

DIET-INDUCED CHANGES TO METABOLIC HEALTH

by

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TABLE OF CONTENTS

1. INTRODUCTION	1
The Obesity Epidemic: A Challenge for Nutrition Research.....	1
The Evolution of Nutrition Research.....	2
Complications of Overnutrition: Adipose Deposition and Metabolic Dysregulation.....	5
Assessing Metabolic Health: Current Practices and Challenges	6
Inflammatory, Gut Microbial, and Metabolomic Contributions to Metabolic Health.....	9
Inflammation.....	10
Gut Microbiota.....	11
Metabolomics.....	13
Dissertation Aims.....	15
References Cited	19
2. TWELVE WEEKS OF DAILY LENTIL CONSUMPTION IMPROVES FASTING CHOLESTEROL AND POSTPRANDIAL GLUCOSE AND INFLAMMATORY RESPONSES—A RANDOMIZED CLINICAL TRIAL.....	28
Contribution of Authors and Co-Authors	28
Manuscript Information	29
Abstract.....	30
Introduction.....	30
Materials and Methods.....	31
Ethics Statement.....	31
Study Population.....	31
Research Design.....	32
Anthropometrics	32
Elevated Postprandial Triglyceride Screening.....	32
Randomization	32
Lentil Sampling and Nutritional Analysis.....	32
Dietary Intervention.....	33
Diet Adherence.....	34
Recent and Habitual Diet Surveys	34
Satiety and Gastrointestinal Symptom Surveys.....	34
Blood Sampling	35
High-Fat Meal Challenge.....	35
Analysis of Blood Markers	35
Analysis of Inflammation Biomarkers.....	35
Statistical Analysis.....	35
Results.....	36

TABLE OF CONTENTS CONTINUED

General Characteristics of Participants	36
Anthropometric Measures.....	38
Habitual Diet Analysis	38
Satiety During the Intervention.....	39
Gastrointestinal Symptoms During the Intervention	39
Fasting Lipid and Glycemic Measures	39
Postprandial Lipid Response.....	40
Postprandial Glycemic Response.....	40
Fasting and Postprandial Inflammation	42
Discussion	42
Conclusion	46
References.....	48
 3. ACUTE LENTIL CONSUMPTION ALTERS THE RELATIONSHIP BETWEEN POSTPRANDIAL BILE ACIDS AND GLUCOSE AND TRIGLYCERIDE METABOLISM: A RANDOMIZED CROSSOVER TRIAL.....	 62
Contribution of Authors and Co-Authors	62
Manuscript Information	63
Abstract.....	64
Introduction.....	65
Materials and Methods.....	68
Ethics Statement.....	68
Study Population.....	68
Research Design.....	68
Anthropometrics	69
Randomization	69
Lentil Nutritional Analysis.....	70
High-Fat Meal Challenge.....	70
Recent and Habitual Diet Surveys	71
Satiety Surveys.....	72
Blood Sampling	72
Analysis of Blood Markers	73
Analysis of Inflammatory Biomarkers.....	73
Bile Acid Analysis.....	73
Statistical Analysis.....	76
Results.....	78
General Characteristics of Participants	78
Habitual Diet Analysis	79
Satiety During the High-Fat Meal Challenge	80
Postprandial Lipid, Glucose, and Inflammatory Responses	80

TABLE OF CONTENTS CONTINUED

Fasting and Postprandial Bile Acids	82
Discussion	87
Conclusions	94
Aknowledgements	95
References	96
 4. POLYPHENOL-RICH ARONIA INTERVENTION ALTERS GLUCOSE AND LIPID METABOLISM AND INFLUENCES MICROBIAL DIVERSITY: A RANDOMIZED CLINICAL TRIAL	 114
Contribution of Authors and Co-Authors	114
Manuscript Information	115
Abstract	116
Introduction	116
Materials and Methods	118
Ethics Statement	118
Study Population	119
Research Design	119
Anthropometrics	120
Randomization	120
Supplement Intervention	121
Aronia Juice LCMS and NMR Analysis	121
Habitual Diet Assessment	123
High-Fat Meal Challenge	123
Blood Sampling	124
Analysis of Blood Markers	124
Analysis of Inflammation Biomarkers	125
Stool Collection	125
Genomic DNA Extraction and Microbial Analysis	125
Statistical Methods	126
Results	128
General Characteristics of Participants	128
Anthropometric Measures	131
Habitual Diet Analysis	131
Fasting and Postprandial Lipid and Glycemic Measures	132
Fasting and Postprandial Inflammation	135
Alpha and Beta Diversity	135
Differential Microbial Taxa	136
Discussion	137
Conclusion	142
References	143

TABLE OF CONTENTS CONTINUED

5. CONCLUSION.....	156
6. CUMULATIVE REFERENCES CITED.....	162

LIST OF TABLES

Table	Page
1. Table 2.1. Overview of intervention meal composition by group.	33
2. Table 2.2. Participant characteristics at baseline, grouped by assigned dietary intervention..	38
3. Table 2.3. Habitual dietary intake as depicted by HEI total and component scores before and after the 12-week intervention....	39
4. Table 2.4. Change in fasting blood markers (post-intervention-pre-intervention), grouped by dietary condition.....	40
5. Table 2.S.1. Nutrient and functional analysis of whole green lentils used in the 12-week intervention.....	59
6. Table 2.S.2. Overview of intervention meals and their nutrient profile.....	60
7. Table 2.S.3. Change in fasting and postprandial inflammation by meal group before and after the 12-week dietary intervention.	61
8. Table 3.1. Identified bile acids and their classifications	76
9. Table 3.2. Participant characteristics (n=24).....	79
10. Table 3.S.1. Nutrient and functional analysis of whole green lentils used in the lentil high-fat meal condition.	109
11. Table 3.S.2. Nutrient profile of lentil and control high-fat meals.....	110
12. Table 3.S.3. Habitual dietary intake (n=24) as represented by the 2015 Healthy Eating Index (HEI) total and component scores	111
13. Table 3.S.4. Fasting inflammation levels prior to high-fat meal challenge by meal group	112
14. Table 3.S.5. Postprandial inflammation by intervention group	113
15. Table 4.1. Carbohydrate and polyphenol content of Aronia juice.	122
16. Table 4.2. Participant characteristics at baseline, grouped by dietary intervention (n=13)	130

LIST OF TABLES CONTINUED

Table	Page
17. Table 4.S.1. List of foods to avoid.....	151
18. Table 4.S.2. Habitual dietary intake as represented by HEI total and component scores by intervention group.	152
19. Table 4.S.3. Change in fasting and postprandial inflammation by intervention group.....	153
20. Table 4.S.4. PERMANOVA results at each taxonomic level for the time and juice interaction.....	154
21. Table 4.S.5. Microbial taxa associated with Aronia juice consumption before multiple testing correction.	155

LIST OF FIGURES

Figure	Page
1. Figure 1.1. Schematic displaying the contribution of metabolomic, inflammation, and gut microbiota assessments in determining the impact of diet on metabolic health.....	18
2. Figure 2.1. A CONSORT flowchart of the study design and enrollment....	37
3. Figure 2.2. Summary plots of individual changes (post-intervention – pre-intervention) in triglyceride area under the curve (dTG _{AUC}).	41
4. Figure 2.3. Summary plots of individual changes (post-intervention – pre-intervention) in glucose area under the curve (dGLU _{AUC}).	41
5. Figure 2.4. Main effect plot for meal group from A) IL-17 _{AUC} and B) IL-1 β _{AUC} linear models.	42
6. Figure 2.S.1. Timeline of study events....	52
7. Figure 2.S.2. Survey questions assessing satiety and gastrointestinal issues	53
8. Figure 2.S.3. Differences in daily fiber intake from pre- to post- intervention by meal group.	54
9. Figure 2.S.4. Self-reported satiety measures over the 12-week intervention by meal group.	55
10. Figure 2.S.5. Self-reported gastrointestinal symptoms over the 12-week intervention by meal group.	56
11. Figure 2.S.6. Postprandial triglyceride (TG) response to a 50-gram, high-fat meal challenge by meal group before and after the 12-week dietary intervention....	57
12. Figure 2.S.7. Postprandial glucose (GLU) response to a 50-gram, high-fat meal challenge by meal group before and after the 12-week dietary intervention.	58
13. Figure 3.1. A CONSORT flowchart of study design and enrollment.	78
14. Figure 3.2. Postprandial A) Triglyceride (TG), B) Glucose (GLU), and C) Insulin (INS) response to the high-fat meal challenge by meal group	81

LIST OF FIGURES CONTINUED

Figure	Page
15. Figure 3.3. Postprandial plasma bile acid responses to the high-fat meal challenge by meal group	83
16. Figure 3.4. Postprandial plasma bile acid concentrations summarized as area under the curve by meal group	84
17. Figure 3.5. Postprandial plasma bile acid responses of the bile acids with the greatest response ($C_{\max}$ – baseline) to the high-fat meal challenge by meal group.	85
18. Figure 3.6. Spearman correlation between postprandial bile acids and postprandial $TGAUC$, $GLUAUC$, and $INSAUC$ in A) Lentil and B) Control meal conditions.....	86
19. Figure 3.7. Spearman correlations between postprandial bile acids and triglycerides summarized as area under the curve (AUC) in Lentil and Control meal conditions.....	87
20. Figure 3.S.1. Questionnaire assessing satiety during the high-fat meal challenge	103
21. Figure 3.S.2. Hourly satiety measures over the 4-hour high-fat meal challenge	104
22. Figure 3.S.3. Summary plot of A) $TGAUC$, B) $GLUAUC$, and C) $INSAUC$ by meal condition.....	105
23. Figure 3.S.4. Fasting plasma bile acid abundance.	106
24. Figure 3.S.5. Correlation heat matrix between fasting bile acids and anthropometric and metabolic markers of participants at baseline (n=24).....	107
25. Figure 3.S.6. Change in plasma bile acid abundance after meal consumption	108
26. Figure 4.1. A CONSORT flowchart of the study design and enrollment.	129
27. Figure 4.2. Radar plot depicting habitual dietary intake of participants by intervention group at baseline as described by average HEI component scores.....	132

LIST OF FIGURES CONTINUED

Figure	Page
28. Figure 4.3. Summary plots of changes (post-intervention – pre-intervention) in fasting blood glucose and lipids.....	133
29. Figure 4.4. Summary plot of individual changes (post-intervention – pre-intervention) in glucose area under the curve (dGLU _{AUC})	134
30. Figure 4.5. Alpha and beta-diversity of the gut microbiome... ..	136
31. Figure 4.S.1. Summary plot of individual changes (post-intervention – pre-intervention) in triglyceride area under the curve (dTG _{AUC}).	148
32. Figure 4.S.2. Postprandial triglyceride (TG) response to a 50-gram, high-fat meal challenge by meal group before and after the 30-day intervention.....	149
33. Figure 4.S.3. Postprandial glucose (GLU) response to 50-gram, high-fat meal challenge by meal group before and after the 30-day intervention.....	150

ABSTRACT

The accumulation of excess body fat, particularly visceral adipose, is linked to metabolic and endocrine disturbances which contribute to a systemic, low-grade, inflammatory state and abnormal glucose and lipid metabolism. These metabolic states are primary drivers of non-communicable, chronic diseases including nonalcoholic fatty liver disease, type 2 diabetes and cardiovascular disease which are amongst the leading causes of morbidity and mortality worldwide. Diet contributes to visceral adiposity and subsequent metabolic dysregulations. As such, healthy dietary changes are a key strategy to prevent and reduce the burden of obesity-associated disease. Assessing changes to metabolism in response to diet is critical to the formation of dietary recommendations to address the obesity epidemic. However, physiological responses to diet are variable and depend on many factors, thereby complicating metabolic health assessment. The inclusion of inflammatory, gut microbial, and metabolomic measures in addition to postprandial measurements of traditional metabolic markers help establish a more accurate picture of metabolic health and response to diet. Changes to postprandial metrics of lipid and glucose metabolism and inflammation in response to chronic lentil consumption were examined in a metabolically at-risk population. The potential of acute lentil intake to favorably alter the postprandial bile acid response as well as postprandial lipid, glucose, and inflammation responses was tested in a crossover trial of overweight and obese individuals. In addition, the impact of polyphenol-rich aronia juice on gut microbial taxonomy and postprandial lipidemic, glycemic, and inflammatory responses was investigated. Chronic lentil consumption improved fasting cholesterol levels as well as postprandial glucose and inflammatory responses. While acute lentil intake did not modify the magnitude of postprandial glucose, lipid, or inflammatory responses, differential relationships between bile acids and postprandial metabolism were identified. Aronia juice supplementation attenuated fasting cholesterol levels, lowered postprandial glucose responses, and altered gut microbial diversity. This body of work demonstrates the contributions of postprandial metabolism, inflammation, and gut microbial taxonomy to metabolic response to dietary interventions and highlights their utility in the accurate assessment of metabolic health.

CHAPTER ONE

INTRODUCTION

The Obesity Epidemic: A Challenge for Nutrition Research

Non-communicable diseases (NCDs) associated with overweight and obesity such as nonalcoholic fatty liver disease, type 2 diabetes, cardiovascular disease, and certain cancers are the leading causes of morbidity and mortality worldwide.¹ In 2016, NCDs accounted for 70% of the 57 million deaths globally. Cardiovascular diseases account for most NCD deaths (17.9 million people annually), followed by cancers (9.3 million), and diabetes (2.0 million). Importantly, NCD deaths are on the rise across all age groups, income levels, and regions.² Increases in NCD morbidity and mortality rates mirror the increased prevalence of overweight and obesity. The World Obesity Federation predicts that over half of the global population, over 4 billion people, will be classified as overweight (BMI ≥ 25 kg/m²) or obese (BMI ≥ 30 kg/m²) by 2035.³ The associated global economic impact of obesity and its related chronic diseases will reach \$4.32 trillion by the same year, representing almost 3% of the global gross domestic product.³ These estimated costs are likely underestimated as they do not capture the increased financial burden of diet-related chronic disease in low- and middle- income countries, which have the highest projected increases in obesity.³

The pathogenesis of obesity is influenced by a multitude of genetic and environmental factors that collectively influence and regulate energy balance.⁴ In addition to genetics, modifiable lifestyle factors such as physical activity and diet are key determinants of obesity.⁵ Diet is a known contributor to increased visceral adiposity and subsequent metabolic

dysregulations characteristic of obesity.⁶⁻⁸ As such, healthy dietary changes represent a key strategy to prevent and reduce the burden of obesity-associated disease. In addition to improvements in anthropometric indicators like weight and visceral adiposity, dietary alterations can improve metabolic health by decreasing glycemic, lipidemic, and inflammatory responses to food consumption.⁹⁻¹¹ Assessing changes to metabolism and improvements to metabolic risk in response to diet is critical to the formation of dietary recommendations to address the obesity epidemic and reduce the burden of obesity-related diseases. However, interindividual differences in response to diet present a challenge to accurately assessing metabolic health responses to dietary interventions. The assessment of reliable biomarkers of metabolic health that help account for interindividual variability is a critical aspect to nutrition research and clinical management of metabolic disease.

The Evolution of Nutrition Research

The relationship between food and health has been recognized for centuries. Philosophers and scholars in ancient Chinese, Greek, Egyptian, and Roman cultures made early observations regarding the impact of food on health.¹² Hippocrates, the Greek philosopher recognized as the figurehead of modern medicine, emphasized the role of diet in health with his famous quote: “Let food be thy medicine and medicine be thy food.” Although the relationship between food and health has been studied for centuries, the field of modern nutrition research is still in its nascent stage. The golden age of nutrition occurred less than 100 years ago with the identification of essential vitamins and minerals and the determination of macronutrient energy values.¹³ These discoveries led to a burgeoning interest in experimental and clinical nutrition and set the stage for modern nutrition science.

As nutrition research developed its roots with a focus on single nutrient discoveries, modern clinical nutrition practice was established with the foundation of the American Dietetic Association in 1917.¹⁴ The original mission of its 58 members was to conserve food resources and improve public health during World War I.¹⁴ In the next 10-20 years, World War II and the Great Depression contributed to widespread food shortages and increased employment of dietitians.¹³ As food security became an international concern, the US government, the League of Nations, and the British Medical Association independently commissioned nutrition scientists to generate minimum dietary requirements.¹³ In 1943, the first recommended dietary allowances (RDAs) were published providing guidelines for total energy requirements as well as select nutrients including calcium, iron, vitamins, and protein.¹³ These events established a precedent for nutrition research and policy recommendations to focus on single nutrients and the prevention of their associated deficiency-related diseases such as scurvy, rickets, and nutritional anemias.

From the 1950s through the 1970s, accelerating economic development and modernization of the agricultural and food processing industries ushered in a transition from deficiency-based nutrition research towards the emergent burden of overnutrition.¹³ In high income countries, changes in food processing and industrialization led to large increases in low-cost, fortified, shelf-stable foods. As a result, calorie malnutrition and micronutrient deficiencies fell sharply. However, these changes also resulted in a shift towards overnutrition characterized by excessive nutrient intake, accelerated fat deposition, and health impairments.¹⁵ This shift marked the beginning of an obesity epidemic and a concomitant increase in obesity-related diseases including type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease.¹⁶

Rising rates of these diseases altered the direction of nutrition research from states of nutritional deficiency towards overnutrition and its associated health concerns.¹⁵ Nutritional policy guidelines also adjusted to address these concerns with the 1980 Dietary Guidelines for Americans largely focused on reduced fat, sugar, and sodium intake.¹³ At the same time, less wealthy countries began to experience a double burden of malnutrition resulting from health concerns associated with both undernutrition and overnutrition – a trend that still persists.¹³

From the late 20th century to present day, the increased disease burden from overnutrition has remained a primary focus of nutrition research. However, advances in research over the past 30 years have established that single nutrient theories are inadequate to explain the effects of diet on chronic disease burden. These advancements pushed the field of nutrition research beyond the single-nutrient focused framework of RDAs towards a more complex examination of foods and diet patterns. The effect of food on health is multifaceted and is influenced by both food-related and interindividual factors. Food-related factors include macronutrient and micronutrient composition, dietary bioactive components, meal structure and food matrix effects, and food preparation and processing methods.¹³ Interindividual influences include physiological, environmental, genetic, and behavioral factors.¹⁷ The complexity of food effects, their interactions with interindividual factors, and the extended duration of chronic disease development are major challenges to nutrition research and the establishment of cause-and-effect relationships between diet and health. Despite these challenges, there is no doubt that diet has a major effect on disease risk. While nutrition research continues to focus on obesity-related chronic disease, these developments provide a new approach that begins to recognize the great complexity between food and health.

Complications of Overnutrition: Adipose Deposition and Metabolic Dysregulation

The accumulation of excess body fat typically occurs gradually, driven by overnutrition - a prolonged energy imbalance between calorie intake and expenditure, where more calories are consumed than burned through physical activity and basal metabolic processes. This surplus energy is stored, mainly as triglycerides, in adipose tissue.⁵ Increases in adiposity are distributed between subcutaneous and visceral adipose tissue. Visceral adipose tissue mass, including omental and mesenteric fat, is mechanistically linked to metabolic and endocrine disturbances and adverse health outcomes.⁵ Increased visceral adiposity is accompanied by an influx of immune cells, particularly macrophages, into adipose tissue, partly due to tissue remodeling processes.^{6,7} Accumulation of adipocytes and immune cells in adipose tissue results in increased secretion of proinflammatory cytokines, hormones, and free fatty acids to systemic circulation. These molecules serve as cellular mediators, initiating a complex interplay, or cross-talk, between adipose and other tissues. Inflammation mediators trigger a cascade of metabolic dysregulation via modulation of many pathways including the sympathetic nervous system, oxidative stress, and nuclear factor kappa B (NF- κ B) signaling.^{6,7,17-19}

Overnutrition also contributes to the accumulation of excess lipid intermediates in non-adipose tissues including skeletal muscle and hepatic tissue. Several mechanisms contribute to the accumulation of intrahepatic fat including increased flux of fatty acids to the liver from adipose tissue, increased de-novo lipogenesis secondary to excess energy intake, and reduced clearance of hepatic fat stores via very low-density lipoprotein secretion or β -oxidation of fatty acids.⁵ Increased intrahepatic fat, or steatosis, results in lipotoxicity and cellular dysfunction and promotes hepatic insulin resistance and inflammation.²⁰ Excessive lipid accumulation within

skeletal muscle disrupts insulin signaling and diminishes glucose uptake and utilization which fosters peripheral insulin resistance.⁵ Moreover, compromised mitochondrial function leads to diminished oxidative phosphorylation which exacerbates energy dysregulation and insulin resistance and impairs muscle function.⁵ These interconnected pathophysiological mechanisms in adipose, hepatic, and skeletal muscle tissues contribute to the dyslipidemia, insulin resistance, and low-grade systemic inflammation commonly observed in individuals classified as overweight and obese and drive the development of obesity-related disease.^{5,7,20}

Assessing Metabolic Health: Current Practices and Challenges

Both clinical and research practices rely on the measurement of biomarkers to define and diagnosis metabolic health. The accurate assessment of metabolic health is imperative to decreasing disease development stemming from metabolic complications. Most commonly, metabolic health is defined by optimal levels of five biomarkers including blood glucose, triglyceride and high-density lipoprotein cholesterol levels as well as blood pressure and central obesity as measured by waist circumference.²¹ In 2001, the National Cholesterol Education Program Adult Treatment Panel defined the presence of sub-optimal levels of any three of these five biomarkers as metabolic syndrome.²² Compared to healthy counterparts, individuals with metabolic syndrome have a 2-fold increased risk of cardiovascular disease, a 5-fold increased risk of type 2 diabetes, and a 1.5-fold increase in all-cause mortality.^{21,23} These biomarkers are commonly used to assess metabolic health and disease risk and to monitor change in metabolic status in response to medical treatments or lifestyle interventions. However, numerous biological mechanisms influence metabolic health beyond these measures and several challenges exist in using these standard biomarkers to define metabolic health.

While blood-based biomarkers used for diagnosis of metabolic syndrome are based on fasting levels, postprandial lipid²⁴⁻²⁶ and glycemic responses²⁷ are established as better predictors of metabolic disease risk. Furthermore, abnormal lipid and glucose responses to food intake are early indicators of metabolic impairment and contribute to metabolic dysregulations such as dyslipidemia, insulin resistance, and low-grade inflammation.^{11,28} Although these measures are more robust in predictive power, their assessment is not yet established in the clinical setting. Adoption in the clinical setting is slow for a variety of reasons including the need for standardization of meal challenges and timing of postprandial testing.²⁹ While postprandial measures of metabolic health are more commonly assessed in the research setting, their measurement is still far from standard practice.

Examining additional biomarkers beyond glucose and lipid metabolism measurements provides further insight into the pathophysiology of metabolic syndrome and can aid in identifying metabolic risk before the development of insulin resistance and dyslipidemia. In particular, the measurement of inflammatory biomarkers is highly informative, as these markers are primary drivers of metabolic disease progression. However, there is no consensus as to which markers of inflammation best represent the low-grade inflammation characteristic of obesity and metabolic conditions. C-reactive protein (CRP) is the most commonly measured inflammation biomarker and elevated levels have been associated with an increased risk of chronic disease.³⁰⁻³³ However, as a non-specific, acute phase protein, CRP may fail to capture subtle changes in inflammatory status or localized inflammation in metabolic tissues.³⁴ Additionally, inflammatory measurements tend to be unstable, influenced by many factors with high variability like physical activity and acute dietary intake that impact concentration at any given time.³² For these reasons,

the singular measurement of one inflammatory biomarker may not accurately reflect inflammatory status.^{32,35}

Measuring both fasting and dynamic concentrations of a combination of inflammatory markers provides a better assessment of metabolic, low-grade inflammation.³⁶ Measuring a wide range of inflammatory mediators provides better sensitivity in identifying the presence of low-grade inflammation and specificity by providing insight into inflammation location. For example, the pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) elicits metabolic effects within skeletal muscle, adipose, and bone tissue³² whereas cytokines interleukin (IL)-23 and IL-17 have pro-inflammatory and regulatory roles in the gut mucosa.³⁷ Similar to postprandial measures of glucose and lipid metabolism, dynamic inflammatory responses to metabolic challenges such as oral glucose or lipid tolerance tests provide a meaningful indication of metabolic health and have been shown to distinguish between healthy individuals and individuals with obesity.^{38,39} In addition, inflammatory responses to meal challenges are sensitive to modulation by pharmaceutical, lifestyle, and dietary interventions making them useful in investigating inflammatory response to diet.³⁹ Currently, the usefulness of these measures is confined to research as a lack of established ranges for “healthy” versus “abnormal” cytokine levels limits clinical utility.

