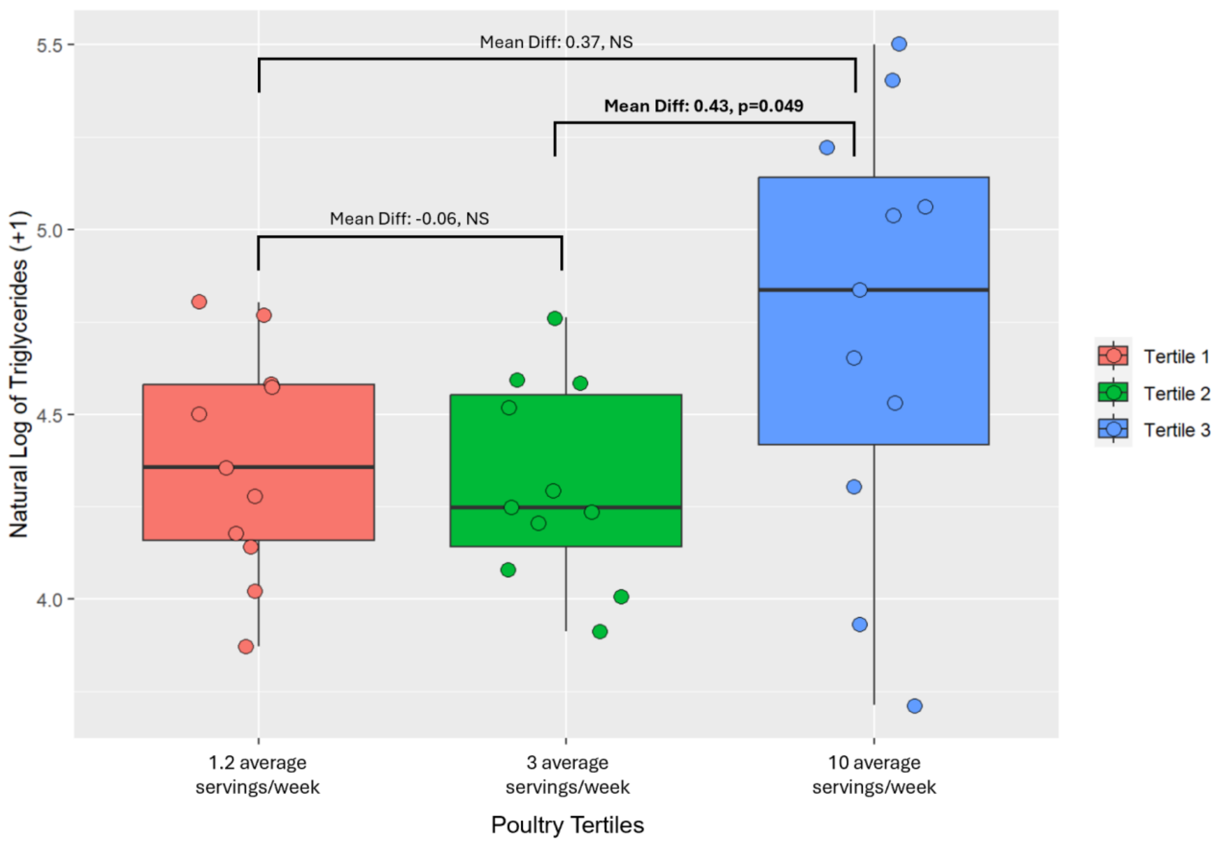

Figure 1. Scatter Box Plot of Triglyceride Concentration ([log] mg/dL +1) by Poultry Servings Tertile.

Additionally, mean hematocrit differed significantly between green bean and pea servings tertiles (p=0.007). Post-hoc analysis revealed the primary difference occurred between tertiles 2 and 3, with the mean hematocrit concentration of tertile 3 being significantly lower than that of tertile 2 (Figure 2). Participants in tertile 3 consumed greater than 0.9 servings (3.4 servings on average) per week of green beans and peas versus participants in tertile 2 who consumed between 0.4 and 0.9 servings (0.64 servings on average) per week. The mean difference between tertile 1 and tertile 3 was also statistically significant according to the post-hoc analysis, with the mean hematocrit concentration of tertile 3 being significantly lower than that of tertile 1. Participants in tertile 1 consumed 0.2 servings or less (0.09 servings on average) of green beans and peas per week.

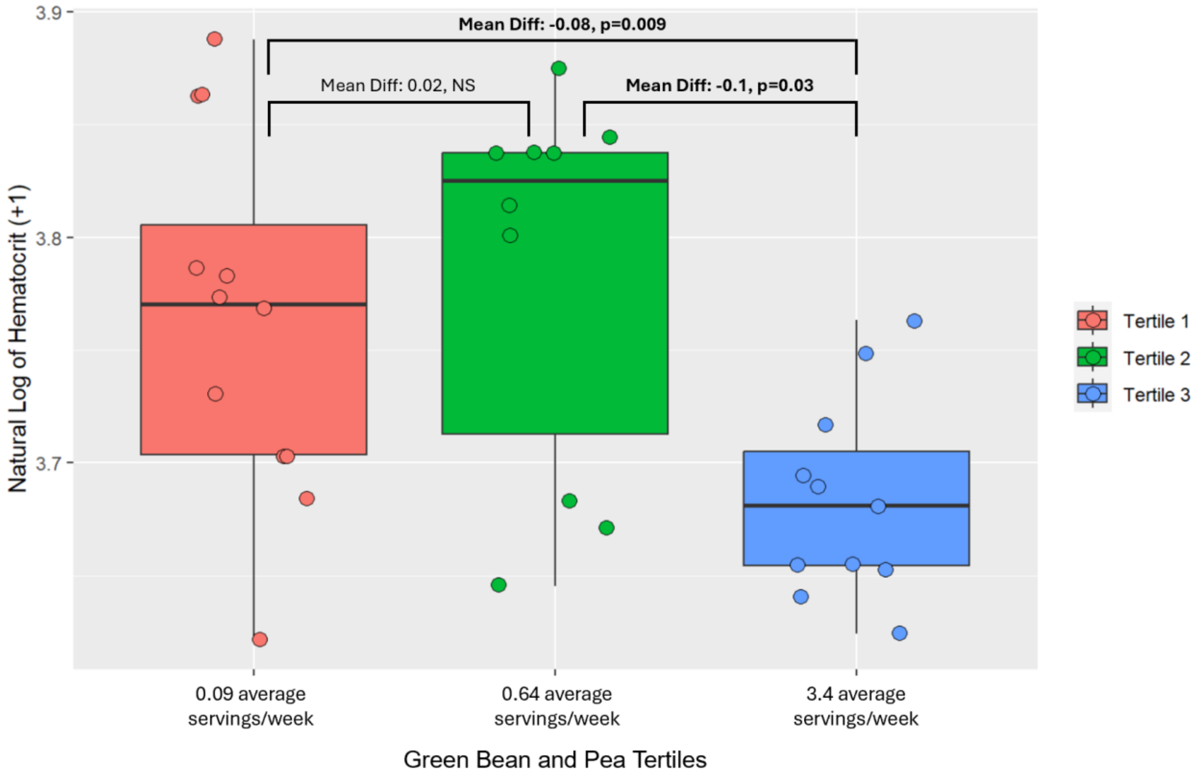

Figure 2. Scatter Box Plot of Hematocrit Concentration ([log] % +1) by Green Bean and Pea Servings Tertile.

No other significant between-tertile differences were identified in other ANOVA tests (Appendix 4).