Perhaps the largest challenge in accurately measuring metabolic health is interindividual variability. It is well-recognized that metabolic health and metabolic response to diet depend on many factors including habitual diet, genetic predisposition, exercise habits, and many other cultural, socioeconomic, and environmental factors. For example, clinical and research findings suggest that metabolic syndrome biomarkers are less accurate measures of metabolic health in

non-White ethnicities as well as children and adolescents.⁴⁰⁻⁴² This recognition has spawned the concept of personalized or precision nutrition, which moves away from a “one-size-fits-all” approach to nutrition and acknowledges interindividual factors when considering ideal diet.^{43,44} While this concept is far from clinical application, it is an active area of research. Perhaps the largest study exploring this concept is the Nutrition for Precision Health initiative from the National Institutes of Health.⁴⁵ This study is actively recruiting 10,000 individuals from diverse backgrounds to examine how a range of factors including genetics, lifestyle, medical history, gut microbial composition, and social health determinants influence individual response to diet. From this data, algorithms that predict response to dietary patterns may be developed and, ultimately, applied to provide customized nutritional guidance.

Inflammatory, Gut Microbial, and Metabolomic Contributions to Metabolic Health

As new insights into metabolic health and its myriad determinants are made, relying solely on traditional biomarkers to delineate the presence or absence of metabolic health may inadequately capture the complexity of contributing factors and contribute to inaccurate assessments. The use of inflammatory biomarkers as well as postprandial measurements of established markers of lipid and glucose metabolism to diagnose, risk stratify, and monitor metabolic health and response to diet intervention are useful in addressing these challenges. However, interindividual variability in response to diet is still poorly understood and represents a major focus of nutrition research. Prominent areas of investigation include inflammatory, gut microbial, and metabolomic contributions to metabolic health and variable responses to diet (**Figure 1**). The assessment of these factors in addition to traditional metabolic screening criteria

enhances understanding of metabolic health and correlations to dietary changes. Understanding interindividual variability in metabolic response to diet represents a critical step towards the development of effective dietary interventions to improve metabolic health.

Inflammation

Dietary patterns exert profound influence on inflammatory status.^{36,46-49} Diets high in saturated fats, refined sugars, and processed foods promote inflammation by triggering immune responses and oxidative stress.⁴⁸ Conversely, anti-inflammatory nutrients like omega-3 fatty acids, polyphenols, and fiber mitigate inflammation by modulating inflammatory signaling pathways and promoting immune regulation.⁴⁸ However, complex interactions among nutrients, inflammation, and metabolic health have led to an expanded scope of focus investigating the impact of dietary patterns on inflammation. One of the most well-studied diets is the Mediterranean diet which is high in several anti-inflammatory nutrients such as omega-3 fatty acids and polyphenols. Several cross-sectional studies have identified decreased CRP, TNF- α , and IL-6 levels in individuals following this diet.^{50,51} Studies also suggest that healthy eating patterns characterized by reduced postprandial glycaemia and lipemia are associated with reduced concentrations of markers of low-grade inflammation.³⁶ Thus, altered dietary intake to mitigate inflammation is useful in the prevention or treatment of obesity and its associated metabolic conditions.

Importantly, the relationship between diet and inflammation is bi-directional. In addition to the influence of diet on inflammation, there is evidence that inflammation modulates metabolic response to diet.^{52,53} Conditions of insulin resistance and dyslipidemia secondary to low-grade inflammation hinder the normal metabolism of ingested nutrients and alter their

impact on host health.⁵² As a result, differential responses to dietary interventions based on inflammatory phenotype may help explain interindividual variability in nutrition trials that identify participants as either responders or non-responders. For example, several studies have demonstrated that high levels of C-reactive protein (CRP) diminish the positive effects of nutrition intervention.⁵³

Gut Microbiota

One of the mechanisms involved in the pro- or anti-inflammatory effects of diet is the impact of diet on the gut microbiota⁵⁴, the bacterial population colonizing the gastrointestinal tract. The human intestine harbors around 100 trillion bacterial cells, and their combined genetic capacity, known as the gut microbiome, surpasses that of the human genome by approximately 100-fold.⁴⁷ The gut microbiota play a crucial role in shaping metabolic health and influencing obesity.^{5,54-56} This complex community of microorganisms interacts with dietary components and host metabolism and impacts many physiological processes including dietary energy harvest and maintenance of gastrointestinal tract barrier integrity.⁵⁴ The gut microbiota have many well-established roles in systemic metabolism and immune function that are key contributors to metabolic health and responses to diet.^{46,47} For example, maintenance of gut barrier function prevents the absorption and translocation of microbially-produced endotoxins which are potent activators of pro-inflammatory pathways and contribute to the development of insulin resistance.⁵⁴

Four major phyla comprise the majority of the gut microbial community in humans: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria.⁵⁷ However, interindividual differences are substantial at lower taxonomic levels.⁵⁷ Although challenging to quantify,

differences in composition at lower taxonomic levels are significant as both symbiotic and pathogenic microbes coexist within higher orders.⁵⁴ Factors contributing to interindividual differences in composition include age, environmental exposures, health status, genetics, socioeconomic status, geography, pregnancy, exercise, and diet.⁵⁸ While individual compositions vary, a healthy gut microbiota is distinguished by high alpha diversity – the number of taxa present and their relative abundances.^{59,60} Functionally, this trait confers high resistance, resilience, and redundancy which measure the degree to which microbial composition remains unchanged in the face of a disturbance, can return to baseline composition after a disturbance, and can maintain functional capacity under different environmental conditions, respectively.⁶¹ Compositional and functional disturbances of the gut microbial community occur in obesity and its associated metabolic diseases.^{54,55} Some of the most characteristic changes in obesity include decreased bacterial diversity⁶² and an increased capacity for energy harvest from dietary intake.⁶³ While the exact composition of an “obesogenic” microbiota is still debated,⁶⁴ distinct patterns of microbial communities, or enterotypes, have been detected for metabolic disorders such as obesity,^{62,63} insulin resistance,⁶⁵ and inflammation.⁶⁶

Diet significantly impacts the composition and function of the gut microbiome, thereby affecting metabolic health.⁶⁷⁻⁶⁹ Both long-term⁷⁰ and short-term diets⁷¹ have been shown to alter the gut microbiota composition in healthy adults. High-fiber diets low in red meat and high in unsaturated fatty acids are associated with a more beneficial microbial composition with increased microbial diversity and higher abundances of health-promoting bacteria such as *Bifidobacteria* and *Lactobacillus*.^{56,72} In contrast, diets characterized by high saturated fat and sugar intake and low fiber intake are strongly associated with decreases in health-promoting

bacteria and increases in pathogenic taxa including *Proteobacteria*.^{73,74} These detrimental compositional changes have been linked to increased gut permeability, endotoxemia, and adipose tissue inflammation.^{56,66,67} Research also indicates that gut microbial data can accurately predict postprandial glycemic responses^{75,76} and significantly contribute to the interindividual variation in postprandial lipid responses.⁷⁷ Taken together, this research indicates that interindividual variation in gut microbial communities might help discriminate between responders and non-responders to dietary interventions.

Metabolomics

Metabolites represent a diverse group of chemical compounds including amino acids, lipids, and organic acids. Thousands of metabolites originate from diet and both microbial and host metabolism. The field of metabolomics is concerned with the detection and quantification of metabolites in a biological sample. Metabolomic measurements provide valuable insights into metabolic health and obesity as metabolites represent the end products of various metabolic pathways and can reflect alterations in biochemical processes associated with obesity and metabolic dysfunction. In addition, metabolomic measurements provide phenotypic insight by integrating combined influences of genetics, environment, diet, and microbiome into one outcome “signature”.^{44,78} Indeed, metabolomic studies have identified distinct metabolic signatures associated with obesity,⁷⁹ insulin resistance,⁸⁰ and other metabolic disorders,⁸¹ offering potential biomarkers for disease diagnosis, prognosis, and treatment monitoring.

The usefulness of measuring the metabolome lies in its ability to capture the complexity of metabolic processes and provide a holistic view of metabolic health. Unlike single biomarkers, which may offer limited insights into metabolic status, metabolomic profiling

enables the identification of multiple biomarkers simultaneously, offering a more comprehensive assessment of metabolic dysfunction.⁸² Additionally, metabolomic data can reveal underlying metabolic pathways dysregulated in obesity and related disorders, facilitating the discovery of novel therapeutic targets and personalized interventions.⁸² Furthermore, metabolomic measurements offer dynamic and real-time information about metabolic status,^{83,84} allowing for the assessment of metabolic responses to dietary interventions and lifestyle modifications. By monitoring changes in metabolite profiles over time, clinicians and researchers can evaluate the efficacy of interventions and tailor treatments to individual metabolic needs.

Importantly, the interaction between nutrition and gut microbiota extends beyond microbial composition to include the production of microbial metabolites, such as short-chain fatty acids (SCFAs), bile acids, trimethylamine-N-oxide (TMAO), and polyphenolic derivatives that are key mediators of the relationship between nutrition, the gut microbiome, and metabolic health.^{54,56} SCFAs, produced through the fermentation of dietary fiber by gut bacteria, exert anti-inflammatory and metabolic effects, promoting glucose homeostasis and reducing adiposity.⁸⁵ Bile acids, modified by gut bacteria, regulate lipid metabolism, energy expenditure, and glucose homeostasis through activation of various signaling pathways.⁸⁶ Diets rich in food sources of choline and carnitine such as red meat and eggs serve as precursors for gut microbial production of TMA. In the liver, TMA is converted to TMAO which has been associated with cardiovascular disease risk, insulin resistance, and non-alcoholic fatty liver disease.⁸⁷ Dietary polyphenolics exert antioxidant, anticarcinogenic, anti-inflammatory, vasodilatory, and immune stimulating effects thereby promoting metabolic health.⁸⁸⁻⁹⁰ However, the bioavailability and bio-efficacy of diet-derived polyphenolics depends, at least in part, on digestive metabolism by gastrointestinal

microorganisms⁹¹⁻⁹³ with health-promoting properties of phenolic derivatives frequently exceeding the parent compound.^{94,95}

In addition to altering gut microbial metabolites, dietary intake influences the composition and concentration of metabolites resulting from host metabolism –providing insight into metabolic health. For instance, consumption of high-fat or high-sugar diets can lead to alterations in lipid and glucose metabolism, resulting in changes in circulating metabolite profiles. Conversely, diets rich in fruits, vegetables, and whole grains can promote metabolic health by modulating circulating plasma levels of metabolites associated with antioxidant capacity, inflammation, and energy metabolism.^{44,56,96} Although many human and microbial metabolites remain unknown, the integration of metabolomic measurements into research and clinical practice holds promise for advancing understanding of metabolic health, obesity, and the role of nutrition in modulating metabolic processes.⁴³

Improved understanding of the intricate interplay between nutrition, inflammation, the gut microbiome, and metabolites is essential for developing strategies to promote metabolic health and combat obesity. The assessment of these factors in addition to traditional metabolic screening criteria improves accuracy in diagnosing metabolic health status. While understanding interindividual variability in response to diet is still an area of active research, gut microbial composition, inflammation phenotype, and metabolomic profiles are established contributors and should be considered when developing effective dietary strategies to improve metabolic health.

Dissertation Aims

Obesity and its associated metabolic disease states are a primary focus of nutrition research. The pathogenesis of obesity is complex with genetic, environmental, and lifestyle

factors all playing a role.^{4,5} Diet is a modifiable risk factor contributing to overnutrition, visceral adiposity, and metabolic dysregulation.⁵⁻⁸ Therefore, healthy dietary changes represent a critical strategy to prevent and reduce the burden of metabolic disease. However, the complexity of interactions between food and interindividual factors present a challenge in developing causal relationships between diet and metabolic health. Differences in a habitual diet, exercise habits, and many other cultural, socioeconomic, and environmental factors all contribute to metabolic response to diet.^{13,17}

High interindividual variability in metabolic responses to diet are poorly understood. However, there is growing recognition for the usefulness of inflammatory, metabolomic, and gut microbial biomarkers to help establish a more accurate picture of metabolic health and variable response to diet. In addition, postprandial measures of commonly utilized fasting metabolic health biomarkers such as triglyceride and glucose levels provide insight into acute responses to food and are more accurately correlated to metabolic disease risk.²⁴⁻²⁷ By assessing postprandial metabolic responses, diverse inflammatory biomarkers, metabolomic, and gut-microbial responses to dietary interventions, this body of work contributes novel information regarding the impact of dietary interventions on metabolic health. Specifically, this dissertation aims to 1) characterize the impact of fiber-rich lentils on fasting and postprandial measures of inflammation, glucose, and lipid metabolism, 2) determine the impact of acute lentil consumption on the postprandial bile acid response, and 3) evaluate how a polyphenol-rich juice may improve host metabolism and alter gut-microbial composition.

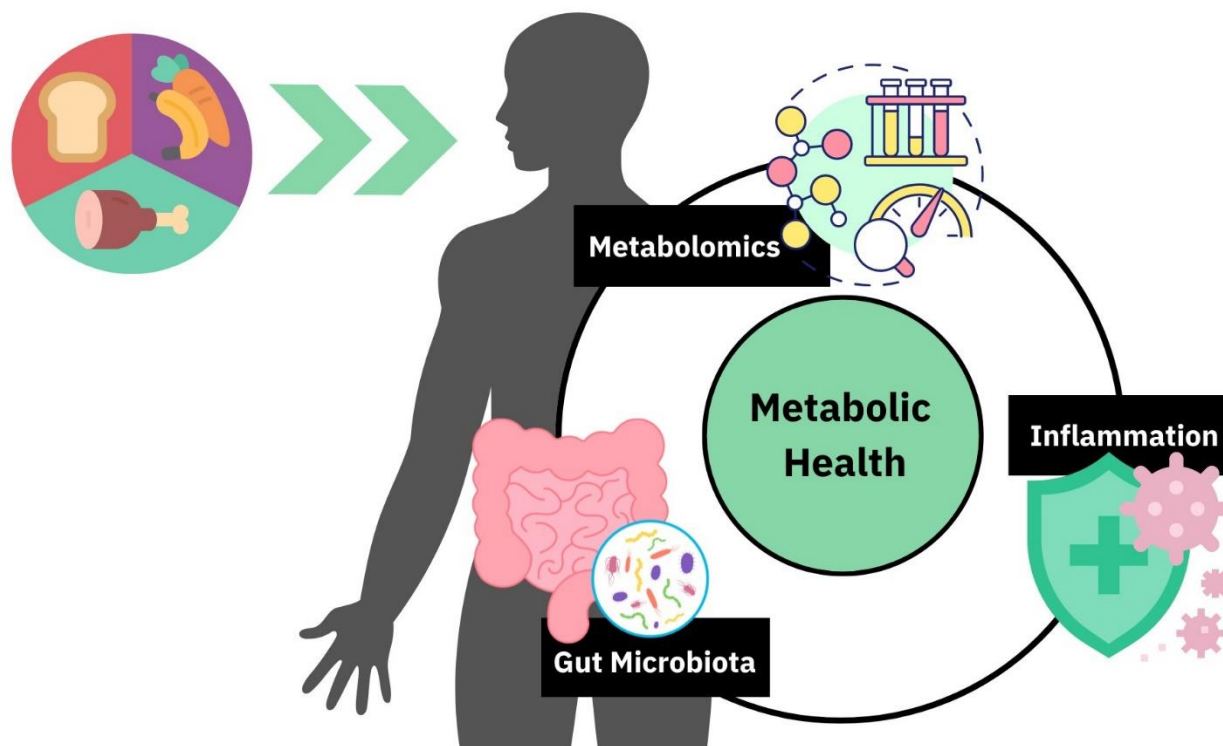
Lentils have potential to improve metabolic health but there are limited randomized clinical trials evaluating their comprehensive impact on metabolism.⁹⁷⁻⁹⁹ In **Chapter 2**, the

impact of chronic lentil consumption on fasting and postprandial measures of inflammation, glucose and lipid metabolism are assessed. Thirty-eight adults with increased risk for metabolic disease, as measured by increased waist circumference and elevated postprandial triglycerides, participated in a 12-week dietary intervention that included seven prepared midday meals totaling either 980 grams or 0 grams of cooked green lentils per week. The importance of assessing postprandial metrics in addition to their respective fasting measures is highlighted as postprandial measures are established as better predictors of metabolic health. In addition, the value of assessing a diverse panel of inflammatory biomarkers is established as differential responses of biomarkers to intervention is demonstrated.

In **Chapter 3**, the potential of acute lentil intake to favorably alter the postprandial bile acid response is examined. Bile acids are powerful regulators of metabolism⁸⁶ and altered bile acid profiles have been identified as biomarkers of impaired metabolism and causative mediators in the pathogenesis of obesity-related metabolic diseases.¹⁰⁰⁻¹⁰⁵ However, research examining the postprandial bile acid response to diet is limited and more research is needed to determine their utility as a biomarker of metabolic health. To address this gap, a randomized crossover trial was conducted in twenty-four adults with overweight or obesity ($\text{BMI} > 27 \text{ kg/m}^2$). Participants completed testing for a lentil (140 grams cooked green lentils) and control meal (0 grams lentils) serving as their own control and bile acid as well as inflammatory, glycemic, and lipidemic responses were measured for four hours postprandially. Postprandial bile acid profiles in response to lentil intake were then correlated with postprandial metabolic measures to elucidate relationships between individual bile acids and metabolic responses to lentils.

Aronia fruits are the richest fruit source of polyphenols and have been shown to improve glucose and lipid metabolism, increase vascular function, and decrease inflammation making them a functional food of interest to combat metabolic disease.¹⁰⁶ The favorable metabolic impacts of polyphenolic consumption are likely mediated by the gut microbiome.⁹¹⁻⁹³ **Chapter 4** examines the impact of a daily dose of 100 mL of aronia juice in a 30-day clinical intervention trial. Changes to gut microbial taxonomy and to host health including fasting and postprandial lipidemic, glycemic, and inflammatory responses are examined. **Chapter 5** discusses the implications from these experiments in both clinical and research settings as well as future directions of personalized nutrition and biomarker use for metabolic health assessment.

Figure 1. Schematic displaying the contribution of metabolomic, inflammation, and gut microbiota assessments in determining the impact of diet on metabolic health.



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CHAPTER TWO

TWELVE WEEKS OF DAILY LENTIL CONSUMPTION
IMPROVES FASTING CHOLESTEROL AND POSTPRANDIAL
GLUCOSE AND INFLAMMATORY RESPONSES—A
RANDOMIZED CLINICAL TRIAL

Contribution of Authors and Co-Authors

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Article

Twelve Weeks of Daily Lentil Consumption Improves Fasting Cholesterol and Postprandial Glucose and Inflammatory Responses—A Randomized Clinical Trial

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Abstract: Lentils have potential to improve metabolic health but there are limited randomized clinical trials evaluating their comprehensive impact on metabolism. The aim of this study was to assess the impact of lentil-based vs. meat-based meals on fasting and postprandial measures of glucose and lipid metabolism and inflammation. Thirty-eight adults with an increased waist circumference (male ≥ 40 inches and female ≥ 35 inches) participated in a 12-week dietary intervention that included seven prepared midday meals totaling either 980 g (LEN) or 0 g (CON) of cooked green lentils per week. Linear models were used to assess changes in fasting and postprandial markers from pre- to post-intervention by meal group. Gastrointestinal (GI) symptoms were assessed through a survey randomly delivered once per week during the intervention. We found that regular consumption of lentils lowered fasting LDL ($F = 5.53, p = 0.02$) and total cholesterol levels ($F = 8.64, p < 0.01$) as well as postprandial glucose ($\beta = -0.99, p = 0.01$), IL-17 ($\beta = -0.68, p = 0.04$), and IL-1 β ($\beta = -0.70, p = 0.03$) responses. GI symptoms were not different by meal group and all symptoms were reported as “none” or “mild” for the duration of the intervention. Our results suggest that daily lentil consumption may be helpful in lowering cholesterol and postprandial glycemic and inflammatory responses without causing GI stress. This information further informs the development of pulse-based dietary strategies to lower disease risk and to slow or reverse metabolic disease progression in at-risk populations.

Keywords: dietary pulses; dietary fiber; postprandial period; metabolic disease

1. Introduction

Obesity-related diseases such as nonalcoholic fatty liver disease, type 2 diabetes, and cardiovascular disease are among the leading causes of morbidity and mortality worldwide [1]. These diseases are characterized by abnormal glucose and lipid metabolism driven by biological and environmental factors including genetic predisposition, lifestyle, and diet [1]. Increased central adiposity, in particular, drives metabolic dysregulation through the promotion of macrophage infiltration, low-grade inflammation, altered lipid metabolism, and insulin resistance [2–4]. Diet is a known contributor to central adiposity and metabolic dysregulation. Therefore, healthy dietary changes represent one strategy to prevent and reduce the burden of metabolic disease.

Pulse crops have potential to improve metabolic health [5–10] as they are a type of legume that offers consumers a low-cost, plant-based protein, rich in fiber with low fat and sodium content. Pulse protein content ranges between 17% and 30% (dry weight) [11]

and provides up to 30 g of fiber per 100 g dry weight, around half of which is insoluble fiber [12]. Fiber offers various metabolic health benefits including increased satiety [13], reduced triglyceride absorption, and improved glycemic control [14,15]. Increased fiber intake has also been linked to blunted systemic inflammation [16,17], which often manifests with altered lipid and glucose metabolism. However, metabolic benefits vary by pulse variety, dosage, and duration of inclusion in the diet [15]. As such, comprehensive dietary intervention studies examining inflammatory and metabolic responses to individual pulse consumption are needed to devise effective dietary recommendations regarding pulse consumption for metabolic health improvements. In addition, gastrointestinal (GI) symptoms are commonly reported as a barrier to pulse consumption [18], yet are rarely reported in pulse intervention studies. Increased transparency of reported GI symptoms may help encourage and increase pulse consumption among consumers.

Lentils are a type of pulse known for their unique nutritional profile. They are high in dietary fiber and protein and contain dietary bioactive compounds such as polyphenols [19]. Randomized clinical trials found improvements to measures of glycemic control including postprandial glucose, glycated hemoglobin (HbA1c), and the homeostatic model of insulin resistance (HOMA-IR) with lentil consumption [8,9,19,20]. However, there is a lack of studies assessing more comprehensive measures of metabolic health including postprandial lipid metabolism and systemic inflammation. Postprandial lipid [21–23] and glycemic responses [24] are better predictors of disease risk than fasting levels and are also causally linked to low-grade inflammation [25,26], which drives metabolic disease progression. Although these measures are more robust in predictive power, their assessment is not yet established in the clinical setting. They are, however, excellent markers to better describe metabolic health in the research setting. Individuals with these conditions therefore represent an ideal target population to examine how dietary interventions may be used to prevent disease progression.