IV. Discussion

In this secondary analysis, statistically significant associations were identified between both plant and animal-based protein intake as a percent of total calories and hematocrit levels. On average, lower plant-based protein intake and higher animal-based protein intake were associated with increased hematocrit levels. This suggests that the relative proportion of these proteins in the diet may meaningfully influence hematocrit and may have further health implications, such as potentially modulating anemia and cardiovascular disease risk.

Diet and Hematocrit

Limited research exists on the relationship between diet and hematocrit. However, a wider range of studies include hemoglobin and iron concentrations as outcomes. Hemoglobin, a protein in red blood cells that is responsible for transporting oxygen in the body, is closely related to hematocrit and has similar health implications.[14] One study involving a prospective cohort of nearly 500,000 found that people with low or no red meat intake generally had lower hemoglobin concentrations and were more likely to be anemic.[27] While this study did not reference hematocrit directly, the findings can reasonably be compared to that of the present analysis in which a similar association was observed.

Alternatively, a 2021 cross-sectional study with a sample over 100,000 identified a specific anemia–inflammation-related dietary pattern, characterized by high intakes of eggs, meat, sugary beverages, and fried and processed foods, that was associated with decreased hemoglobin, hematocrit, and red blood cells.[28] The authors note that these foods can contribute to low-grade inflammation, leading to a higher incidence of anemia.[28] These results conflict with findings of the present analysis, which suggest that higher intake of meat and eggs is associated with increased hematocrit. However, the authors did not assess these foods in isolation to confirm their inflammatory effect.[28]

Hematocrit and Iron-Deficiency Anemia

Low levels of hematocrit can indicate iron-deficiency anemia, the most common nutritional deficiency that can be caused by inadequate dietary iron intake.[29] Dietary iron occurs in two forms, heme and non-heme.[29] Heme iron can only be derived from meat and other animal products and is more bioavailable, with up to 30% absorbed by the human body.[29] Non-heme iron is found in both plant and animal products, but absorption ranges from 1 to 10%.[29] The presence of heme iron in animal products may presumably make consumption of animal protein more effective than plant protein in preventing or treating iron-deficiency anemia, however the research is mixed.

A 2016 systematic review found that, of seven studies, five (three of three randomized trials and two of four observational studies) showed a positive association between animal flesh intake and iron status, however the authors note that the ideal quantity or frequency of intake is unclear.[30] Other studies suggest that sex may play a prominent role in influencing consumption patterns and iron status. A 2021 study assessing iron status among healthy Norwegian vegans, vegetarians and pescatarians found that the majority of participants had sufficient iron status and one of the strongest predictors of decreased iron was the female sex.[31] The authors of a 2022 systematic review discuss certain gender-dependent food preferences that may influence overall intake of iron, including a generally lower preference towards meat and higher likelihood of consuming non-heme iron sources, such as legumes, among females.[29]

Physiological differences between the sexes, including increased lean body mass and potentially increased protein needs among males, may influence intake but may not be significant.[32] Most adults need around 0.75 grams of protein per kilogram of body weight per day, and this equates to about 45 grams for the average female and 55 grams for the average male.[33] A 2025 cross-sectional study, which found meat and processed meat consumption to be significantly higher in men and soy consumption to be significantly higher among women, suggests that these choices are influenced by cultural norms, economic factors, and health perceptions rather than physiological needs alone.[34]

Findings of the present analysis reflected a significant inverse association between female sex and hematocrit ($p<0.001$). The ranges of hematocrit among males and females in the sample were within clinically normal values, which differ based on sex. Additionally, female participants had lower animal protein intake, on average, than male participants. However, the sample included a larger proportion of female participants (64%), which may have influenced results. Additionally, this analysis did not account for the timing of menstrual cycles among female participants, which may meaningfully influence hematocrit levels.[35]

Hematocrit and Cardiovascular Disease

Elevated hematocrit can also pose health risks, including increased risk of CVD. A 2014 study involving middle-aged adults in China found that elevated hematocrit may be positively associated with CVD risk factors such as hypertension, diabetes, and dyslipidemia.[17] A 2015 prospective cohort study within the Hisayama Study followed over 2,500 Japanese subjects for 19 years, and findings suggest that both elevated and decreased hematocrit levels are associated with an increased risk of CVD.[18] The authors compared findings to a study with a prior cohort in the same town and noted that mean hematocrit levels of the more recent cohort were significantly higher. They explain that the rise in hematocrit levels among Japanese individuals may be due to the Westernization of dietary habits, which is characterized by high intakes of processed foods, red meat, sugary drinks, and saturated fats, and low intakes of fruits, vegetables, and dietary fiber.[18,36] This suggests that while higher meat intake may decrease the risk of iron-deficiency anemia, it may increase CVD risk and thus, identifying the ideal level of intake should be prioritized, especially given meat may displace other healthful foods in the diet, such as fruits and vegetables.

Non-significant Trends

All other outcome measures analyzed to address primary research questions, including LDL cholesterol, triglycerides, and albumin, were not significantly associated with plant or animal protein intake. However, the regression lines associated with LDL cholesterol and triglycerides were in the hypothesized direction. The lack of significance in these associations may be related to the small sample size or the influence of other non-protein dietary factors, such as fat content, as saturated fat intake has been associated with LDL cholesterol and triglycerides, while hematocrit may be more closely related to protein intake. Alternatively, the regression line associated with albumin concentration was not in the hypothesized direction. However, the confidence level of this hypothesis was low due to the lack of previous evidence demonstrating an association between dietary intake and albumin.

Exploratory Findings
Exploratory analysis revealed a significant difference in mean triglyceride concentrations between poultry servings tertiles (p=0.04). The mean triglyceride concentration of the largest servings tertile was significantly higher than that of the middle servings tertile. This suggests that higher poultry intake may be associated with increased triglyceride concentrations. However, this analysis did not account for other foods consumed alongside poultry that can affect triglycerides or methods of preparation, e.g. fried vs boiled.

Elevated triglycerides are associated with increased risk of CVD, but few studies have assessed whether consumption of poultry may be associated with triglycerides or other CVD risk factors. A 2023 narrative review found, based on limited evidence, that the consumption of lean unprocessed chicken as a primary dietary protein source has either beneficial or neutral effects on risk factors for CVD, but additional research is needed.[37] The findings of the present analysis conflict with the limited available evidence. This may be related to the small overall sample size and subgroup sizes.

A significant mean difference was also identified in hematocrit levels among green bean and pea servings groups (p=0.007). The mean hematocrit of the largest servings group was significantly lower than that of the middle servings group. This result may be related to the previously discussed inverse association between plant protein intake and hematocrit, however, the difference in average servings per week between groups was particularly small, which limits the validity of the result.

All other protein foods included in exploratory analysis did not yield significant results. Notable differences in the average weekly servings between protein foods may have influenced results, with average intake of plant foods significantly lower than that of animal flesh foods among the sample.

Strengths and Limitations
A strength of this secondary analysis is the use of existing datasets, which allowed for the conduct of comprehensive analysis without the time and financial constraints associated with primary data collection. Also, the data were originally collected using validated and reliable methods and underwent batch processing prior to the analysis, which included automated error checking, increasing the accuracy of the source data and limiting the opportunities for human error.

This analysis has several limitations. The relatively small overall sample size and subgroup sizes limit the statistical power of the tests performed. Additionally, because the participants were relatively young and generally healthy, and the majority of their biomarker concentrations were within normal ranges, this may limit the validity and generalizability of results. The analysis also relied on self-reported dietary data, which can be subject to recall bias. Significant differences in mean intake of plant versus animal protein limited the ability to appropriately compare and interpret results. Possible confounders that were not accounted for include genetic factors and the social determinants of health, which may meaningfully influence biomarker concentrations. Finally, the cross-sectional nature of this analysis is a limitation due to the inability to study changes in diet and clinical chemistries over time.

V. Conclusion

This analysis found that lower plant-based protein intake and higher animal-based protein intake, on average, were associated with increased hematocrit levels. These findings suggest that the proportion of animal and plant-based protein in the diet may meaningfully influence hematocrit levels. Additionally, the results are consistent with previously published data suggesting that females generally have lower hematocrit than males and also indicate potential differences in dietary preferences between the sexes with respect to dietary protein sources. However, large, randomized controlled trials including samples representative of the general population and comparing various protein sources and intake levels are needed to confirm these findings.

Low hematocrit can reflect iron-deficiency anemia, and thus it may be important to closely monitor hematocrit levels in women and those eating a higher proportion of plant-based protein. Elevated hematocrit should also be addressed given the apparent association with CVD risk. Incorporating a variety of proteins in the diet from both plant and animal sources may help maintain ideal hematocrit levels. Registered dietitians may be a valuable resource in helping at-risk clients identify foods and intake quantities to achieve this balance, but recommendations should be based on current health status, including hematological status.

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VII. <u>Appendix</u>

Appendix 1: Eligibility Criteria

Healthy adult volunteers who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study. All races/ethnicities are eligible to participate.

1.1 Inclusion Criteria

1. Healthy adults
2. Age 18 years or older
3. Willing to come to the Fred Hutch campus 16 times during the study
4. BMI 18.5-39.9 kg/m^2

1.2 Exclusion Criteria

1. History of gastrointestinal disorders (e.g., ulcerative colitis, Crohn disease, celiac sprue, HNPCC, familial adenomatous polyposis, pancreatic disease, liver disease)
2. Bleeding disorder that precludes blood draws
3. Previous gastrointestinal resection or bariatric surgery
4. Recent hospital admissions (in past 6 months) for heart disease (MI/CVA or CHF) or other CVD/CAD condition under physician guided therapy that is not medically stable.
5. Cancer under active radiation or chemotherapy treatment-(post -6 mos)
6. Pregnant or lactating (self-reported)
7. Weight change (±5% in 3 months)
8. Regular alcohol intake of >2 drinks/day (2 drinks being equivalent to 720 ml beer, 240 ml wine, or 90 ml spirits) and unwilling to abstain during feeding periods
9. Use of tobacco and/or marijuana, hookahs, e-cigarettes (e-cigs, vapes, etc) and not willing to abstain during feeding periods.
10. Use of illicit drugs and not willing to abstain during feeding periods.
11. BMI >40 kg/m^2
12. Seated blood pressure > 140/90 mm Hg
13. Fasting clinical lab tests outside acceptable value as ascertained at a screening blood draw*
14. Food allergies/intolerances or major dislikes to foods used in the study menus; unwilling to consume study foods.
15. Current use of specific prescription medication**
16. Regular (daily to weekly) use of over-the-counter weight-loss aids, anti-inflammatories, and unable to stop taking these during feeding periods
17. Unwilling to stop taking OTC dietary supplements that interfere with the test foods being studied, including pills, chewables, liquids or powders for the following: protein supplements, soy, fiber, flaxseed, fish oil (including cod liver oil), probiotics, glucosamine and chondroitin (if vitamin

supplement is MD prescribed – may continue; study staff will review supplements to determine eligibility)

18. Oral or IV antibiotic use in the past 3 months (may defer participation until 3 months post completion of course of antibiotics)

19. Inability to freely give informed consent.

***Fasting clinical lab tests outside acceptable value as ascertained at a screening blood draw:**

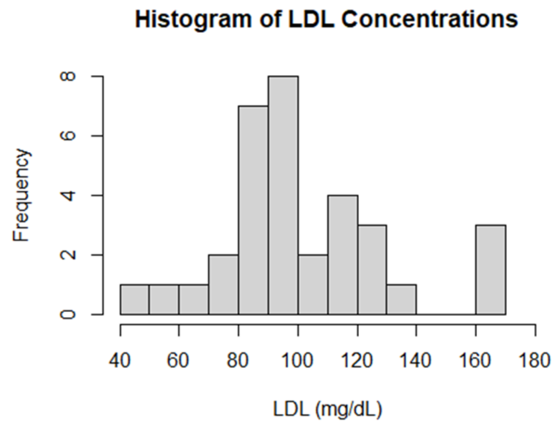
	Description	Normal Values	Acceptable Values
Comprehensive Metabolic Panel			
Glucose (Fasting)	Serum Glucose	54-118mg/dl	54-125 mg/dl
Urea	BUN	9-25 mg/dl	6-50mg/dl
Creatinine	Serum Creatinine	0.7-1.3 mg/dl	0.4-1.3 mg/dl
eGFR	estimated GFR	>60ml/min	>60ml/min
Sodium	Serum Sodium	136-142 mmol/L	133-146 mmol/L
ALT/GPT	Liver Enzyme	7-52 U/L	5-60 U/L
AST/GOT	Liver Enzyme	9-30 U/L	5-40 U/L
Alk Phos	Liver Enzyme	36-118 U/L	20-135 U/L
Total Bili	Liver Function	0.2-1.2 mg/dl	0.0-1.9 mg/dl
Total Protein	Serum Protein	6.0-8.0 g/dl	5-9.0 g/dl
Albumin	Serum Protein	3.7-5.4 g/dl	3.5-5.9 g/dl
LDL cholesterol		<130 mg/dl	<160 mg/dl
Triglycerides		<150 mg/dl	<500 mg/dl
Complete Blood Count			**EDTA, 3mL**
WBC	White Blood Cells	4-10 KI/uL	3-10.5 K/uL
HCT (women)	Hematocrit	36-48%	35-48%
HCT (men)	Hematocrit	38-49%	37.5-49%

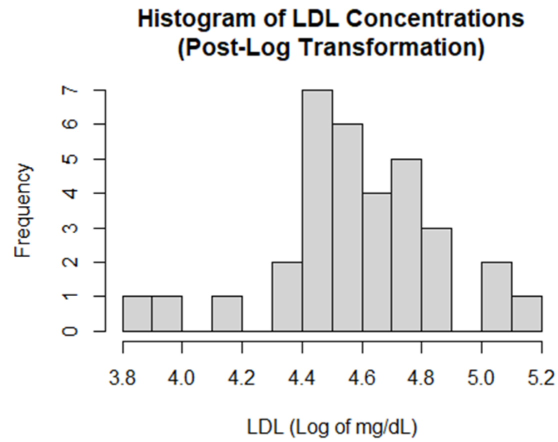
**Medication use for exclusion:

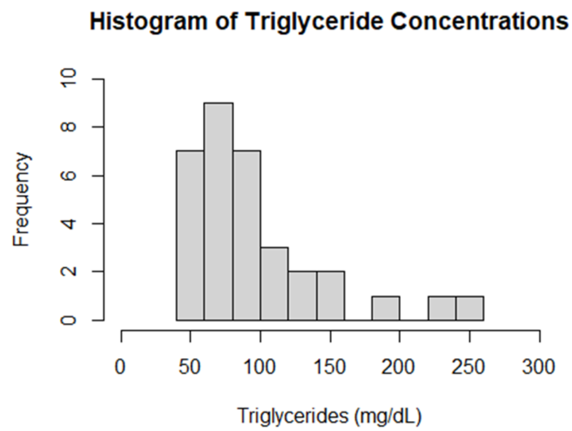
1. Diuretics
2. Steroids (oral): daily oral any dose within 1 month of study, except OCP as noted below
3. Opiates: any use within 1 month of study
4. Anti-lipemics that affect GI or renal function (ie. Fibrates)