We strategically conducted this clinical trial with individuals at high-risk for metabolic disease as defined by an elevated waist circumference, as an accepted proxy for central adiposity [4], and increased postprandial triglyceride response. The aim of this study was to determine the impact of 12 weeks of lentil consumption on fasting and postprandial measures of lipid metabolism, glycemic control, and inflammation. Few studies have investigated the long-term impact of lentil consumption at the recommended dose set by the US Department of Agriculture (USDA), which suggests approximately 1.5 cups (~300 g) of cooked pulses per week for adults with a daily caloric intake of 2000 calories (kcal) [7,27]. For this reason, we investigated a daily lentil dose exceeding this recommendation at 980 g per week which aligns with study doses from publications reporting improvements in blood lipid profiles, inflammation biomarkers, and other metabolic markers from daily pulse consumption [7]. In addition, we conducted weekly surveys during the intervention to assess how lentil consumption at this dose impacts GI symptoms and satiety. This is the first study that we know of to assess dynamic lipidemic, glycemic, and inflammation responses in this population to demonstrate new and potentially more meaningful health impacts of long-term lentil consumption.

2. Materials and Methods

2.1. Ethics Statement

The study protocol was approved by the Institutional Review Board at Montana State University (2020-131-MM010320-FCR). The informed consent was reviewed verbally in-person, allowing time for questions and clarifications. Written informed consent was obtained from all participants prior to their participation. This study was prospectively registered with ClinicalTrials.gov (NCT04283448).

2.2. Study Population

Potential participants were recruited via advertisement from June 2020 to the end of August 2023 and screened for eligibility using REDCap (version 13.10.6) [28,29] electronic

data capture tools hosted at Montana State University, Bozeman. Inclusion criteria included being between 18 and 70 years old, having a waist circumference ≥ 40 inches for males or ≥ 35 inches for females, and having a non-fasting serum triglyceride concentration greater than 1.69 mmol/L (150 mg/dL). These criteria were implemented in an effort to recruit individuals with a greater risk of chronic disease development [4]. Potential participants were excluded if they had taken oral antibiotics within 90 days of study enrollment, regularly used anti-inflammatory, oral contraceptive, or lipid lowering medications, had wheat, dairy, or legume allergies or were vegetarian, were pregnant or lactating, had been diagnosed as diabetic or prediabetic, or had any musculoskeletal, cardiovascular, gastrointestinal, or immunological condition that could interfere with this study.

2.3. Research Design

This study was a randomized clinical trial with 0 or 980 g of cooked whole green lentils seven days a week as a mid-day meal for 12 weeks. Testing for the human subject cohort took place in the Nutrition Research Laboratory at Montana State University. Participants reported to the lab for data collection over four visits. Subject consent and anthropometric measurements were completed at the first visit. Postprandial serum triglycerides were tested during visit 2 to ensure levels met inclusion criteria of ≥ 150 mg/dL (1.69 mmol/L). During visits 3 and 4, participants completed a high-fat meal challenge (50 g oral fat load) with blood collected at fasting and hourly timepoints for 5 h postprandially for blood marker assessment. Participants completed a 12-week dietary intervention between visits 3 and 4. See Supplementary Figure S1 for a summary of the study design.

2.4. Anthropometrics

All anthropometric measurements were collected from participants at baseline and after the 12-week intervention. Subjects were directed not to exercise, drink, or eat in the 3 h prior to testing. A validated [30] segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) was utilized to assess body mass index (BMI) and visceral adipose. Waist circumference was measured in triplicate using an anthropometric tape measure, with the average measurement used for analysis. Blood pressure was taken in the morning after subjects had been seated for at least 15 min. An automated measurement was taken in duplicate with the mean used for analysis.

2.5. Elevated Postprandial Triglyceride Screening

High-fat meal challenges containing 40 to 100 g of dietary fat are an established method to induce postprandial triglyceride and inflammation responses [25]. Participants were provided with a list of suggested breakfast and lunch meals containing approximately 50 g of fat. They were asked to consume a high-fat breakfast or high-fat lunch three to four hours before a visit with a single blood sample collection by a trained technician. Elevated postprandial triglycerides (ppTG) were defined as blood triglycerides greater than 150 mg/dL (1.69 mmol/L). Blood samples were run on-site using Piccolo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, CA, USA). Participants who met ppTG criteria were enrolled into this study and continued to the dietary intervention.

2.6. Randomization

Block randomization was performed using the blockrand function in the R blockrand package (version 1.5) [31]. Fourteen blocks of six with two equally numbered levels were created, with the seed for each block individually set based on the last five integers returned via the sys.time (version 4.3.1) function. Participants were randomized to lentil (LEN) or control (CON) meal condition after confirmation of elevated ppTG to allow adequate time for preparation of their first week of study meals. Volunteers and clinical staff aiding in data collection during research visits were masked to treatment allocation. However, investigators performing research visits were aware of treatment allocation as they were

the same individuals responsible for allocation of meals to participants for the duration of the study. Similarly, these investigators also analyzed the study data and were not masked.

2.7. Lentil Sampling and Nutritional Analysis

Medium green lentils (CDC Richlea cultivar) used in the study diet were provided by AGT Foods Canada and sent to Medallion Labs (Minneapolis, MN, USA) for analytical testing. Functional properties and nutrient breakdown are provided in Supplementary Table S1. Dietary fiber was determined through the analytical method AOAC 991.43 [32].

2.8. Dietary Intervention

The 12-week dietary intervention consisted of 7 prepared mid-day meals (Table 1) each week that summed to either 0 g (CON) or 980 g (LEN) of cooked whole green lentils (provided by AGT Foods Regina, SK, Canada). This amount is equivalent to a daily dose of 140 g (0.7 cups) [33] of lentils which aligns with past studies reporting metabolic improvements from daily pulse consumption of 150 g (minimum–maximum: 54–360 g/day; cooked) [7].

Table 1. Overview of intervention meal composition by group.

Meal	CON—0 g/week	LEN—980 g/week
Bolognese	Turkey Bolognese + ½ cup cooked rotini pasta	Lentil Bolognese + ½ cup cooked rotini pasta
Curry	Chicken curry + 1/3 cup cooked basmati rice	Lentil curry + 1/3 cup cooked basmati rice
Loaf	Turkey loaf + ¼ cup mashed potatoes + ½ cup cooked zucchini	Lentil loaf + ¼ cup mashed potatoes + ½ cup cooked zucchini
Taco	Turkey taco filling + ½ oz shredded cheddar cheese + 1 Tbsp salsa + 1 Tbsp sour cream + 2 street flour tortillas	Lentil taco filling + ½ oz shredded cheddar cheese + 1 Tbsp salsa + 1 Tbsp sour cream + 2 street flour tortillas
Soup	Chicken soup + 3 packets saltine crackers	Lentil soup
Shepherd's Pie	Chicken shepherd's pie + 2 dinner rolls + 1 pat butter	Lentil shepherd's pie + 1 dinner roll + 2 pats butter
Chili	Turkey chili + 3 packets saltine crackers	Lentil chili + 1 packet saltine crackers

Provided meals were prepared by a registered dietitian and professional chef on the research team. Meals were prepared in plastic to-go containers, labelled with heating instructions, and frozen for meal pick-up. Meal options included shepherd's pie, soup, loaf with mashed potatoes, curry with basmati rice, street tacos, pasta with Bolognese sauce, and chili. CON and LEN meals were designed to have similar protein (g) and energy (kcal) content based on consumption of one of each meal per week with CON meals containing ground turkey or chicken instead of lentils. LEN meals had higher fiber and carbohydrate content, and less fat than CON meals. These compositional differences are representative of a diet substitution of lentils in lieu of meat (Supplementary Table S2). Because the dietary intervention followed a whole-foods approach, participants were able to identify that they were consuming either lentil-based meals or meat-based meals. However, participants were informed only that the purpose of this study was to study “how certain foods” influence the variables being assessed in the current study. Thus, while this study was not formally blinded so that a whole-foods approach could be utilized, participants were not aware that lentils were being compared to meals with animal protein.

Participants were instructed to consume the provided meals during their regular lunch time—no specific time was required. Participants were asked to consume one of each of the seven meal options within their meal group the first week of the intervention. To increase satisfaction and diet adherence, participants were then allowed to select their meals based on preference for the remaining weeks. Other than consumption of the daily intervention meal, participants were instructed to maintain their normal dietary and physical activity patterns.

2.9. Diet Adherence

Adherence to daily consumption of intervention meals was monitored during meal pick-ups which occurred once per week or once every two weeks depending on participant preference. Researchers recorded type and number of meals provided to participants at every meal pick-up as well as any meals not consumed during the provided meal period. Failure to consume three or more meals in a one-week period resulted in a one-week extension of the intervention. In addition, participants could not miss any meals in the one-week period prior to their final visit. Any missed meals during the last week resulted in a one-week extension of the intervention period. To maintain equitable intervention time periods, participants were excluded from study analysis if adherence failures resulted in an intervention period exceeding 13 weeks.

2.10. Recent and Habitual Diet Surveys

Participants were also asked to provide information on recent and habitual dietary intake while seated during the high-fat meal challenge. Recent dietary intake was assessed through a 24 h dietary recall collected and analyzed with the Automated Self-Administered 24 h (ASA24) Dietary Assessment Tool, version (2020), developed by the National Cancer Institute, Bethesda, MD. The ASA24 was orally administered by research staff to participants. The generated report including portion sizes and specific food items consumed in the 24 h prior to the high-fat meal challenge to match food and beverage intake before repeat testing at the end of the nutrition intervention.

Habitual dietary intake was collected using the validated diet history questionnaire (DHQ III), offered by the National Cancer Institute. Participants completed the online survey using a lab-provided laptop. A 12-month recall was completed during visit 3 before the intervention and a 1-month recall was completed during visit 4 following the 12-week intervention. Participants were encouraged to give their best estimates of habitual intake during the recall with the acknowledgment that exact recollection of portion sizes for all food and beverage intake is unlikely. The DHQ III consists of 135 questions assessing food and beverage intake and 26 supplement questions assessing supplement intake. Questions address both dietary frequency and portion sizes. Healthy Eating Index (HEI, 2015) scores were obtained through DHQ III survey data output and included nine components based on adequacy (total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, and fatty acids) and four components based on moderation (refined grains, sodium, added sugars, and saturated fats), as well as total HEI score (sum of the thirteen components). These scores are a measure of diet quality in compliance with the 2015–2020 US Dietary Guidelines for Americans [34,35]. In addition to HEI component scores, diet components expected to change with a lentil-based intervention were also analyzed as a dietary adherence check. These items included total fiber, g; insoluble fiber, g; and legumes, cups. Overall energy intake was also assessed.

2.11. Satiety and Gastrointestinal Symptom Surveys

To understand how participants respond to our lentil intervention, two surveys addressing satiety and gastrointestinal symptoms were delivered through email using REDCap (version 13.10.6) [28,29] on a randomly selected day, once a week for each week of the 12-week (or 13-week, if applicable) dietary intervention. At 4:00 p.m., participants were asked to rate their levels of hunger, fullness, satisfaction, desire to eat, and quantity of food they could eat in relation to the study meal they consumed mid-day. At 8:00 p.m., participants received a survey asking them to rank how they felt overall that day regarding common GI symptoms including abdominal discomfort, bloating, flatulence, and cramping. Survey questions and response scales are outlined in Supplementary Figure S2. Participant responses to both surveys were analyzed if they completed at least 8 of the 12 (or 13 if applicable) weekly surveys.

2.12. Blood Sampling

Participants were instructed not to participate in strenuous physical activity or consume alcohol in the 24 h prior to their visit and to complete a 10–12 h overnight fast before blood collection. Venous blood samples were collected via an intravenous catheter from the antecubital vein after a 3 mL waste withdrawal and then followed by a sterile saline flush. The fasting sample was drawn 20 min after catheter insertion. After fasting blood collection, participants consumed the high-fat meal followed by blood collection every hour for 5 h after meal ingestion. Blood was collected into serum separating and heparinized vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA). Serum tubes were allowed to clot for 15 min at room temperature before centrifugation (1200 RPM, 15 min). Serum aliquots were frozen at -80°C until analysis. Whole blood from the heparinized tube was immediately utilized for blood marker analysis.

2.13. High-Fat Meal Challenge

The meal consisted of 58.3 g of salted butter (Tillamook, OR, USA) on three pieces of toasted whole wheat bread (Wheat Montana, Three Forks, MT, USA). The meal contained 50 g fat, 54 g carbohydrate, and 12 g protein with a total energy content of 714 kcal. Fat comprised approximately 43.1% of caloric content with 57% of total fat load from saturated fats. The meal contained approximately 9 g of dietary fiber. Participants were provided with water and caffeinated earl grey black tea (Bigelow, Fairfield, CT, USA) if they identified as habitual coffee consumers. Timing for the postprandial period began when participants started eating and they were given 15 min to consume the meal.

2.14. Analysis of Blood Markers

Blood markers were determined from whole blood collected in a sodium heparin tube run on Piccolo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, CA, USA) which included total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and glucose (GLU). Glycated hemoglobin was determined using the Affinon2 analyzer (Abbott, Princeton, NJ, USA) from whole blood collected in an EDTA additive tube. Serum insulin was determined through ELISA (MP Biomedicals, Irvine, CA, USA) performed according to manufacturer instructions, with the average used for analysis. Fasting blood GLU and insulin (INS) were used to determine the homeostatic model of insulin resistance (HOMA-IR), a measure of hepatic INS resistance at fasting calculated with the original HOMA-IR formula [36].

2.15. Analysis of Inflammation Biomarkers

Measurement of cytokine inflammation biomarkers was completed using high-sensitivity multiplexing technology (Bio-Rad Bio-Plex[®] 200 HTS, Hercules, CA, USA) following procedures by Millipore (EMD Millipore Corporation, Billerica, MA, USA). Selected cytokines included interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), granulocyte macrophage colony stimulating factor (GM-CSF), and five interleukins (IL) including IL-1 β , IL-6, IL-10, IL-17, and IL-23. Plasma samples at each time point were run in duplicate and the mean was used for analysis. Sample values that were under the detectable limit were replaced with $\frac{1}{2}$ the minimum detectable concentration for the respective cytokine for analysis [37].

2.16. Statistical Analysis

Analysis and visualizations were conducted in RStudio (2023.06.1) running R 4.3.1, with data visualized using ggplot2 (version 3.4.4) [38] and effects (version 4.2.2) [39] packages. Power for this study was calculated a priori using preliminary data for ppTG responses and 50% effect sizes consistent with published findings in animal models. Based on a two-sample *t*-test comparing pre- and post-intervention values, it was estimated that 18–24 participants would be required per group to achieve a power of 0.76 to 0.86 at $\alpha = 0.05$.

Habitual dietary patterns, represented by DHQ-III HEI scores, were analyzed at baseline through general linear models to detect existing dietary differences between meal groups. Diet components expected to change with a lentil-based intervention, including total fiber g, insoluble fiber g, and legumes (cups), as well as total energy intake (kcal), were also assessed for baseline differences. To determine if intervention meal consumption changed participant dietary intake, linear mixed effects models were used to assess dietary components and HEI scores with an interaction predictor between meal and time (pre- or post-intervention) and a random effect for subject. Post-hoc multiple pairwise comparisons were performed to detect significant differences by group using Tukey's HSD.

Linear mixed effects models were used to assess the impact of an interaction of time (week) and meal (LEN, CON) on satiety measures. Linear mixed effects models were used to assess GI symptom severity using symptom type and a week and meal interaction as predictors. Models for GI symptom severity and satiety included a random effect for subject.

General linear models were used to determine if physical and biological characteristics differed between meal groups before the intervention began. Descriptive summary statistics were performed to summarize participant characteristics as the average by meal group at baseline. General linear models were used to assess the impact of the dietary intervention on the following categories of variables: anthropometric measures (weight, fat mass percent, visceral adipose, and waist circumference); fasting blood lipid measures (CHOL, HDL, LDL, and TG); fasting glycemic measures (GLU, INS, and HOMA-IR); and fasting inflammation markers (GM-CSF, IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-17, and IL-23). Fasting inflammation markers were normalized using R package *bestNormalize* (version 1.9.1) [40] prior to statistical analysis. Dependent variables were summarized as the change in values from baseline (post-intervention–pre-intervention). Predictor variables were common to each category of dependent variable as follows: anthropometric measures—meal group; blood lipid measures—meal group, visceral adipose tissue; glycemic measures—meal group, visceral adipose tissue, change in BMI, and HbA1c; inflammation measures—meal group, visceral adipose tissue. Predictor variables for glycemic measures were selected based on findings from Zeevi and colleagues [41]. Visceral adipose was included as a predictor variable in inflammation models because it represents a common difference between genders and is a key contributor to systemic inflammation levels [42]. It was also included in models examining blood lipid levels based on findings from Couillard and colleagues indicating its inclusion helps mitigate sex effects on blood lipid measures [43].

Analysis of postprandial metabolic (TG, GLU, and INS) and inflammatory (GM-CSF, IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-17, and IL-23) measures were conducted by summarizing each makers' measurements at timepoints 0 (fasting) and 1, 2, 3, 4, and 5 h postprandially as net area under the curve (AUC) using the *auctime* (version 2.0.0) [44] R package. Each dependent variable was then summarized as the change in AUC (post-intervention–pre-intervention). AUC values for inflammation markers were normalized using the R package *bestNormalize* (version 1.9.1) [40] prior to statistical analysis. General linear models were used to assess the impact of intervention meal group on each dependent variable. Predictor variables were identical to variables utilized in corresponding fasting models.

3. Results

3.1. General Characteristics of Participants

A total of 290 participants were screened for study participation of which 242 were excluded for not meeting inclusion criteria, lost to follow-up, or withdrew for non-intervention related reasons. Of the 78 individuals who completed screening for elevated postprandial triglycerides, 28 individuals (36%) were below the 1.69 mmol/L criteria, though all had met elevated waist circumference criteria. Forty-seven individuals were randomized to a meal intervention with a total of nine individuals lost for various reasons (Figure 1). Only one participant randomized to a diet withdrew from this study due to dislike of study

meals. A total of 38 overweight and obese adults (mean $\pm$ SD); age (47.2 ± 13.1 years); BMI (34.4 ± 6.5 kg/m²) with elevated waist circumference (107.1 ± 13.9 cm females, 117.4 ± 0.7 cm males) completed the 12-week intervention. A total of 14 of the 38 participants completed an extra week of intervention meals (13 weeks total) due to reported missed meals in diet adherence checks.

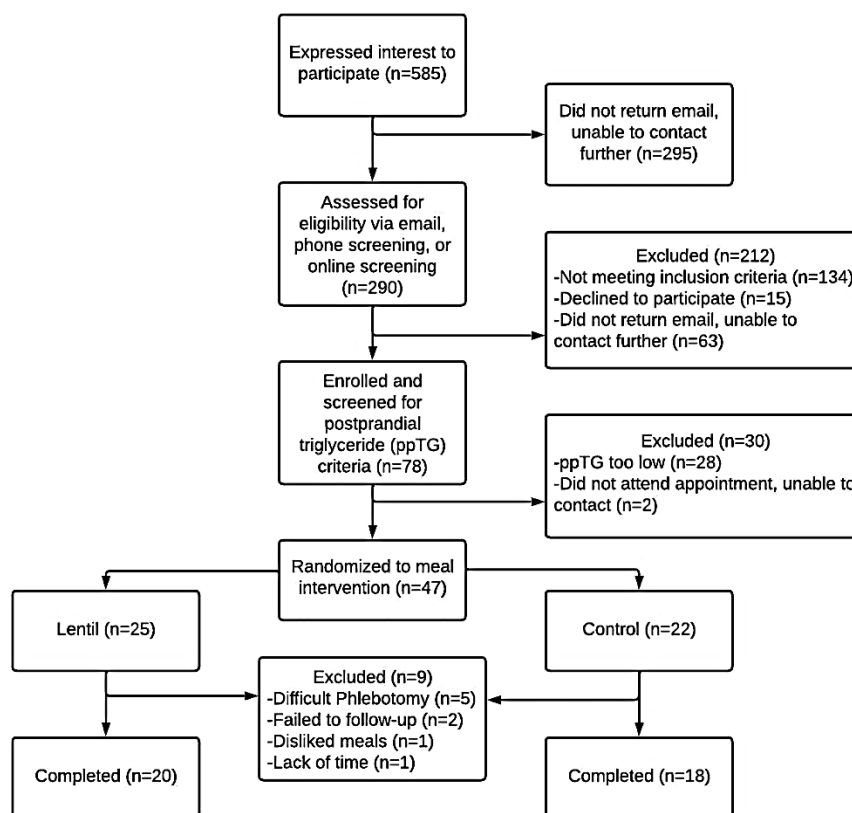


Figure 1. A CONSORT flowchart of the study design and enrollment. Flyers and emails were used to recruit interested individuals. Interested individuals contacted the research team, who sent additional information about this study. Two hundred and ninety individuals completed an initial eligibility screening with the research team. Forty-seven individuals met study requirements and were randomly allocated to CON or LEN experimental groups with varying weekly doses of lentils: CON, control (0 g/week) and LEN, lentil (980 g/week).

Between meal groups at baseline, participants had similar anthropometric values as well as blood measures for HbA1c, GLU, HOMA-IR, TG, and blood pressure (Table 2). Fasting blood GLU and HbA1c were normal on average across meal groups and TG levels were optimal in the LEN group and intermediate in the CON group as compared to standard reference ranges. Fasting total CHOL and HDL cholesterol were different between groups at baseline, though CHOL values were optimal and HDL intermediate in both groups. The proportion of participants with metabolic syndrome was higher in the CON group compared to LEN with 61% and 20% meeting diagnostic criteria, respectively.

Table 2. Participant characteristics at baseline, grouped by assigned dietary intervention ($n = 38$). Blood profile determined from fasting venous blood sample. Bold p -values indicate strong evidence of a difference between meal groups.

	CON ($n = 18$)	LEN ($n = 20$)	p -Value
Age (years)	43.2 (14.0)	50.6 (11.5)	0.08
Sex (M/F)	1/17	2/18	0.58
BMI (kg/m^2)	35.9 (7.6)	33.1 (5.1)	0.19
Fat mass (%)	45.1 (6.2)	44.0 (6.1)	0.57
Visceral adipose (L)	3.6 (2.1)	3.1 (1.8)	0.45
MetS Presence (Y/N) ¹	11/7	4/16	<0.01
HbA1c (%)	5.4 (0.3)	5.5 (0.2)	0.48
Fasting Glucose (mmol/L)	5.5 (0.5)	5.4 (0.3)	0.43
HOMA-IR	3.8 (2.6)	4.2 (8.2)	0.86
Total Cholesterol (mmol/L)	4.4 (0.7)	5.1 (0.7)	0.01
HDL Cholesterol (mmol/L)	1.2 (0.2)	1.5 (0.4)	0.01
LDL Cholesterol (mmol/L)	2.85 (0.64)	3.28 (0.64)	0.05
Triglycerides (mmol/L)	1.7 (0.8)	1.6 (0.7)	0.52
Blood Pressure (mmHg)			
Systolic	117.8 (11.9)	111.2 (11.4)	0.09
Diastolic	82.6 (10.8)	76.5 (9.4)	0.08

¹ Criteria were based on the National Cholesterol Education Program Adult Treatment Panel III definition of metabolic syndrome which includes criteria on central obesity, hypertension, insulin resistance, and dyslipidemia. Data represent mean (standard deviation). Sex and MetS Presence p -values determined through 2-sample test for given proportions; all other p -values determined with ANOVA. Abbreviations: control, CON; lentil, LEN; body mass index, BMI; Metabolic Syndrome, MetS; hemoglobin A1c, HbA1c; homeostatic model of insulin resistance, HOMA-IR; high-density lipoprotein, HDL; low-density lipoprotein, LDL.

3.2. Anthropometric Measures

We did not observe meal group differences in the change in waist circumference ($F = 0.2$, $p = 0.65$), fat mass percentage ($F = 1.13$, $p = 0.30$), body weight ($F = 0.06$, $p = 0.80$), or visceral adipose tissue ($F < 0.001$, $p = 0.98$) over the 12-week intervention. Furthermore, these anthropometric metrics did not change in either meal group from pre- to post-intervention.

3.3. Habitual Diet Analysis

Participants had similar habitual diets at baseline as determined by meal group comparisons of several DHQ-III measures including the following: total and component HEI scores, total daily energy intake (kcal), legume consumption (cups), and total, soluble, and insoluble fiber consumption (grams). Of note, total fiber consumption averaged 17.3 g in the LEN group and 22.9 g in the CON group which both represent deficit intakes according to the 2020–2025 dietary guidelines for Americans [27]. In examining differences from the intervention, we found that LEN participants reported increased sodium intake over the intervention ($t = 3.42$, $p < 0.01$), and CON participants reported decreased dairy ($t = 2.85$, $p = 0.04$) intake and increased refined grain ($t = 2.82$, $p = 0.04$) intake. Table 3 summarizes HEI scores from pre- and post-intervention by group.

As expected, participants in the LEN group had a significant increase in legume consumption ($\beta = 0.53$, $p < 0.001$) from a baseline daily average of 0.1 cups to 0.6 cups whereas average legume intake for CON remained unchanged at 0.2 cups. We also observed increases in the LEN group in the four HEI components that legume consumption is allocated to: total protein foods, seafood and plant proteins, total vegetables, and greens and beans. The LEN group also had an increase in total fiber (g) intake ($\beta = 9.5$, $p < 0.01$), insoluble fiber (g) intake ($\beta = 6.6$, $p < 0.01$), and soluble fiber (g) intake ($\beta = 2.9$, $p = 0.01$) over the course of the intervention whereas the CON group experienced a non-significant decrease in all fiber categories from baseline values (Supplementary Figure S3).

Table 3. Habitual dietary intake as depicted by HEI total and component scores before and after the 12-week dietary intervention. HEI component scores were calculated through an online DHQ III in which participants self-reported the types and frequency of foods consumed.

Component	Maximum Points	Score			
		CON (<i>n</i> = 18)		LEN (<i>n</i> = 20)	
		Pre	Post	Pre	Post
Adequacy					
Total fruits	5	3.6 ± 1.4	2.8 ± 1.5	3.3 ± 1.6	3.2 ± 1.6
Whole fruits	5	4.4 ± 1.0	3.9 ± 1.5	4.0 ± 1.5	3.9 ± 1.6
Total vegetables	5	4.0 ± 1.3	4.1 ± 1.0	3.9 ± 1.3	4.5 ± 1.0 *
Greens and beans	5	4.3 ± 1.2	4.2 ± 1.4	4.0 ± 1.5	4.8 ± 0.8
Whole grains	10	2.7 ± 1.1	3.3 ± 1.5	3.3 ± 1.6	2.9 ± 2.2
Dairy	10	7.4 ± 2.0	6.3 ± 2.0 *	6.8 ± 2.1	5.9 ± 2.5
Total protein foods	5	4.9 ± 0.4	4.9 ± 0.2	4.7 ± 0.9	5.0 ± 0.2
Seafood and plant proteins	5	4.4 ± 1.0	4.7 ± 0.7	4.2 ± 1.1	4.8 ± 0.8
Fatty acids	10	3.7 ± 2.4	4.1 ± 2.2	4.9 ± 2.5	5.0 ± 2.5
Moderation					
Refined grains	10	9.1 ± 1.4	7.1 ± 2.4 *	8.5 ± 2.2	8.2 ± 2.7
Sodium	10	3.4 ± 3.3	1.9 ± 1.8	4.1 ± 2.7	2.2 ± 2.1 *
Added sugars	10	8.0 ± 2.3	8.5 ± 1.6	7.9 ± 2.5	8.7 ± 1.5
Saturated fats	10	3.3 ± 2.9	4.0 ± 2.0	4.7 ± 2.9	5.5 ± 2.8
Total	100	63.1 ± 8.1	59.6 ± 7.7	64.1 ± 10.8	64.4 ± 7.5

Data represent mean ± sd. Linear mixed modeling with Tukey's HSD used to determine differences between group means. Difference ($p < 0.05$) between pre and post scores identified with *. Abbreviations: LEN, lentil; CON, control; and DHQ, diet history questionnaire.

3.4. Satiety during the Intervention

The overall response rate to the 4:00 p.m. survey on satiety was 90.2% ($n = 35$). The mean response rates were 89.6% and 90.8% for CON and LEN, respectively. Satiety measures were not different by meal group over the 12-week intervention (Supplementary Figure S4). Both groups self-reported a decrease in hunger ($\beta = -0.07$, $p = 0.02$) and desire to eat ($\beta = 0.07$, $p = 0.02$) over the course of the intervention period which corresponded to an increase in fullness ($\beta = 0.09$, $p < 0.01$). No change was observed in meal satisfaction over the 12 weeks with a mean score of 6.2 (CON) and 6.3 (LEN) out of 10. Only in the rating participants assigned to the amount of food they felt they could eat was a meal main effect ($p = 0.04$) observed with a lower average rating in the LEN (3.9) group than the CON (4.3) group.

3.5. Gastrointestinal Symptoms during the Intervention

The overall mean response rate to the 8:00 p.m. survey on GI symptoms was 95.4% ($n = 34$). Mean response rates for the GI survey were 89.0% and 89.4%, respectively, for LEN and CON. There was no difference in symptom severity by meal group over the 12 weeks ($p = 0.32$). Symptom severity responses for both groups throughout the entire intervention were predominantly rated as none or mild (87.7%) with only 10% rated as moderate and 2.3% as severe. We observed a main effect for symptom type in our mixed effects modelling, with average reported flatulence as the GI symptom with the highest severity, though it was rated as mild throughout the duration of this study ($p < 0.001$). Flatulence was followed sequentially in symptom severity by bloating, abdominal discomfort, and cramping, with average ratings of 0.8, 0.68, 0.36, and 0.27, respectively, on a scale from 0 (none) to 4 (severe). Weekly fluctuations in symptom severity are visualized in Supplementary Figure S5.

3.6. Fasting Lipid and Glycemic Measures

We observed decreased cholesterol measures with regular lentil consumption (Table 4). Specifically, we detected differences between groups in fasting total CHOL ($F = 8.64$,

$p < 0.01$) and LDL cholesterol ($F = 5.53$, $p = 0.02$) at the post-intervention time point. The LEN group averaged a 0.11 mmol/L decrease in total CHOL and a 0.03 mmol/L decrease in LDL whereas the CON group averaged a 0.36 mmol/L increase in total CHOL and a 0.29 mmol/L increase in LDL. We observed that fasting HDL differed by meal group ($F = 14.08$, $p < 0.001$) with LEN decreasing by 0.07 mmol/L and CON increasing by 0.11 mmol/L.

Table 4. Change in fasting blood markers (post-intervention–pre-intervention), grouped by dietary condition. Blood profile determined from fasting venous blood sample. Bold p -values indicate strong evidence of a difference between meal groups.

	CON (n = 18)	LEN (n = 20)	p -Value
Δ Total Cholesterol (mmol/L)	0.36 (0.43)	−0.11 (0.50)	<0.01
Δ HDL Cholesterol (mmol/L)	0.10 (0.14)	−0.07 (0.15)	<0.001
Δ LDL Cholesterol (mmol/L)	0.30 (0.36)	−0.03 (0.43)	0.02
Δ Triglycerides (mmol/L)	−0.21 (0.54)	−0.06 (0.73)	0.51
Δ GLU (mmol/L)	−0.05 (0.44)	−0.06 (0.34)	0.56
Δ INS (mmol/L)	−2.40 (8.84)	−3.83 (22.35)	0.85
Δ HbA1c (%)	0.06 (0.38)	0.03 (0.17)	0.56
Δ HOMA-IR	−0.59 (2.29)	−0.95 (5.23)	0.87

Data represent mean change (Δ) in fasting marker (post-intervention–pre-intervention) (standard deviation). p -values determined with ANOVA. Abbreviations: control, CON; lentil, LEN; high density lipoprotein, HDL; low density lipoprotein, LDL; glucose, GLU; insulin, INS; hemoglobin A1c, HbA1c; and homeostatic model of insulin resistance, HOMA-IR.

Participants entered the high-fat meal challenge with fasting TG concentrations considered normal (<1.7 mmol/L) to moderately elevated (2.0–5.6 mmol/L). We did not detect differences in fasting blood TG ($F = 0.18$, $p = 0.68$) between meal groups at the post-intervention time point.

Fasting GLU concentrations were normal (<5.5 mmol/L) prior to the high-fat meal challenge. Most participants maintained blood glucose levels under 7.8 mmol/L at the 2 h postprandial mark indicating a normal blood glucose response to a mixed meal, except for one participant who had a level of 7.88 mmol/L indicative of borderline prediabetes. We did not detect differences between meal groups in fasting blood GLU ($F = 0.35$, $p = 0.56$), fasting INS ($F = 0.03$, $p = 0.85$), HOMA-IR ($F = 0.03$, $p = 0.87$), or HbA1c ($F = 0.34$, $p = 0.56$). Changes to fasting blood markers from pre- to post-intervention are summarized in Table 4.

3.7. Postprandial Lipid Response

To better understand the impact of lentil consumption on postprandial metabolism, we examined postprandial lipid and glucose responses to a high-fat meal challenge. There was no effect of meal ($F = 0.42$, $p = 0.52$) on the change in TG AUC (dTG_{AUC}) with an average dTG_{AUC} of 0.24 mmol/L for CON and −0.08 mmol/L for LEN. There was substantial variation between individuals in the dTG_{AUC} in both meal groups with responses ranging from a 2.7 mmol/L decrease to a 3.2 mmol/L increase from pre- to post-intervention. Variability in the change in TG AUC for each participant is summarized in Figure 2. During the high-fat meal challenge, 35 of the 38 participants reached the threshold of 1.98 mmol/L (175 mg/dL) for hypertriglyceridemia diagnosis in non-fasting states [45]. See Supplementary Figure S6 for a summary of the postprandial triglyceride response by meal group.

3.8. Postprandial Glycemic Response

We found that regular lentil consumption affected postprandial glucose but not insulin responses. Specifically, regular lentil consumption significantly lowered glucose AUC (GLU_{AUC}) ($\beta = -0.99$, $p = 0.01$) with an average (mean) increase of 0.10 mmol/L in the CON group and a 0.67 mmol/L decrease in the LEN group, accounting for visceral adipose and change in BMI. The effect of intervention meal on the change in GLU_{AUC} ($dGLU_{AUC}$)

values for each participant is displayed in Figure 3. Lentil consumption did not impact insulin AUC (INS_{AUC}) ($F = 1.00$, $p = 0.32$). Average glucose concentrations from the high-fat meal challenge before and after the intervention are displayed in Supplementary Figure S7.

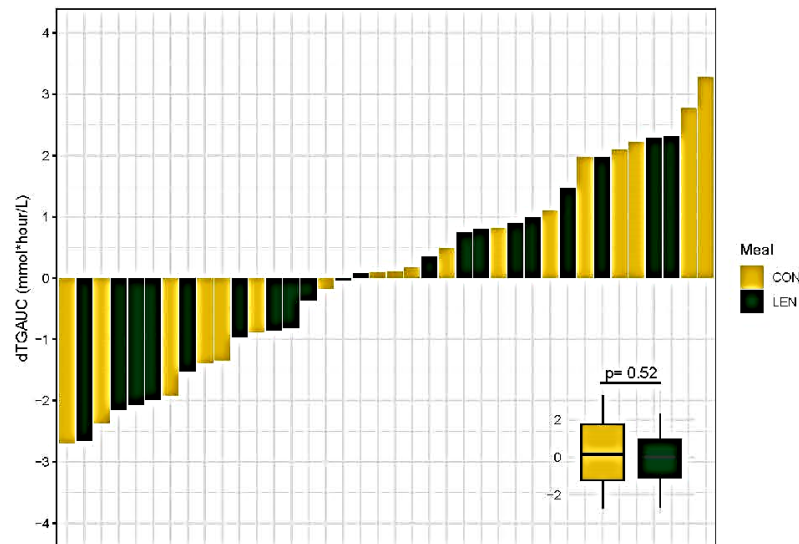


Figure 2. Summary plots of individual changes (post-intervention–pre-intervention) in triglyceride area under the curve (dTG_{AUC}). dTG_{AUC} values represent the change in the sum of values from fasting and hourly timepoints for 5 h post high-fat meal ingestion. Each bar is representative of the value for one participant ($n = 38$). Difference in dTG_{AUC} between meal groups determined with ANOVA and displayed in inset. CON, control and LEN, lentil.

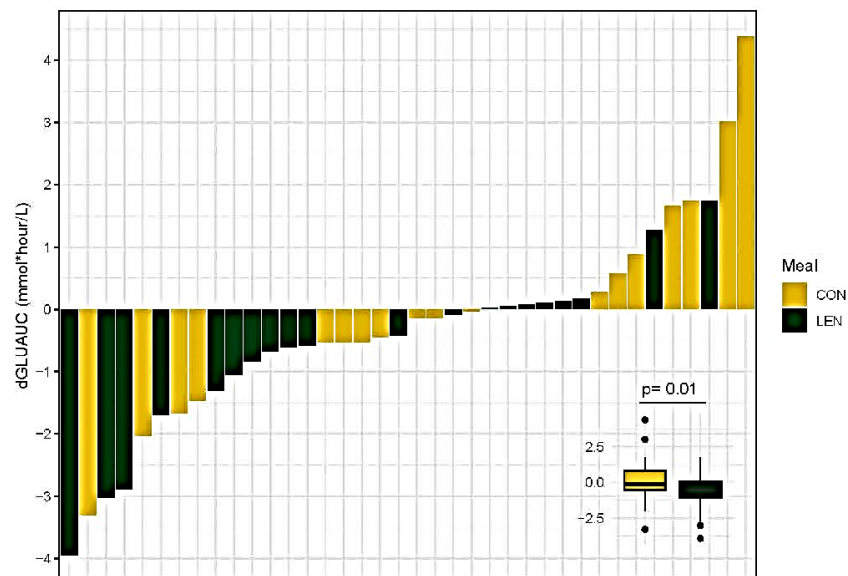


Figure 3. Summary plots of individual changes (post-intervention–pre-intervention) in glucose area under the curve (dGLU_{AUC}). dGLU_{AUC} values represent the change in the sum of values from fasting and hourly timepoints for 5 h post high-fat meal ingestion. Each bar is representative of the value for one participant ($n = 38$). Difference in dGLU_{AUC} between meal groups determined with ANOVA and displayed in inset. CON, control and LEN, lentil.

3.9. Fasting and Postprandial Inflammation

As high-fat meals can induce an inflammatory response, we also examined a range of inflammatory markers. We found that regular lentil consumption decreased postprandial inflammation as measured with IL-1 β _{AUC} ($F = 5.4$, $p = 0.03$) and IL-17_{AUC} ($F = 4.80$, $p = 0.04$) (Figure 4). Though not significant at $\alpha = 0.05$, postprandial measures of IL-6, IL-10, TNF- α , GM-CSF, and IFN γ decreased in the LEN group and increased in the CON group from pre- to post-intervention, whereas both meal groups had a decrease in IL-23 (Supplementary Table S3). We did not detect a meal effect on changes in fasting inflammation markers, accounting for visceral adipose tissue (Supplementary Table S3).

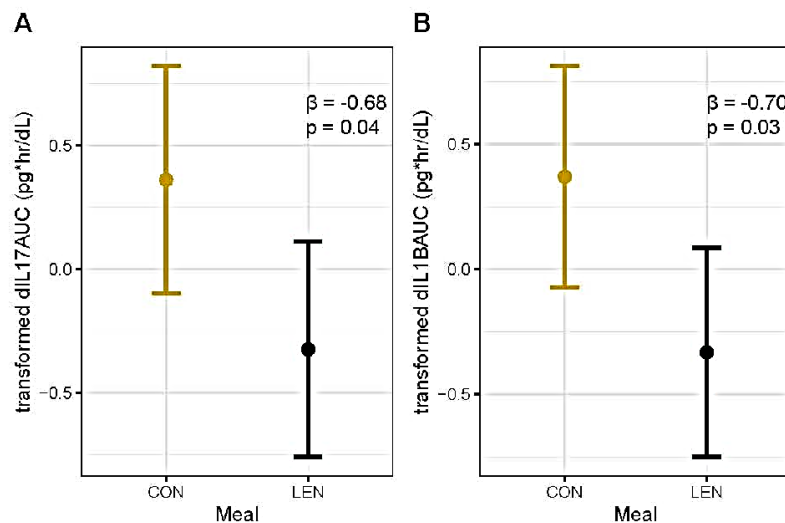


Figure 4. Main effect plot for meal group from (A) IL-17_{AUC} and (B) IL-1 β _{AUC} linear models. Points indicate the average change in each marker as determined with the general linear model, and bars indicate 95% confidence intervals. Y-axis represents transformed values for each marker: orderNorm (IL-17_{AUC}) and double reverse log (IL-1 β _{AUC}). β coefficient and p -value for LEN meal from model displayed in upper right corner. IL-1 β , Interleukin 1 beta; IL-17, Interleukin 17; CON, control; and LEN, lentil.

4. Discussion

Increased adiposity [2–4] and postprandial lipids [21–23,46] are established predictors of disease risk and are causally linked to low-grade inflammation and metabolic disease [25,26]. We recruited adults with high metabolic risk, marked by increased central adiposity and a high postprandial triglyceride response, and assessed the impact of regular lentil consumption compared to meat-based meals on fasting and postprandial lipid, glucose, and inflammatory measures. In addition, we surveyed participants about gastrointestinal (GI) symptoms throughout the 12-week intervention to assess the potential GI impacts of lentil consumption. Key findings of this study include decreased or maintained fasting cholesterol levels with lentil consumption and increased levels with consumption of meat-based meals. In addition, we observed improved postprandial glucose and inflammation responses to a high-fat meal challenge with long-term lentil consumption. Self-reported GI symptoms were not different between lentil and control groups, and all symptoms were reported as none or mild for the duration of the intervention. Importantly, metabolic improvements occurred independent of changes in anthropometric measures which suggests a direct impact of lentil consumption on metabolism.

In our study, fasting measures of lipid metabolism, including total and LDL cholesterol, were decreased with 12 weeks of daily lentil consumption. There are mixed results in the literature examining the impact of pulse consumption on fasting lipid markers.

In a study examining the impact of eight weeks of mixed pulse (chickpeas, lentils, yellow split peas, and navy beans) intake at an average of 896 g/week on metabolism in adults with metabolic syndrome, no statistically significant changes were found in fasting triglyceride, total, or LDL cholesterol levels [47]. However, a study utilizing similar mixed pulses (lentils, chickpeas, peas, and beans) and an average weekly dose of 1050 g (dry weight) for eight weeks identified improvements to fasting cholesterol levels with pulse consumption in older adults [48]. Improvements to cholesterol with lentil consumption in our study may potentially be explained by the ability of fiber to bind bile acids, which decreases the return of bile acids to the liver and stimulates production of hepatic bile acids. Decreased hepatic cholesterol levels are replenished through cholesterol uptake from the blood which decreases serum cholesterol levels [49]. Lentils are also a rich source of saponins, bioactive compounds with a demonstrated ability to lower cholesterol levels via a variety of mechanisms including regulation of lipid metabolism and prevention of cholesterol absorption [50,51]. Habitual lentil consumption could also lower total saturated fat intake, a dietary component associated with cholesterol levels. However, this is not supported by our dietary survey data which indicate comparable saturated fat intake at pre- and post-intervention timepoints. Clinical trials investigating the impact of lentil consumption on lipid metabolism and bile acids are needed to provide more insight regarding the cholesterol lowering capability of lentils.

Long-term lentil consumption did not improve fasting markers of glycemic control including HbA1c and fasting glucose. There are mixed results in the literature concerning the impact of pulse consumption on glycemic control. Some randomized clinical trials found improvements to HbA1c and HOMA-IR with lentil consumption [9,20]. However, a recent meta-analysis of 28 long-term (3–72 weeks) randomized controlled trials examining the effects of pulse intake on glycemic control found that while long-term incorporation of pulses into dietary eating patterns attenuated fasting glucose in normoglycemic adults and lowered fasting glucose in participants with type 2 diabetes, consumption had no significant effect on fasting insulin, HOMA-IR, or HbA1c [8]. Importantly, only five studies examined the impact of single pulses and only three of these utilized the pulse in its whole food form. Because the metabolic benefits of pulse consumption vary by pulse variety, dosage, and duration of inclusion in the diet [15], more randomized controlled trials examining the impact of long-term consumption of single pulses on metabolic health are needed to clearly define their impact on glucose metabolism.

To better understand the impact of lentil consumption on postprandial metabolism, we examined postprandial glycemic and lipid responses to a high-fat meal challenge. We found that long-term, daily lentil consumption lowers postprandial glucose in a manner independent to the “same-meal” or acute effect of lentil consumption, as no lentils were consumed the day of the meal challenge. The mechanism of this effect is still debated, but lowered blood glucose with acute lentil ingestion has been associated with both fiber- and protein-dependent changes to glucose metabolism [52]. While acute legume intake has been found to lower postprandial glucose levels [8,52], few studies have examined the impact of long-term pulse consumption on postprandial glucose [6]. A review [6] of randomized controlled trials ($n = 18$) investigating long-term legume consumption (>6 weeks) on markers of glycemic control identified only one study [53] examining postprandial glucose responses which found that 6 weeks of mixed bean consumption lowered 2 h postprandial glucose levels in individuals with diabetes. A number of factors impact the postprandial glycemic response including baseline glycemic status and insulin secretion [54]. However, we found no changes to fasting glycemic variables or postprandial insulin responses in response to long-term lentil consumption. Our results may be explained by short chain fatty acid (SCFA) production during bacterial fermentation of indigestible carbohydrates [55]. SCFA's modulate metabolism in several ways including decreased hepatic glucose output [56]. This process modulates postprandial glycemia independent to mechanisms attributed to acute meal effects due to the delayed appearance of SCFA's in the blood, secondary to meal ingestion and bacterial fermentation [57]. In this way, increased dietary fiber from

lentil consumption may have reduced postprandial glucose concentrations. More studies are needed to decipher the relationship between long-term lentil intake and improved postprandial metabolism. This relationship is important to identify as long-term studies are a better indicator of the metabolic impacts of frequently applied dietary strategies to improve health.

Contrary to our hypothesis, fasting and postprandial triglyceride responses were not improved with 12 weeks of lentil consumption in our cohort of individuals with established elevated postprandial triglyceride responses. On average, we did observe a trend of small decreases in the lentil group as opposed to small increases in the meat-based control group. These results are in contrast to studies demonstrating modified postprandial lipemia responses with habitual diet changes [58] and studies demonstrating the ability of legume consumption to decrease blood triglyceride levels [9,59–61]. However, we are not aware of any studies specifically assessing long-term lentil intake on postprandial triglyceride responses. Our ability to detect changes with lentil consumption may have been masked by high interindividual variability in triglyceride responses both at baseline and after the intervention. Large interindividual variability in blood triglyceride responses were found in the large PREDICT 1 cohort in which factors such as gut microbiome composition influenced postprandial lipemia more than macronutrient composition of the meal [62]. In addition, lifestyle factors such as meal timing, physical activity [63], and sleep may also predict individual responses [62]. These results may support the use of personalized nutrition approaches in the application of any dietary strategy to improve metabolic health.