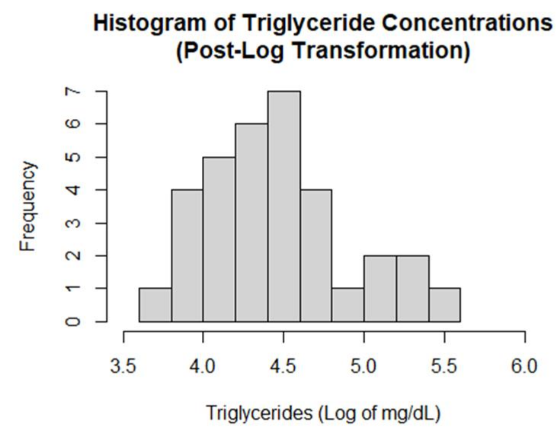
5. Anti-glycemics other than metformin (ie. insulin, SGLT2 inhibitor, α-glucosidase inhibitor)
6. Psychiatric that affect metabolism/renal function (anti-psychotics, lithium)
7. Biologics/immune modulators (ie. RA, psoriasis, other rheumatologic/hematologic active disease)
8. Anti-coagulants (coumadin, heparin, Eliquis, etc.)
9. HIV/HAART, etc. (dyslipogenic)

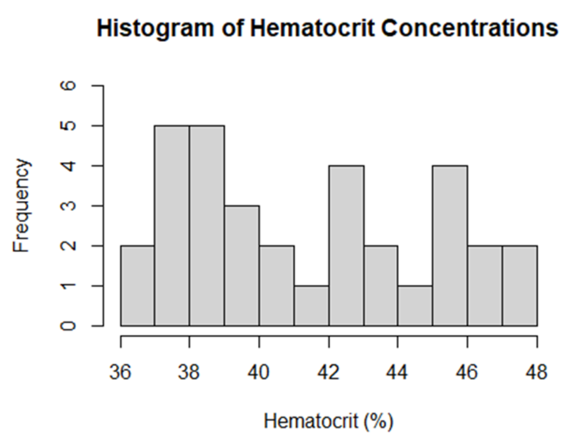
Appendix 2: Data Distributions (Pre and Post-Log Transformation)

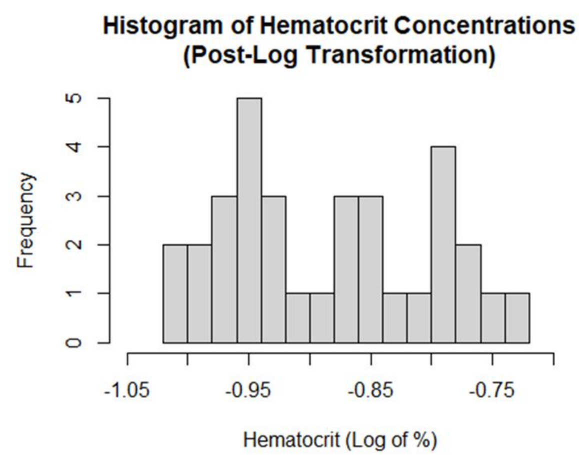


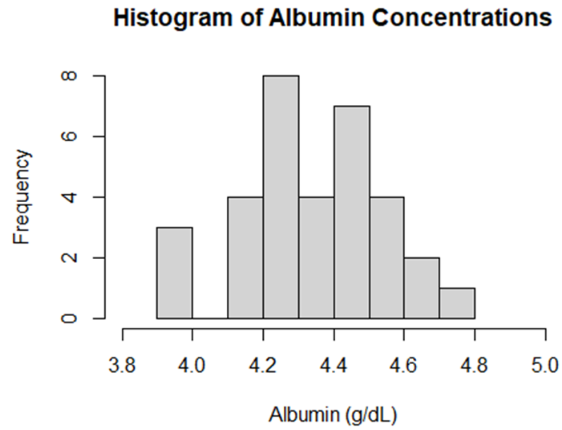


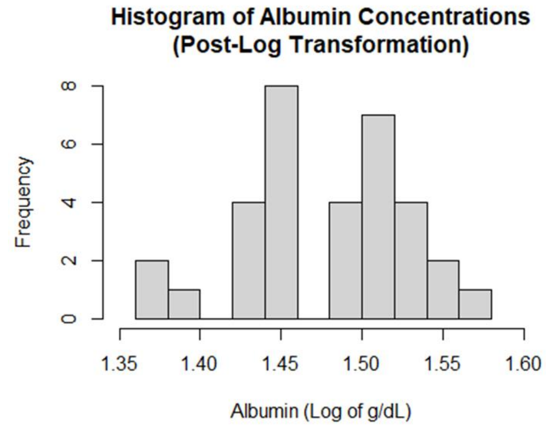


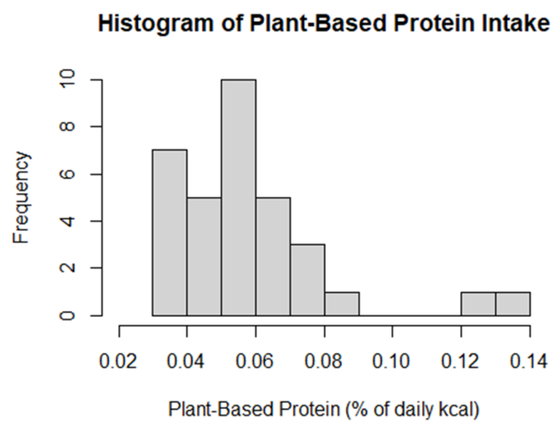


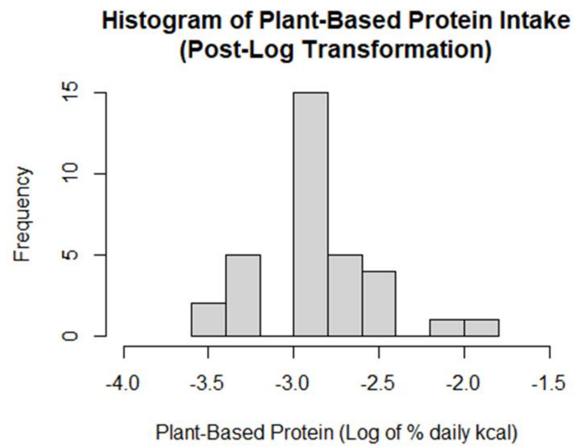


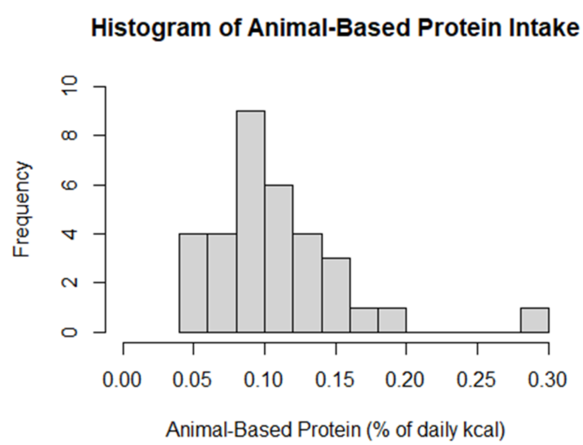


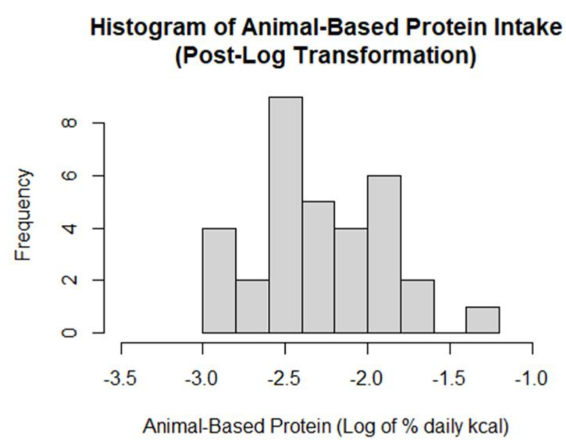

Histogram of Albumin Concentrations
Frequency
0
2
4
6
8
3.8
4.0
4.2
4.4
4.6
4.8
5.0
Albumin (g/dL)