We found that long-term consumption of lentil-based meals reduced postprandial IL-1 β and IL-17 responses to a high-fat meal challenge compared to the meat-based meals. As IL-17 is a colonic inflammation marker, lowered IL-17 may be mediated by changes to the GI microbiome and microbial metabolites from habitual lentil consumption. Increased fiber consumption promotes the growth of microbiota-accessible carbohydrate degrading bacteria [64] and increases metabolic capacity to utilize carbohydrates by increasing carbohydrate active enzymes [65]. These changes promote microbial production of fiber fermentation derivatives such as SCFAs. SCFAs beneficially influence gut health in many ways including reducing colonic inflammation and improving gut epithelial barrier integrity. Anti-inflammatory effects of SCFAs include enhancing activity of immunosuppressive regulatory T-cells [66] and inhibiting proinflammatory cytokine production [67,68]. A lentil supplementation study in the murine model identified decreased colonic proinflammatory cytokines and increased expression of the tight junction protein occludin [69], which suggests lentils may confer a protective effect against colonic inflammation through improved barrier function. Though IL-1 β is not an intestinal inflammation marker, previous work has shown that postprandial increases in IL-1 β secretion via peritoneal macrophages occur in a glucose dependent manner [70]. It is therefore possible that the decrease in IL-1 β in the lentil group may be a reflection of their lower postprandial glucose responses. We did not observe changes in fasting markers between meal groups which echoes previous findings in eight-week legume feeding trials in humans [71,72]. Further research on postprandial inflammation may consider the inclusion of gastrointestinal health markers and relationships with glucose metabolism.

Given the impacts of lentils on GI health, it is important to note that microbial responses to fiber depend on the fiber type and the host GI microbiome [73]. The broad fiber analysis of our study lentils limits our ability to speculate based on fiber type, but we might reasonably expect a fiber composition of 3% oligosaccharides (dry weight), 44% total starch content, and 11% of total starch as resistant starch based on chemical analysis of other green lentil cultivars [74]. Resistant starch displays low-to-intermediate specificity, meaning it can be metabolized by many bacteria. As substrate usage is not taxa-specific, individuals display high interindividual variability in microbial responses to low-specificity fiber types based on their initial GI communities [73]. Microbiome response variability may also contribute to variable immunological profiles after fiber consumption [65]. Thus, it is possible that our participant microbiomes had personalized compositional and metabolic responses

to fiber consumption which contributed to the observed postprandial responses in this study. Future studies assessing the impact of lentil consumption on inflammation should consider the contributions from microbial fermentation, with particular care given to the fermentability of fiber types and fermentation indicators such as fecal pH or serum SCFA.

Importantly, improvements in metabolism with the study dose of 980 g of lentils per week were not associated with increased GI symptoms as compared to control meals. This finding is impactful as GI symptoms are infrequently reported in pulse interventions, and we are aware of only one study exclusively examining lentils that reported symptomology, which was a study conducted by our research group [20]. Additionally, legume consumption by US adults is low, with an estimated 15% of the population consuming legumes at the amount (≥ 87.5 g/d) recommended to achieve disease prevention benefits [75]. Consumers commonly report GI symptoms as a barrier to increased consumption [18]. In this study, the GI symptom with the highest average severity was flatulence; however, severity was still reported as mild throughout the duration of the intervention. Furthermore, severity was not different between meal groups. GI discomfort largely arises from bacterial fermentation of indigestible complex carbohydrates such as soluble fiber and resistant starch [76–78]. Lentils are primarily rich in insoluble fiber [79] but are also high in starch content (~ 36 g/100 g) (Supplementary Table S1). The analysis performed on the lentils used in this study did not include further characterization of starch type, so it is unclear if the lack of GI discomfort with lentil intake was due to inadequate substrate for increased bacterial fermentation and secondary gas production or substrate adaptation. However, because our intervention dose was over the current USDA recommendation for pulse consumption, we have effectively demonstrated that USDA pulse consumption recommendations may be met with lentil intake without an increase in gastrointestinal symptoms.

We recognize there are additional factors and limitations which may have influenced changes to metabolic and inflammatory markers that were not measured in the current study. First, the interpretation of study results is constrained to individuals who are representative of the study sample. This sample comprises individuals at an increased metabolic risk, as indicated by elevated waist circumference and postprandial triglyceride levels. In addition, while intervention meals were matched for protein and energy content, the carbohydrate, fiber, and fat content were different between meals. This variance in macronutrient composition represents the difference between lentil-based meals and meat-based meals and is the basis of our study design and statistical analysis. However, we cannot exclude the possibility that these differences impacted outcome measures in a manner independent to lentil consumption. Although participants were asked to maintain their regular dietary and physical activity patterns for the duration of this study, it is possible that alterations to eating and exercise patterns during the 12-week period may have impacted study measures. However, as anthropometric measures did not change between groups during this study, large lifestyle shifts do not seem likely. In addition, total HEI scores were not different for either meal group from pre- to post-intervention, indicating a relatively stable diet intake. The decrease in dairy intake and increase in refined grain intake in the control group as well as the increase in sodium intake in the lentil group may be due to changes in diet in response to the intervention meal. For example, single-serving yoghurt and cheese are commonly consumed lunch foodstuffs which would be replaced by the intervention meals which were all low in dairy content. The intervention meals were also low in refined grain and sodium content, so the increase in these scores may be reflective of compositional changes to the next meal consumed. It is also possible that changes to HEI component scores are reflective of estimation errors common to all self-reported dietary intake questionnaires. There is some evidence to indicate that sex differences are an important variable to consider regarding fasting and postprandial lipid and glycemic measures [62], but we were unable to do so with the limited sample size of male participants. Our male participants were not detected as outliers in models examining metabolic and inflammatory changes. The survey used to collect GI symptom and severity data was self-reported. This method has been used and reported as

an effective measure of gastrointestinal symptoms in various studies [80–82]. However, the survey results could be further validated with external observations and ratings by trained medical professionals. Lastly, lentils are a rich source of dietary bioactives which represent an additional mechanism for improvement to metabolic health measures. However, these nutrients were not analyzed so we cannot define their impact on study results.

In an effort to comprehensively measure the impact of our lentil dose on metabolic health, we measured postprandial lipid, glucose, and inflammation responses in addition to corresponding fasting metrics. While the importance of postprandial measures in relation to metabolic health is well-established, these measures are not consistently measured in pulse intervention studies—particularly in non-acute intervention studies. The completion of these measures in our study more comprehensively characterized the impact of long-term lentil intake on metabolic health. Other strengths of this study include the use of a whole-food based intervention in free-living adults with meals matched in protein and calorie content. This approach is most representative of dietary changes likely to be made by individuals wanting to incorporate more pulses into their diet. In addition, only one pulse, lentils, were tested as opposed to studies examining increased pulse intake via incorporation of multiple pulses. Because the impact of pulse intake on metabolic health is both dose and pulse-specific, randomized controlled trials examining individual pulses are necessary to fully characterize their impact on metabolic health and establish dietary recommendations. Lastly, the dietary dose of 980 g/week meets, and exceeds, the recommendation set for pulse intake by the USDA and is reflective of long-term lentil intake with a 12-week intervention period—criteria which are less frequently examined in pulse intervention studies. In this manner, our study results are highly applicable towards the development of dietary recommendations for lentil consumption to improve metabolic health. Gastrointestinal (GI) symptoms associated with pulse intake are also infrequently measured in the literature. Our study directly contributes to this knowledge gap by collecting questionnaires from participants on a weekly basis during the intervention period regarding commonly reported GI symptoms associated with the intervention meals.

5. Conclusions

In conclusion, our findings provide evidence that long-term lentil consumption in individuals with increased risk for metabolic disease, as defined by elevated waist circumference and postprandial triglyceride responses, can mitigate increases in fasting cholesterol levels as well as postprandial glucose and inflammatory responses. Our findings help address the current limited body of research regarding the impact of legume consumption on gastrointestinal symptomology with the important finding that lentil consumption over the USDA recommended dose is not associated with increased GI distress. These results implicate daily consumption of lentils as a safe and effective dietary strategy to improve metabolic health which may help reduce barriers to increased consumption for health benefit. This information further informs the development of pulse-based dietary strategies to lower disease risk and to slow or reverse metabolic disease progression in at-risk populations. Further investigation of the long-term impact of other pulse crops on fasting and postprandial metabolism and systemic inflammation is warranted.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu16030419/s1>. Supplementary Figure S1. Timeline of study events. Baseline anthropometric and screening questionnaires completed at visit 1 followed by testing for elevated postprandial triglycerides (>1.98 mmol/L) at visit 2. Individuals with elevated postprandial triglycerides were enrolled in this study and completed a 5 h high-fat meal challenge, dietary questionnaires, and stool collection at visit 3. Participants were randomly allocated to CON or LEN experimental groups with varying weekly doses of lentils, CON, control (0 g/week) and LEN, lentil (980 g/week), for 12 weeks. Repeat testing of visit 3 metrics and anthropometric testing was completed at visit 4. Supplementary Figure S2. Survey questions assessing satiety and gastrointestinal issues. Online surveys were delivered electronically once a week each week of the 12-week dietary intervention. Satiety was scored on a scale from 0 to 10 as indicated below.

Gastrointestinal symptom severity was scored as follows: 0 = None, 1 = Mild, 2 = Moderate, and 3 = Severe. Supplementary Figure S3. Differences in daily fiber intake from pre- to post-intervention by meal group. Significance determined using paired *t*-test with * indicating significant effect of time (pre- to post-intervention) at a level of $\alpha = 0.05$. Supplementary Figure S4. Self-reported satiety measures over the 12-week intervention by meal group. An online satiety survey was electronically delivered at 4 p.m. once a week for each week of the 12-week dietary intervention. Participants were asked to self-report hunger, fullness, satisfaction, desire to eat, and amount of food that could be eaten (see Supplementary Figure S2 for survey questions). All measures on 10-point scale. Points indicate group means and bars represent $\pm$ standard error. LEN, lentil and CON, control. Supplementary Figure S5. Self-reported gastrointestinal symptoms over the 12-week intervention by meal group. An online survey addressing gastrointestinal symptoms was electronically delivered at 8 p.m. once a week for each week of the 12-week dietary intervention. Participants were asked to self-report symptoms of flatulence, bloating, cramping, and abdominal discomfort (see Supplementary Figure S2 for survey questions). All measures on 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). Points indicate group means and bars represent $\pm$ standard error. LEN, lentil and CON, control. Supplementary Figure S6. Postprandial triglyceride (TG) response to a 50 g, high-fat meal challenge by meal group before and after the 12-week dietary intervention. Points indicate group means and bars represent $\pm$ standard error. LEN, lentil and CON, control. Supplementary Figure S7. Postprandial glucose (GLU) response to a 50 g, high-fat meal challenge by meal group before and after the 12-week dietary intervention. Points indicate group means and bars represent $\pm$ standard error. LEN, lentil and CON, control. Supplementary Table S1. Nutrient and functional analysis of whole green lentils used in the 12-week intervention. Supplementary Table S2. Overview of intervention meals and their nutrient profile. Profile based off the first week of study meals, where participants received one of each of the seven study meals. Macronutrient totals were rounded to nearest whole number. Supplementary Table S3. Change in fasting and postprandial inflammation by meal group before and after the 12-week dietary intervention. Fasting values represent timepoint 0 (fasting) collected prior to 50 g, high-fat meal challenge. Postprandial values summarized as net area under the curve (AUC) using fasting and 1-, 2-, 3-, 4-, and 5-h postprandial measurements. Delta values calculated as (post-intervention–pre-intervention) and expressed by Δ Baseline to represent changes in fasting inflammation and Δ AUC to represent changes in postprandial inflammation. Values as mean (standard error). Bold *p*-values indicate strong evidence of a difference between meal groups.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board at Montana State University (2020-131-MM010320-FCR) on 2/24/2020. This study was prospectively registered with ClinicalTrials.gov (NCT04283448).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to participant confidentiality.

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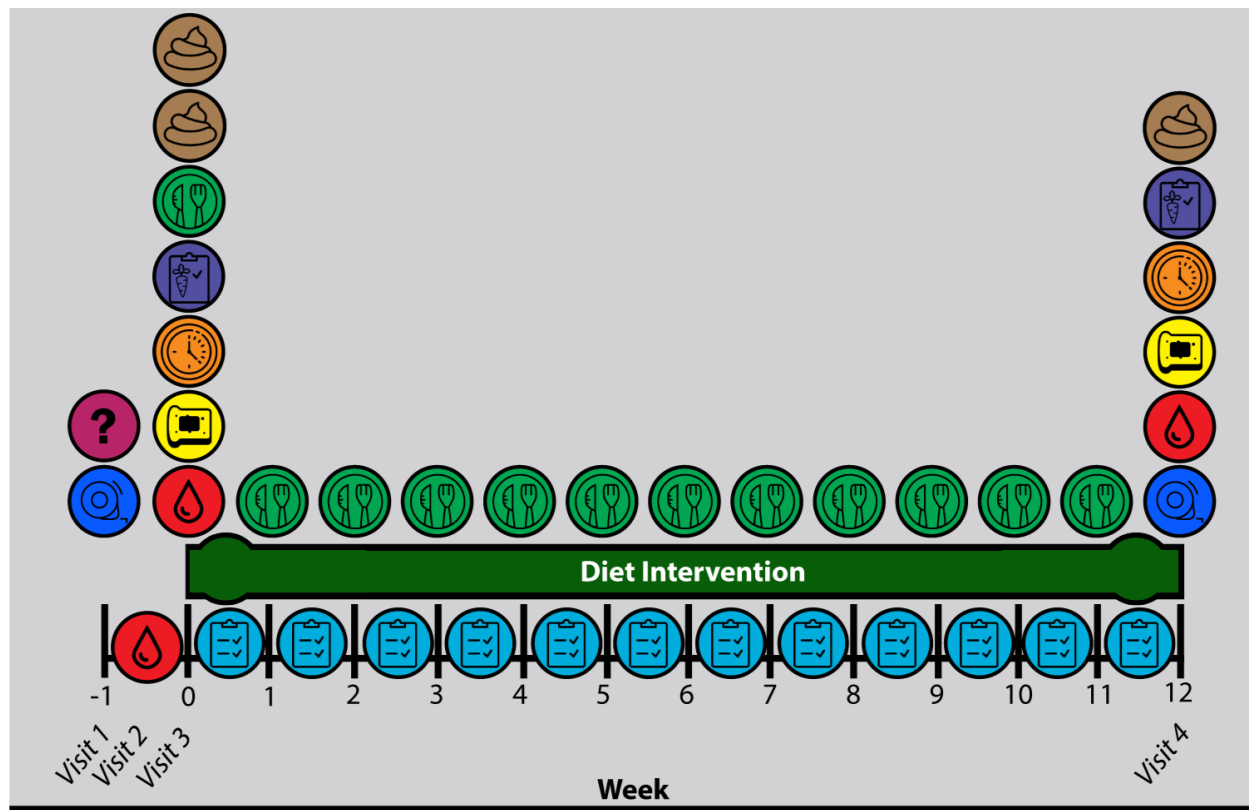
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Supplementary Figure 1. Timeline of study events. Baseline anthropometric and screening questionnaires completed at Visit 1 followed by testing for elevated postprandial triglycerides (>1.98 mmol/L) at Visit 2. Individuals with elevated postprandial triglycerides were enrolled in the study and completed a 5-hour high-fat meal challenge, dietary questionnaires, and stool collection at Visit 3. Participants were randomly allocated to CON or LEN experimental groups with varying weekly doses of lentils: CON, control (0 g/week); LEN, lentil (980 g/week) for 12 weeks. Repeat testing of Visit 3 metrics and anthropometric testing was completed at Visit 4.



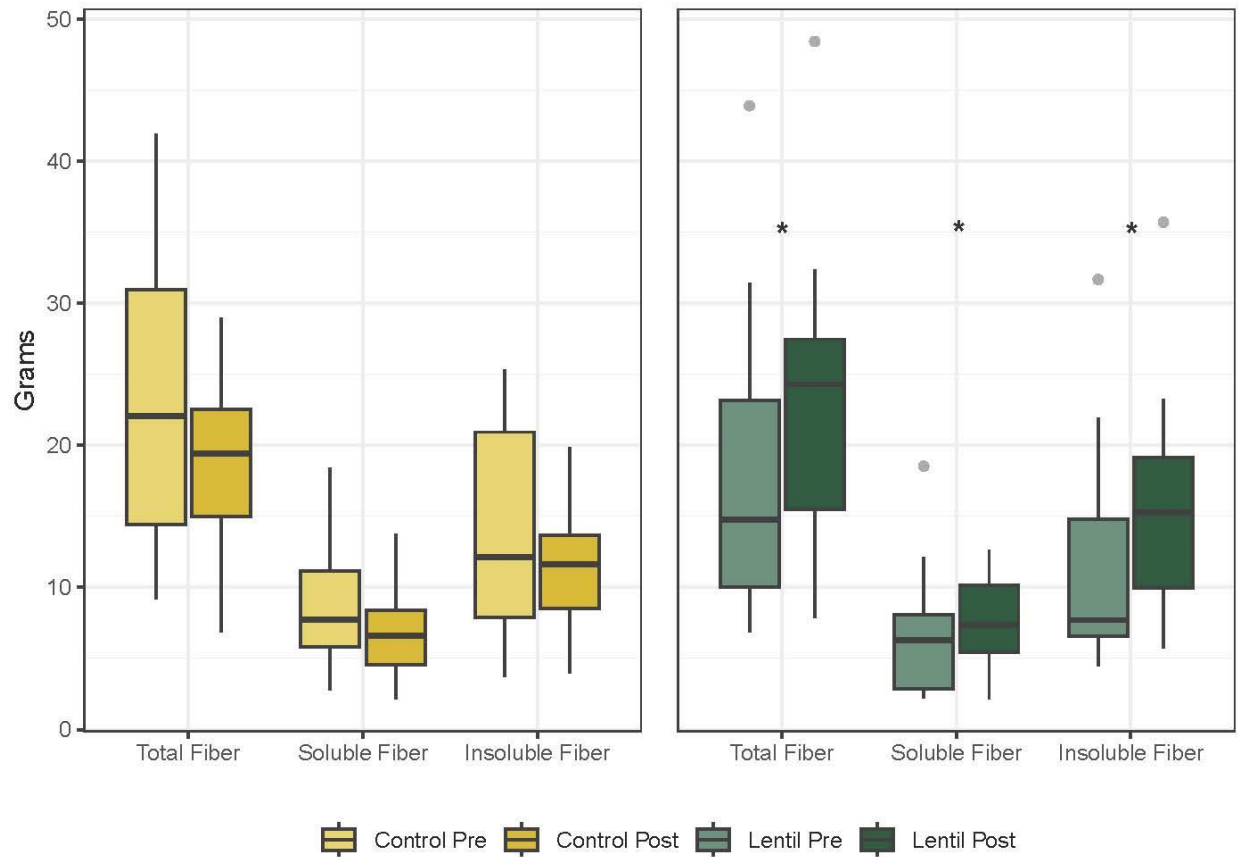
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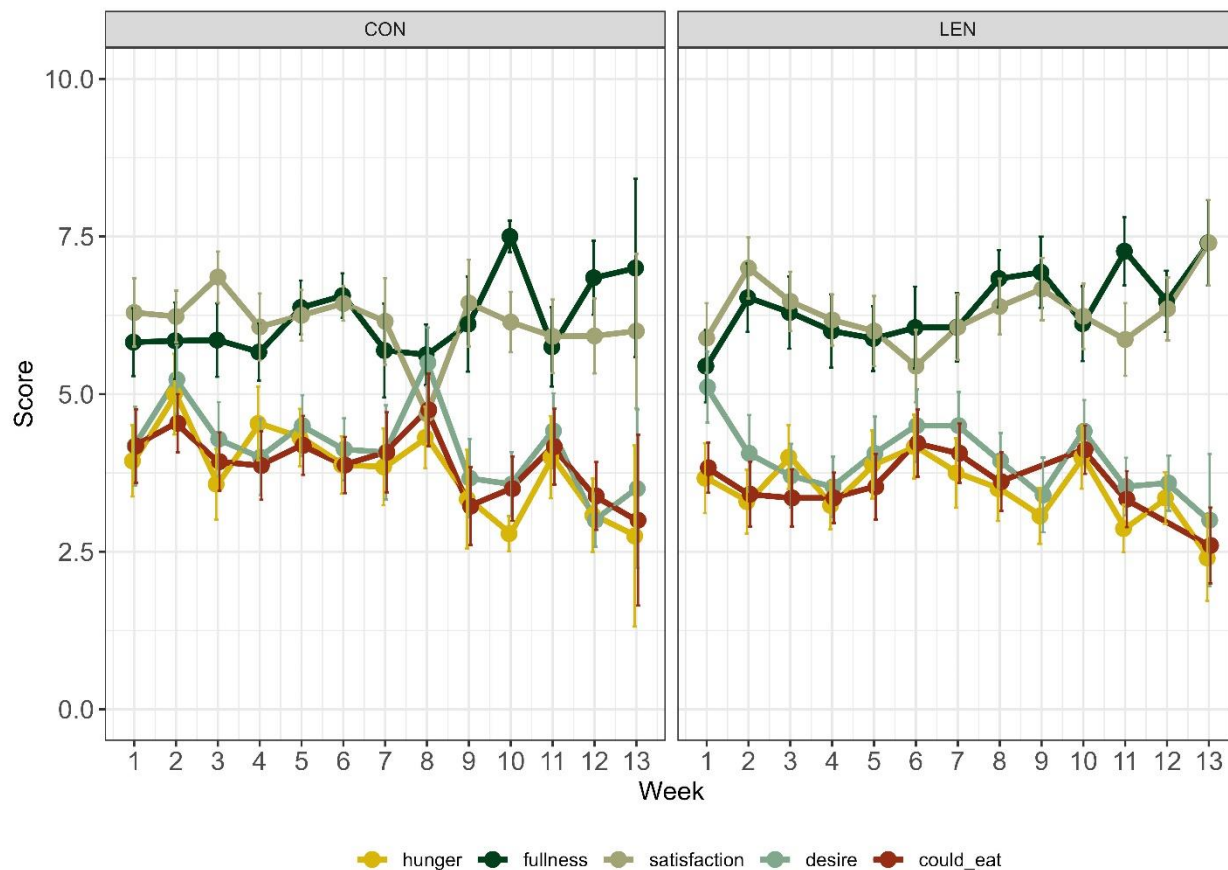
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|--|---|--|---|--|----------------------------|
| | Questionnaires
- Health Screening
- Physical Activity
- Race and Ethnicity | | High-fat meal challenge | | Anthropometrics |
| | Blood Collection | | Meal Pick-up
- 7 meals per week | | Diet History Questionnaire |
| | 24-hour Diet Recall | | Evening Surveys
- 4 PM: Satiety
- 8 PM: GI Issues | | Stool Sample Collection |

Supplementary Figure 2. Survey questions assessing satiety and gastrointestinal issues. Online surveys were delivered electronically once a week each week of the 12-week dietary intervention. Satiety was scored on a scale from 0 – 10 as indicated below. Gastrointestinal symptom severity was scored as follows: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe.

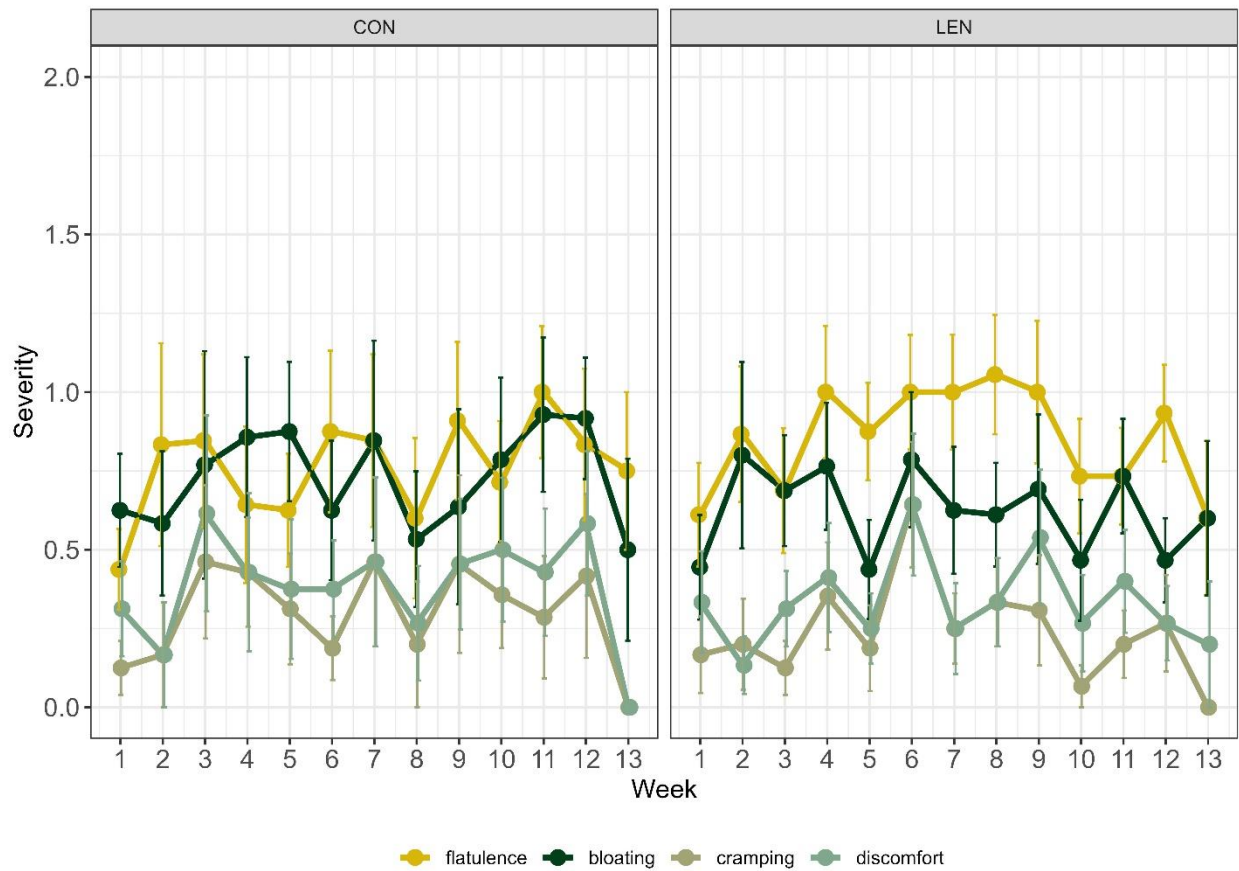
Question	Response
4:00 PM Satiety	Ordinal
How hungry are you?	0 = not at all, 10 = extremely
How full are you?	0 = not at all, 10 = extremely
How satisfied do you feel?	0 = not at all, 10 = extremely
How strong is your desire to eat?	0 = very weak, 10 = very strong
How much do you think you could (or would want to) eat right now?	0 = nothing, 10 = A lot
8:00 PM Gastrointestinal Issues	Ordinal
Please rate below how on how you feel OVERALL today.	
Flatulence (having excessive stomach or intestinal gas)	None, Mild, Moderate, Severe
Bloating (the abdomen feels full and tight, often interpreted as excessive intestinal gas)	None, Mild, Moderate, Severe
Cramping (a type of pain that comes and goes. Can be very uncomfortable but is relieved by passing gas or having a bowel movement)	None, Mild, Moderate, Severe
Abdominal Discomfort (a feeling of discomfort or pain located in the upper or lower abdominal area)	None, Mild, Moderate, Severe

Supplementary Figure 3. Differences in daily fiber intake from pre- to post- intervention by meal group. Significance determined using paired t-test with * indicating significant effect of time (pre- to post- intervention) at a level of $\alpha=0.05$.

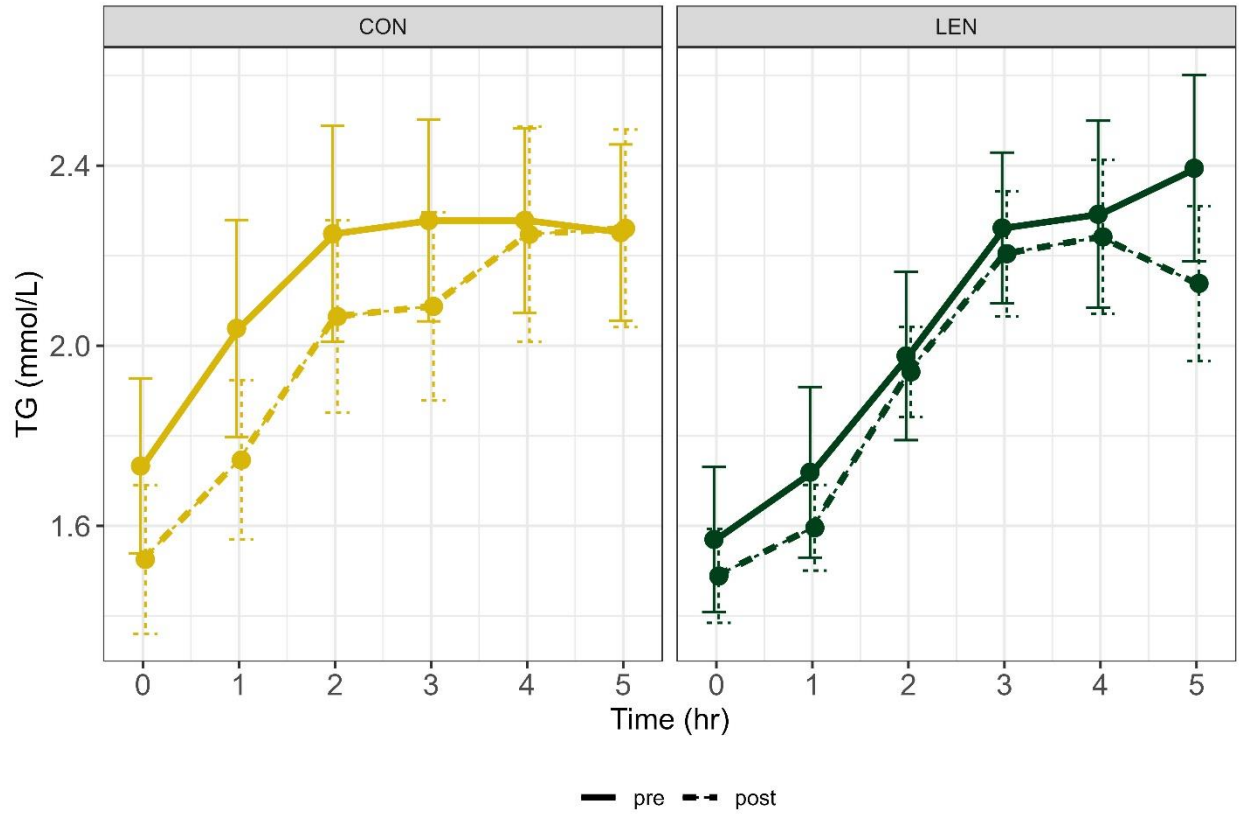




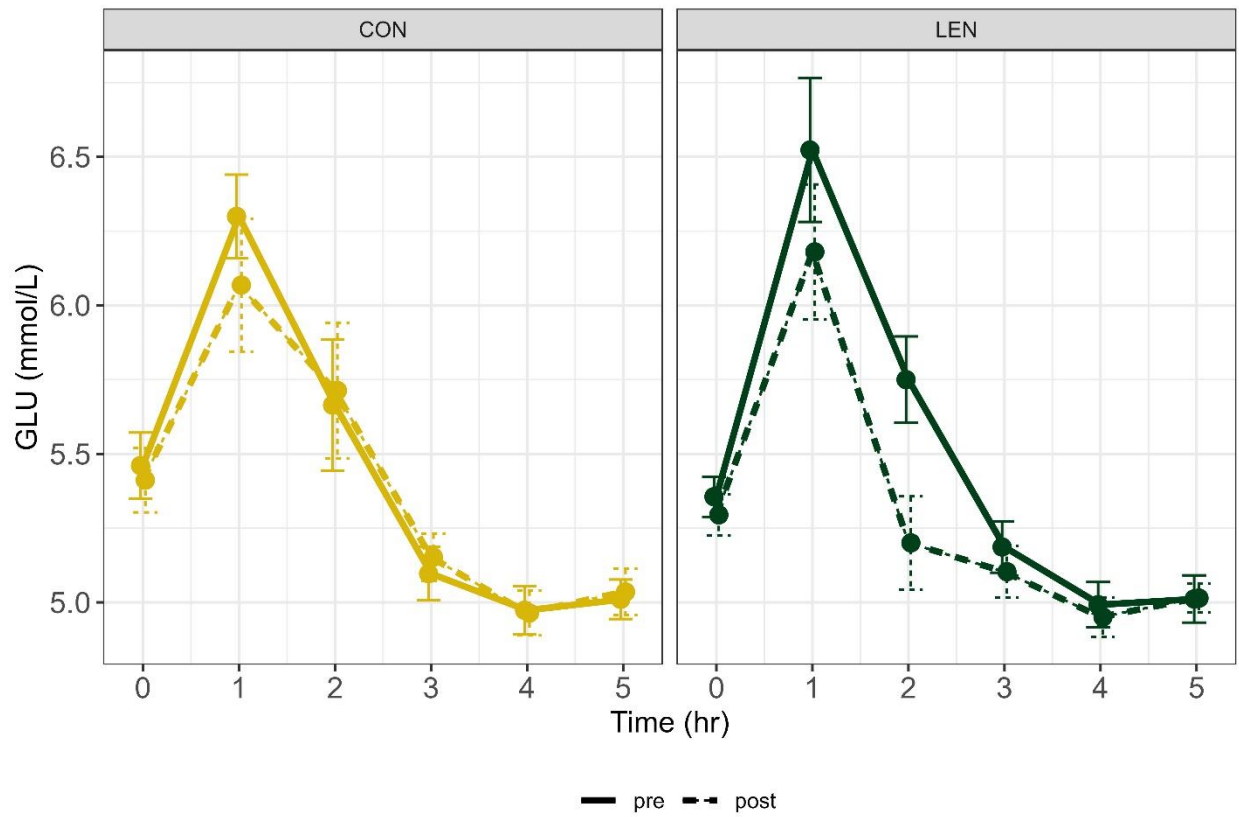
Supplementary Figure 5. Self-reported gastrointestinal symptoms over the 12-week intervention by meal group. An online survey addressing gastrointestinal symptoms was electronically delivered at 8 pm once a week for each week of the 12-week dietary intervention. Participants were asked to self-report symptoms of flatulence, bloating, cramping, and abdominal discomfort (see Supplemental Figure 2 for survey questions). All measures on 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe). Points indicate group means, bars represent $\pm$ standard error. LEN, lentil; CON, control.



Supplementary Figure 6. Postprandial triglyceride (TG) response to a 50-gram, high-fat meal challenge by meal group before and after the 12-week dietary intervention. Points indicate group means, bars represent $\pm$ standard error. LEN, lentil; CON, control.



Supplementary Figure 7. Postprandial glucose (GLU) response to a 50 gram, high-fat meal challenge by meal group before and after the 12-week dietary intervention. Points indicate group means, bars represent $\pm$ standard error. LEN, lentil; CON, control.



Supplementary Table 1. Nutrient and functional analysis of whole green lentils used in the 12-week intervention.

Analysis	Amount
Nutrient (per 100g)	
Calories (kcal)	362
Moisture (g)	9.5
PRO (g)	23.4
Fat (g)	2.1
Ash (g)	2.7
Total CHO (g)	62.3
Soluble Fiber (%)	0.4
Insoluble Fiber (%)	13.3
Starch (g)	36.27
Functional	
Foam Expansion (%) (5% w/w)	218
Water Holding Capacity (g/g)	1.3
Foam Stability (%)	94
pH (5% w/w)	6.3

Abbreviations: Kcal, kilocalories; PRO, protein; g, grams; CHO, carbohydrate.

Supplementary Table 2. Overview of intervention meals and their nutrient profile. Profile based off the first week of study meals, where participants received one of each of the seven study meals. Macronutrient totals were rounded to nearest whole number.

	CON					LEN				
	Kcal	PRO g	CHO g	FAT g	Fiber g	Kcal	PRO g	CHO g	FAT g	Fiber g
Bolognese	260	18	31	16	3	384	23	57	9	10
Curry	555	28	63	20	2	413	21	50	9	9
Loaf	396	23	38	19	4	482	25	66	9	11
Taco	455	25	33	26	3	604	34	76	13	11
Soup	272	20	25	5	1	251	18	42	2	7
Shepherd's Pie	587	34	53	31	4	549	23	73	21	10
Chili	234	14	24	10	2	259	17	36	3	8
TOTALS	2,759	162	267	127	19	2,942	161	400	66	66

Abbreviations: CON, control; LEN, lentil.; Kcal, kilocalories; PRO, protein; g, grams; CHO, carbohydrate.

Supplementary Table 3. Change in fasting and postprandial inflammation by meal group before and after the 12-week dietary intervention. Fasting values represent timepoint 0 (fasting) collected prior to 50 gram, high-fat meal challenge. Postprandial values summarized as net area under the curve (AUC) using fasting and 1-, 2-, 3-, 4-, and 5- hour postprandial measurements. Delta values calculated as (post-intervention – pre-intervention) and expressed by Δ Baseline to represent changes in fasting inflammation and Δ AUC to represent changes in postprandial inflammation. Values as mean (standard error). Bold p-values indicate strong evidence of a difference between meal groups.

Cytokine	CON (n=18)		LEN (n=20)		p-value	
	Δ Baseline	Δ AUC	Δ Baseline	Δ AUC	Δ Baseline	Δ AUC
IL-6	0.26 $\pm$ 1.79	0.09 $\pm$ 3.93	2.20 $\pm$ 5.52	-12.42 $\pm$ 31.92	0.63	0.10
IL-1 β	0.04 $\pm$ 0.78	1.09 $\pm$ 3.92	0.65 $\pm$ 2.05	-4.06 $\pm$ 9.94	0.19	0.03
IL-17	-0.84 $\pm$ 4.93	13.82 $\pm$ 31.64	3.56 $\pm$ 12.62	-27.07 $\pm$ 83.07	0.91	0.04
IL-23	-12.27 $\pm$ 129.20	122.67 $\pm$ 731.23	-28.54 $\pm$ 527.21	-234.36 $\pm$ 941.87	0.74	0.14
IL-10	0.62 $\pm$ 12.72	8.69 $\pm$ 38.50	3.46 $\pm$ 12.93	-43.64 $\pm$ 133.11	0.25	0.10
TNF- α	-0.76 $\pm$ 1.88	3.07 $\pm$ 9.37	1.47 $\pm$ 4.17	-7.59 $\pm$ 21.85	0.50	0.29
GM-CSF	-4.53 $\pm$ 17.42	-77.16 $\pm$ 482.39	3.00 $\pm$ 24.99	-24.35 $\pm$ 67.97	0.11	0.14
IFN- γ	11.67 $\pm$ 85.84	6.99 $\pm$ 434.06	9.69 $\pm$ 143.72	-27.20 $\pm$ 438.32	0.44	0.88

Data represent mean $\pm$ standard error. Linear mixed modeling used to determine differences ($p < 0.05$) between group means. Abbreviations: CON, control; LEN, lentil.; IL, interleukin; TNF- α , tumor necrosis factor alpha; GM-CSF, granulocyte macrophage colony stimulating factor; IFN- γ , interferon-gamma; AUC, area under the curve.

CHAPTER THREE

ACUTE LENTIL CONSUMPTION ALTERS THE
RELATIONSHIP BETWEEN POSTPRANDIAL BILE ACIDS
AND GLUCOSE AND TRIGLYCERIDE METABOLISM: A
RANDOMIZED CROSSOVER TRIAL

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Conducted the study; curated the data; generated visualizations; performed statistical analyses; wrote the manuscript; read, revised, and approved final manuscript.

Co-Author: B. Sather

Contributions: Methodology development; curated the data; read, revised, and approved final manuscript.

Co-Author: G. Cooper

Contributions: Methodology development; curated the data; read, revised, and approved final manuscript.

Co-Author: S.M.G. Wilson

Contributions: Methodology development; read, revised, and approved final manuscript.

Co-Author: B. Bothner

Contributions: Study conceptualization; funding acquisition; methodology development; study validation; read, revised, and approved final manuscript.

Co-Author: M.P. Miles

Contributions: Study conceptualization; funding acquisition; methodology development; study validation; read, revised, and approved final manuscript.

Manuscript Information

M.L. Chamberlin, B. Sather, G. Cooper, S.M.G Wilson, B. Bothner, and M.P. Miles

Nutrition Research

Status of Manuscript: [Put an x in one of the options below, then delete instruction in brackets]

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-reviewed journal
- ☐ Accepted by a peer-reviewed journal
- ☐ Published in a peer-reviewed journal

Elsevier

Abstract

Lentils have potential to improve bile acid metabolism, but few clinical trials evaluate the impact of acute lentil consumption on the postprandial bile acid response. The aim of this randomized crossover study was to assess the impact of a high-fat meal with (LEN) and without (CON) 140 g of cooked green lentils on the postprandial bile acid response as well as postprandial glucose, triglyceride, and inflammation responses in individuals with elevated metabolic risk. Twenty-four adults with overweight or obesity ($\text{BMI} > 27 \text{ kg/m}^2$) completed testing for both meal conditions, serving as their own control. Blood was collected at fasting and half-hour time points for four hours postprandially for blood marker assessment. A total of 12 plasma bile acids were identified for analysis using liquid chromatography-mass spectrometry. Linear models were used to assess the impact of meal on postprandial measures. Spearman correlation tests were used to assess the relationship between postprandial bile acid, triglyceride, glucose, and insulin responses. Both meals stimulated postprandial responses, but we did not detect a difference between LEN and CON meals on triglyceride ($F = 0.02$, $p = 0.60$), glucose ($F = 3.57$, $p = 0.07$), insulin ($F = 0.08$, $p = 0.78$), or total bile acid ($F = 1.6$, $p = 0.22$) responses. However, inverse correlations between bile acids and postprandial glucose and triglyceride responses were identified between meal conditions. In LEN, positive correlations between chenodeoxycholic acid ($r = 0.46$, $p = 0.02$) and deoxycholic acid ($r = 0.40$, $p = 0.04$) and triglyceride responses were identified. In CON, a negative correlation between tauroursodeoxycholic acid ($r = -0.63$, $p = 0.01$) and triglycerides and positive correlations between glycocholic acid ($r = 0.56$, $p < 0.01$), glycochenodeoxycholic acid ($r = 0.5$, $p = 0.01$), and glycodeoxycholic acid ($r = 0.52$, $p = 0.01$) and postprandial glucose were identified. Our

results suggest an interaction between fiber and bile acids on postprandial glucose and lipid metabolism. Further study regarding the impact of acute fiber intake on bile acids and their relation to postprandial metabolism is warranted.

Introduction

Obesity, particularly abdominal adiposity, increases the risk of metabolic diseases including non-alcoholic fatty liver disease, type 2 diabetes, cardiovascular disease, and certain types of cancer.¹ These diseases are a leading cause of morbidity and mortality worldwide and are characterized by metabolic abnormalities including dyslipidemia, low-grade inflammation, and insulin resistance.² Bile acids (BAs) are cholesterol-derived molecules best known for their role in fat emulsification and absorption. However, they have been increasingly recognized as powerful regulators of metabolic health with roles in energy, glucose, and lipid homeostasis as well as gut and systemic immunity.^{3,4} Changes in the size and composition of the BA pool have been reported in obesity⁵⁻⁷ and associated metabolic disease states including type 2 diabetes^{5,8,9}, insulin resistance¹⁰, and nonalcoholic steatohepatitis.¹¹ Accordingly, altered BA profiles may serve as markers of impaired metabolism or causative mediators of metabolic disturbances.^{5-10,12} Therefore, healthy changes to BA profiles represent one strategy to improve metabolism and lessen progression of metabolic diseases.

Most regulatory functions exerted by BAs occur via receptor interactions within enterohepatic circulation as BA cycling during a meal is highly efficient with <10% of BAs reaching systemic circulation.¹³ However, BA receptors are expressed in a plethora of extrahepatic tissues which allows the fraction in systemic circulation to influence metabolic processes.³ Metabolically unhealthy states have been associated with decreased hepatic

resorption of BAs and increased spillover to systemic circulation.³ Elevated fasting and postprandial BA concentrations in systemic circulation allow for increased systemic BA signaling which may contribute to metabolic disturbances.^{5-10,12} Compositional changes in BA profiles have also been identified in metabolic pathologies. In particular, increased concentrations of 12 α -hydroxylated BAs including cholic acid (CA), deoxycholic acid (DCA), and their conjugated species have been identified in individuals with obesity⁶ as well as insulin resistance^{5,14,15}, type 2 diabetes⁸, and non-alcoholic steatohepatitis.¹⁶ Activation of receptors is specific to each BA based on binding affinity and receptor agonism or antagonism. For this reason, these compositional changes in the BA pool can also contribute to BA-mediated metabolic irregularities.

Accumulating evidence suggests that dietary fiber intake can beneficially modify the composition and concentration of BA profiles¹⁷⁻²⁴ with subsequent improvements to glucose and lipid metabolism.^{18,19} Interactions between dietary fiber and BAs in the small intestine result in lower reabsorption rates, increased passage to the colon, and subsequent loss of BAs through fecal excretion.²⁵ Increased excretion rates decrease the size of the BA pool²⁴ and promote increased de-novo synthesis of BAs from cholesterol in hepatocytes, thereby altering BA composition.²⁵ Fiber consumption also alters gastrointestinal microbial-BA interactions. BAs reaching the distal small intestine and colon are subject to microbial enzymatic activity resulting in the formation of secondary BA species.²⁶ Fiber has been shown to alter these conversion reactions resulting in BA compositional changes.²⁴ These fiber-induced modifications to BA circulation resemble those that occur in response to therapeutic BA sequestrant therapies which are utilized for their hypoglycemic and hypolipidemic effects.²⁷ Pulse crops such as beans, peas,

and lentils are excellent sources of dietary fiber providing up to 30 g per 100 g dry weight, around half of which is insoluble.²⁸ A variety of pulses including pinto beans, black-eyes peas, lentils, and chickpeas have been shown to increase fecal BA excretion and decrease hepatic and systemic cholesterol levels in humanized mouse models²⁹ demonstrating the favorable effects of pulses on BA metabolism. However, studies directly measuring the interactions between dietary pulses and BAs are limited in humans. Furthermore, the impact of acute pulse consumption on postprandial bile acid responses has not been examined.

Lentils are a type of pulse that are high in dietary fiber and protein and also contain dietary bioactive compounds such as saponins and polyphenols.³⁰ To our knowledge, there are no randomized controlled trials investigating the efficacy of acute dietary lentil consumption to lower or modify composition of serum BAs in humans. We completed a randomized, crossover trial in which participants consumed a high-fat meal with and without lentils. In addition to stimulating BA secretion, high-fat meals stimulate postprandial triglyceride³¹, glucose³¹, and inflammation³² responses which are established predictors of metabolic health³³⁻³⁶. We measured these postprandial responses to characterize the metabolic response to acute lentil ingestion and the relationship of BAs with these measures. This is the first study that we know of that comprehensively measures the impact of acute lentil consumption on postprandial lipidemic, glycemic, and inflammatory responses and examines the “same-meal” effect of fiber consumption on postprandial bile acid responses. Therefore, this study provides new findings to inform the development of fiber-based strategies to modify bile acid profiles and improve metabolic health.

Materials and Methods

Ethics Statement

The protocol was approved by the Institutional Board at Montana State University (2020-132-MM110520-FCR). The informed consent was reviewed verbally in-person, allowing time for questions and clarifications. Written informed consent was obtained from all participants prior to their participation. The study was prospectively registered with ClinicalTrials.gov (NCT05245357).