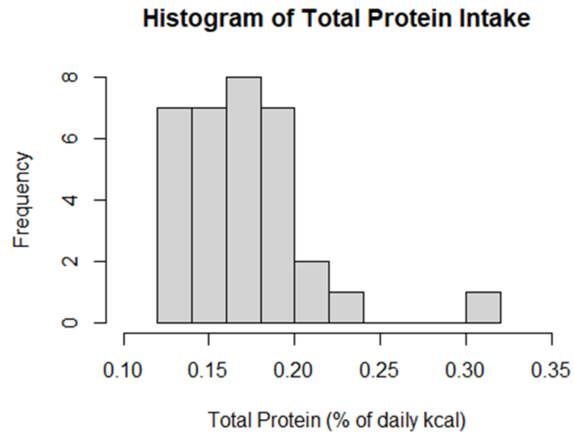

Histogram of Albumin Concentrations
(Post-Log Transformation)
Frequency
0
2
4
6
8
1.35
1.40
1.45
1.50
1.55
1.60
Albumin (Log of g/dL)

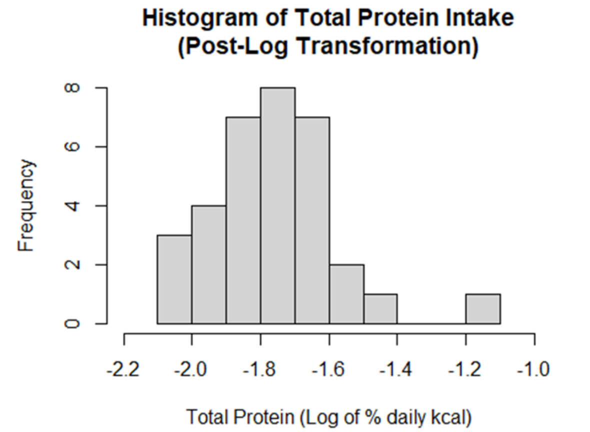

Histogram of Plant-Based Protein Intake
Frequency
0
2
4
6
8
10
0.02
0.04
0.06
0.08
0.10
0.12
0.14
Plant-Based Protein (% of daily kcal)

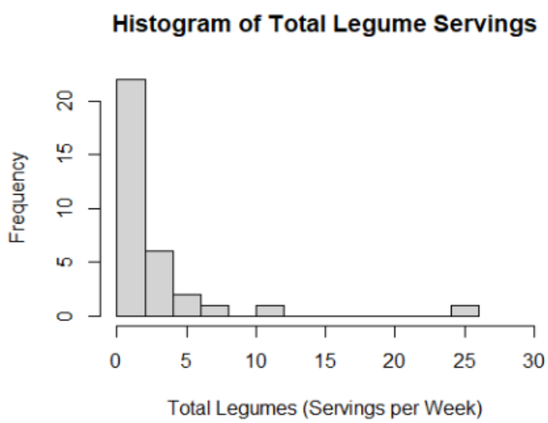

Histogram of Plant-Based Protein Intake
(Post-Log Transformation)
Frequency
0
5
10
15
-4.0
-3.5
-3.0
-2.5
-2.0
-1.5
Plant-Based Protein (Log of % daily kcal)

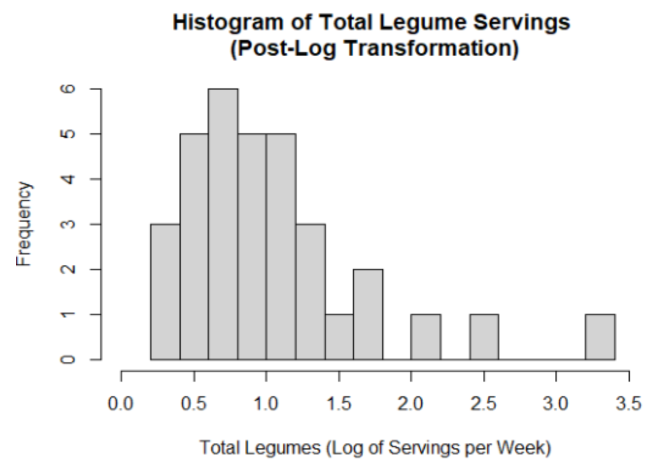

Histogram of Animal-Based Protein Intake
Frequency
0
2
4
6
8
10
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Animal-Based Protein (% of daily kcal)

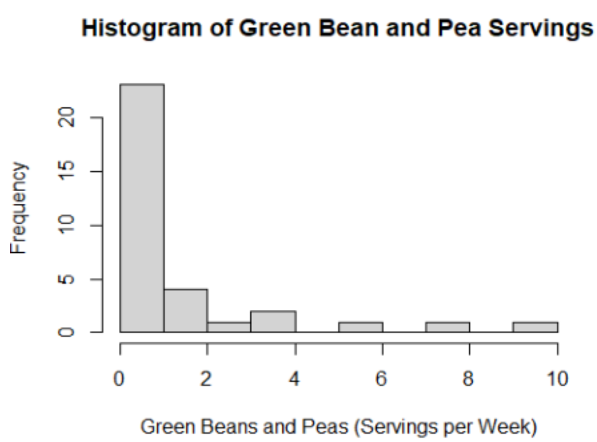

Histogram of Animal-Based Protein Intake
(Post-Log Transformation)
Frequency
0
2
4
6
8
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
Animal-Based Protein (Log of % daily kcal)

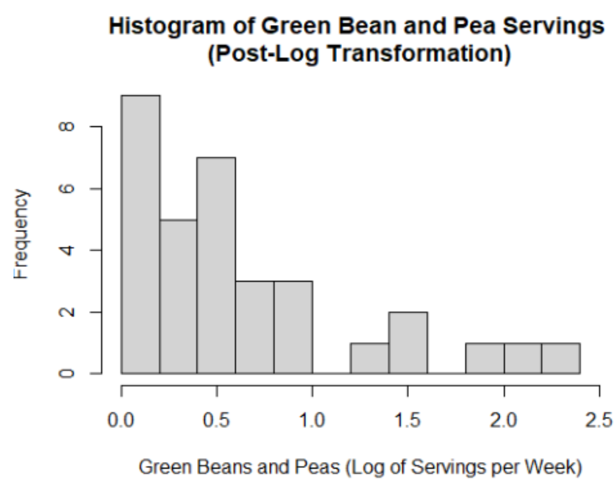

Histogram of Total Protein Intake
Frequency
0 2 4 6 8
0.10 0.15 0.20 0.25 0.30 0.35
Total Protein (% of daily kcal)

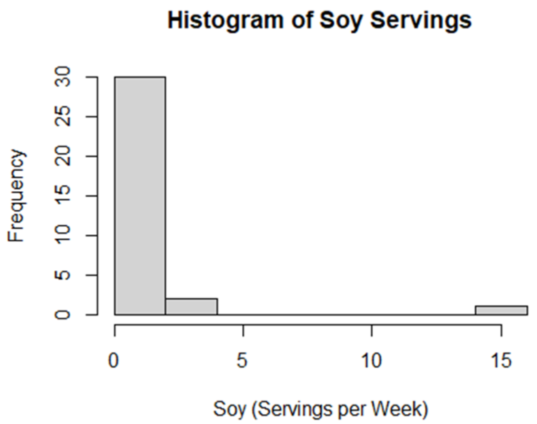

Histogram of Total Protein Intake
(Post-Log Transformation)
Frequency
0 2 4 6 8
-2.2 -2.0 -1.8 -1.6 -1.4 -1.2 -1.0
Total Protein (Log of % daily kcal)

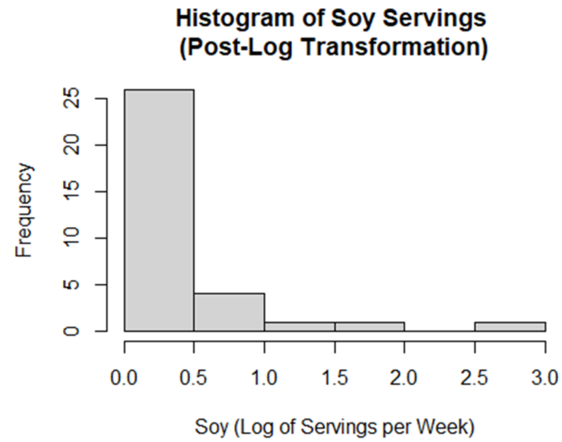

Histogram of Total Legume Servings
Frequency
0 5 10 15 20
0 5 10 15 20 25 30
Total Legumes (Servings per Week)

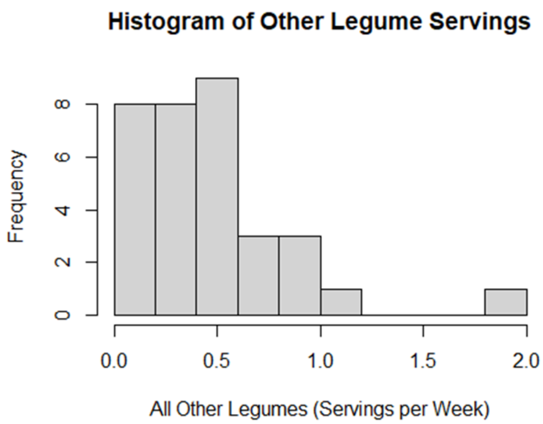

Histogram of Total Legume Servings
(Post-Log Transformation)
Frequency
0 1 2 3 4 5 6
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Total Legumes (Log of Servings per Week)

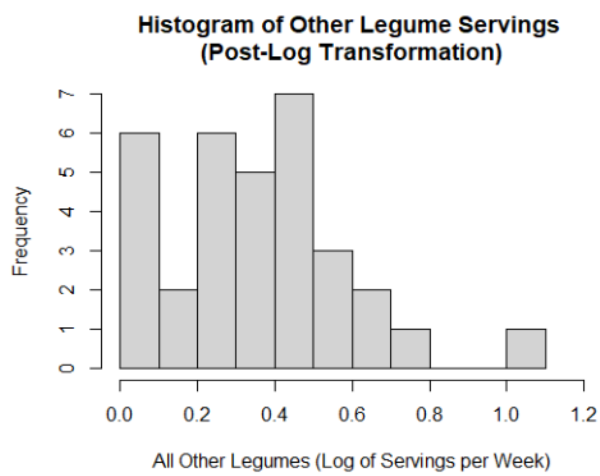

Histogram of Green Bean and Pea Servings
Frequency
0 5 10 15 20
0 2 4 6 8 10
Green Beans and Peas (Servings per Week)

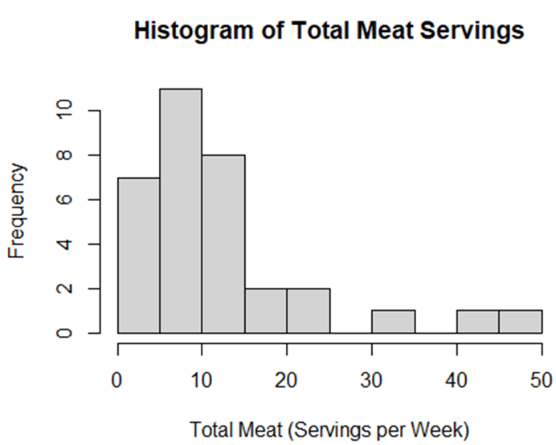

Histogram of Green Bean and Pea Servings
(Post-Log Transformation)
Frequency
0 2 4 6 8
0.0 0.5 1.0 1.5 2.0 2.5
Green Beans and Peas (Log of Servings per Week)

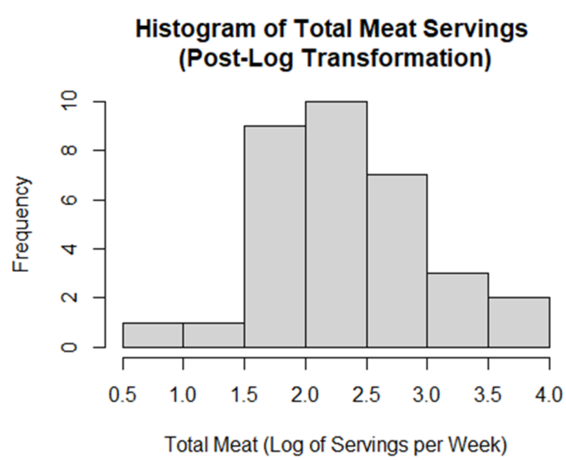

Histogram of Soy Servings
Frequency
0 5 10 15 20 25 30
0 5 10 15
Soy (Servings per Week)

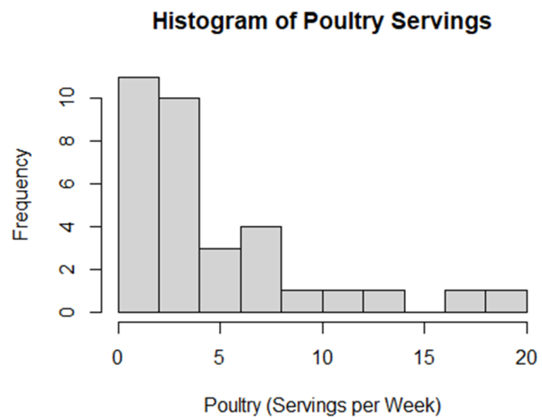

Histogram of Soy Servings
(Post-Log Transformation)
Frequency
0 5 10 15 20 25
0.0 0.5 1.0 1.5 2.0 2.5 3.0
Soy (Log of Servings per Week)

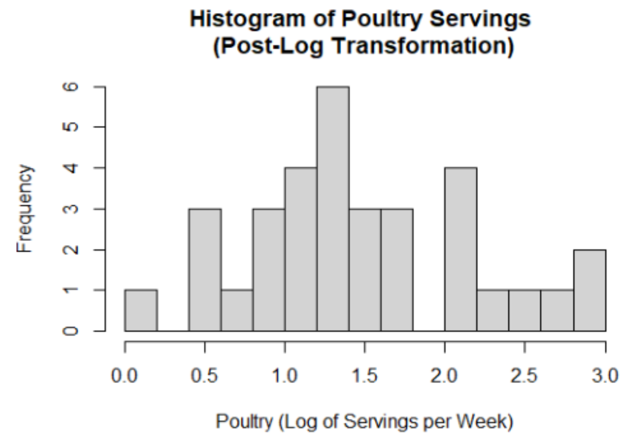

Histogram of Other Legume Servings
Frequency
0 2 4 6 8
0.0 0.5 1.0 1.5 2.0
All Other Legumes (Servings per Week)