Study Population

Potential participants were recruited via advertisement from February 2022 through September 2023 and screened for eligibility using REDCap^{37,38} electronic data capture tools hosted at Montana State University, Bozeman. Inclusion criteria included being between 18 and 65 years old with a body mass index (BMI) ≥ 27 kg/m². Potential participants were excluded if they had taken oral antibiotics within 90 days of study enrollment, regularly used anti-inflammatory, oral contraceptive, or lipid lowering medications, had wheat, dairy, or legume allergies, were pregnant or lactating, had an existing gallbladder condition or gallbladder removal, had any musculoskeletal, cardiovascular, gastrointestinal, or immunological condition that could interfere with the study, had previously been diagnosed as diabetic/pre-diabetic or had an HbA1c $\geq 6.5\%$ during testing at their first study visit.

Research Design

The study was a double-blind randomized crossover trial with a high-fat meal challenge containing 0 or 140 g of cooked green lentils. Testing for the human subject cohort took place in

the Nutrition Research Laboratory at Montana State University. Participants reported to the lab for a total of three visits. Subject consent, anthropometric measurements, and diabetic screening as measured by glycated hemoglobin (HbA1c) via venipuncture or finger stick were completed at the first visit. Participants completed a high-fat meal challenge (40 g oral fat load) with 140 g lentils (LEN) and 0 g (CON) lentils on two separate occasions with blood collected at fasting and half-hour time points for four hours postprandially for blood marker assessment. Stool samples were collected for bile acid analysis. Each participant completed this protocol a total of two times, once for each meal condition. Participants were randomized to initial meal condition and completed a 7-day washout period between visits. Participants were instructed to consume a pulse-free diet for one week prior to their first blood collection and for the duration of the study period.

Anthropometrics

During the first visit, a validated³⁹ segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) was utilized to assess body mass index (BMI) and visceral adipose. Subjects were instructed to refrain from eating, drinking, or exercising in the 3 hours prior to testing. Blood pressure was taken in the morning of the second and third visits after subjects had been seated for at least 15 minutes. Automated measurements were taken in duplicate with the mean used for analysis.

Randomization

Block randomization was performed using the *blockrand* function in the R *blockrand* package.⁴⁰ Nine blocks of four with two equally numbered levels were created, with the seed for each block individually set based on the last five integers returned by the *sys.time* function. Meals

were prepared by a registered dietitian and chef on the research team who did not participate in data collection. Meals were coded so that researchers completing randomization and conducting data collection visits were masked to treatment allocation. Participants were randomized to consume either the LEN or CON meal for their first blood collection visit (Visit 2). The opposite meal condition was then consumed for their second collection visit (Visit 3). After data collection was completed, meals were de-coded for data analysis by the same researchers who conducted research visits.

Lentil Nutritional Analysis

Medium green lentils (CDC Richlea cultivar) used in the lentil high-fat meal condition were sent courtesy of AGT Foods Canada to Medallion Labs (Minneapolis, MN). Analytical testing with the nutrient breakdown and functional properties are provided in **Supplementary Table 1**. Dietary fiber was determined through the analytical method AOAC 991.43.

High-Fat Meal Challenge

The high-fat meal was designed to stimulate BA secretion as well as postprandial inflammatory, triglyceride, and glucose responses. It consisted of savory pancakes containing salted butter (49 g, Tillamook) and either 140 g of lentils (LEN) or 0 g of lentils (CON). Total energy content of the lentil meal was 529 kcal, with 70.2% from fat, with a macronutrient breakdown of 40.5 g fat, 28.1 g carbohydrate, 13.0 g protein and 14.5 g fiber. The control pancake utilized white rice and egg whites to match protein and carbohydrate content of lentils without adding fiber (**Supplementary Table 2**). All other ingredients were identical to the lentil pancake. Water was provided with the meal; caffeinated black tea was provided for participants

who identified as habitual coffee consumers. Participants were asked to consume the meal in 15 min, and the postprandial period timing began when participants started the meal.

Recent and Habitual Diet Surveys

Participants were also asked to provide information on recent and habitual dietary intake at Visit 2. Recent dietary intake was assessed through a 24-hour dietary recall collected and analyzed with the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version (2020), developed by the National Cancer Institute, Bethesda, MD. The ASA24 was orally administered by research staff to participants. The generated report includes portion sizes and specific food items consumed in the 24 hours prior to the high-fat meal challenge. A physical copy of the report was provided to the participant to match food and beverage intake before repeat testing with the other high-fat meal condition.

Habitual dietary intake was collected using the validated diet history questionnaire (DHQ III), a web-based survey offered by the National Cancer Institute. Participants were asked to complete a recall assessing intake in the month prior to study enrollment. Participants were encouraged to give their best estimates of habitual intake during the recall as exact recollection of portion sizes for all food and beverage intake is unlikely. The DHQ III consists of 135 food and beverage and 26 supplement questions to assess dietary frequency and portion sizes. Through built-in analysis of DHQ III surveys, we obtained a calculated Healthy Eating Index (HEI, 2015) score, which is a measure of diet quality in compliance with the 2015 through 2020 US Dietary Guidelines for Americans.^{41,42} Total HEI score is the sum of four components based on moderation (refined grains, sodium, saturated fats, added sugars) and nine components based on adequacy

(total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids).

Satiety Surveys

A satiety survey assessing hunger, fullness, satisfaction, desire to eat, and amount of food desired was provided to participants before consumption of the high-fat meal as well as at hourly timepoints after meal consumption (1, 2, 3 and 4 hours postprandially). A visual analogue scale was used for participants to indicate responses. Scores for each survey question were calculated as described by Weigl and colleagues.⁴³ Satiety questions and response scales are outlined in **Supplementary Figure 1.**

Blood Sampling

Participants were instructed to avoid strenuous physical activity and alcohol consumption for 24 hours and fast (10-12 hours) overnight prior to their blood collection visit. Venous blood samples were collected via an intravenous catheter from the antecubital vein after a 3-mL waste withdrawal and then followed by a sterile saline flush. The fasting sample was drawn 20 min after catheter insertion. After fasting blood collection, participants consumed the high-fat meal followed by blood collection every half hour for 4 hours after meal ingestion, totaling 9 blood collections. Whole blood was collected in two sodium heparin tubes (BD Vacutainer, Franklin Lakes, New Jersey, USA). One tube was kept on ice and allowed to clot for 15 minutes before centrifugation (1200 RPM for 15 minutes). Separated plasma was immediately aliquoted and stored at -80°C for bile acid analysis as well as inflammation and insulin analysis. Whole blood from the other sodium heparin tube was immediately utilized for blood lipid and glucose analysis.

Analysis of Blood Markers

Whole blood collected in a sodium heparin tube at fasting and hourly postprandial timepoints was run on Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, USA) which included total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and glucose (GLU). Glycated hemoglobin (HbA1c) was determined using the Affinion2 analyzer (Abbott, United States) from whole blood. Plasma insulin was determined at fasting and hourly postprandial timepoints through ELISA (MP Biomedicals, United States) performed according to manufacturer instructions, with the average used for analysis.

Analysis of Inflammation Biomarkers

Cytokine measurement on plasma samples from fasting and hourly postprandial timepoints was performed using high-sensitivity multiplexing technology (Bio-Rad Bio-Plex® 200 HTS) following procedures by Millipore (EMD Millipore Corporation, Billerica, USA). Selected cytokines included granulocyte macrophage colony stimulating factor (GM-CSF), interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and five interleukins (IL) including IL-1 β , IL-6, IL-10, IL-17, IL-23. Samples were run in duplicate, and the mean used for analysis. Sample values that were under the detectable limit were replaced with ½ the minimum detectable concentration for the respective cytokine for analysis.⁴⁴

Bile Acid Analysis

Plasma Extraction. Plasma samples from fasting and postprandial half-hour timepoints were removed from -80°C storage and allowed to thaw in groups of 48. 100 μ L of thawed

plasma was removed and placed in a clean vial after which 400 μ L of cold acetone was added to precipitate protein. Samples were then stored at -80°C overnight to aid precipitation.

Precipitated samples were spun in a centrifuge for 10 min at $20,000\times g$. The resulting supernatant was removed and placed in a clean vial while the remaining protein pellet was discarded. The metabolite-rich supernatant was concentrated under negative pressure in a Concentrator Plus (Eppendorf, Hamburg, Germany) to dryness. Dried samples were stored at -80°C until ready for LCMS analysis, at which time the samples were reconstituted with 100 μ L of MeOH:H₂O (51) and placed in a clean mass spectrometry vial. Upon resuspension, pooled samples were generated using two 5 μ L aliquots for either a treatment specific pool, control or lentil, and a super pool. Additionally, extraction blanks of the solvents without sample were run through the same pipeline for every 48 samples.

Untargeted Liquid Chromatography Mass Spectrometry (LCMS) Metabolomics Analysis.

LCMS untargeted analysis was completed on a Waters I-Class UHPLC coupled to a Waters Synapt-XS Q-IMS-TOF. Initial sample separation was completed on an Agilent Eclipses Plus C18 RRHD 1.8 μm (2.1 x 100mm) column. A 10-minute gradient was employed using UHPLC water and methanol, both with 10mM ammonium acetate, as mobile phases A and B, respectively. Separation began with 98% A for 1 minute, at which time B was increase linearly to 45% at 5 minutes. At 5 minutes, B was decreased to 50% and held for 1 minute. At 6.1 minutes, a linear gradient continued to 80% B for 2 minutes. At 8.1 minutes, B was increased to 98% and held there until the run finished at 10 minutes. The column compartment was kept at 40°C with a flow rate at 0.6 mL/minute.

MS acquisition only in negative mode with the Waters instrument set to the sensitivity function was used for every sample to collect spectra. In addition to MS, MS^e was used to fragment the pooled samples for use in identification. A Metabolomics Bile Acid Mixture was purchased from Cambridge Isotope Laboratories, Inc. with 16 total bile acid standards and spiked into serum to verify retention time values for the 16 standards.

Metabolomic Data Processing. After MS analysis, data was processed (deconvolution, alignment, peak peaking, normalization, and scaling) using Progenesis QI software to aid in reliable detection of metabolite differences. A mol file-based SDF database was generated based upon the 16 bile acids and used for theoretical fragmentation spectra referencing. Metabolite data was processed using Progenesis MetaScope with a 5 ppm error identification search parameter for m/z, isotopic distributions, and theoretical fragmentation. Bile acid identifications were then generated and verified using both the referenced retention time values from the spiked serum as well as the annotated identifications found using the SDF database. The bile acids identified as well as their classifications are listed in **Table 1**.

Table 1. Identified bile acids and their classifications.

Bile Acid	Abbreviation	Classification
Chenodeoxycholic acid	CDCA	Primary, unconjugated
Glycocholic acid	GCA	Primary, conjugated, 12 α -OH
Taurocholic acid	TCA	Primary, conjugated, 12 α -OH
Glycochenodeoxycholic acid	GCDCA	Primary, conjugated
Taurochenodeoxycholic acid	TCDCa	Primary, conjugated
Deoxycholic acid	DCA	Secondary, unconjugated, 12 α -OH
Taurodeoxycholic acid	TDCA	Secondary, conjugated, 12 α -OH
Glycodeoxycholic acid	GDCA	Secondary, conjugated, 12 α -OH
Glycolithocholic acid	GLCA	Secondary, conjugated
Ursodeoxycholic acid	UDCA	Tertiary, unconjugated
Glycoursodeoxycholic acid	GUDCA	Tertiary, conjugated
Tauroursodeoxycholic acid	TUDCA	Tertiary, conjugated

Abbreviations: 12 α -hydroxylated, 12 α -OH

Statistical Analysis

Analysis and visualizations were conducted in RStudio (2023.06.1) running R 4.3.1, with data visualized using *ggplot2*⁴⁵ and *effects*⁴⁶ packages.

Power. Power for this study was calculated apriori using results from Sonne et al. (2016) that compare normal adults with those with type 2 diabetes on the total bile acid AUC for a low-fat meal⁸, based on techniques discussed in Wan et al. (2014) and using Champely (2020).^{47,48} This provided an effect size suggesting that 13 subjects were needed to attain 80% power with a one-sided test from a paired t-test. Because it is not possible to guarantee such a large effect, we enrolled 24 subjects to provide power of 0.8 for a Cohen's effect size of just over 0.5.

Anthropometric and Postprandial Metabolic Measures. Descriptive summary statistics were performed for participant anthropometric and biological characteristics. Analysis of postprandial metabolic (TG, GLU, INS) and inflammatory (GM-CSF, IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-17, IL-23) measures were conducted by summarizing each markers measurements at timepoints 0 (fasting) and 1, 2, 3, and 4 hours postprandially as net area under the curve (AUC_{net}) using the *auctime*⁴⁹ R package. Postprandial TG, GLU, and INS were also summarized as peak concentration (C_{max}) and time to C_{max}. Linear mixed effects models were used to assess the impact of intervention meal on each dependent variable with subject as the random effect. Predictor variables were shared within each category of variables and are as follows: blood lipid measures –meal, visceral adipose tissue; glycemic measures –meal, visceral adipose tissue, BMI, HbA1c; inflammation measures –meal, visceral adipose tissue. Predictor variables for glycemic measures were chosen based on previous findings by Zeevi and colleagues.⁵⁰

Bile Acids. Correlations of fasting plasma bile acids with baseline anthropometric and fasting glucose and lipid markers were calculated using the Spearman rank test and adjusted for multiple comparisons using false discovery rate (FDR) correction. Analysis of plasma postprandial BAs were conducted by summarizing each BAs measurements at timepoints 0 (fasting) and every half-hour afterwards for 4 hours postprandially as AUC_{net} using the *auctime*⁴⁹ R package. Change in BA concentrations was also calculated as the difference between peak concentration and fasting concentration. Linear mixed effects models were used to assess the impact of intervention meal on individual BAs and grouped BA classifications (primary, secondary, tertiary, conjugated, unconjugated, 12- α hydroxylated, total) with subject as the

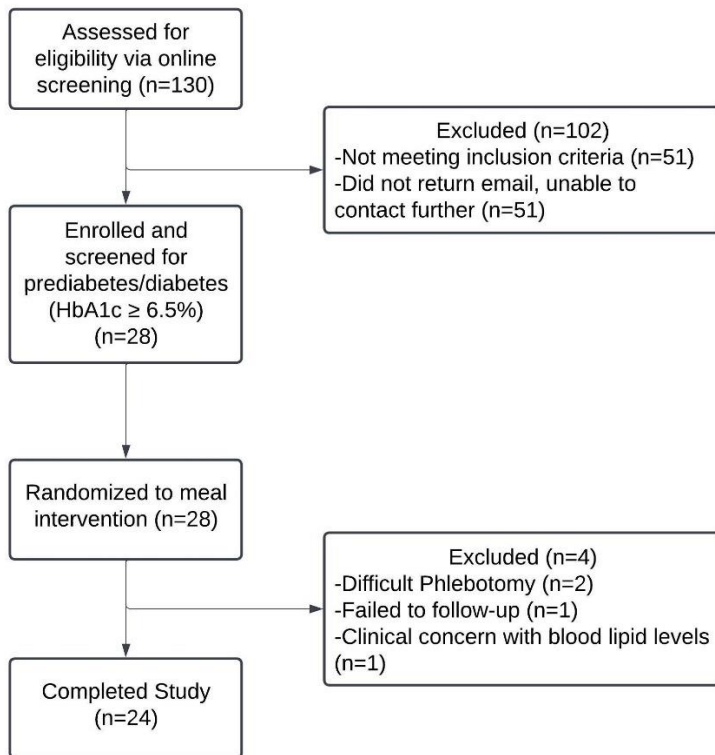
random effect. Correlations of postprandial BAs with postprandial TG, GLU, and INS metabolic blood markers were calculated by using the Spearman rank test with FDR correction.

Results

General Characteristics of Participants

A total of 130 participants were screened for study participation with a total of 102 excluded for not meeting inclusion criteria or lost to follow-up. Twenty-eight individuals were enrolled and screened for prediabetic and/or diabetic status as determined by $\text{HbA1c} \geq 6.5\%$. All 28 individuals met HbA1c criteria and were randomized to an initial meal intervention. A total of 4 individuals were lost after study enrollment for various reasons (**Figure 1**).

Figure 1. A CONSORT flowchart of study design and enrollment. Flyers and emails were used to recruit interested individuals. Interested individuals completed an online eligibility screening.



A total of 24 overweight and obese adults (2 males, 22 females) ((mean $\pm$ SD); age (37.6 $\pm$ 11.7, years); BMI (31.3 $\pm$ 5.7) visceral adipose (2.5 $\pm$ 1.6, liters) completed the study. At baseline, participants fasting blood GLU and HbA1c were normal. Fasting CHOL and TG levels were optimal and HDL intermediate. Participant characteristics are summarized in **Table 2**.

Table 2. Participant characteristics (n=24). Blood profile determined from fasting venous blood sample.

Characteristic	Mean $\pm$ sd
Age	37.6 (11.7)
Sex (M/F)	2/22
BMI (kg/m ²)	31.3 (5.7)
Visceral adipose (L)	2.5 (1.6)
HbA1c (%)	5.40 (0.27)
GLU (mmol/L)	5.47 (0.56)
CHOL (mmol/L)	4.69 (0.85)
HDL (mmol/L)	1.52 (0.32)
TG (mmol/L)	1.48 (0.78)

Abbreviations: body mass index, BMI; hemoglobin A1c, HbA1c; GLU, glucose; cholesterol, CHOL; high density lipoprotein, HDL; triglycerides, TG; standard deviation, sd.

Habitual Diet Analysis

Participants habitual diet was analyzed via HEI 2015 scores derived from the DHQ III questionnaire. The average total HEI score for participants was 67.93 with a standard deviation of 7.32 out of a possible score of 100. Participants lowest average HEI scores included whole grains (3.3/10), sodium (4.5/10), and saturated fat (5.5/10). Scores for variables of adequacy examining fruit, vegetable, and protein intake were high with average scores at or above 80%

(mean score/total HEI score possible). Reported legume intake for participants was low with an average daily intake of 0.1 cups, suggesting inadequate intake compared to the recommended amount of 3 cups/week.⁵¹ Participant mean scores for each HEI category are summarized in **Supplementary Table 3**.

Satiety During High-Fat Meal Challenge

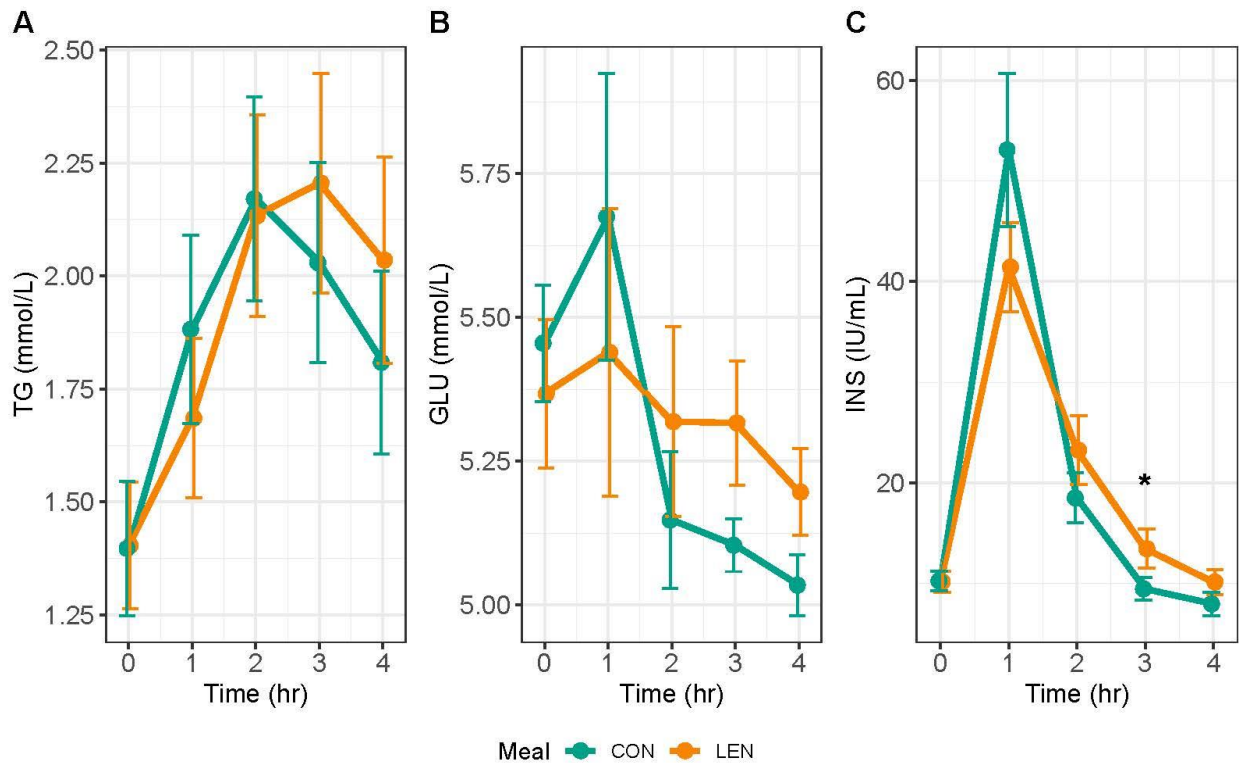
Participants reported higher fullness one hour after consuming the lentil meal compared to one hour after consuming the control meal ($t = -2.16$, $p = 0.042$). There were no other meal group differences in survey responses for fullness, hunger, satisfaction, desire to eat, or amount of food desired over the four-hour postprandial period. Participants reported highest scores of fullness and satisfaction and lowest scores for hunger, desire to eat, and amount of food that could be eaten one hour after meal consumption. This trend was identified in both meal conditions. A summary of satiety measures over the four-hour postprandial period for both meal conditions is provided in **Supplementary Figure 2**.

Postprandial Lipid, Glucose, and Inflammatory Responses

During the high-fat meal challenge, 13 of the 24 participants reached the threshold of 1.98 mmol/L (175 mg/dL) for hypertriglyceridemia diagnosis in non-fasting states (**Figure 2A**).⁵² All of these participants reached the cut-off for both meal conditions. While the incorporation of dietary fiber in a meal can reduce fat absorption, we found no effect of meal ($F = 0.02$, $p = 0.60$) on TG_{AUC} concentrations during the high-fat meal challenge with an average AUC of 2.10 mmol/L in CON and 2.13 mmol/L in LEN (**Supplementary Figure 3A**). Similarly, no difference in C_{max} of postprandial TG was found between meal groups ($F = 0.22$, $p = 0.64$) with an average of 2.3 mmol/L in both groups. However, time to TG C_{max} was increased in LEN

compared to CON ($F = 26.01$, $p = <0.001$) with an average time of 2.8 hours and 1.87 hours, respectively.

Figure 2. Postprandial A) Triglyceride (TG), B) Glucose (GLU), and C) Insulin (INS) response to the high-fat meal challenge by meal group. Points indicate group means, bars represent $\pm$ standard error. Significance between meal groups at $\alpha=0.05$ indicated with *. CON, control; LEN, lentil.



Average blood GLU excursions above and below fasting levels were more extreme in CON compared to LEN (**Figure 2B**) with an average range of -0.4 to 2.7 and -0.2 to 2.2 mmol/L, respectively. However, average increases from baseline were not different by group ($F = 0.38$, $p = 0.55$) and were modest in both groups (CON, 0.42 mmol/L; LEN, 0.48 mmol/L). Additionally, no difference in GLU $C_{\max}$ ($F = 0.03$, $p = 0.86$), time to GLU $C_{\max}$ ($F = 3.83$, $p = 0.06$), or GLU_{AUC} ($F=3.57$, $p=0.07$) was identified between meal groups. Average maximum GLU

concentrations were 5.87 mmol/L in CON and 5.85 in the LEN occurring at 1.4 and 1.9 hours, respectively. Average GLU_{AUC} values are summarized by meal group in **Supplementary Figure 3B**. Insulin concentrations three hours post meal ingestion were lower in the CON compared to LEN ($t = 2.16$, $p = 0.04$) with means of 9.5 and 13.5 IU/mL, respectively (**Figure 2C**). However, total postprandial insulin responses summarized as INS_{AUC} was not different ($F = 0.08$, $p=0.78$) between meal conditions (**Supplementary Figure 3C**).