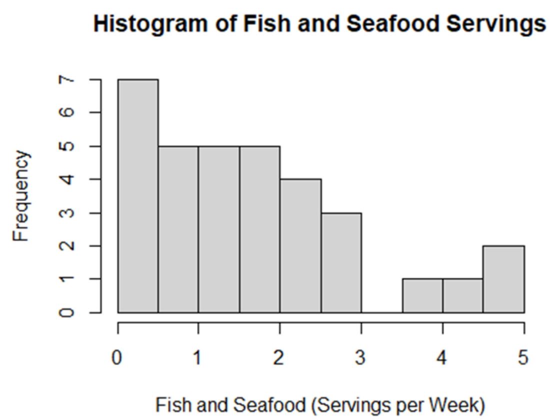

Histogram of Other Legume Servings
(Post-Log Transformation)
Frequency
0 1 2 3 4 5 6 7
0.0 0.2 0.4 0.6 0.8 1.0 1.2
All Other Legumes (Log of Servings per Week)

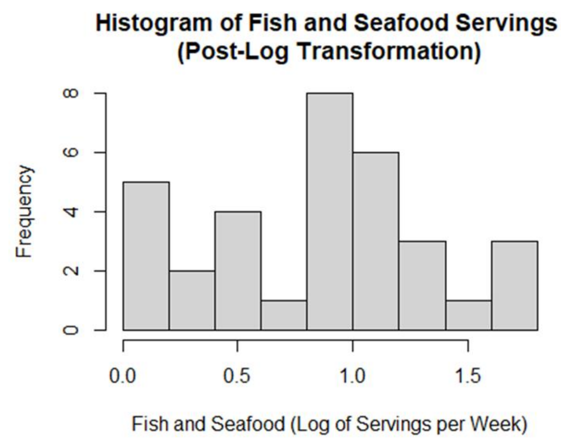

Histogram of Total Meat Servings
Frequency
0 2 4 6 8 10
0 10 20 30 40 50
Total Meat (Servings per Week)

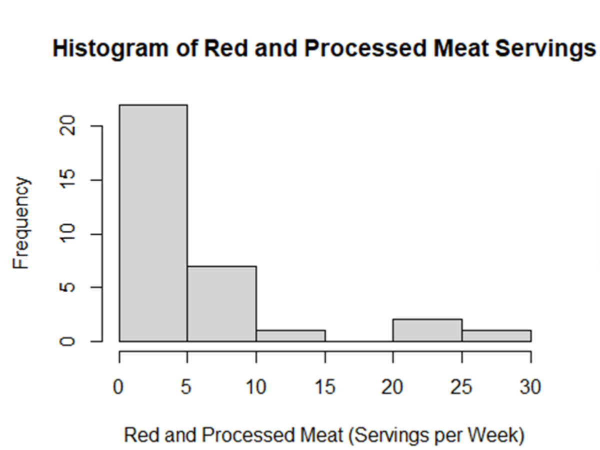

Histogram of Total Meat Servings
(Post-Log Transformation)
Frequency
0 2 4 6 8 10
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
Total Meat (Log of Servings per Week)

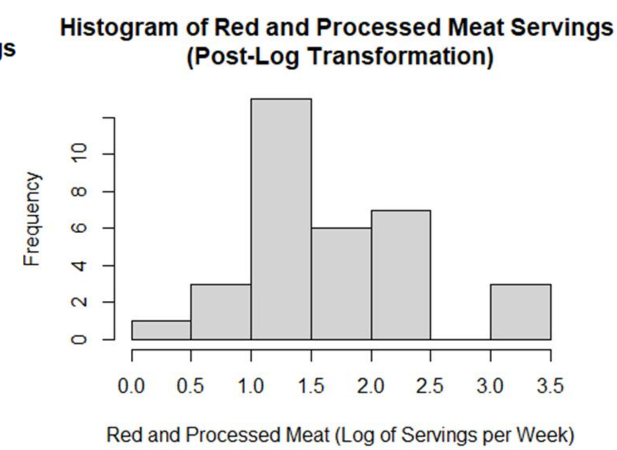

Histogram of Poultry Servings
Frequency
Poultry (Servings per Week)


Histogram of Poultry Servings
(Post-Log Transformation)
Frequency
Poultry (Log of Servings per Week)

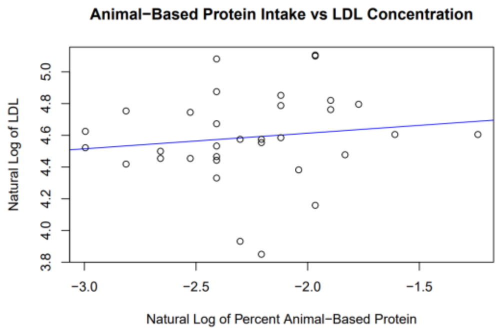

Histogram of Fish and Seafood Servings
Frequency
Fish and Seafood (Servings per Week)

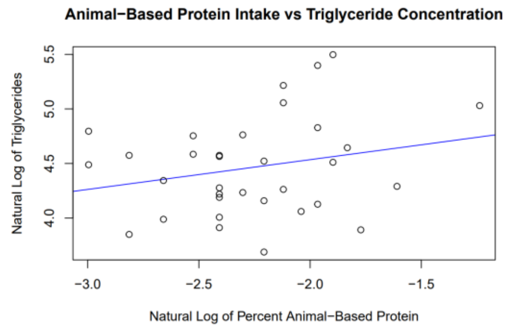

Histogram of Fish and Seafood Servings
(Post-Log Transformation)
Frequency
Fish and Seafood (Log of Servings per Week)

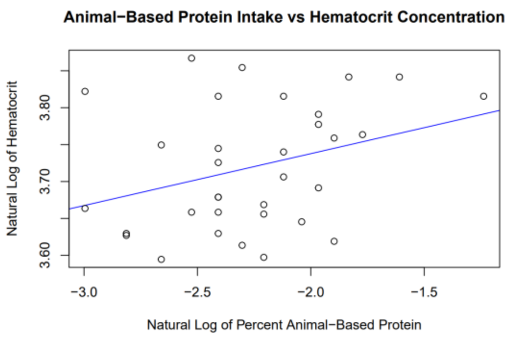

Histogram of Red and Processed Meat Servings
Frequency
Red and Processed Meat (Servings per Week)

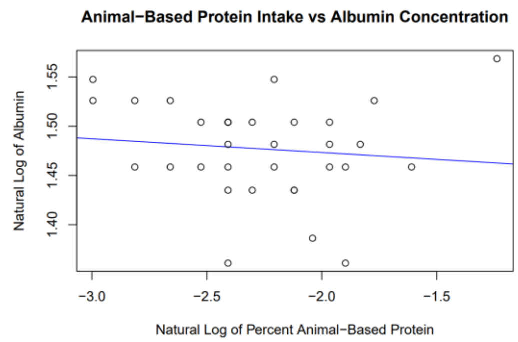

Histogram of Red and Processed Meat Servings
(Post-Log Transformation)
Frequency
Red and Processed Meat (Log of Servings per Week)

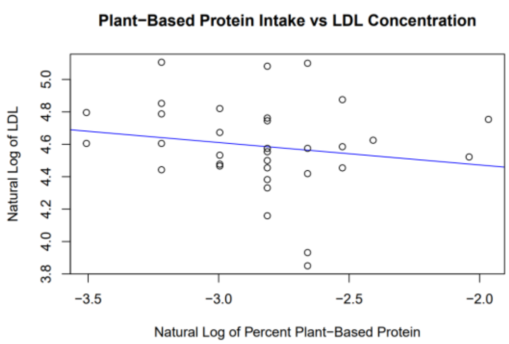
Appendix 3: Linear Regression (Model 1) Results - Scatter/Line Charts

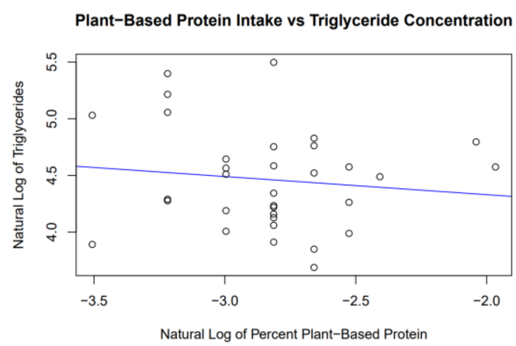


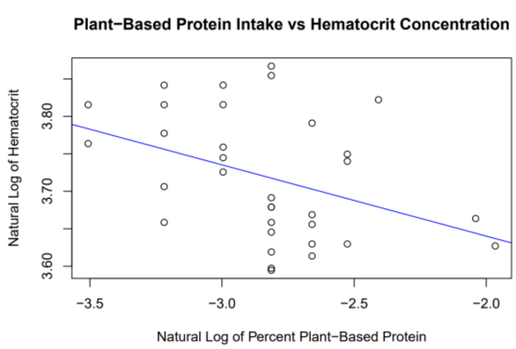


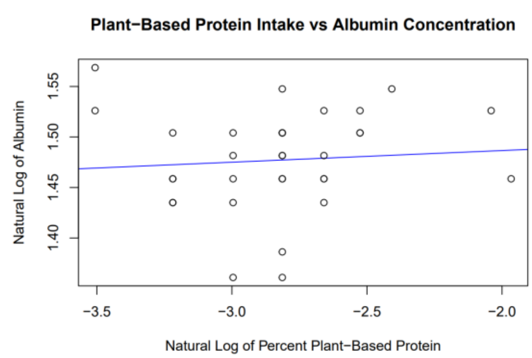












Appendix 4: ANOVA Test Results

Table 7. Biomarker Variance between Servings Tertiles of Eight Protein Foods (ANOVA), R Output

		Degrees of Freedom	***Sum of Squares***	***Mean Sum of Squares***	***F-value***	***p-value***	***Significance code***
Total Legume vs LDL	Total Legume	2	0.27	0.13	1.78	0.185	
	Residuals	30	2.2	0.074			
Total Legume vs Triglycerides	Total Legume	2	0.35	0.18	0.889	0.422	
	Residuals	30	5.9	0.2			
Total Legume vs Hematocrit	Total Legume	2	0.024	0.012	1.86	0.174	
	Residuals	30	0.19	0.0064			
Total Legume vs Albumin	Total Legume	2	0.0033	0.0016	1.02	0.371	
	Residuals	30	0.048	0.0016			
Green Beans/Peas vs LDL	Green Beans/Peas	2	0.44	0.22	3.26	**0.0526**	.
	Residuals	30	2.05	0.068			
Green Beans/Peas vs Triglycerides	Green Beans/Peas	2	1.1	0.56	3.24	**0.0534**	.
	Residuals	30	5.2	0.17			
Green Beans/Peas vs Hematocrit	Green Beans/Peas	2	0.061	0.03	5.93	**0.00668**	**
	Residuals	30	0.15	0.0051			
Green Beans/Peas vs Albumin	Green Beans/Peas	2	0.0011	0.0006	0.333	0.719	
	Residuals	30	0.05	0.0017			
Soy vs LDL	Soy	2	0.072	0.036	0.446	0.644	
	Residuals	30	2.42	0.081			
Soy vs Triglycerides	Soy	2	0.077	0.039	0.187	0.830	
	Residuals	30	6.2	0.21			
Soy vs Hematocrit	Soy	2	0.027	0.013	2.12	0.138	
	Residuals	30	0.19	0.0063			
Soy vs Albumin	Soy	2	0.0005	0.0002	0.138	0.872	
	Residuals	30	0.051	0.0017			
All Other Legumes vs LDL	All Other Legumes	2	0.17	0.087	1.13	0.337	
	Residuals	30	2.3	0.077			
All Other Legumes vs	All Other Legumes	2	0.58	0.29	1.53	0.233	

Triglycerides	Residuals	30	5.7	0.19			
All Other Legumes vs Hematocrit	All Other Legumes	2	0.0017	0.0008	0.116	0.891	
	Residuals	30	0.21	0.0071			
All Other Legumes vs Albumin	All Other Legumes	2	0.0033	0.0017	1.03	0.368	
	Residuals	30	0.048	0.0016			
Total Meat vs LDL	Total Meat	2	0.19	0.093	1.2	0.315	
	Residuals	30	2.3	0.077			
Total Meat vs Triglycerides	Total Meat	2	0.096	0.048	0.235	0.792	
	Residuals	30	6.2	0.21			
Total Meat vs Hematocrit	Total Meat	2	0.012	0.006	0.885	0.423	
	Residuals	30	0.2	0.0068			
Total Meat vs Albumin	Total Meat	2	0.0088	0.0044	3.08	**0.0608**	.
	Residuals	30	0.043	0.0014			
Poultry vs LDL	Poultry	2	0.16	0.08	1.02	0.372	
	Residuals	30	2.3	0.078			
Poultry vs Triglycerides	Poultry	2	1.2	0.61	3.6	**0.0397**	*
	Residuals	30	5.05	0.17			
Poultry vs Hematocrit	Poultry	2	0.02	0.01	1.55	0.23	
	Residuals	30	0.19	0.0065			
Poultry vs Albumin	Poultry	2	0.0023	0.0011	0.699	0.505	
	Residuals	30	0.049	0.0016			
Fish and Seafood vs LDL	Fish and Seafood	2	0.092	0.046	0.573	0.57	
	Residuals	30	2.4	0.08			
Fish and Seafood vs Triglycerides	Fish and Seafood	2	0.052	0.026	0.126	0.882	
	Residuals	30	6.2	0.21			
Fish and Seafood vs Hematocrit	Fish and Seafood	2	0.032	0.016	2.66	**0.0868**	.
	Residuals	30	0.18	0.0061			
Fish and Seafood vs Albumin	Fish and Seafood	2	0.007	0.0035	2.35	0.113	
	Residuals	30	0.045	0.0015			
Red and Processed Meat vs LDL	Red and Processed Meat	2	0.34	0.17	2.38	0.11	

	Residuals	30	2.2	0.072			
Red and Processed Meat vs Triglycerides	Red and Processed Meat	2	0.078	0.039	0.19	0.828	
	Residuals	30	6.2	0.21			
Red and Processed Meat vs Hematocrit	Red and Processed Meat	2	0.019	0.0094	1.44	0.254	
	Residuals	30	0.2	0.0065			
Red and Processed Meat vs Albumin	Red and Processed Meat	2	0.0025	0.0012	0.752	0.48	
	Residuals	30	0.049	0.0016			

Significance Codes: . p<0.1, *p<0.05, **p<0.01