As high-fat meals can induce an inflammatory response³², we also examined the impact of lentil incorporation into a high-fat meal on postprandial inflammation. Participants began each high-fat meal challenge with similar fasting inflammation levels (**Supplementary Table 4**). We did not detect an effect of intervention meal on postprandial inflammation markers (**Supplementary Table 5**).

Fasting and Postprandial Bile Acids

Analysis of fasting bile acid abundance revealed no difference between meal conditions which were separated by a 7-day period (**Supplementary Figure 4**). Conjugated and primary BAs were the predominant species in fasting plasma followed by secondary and tertiary species. Spearman correlation analysis of fasting BAs with anthropometric (weight, BMI, age, waist circumference) and metabolic (HbA1c, CHOL, HDL, TG, GLU, insulin) biomarkers at baseline revealed no statistically significant correlations after correction for multiple comparisons (**Supplementary Figure 5**).

Both meals stimulated a bile acid response, with the largest plasma increases ($C_{\max}$ - fasting) in primary, secondary, and conjugated species (**Figure 3**). In contrast, tertiary and unconjugated species remained near fasting levels for the duration of the four-hour postprandial

period. Increases in individual and total plasma BA abundances were not different by meal group and total plasma BA increases were primarily due to glycine conjugated species, GCDCA, GDCA, and GCA (**Supplementary Figure 6**). CDCA was the only BA to have a statistically significant ($\alpha < 0.05$) difference in $C_{\max}$ between meal groups ($F = 4.69$, $p = 0.04$) with increased abundance in the LEN condition. No effect of meal was identified on postprandial BA abundance as summarized by area under the curve (**Figure 4**). Though not significant at $\alpha = 0.05$, total postprandial BAs were increased in LEN compared to CON ($F = 1.6$, $p = 0.21$) and a trend was present for increased abundance of primary, secondary, tertiary, unconjugated, and 12α -hydroxylated species in response to the LEN meal.

Figure 3. Postprandial plasma bile acid responses to the high-fat meal challenge by meal group. Points indicate group means, bars represent $\pm$ standard error.

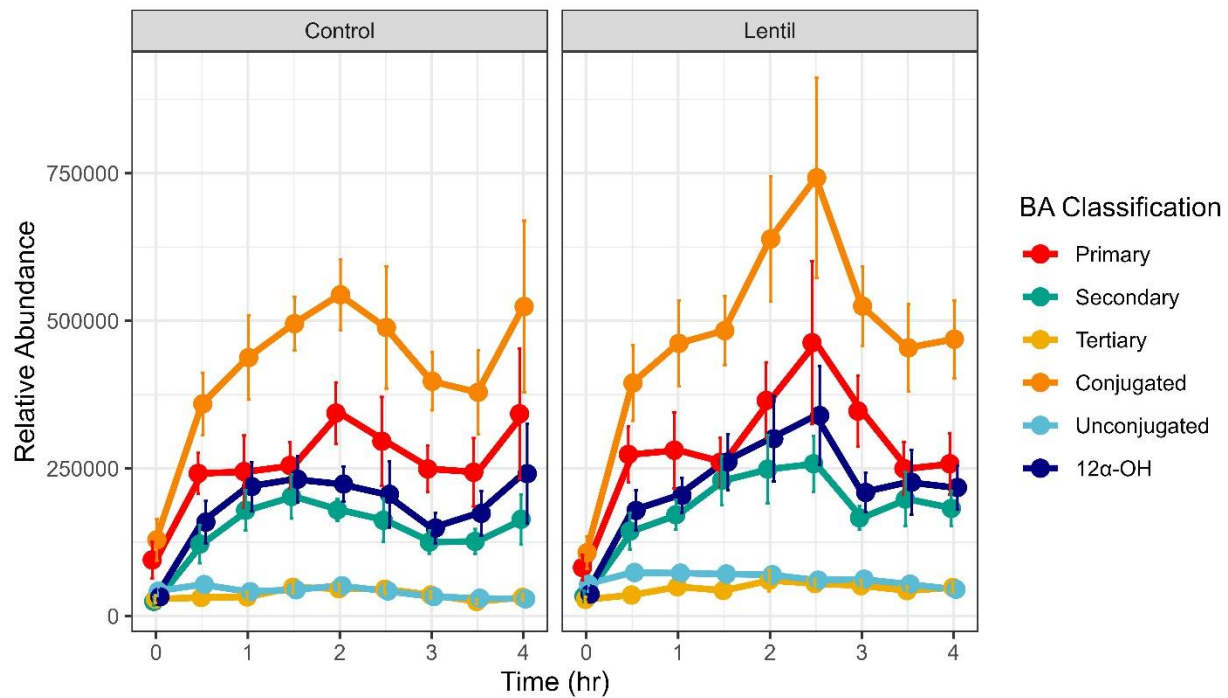
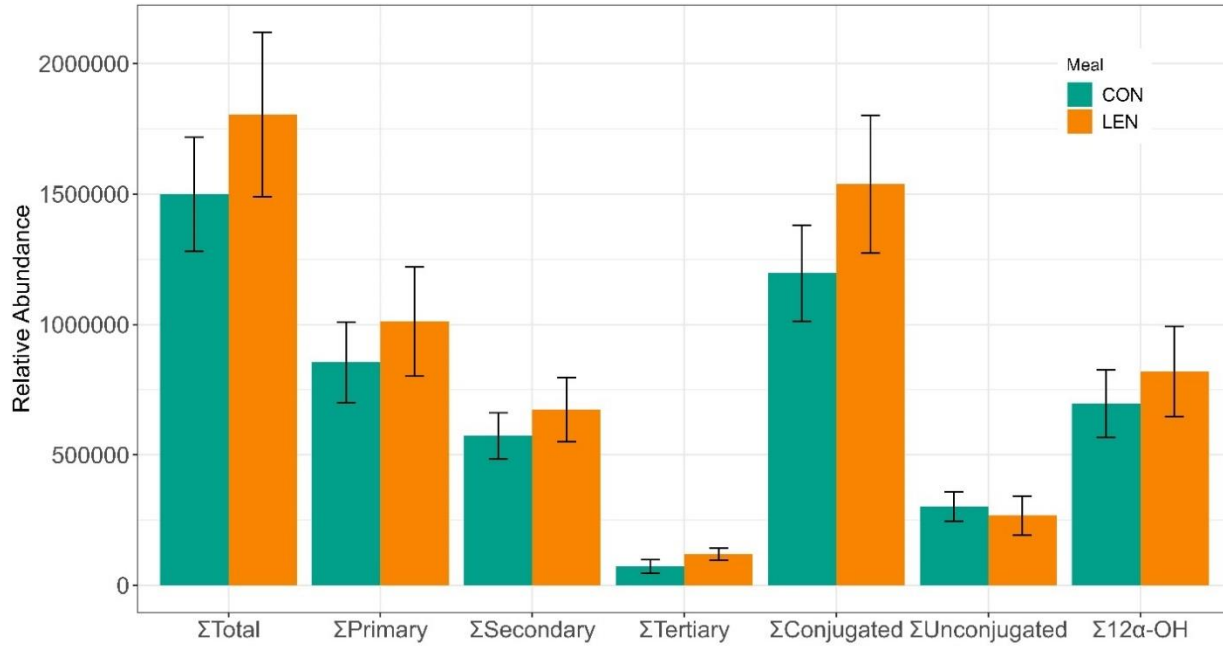
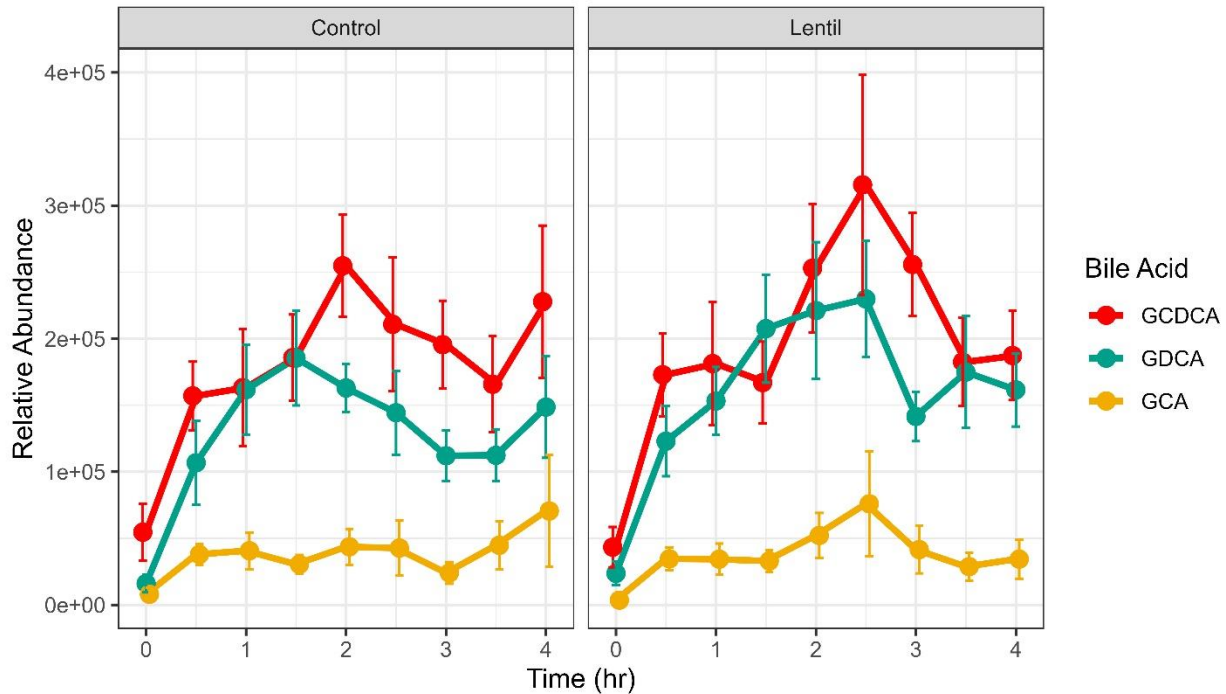


Figure 4. Postprandial plasma bile acid concentrations summarized as area under the curve by meal group. Σ indicates sum of all individual BAs belonging to category. Bar height indicates group means, bars represent $\pm$ standard error. Significance at $\alpha=0.05$ indicated with *. CON, control; LEN, lentil.



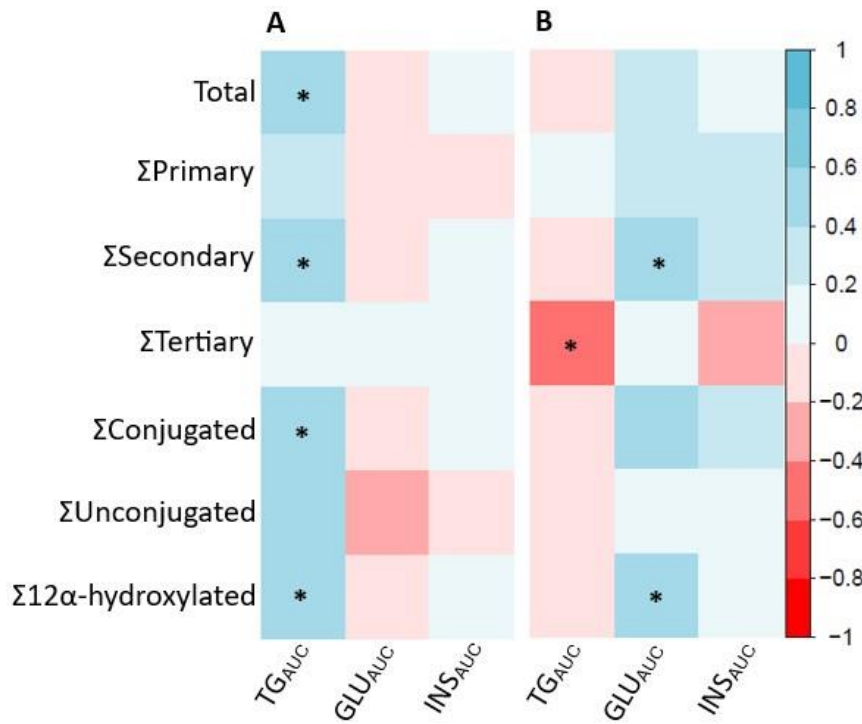
To better characterize the BA postprandial response, the abundance of primary contributors to plasma BA increases - GCDCA, GDCA, and GCA, were examined over the 4-hour postprandial period (**Figure 5**). Increases in GCDCA and GDCA were the largest in both meal conditions with a smaller contribution from GCA. Peak abundance of each bile acid was delayed after consumption of the lentil meal compared to the control meal with peak concentrations at 2.5 hours in LEN compared to 1.5 to 2 hours in CON. This delay in peak abundance echoes that seen with BA groupings of primary, secondary, conjugated, and 12 α -hydroxylated BAS seen in **Figure 3**.

Figure 5. Postprandial plasma bile acid responses of the bile acids with the greatest response ($C_{\max}$ – baseline) to the high-fat meal challenge by meal group. Points indicate group means, bars represent $\pm$ standard error.



We performed spearman correlation testing to assess the relationship between plasma BAs and glucose and lipid metabolism markers during the high-fat meal challenge. This analysis revealed that BAs are differentially correlated with triglyceride and glucose levels according to meal condition. In the LEN meal, BAs were positively correlated with blood TG and negatively correlated with blood GLU levels (**Figure 6A**). These relationships were inversed in the CON meal (**Figure 6B**).

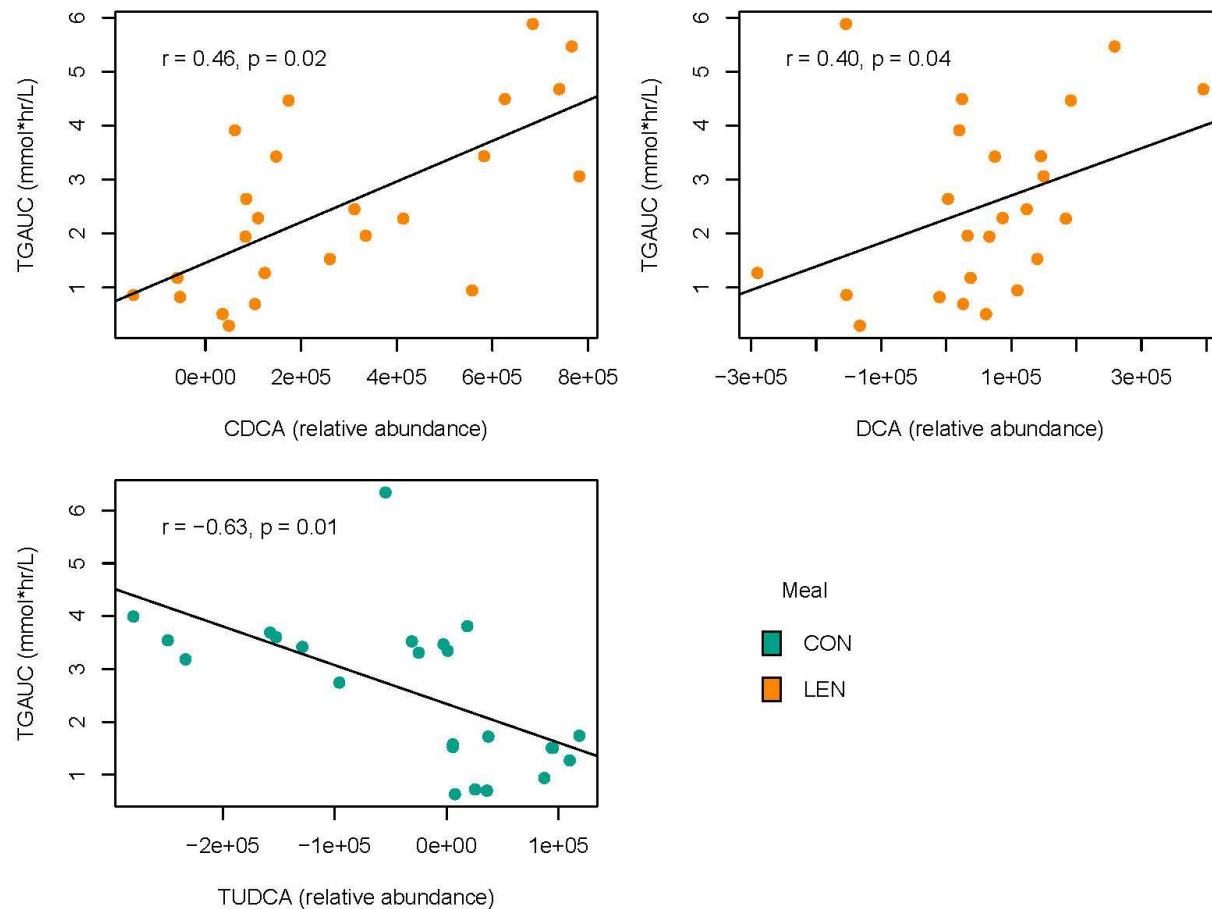
Figure 6. Spearman correlation between postprandial bile acids and postprandial TG_{AUC} , GLU_{AUC} , and INS_{AUC} in **A)** Lentil and **B)** Control meal conditions. Postprandial bile acid concentrations were summarized as area under the curve and summed (Σ) by classification. The color of each spot in the heatmap corresponds to the r value of the spearman correlation analysis. Significance at $\alpha=0.05$ indicated with *.



After correction for multiple comparisons, significant relationships between TG_{AUC} and BAs in the LEN were as follows: total ($r = 0.51$, $p = 0.01$); secondary ($r = 0.45$, $p = 0.03$); conjugated ($r = 0.45$, $p = 0.03$); and 12 α -hydroxylated ($r = 0.46$, $p = 0.02$). These relationships were primarily due to strong correlations with CDCA ($r = 0.46$, $p = 0.02$) and DCA ($r = 0.40$, $p = 0.04$) (**Figure 7**). In the CON condition, TG_{AUC} was negatively correlated to tertiary BAs ($r = -0.56$, $p = <0.01$) due to a strong negative correlation with TUDCA ($r = -0.63$, $p = 0.01$) (**Figure 7**). GLU_{AUC} was positively correlated to secondary ($r = 0.41$, $p = 0.04$), conjugated ($r = 0.43$, $p = 0.03$) and 12 α -hydroxylated ($r = 0.43$, $p = 0.03$) bile acids with significant relationships to GCA

($r = 0.56$, $p < 0.01$), GCDCA ($r = 0.50$, $p = 0.01$), and GDCA ($r = 0.52$, $p = 0.01$). No significant correlations were present in either meal condition between BAs and INS_{AUC} (**Figure 6**).

Figure 7. Spearman correlations between postprandial bile acids and triglycerides summarized as area under the curve (AUC) in Lentil and Control meal conditions.



Discussion

Incorporation of dietary fiber in a meal can improve postprandial metabolism by lowering blood glucose and triglyceride responses. Fiber consumption has also been shown to alter bile acid profiles which can further impact postprandial metabolism. We recruited adults with

overweight or obesity to measure the impact of acute lentil consumption on postprandial BA, lipidemic, glycemic, and inflammatory responses. This is the first study we are aware of to examine the “same-meal” effect of fiber on postprandial BA responses in humans. In addition, the acute impact of lentil ingestion on postprandial lipidemic and glycemic responses is not established. The key finding of this study is a differential impact of meal on the relationship between postprandial bile acids and triglyceride and glucose responses. Furthermore, these inverse relationships are identified in the context of comparable magnitudes of bile acid, glucose, and triglyceride responses between meal conditions, despite a 14 g difference in fiber content derived from 140 g of cooked lentils.

High-fat meal challenges containing 40 to 100 g of dietary fat are an established method to induce postprandial triglyceride and inflammation responses.³² Our study meals with ~40 g of fat did elicit a triglyceride response, however, responses were not reduced with acute lentil consumption in our cohort. We did observe an increase in time to maximum triglyceride concentrations with lentil consumption compared to the control meal. This finding is explained by slowed rates of gastric emptying and intestinal transit time with the addition of fiber to fat-rich meals.^{53,54} These changes to meal processing align with our finding of increased feelings of fullness that were self-reported by participants after lentil meal consumption. Acute fiber intake can reduce the magnitude of postprandial lipid responses⁵⁵⁻⁵⁷, however other studies found no change⁵⁸. Variation in study results may be due to variable fat and fiber content of test meals as well as fiber type.⁵⁹

Study meals did not elicit significant inflammation responses. Our research group has demonstrated increases in identical inflammatory biomarkers with a similar challenge meal⁶⁰,

however, study participants were at increased metabolic risk as defined by elevated waist circumferences (≥ 40 inches males, ≥ 35 inches female) and elevated postprandial triglycerides (>1.69 mmol/L). While the current study's participants had elevated BMI, they were otherwise metabolically healthy as characterized by normal fasting measures of glucose and lipid metabolism. The metabolic health of this cohort may contribute to a lack of inflammation response as there is evidence that inflammatory responses to high-fat meals may be less pronounced in metabolically healthy individuals^{61,62} compared to individuals with components of metabolic syndrome.⁶³

The 14-gram difference in fiber content between lentil and control meals did not impact postprandial glucose or insulin responses in our cohort. This result contradicts a review examining the relationship between acute lentil consumption and postprandial glucose responses which identified a minimum effective serving of ~ 110 g cooked lentils to reduce postprandial glucose responses by 20%.⁶⁴ While our serving of 140 g of lentils meets this criteria, lentil preparation for our study meals was achieved with overnight soaking, not cooking. As processing techniques such as soaking and cooking modify the composition and structure of polysaccharide content in lentils⁶⁵, it is possible that processing variations contributed to the lack of glucose modification seen in our study. In addition, the high fat and protein (~ 13 grams) content of both study meals may contribute to glycemic responses in a manner overpowering the variable fiber content between meal conditions⁶⁶.

Similar to findings of other studies^{67,68}, the fasting BA profiles in our participants were predominantly composed of primary BAs followed by equitable amounts of secondary and tertiary species with conjugated species present in greater abundance than unconjugated. In our

study, neither individual nor grouped BAs correlated with baseline characteristics of participants including anthropometric and blood glucose and lipid values. This is in contrast to other studies which have identified significant correlations between individual and grouped BAs and metabolic characteristics of participants.^{23,67,69} However, correlations are highly variable between these studies which may be due, in part, to variable participant populations. The participants in our study were metabolically healthy and relatively homogenous in factors often correlated with BA profiles such as BMI and age which may have impacted our ability to detect correlations.

Our study meals did elicit significant increases in plasma bile acid abundances. Several studies⁶⁹⁻⁷¹ corroborate our finding that increased plasma BA levels were predominantly due to an increased abundance of conjugated BAs. Similar to the present study, a study examining postprandial BA responses to an oral fat challenge also found that unconjugated BAs do not contribute substantially to the overall rise in postprandial total BAs.⁶⁸ In addition, the predominant increase in glycine-conjugated BA species in our study after meal consumption is supported by the preferable conjugation of BAs with glycine versus taurine in humans.⁷² Contrary to our hypothesis, the incorporation of 140 g of lentils into the high-fat meal did not impact the abundance or composition of postprandial plasma BA response compared to a meal with negligible fiber content. However, a consistent trend for delayed timing to peak BA concentrations with the lentil meal was present. This finding aligns with delayed time to peak plasma triglyceride concentrations due to delayed gastric emptying and intestinal transit time as slowed entry of dietary fat into the small intestine would delay BA response.

The bile acid binding ability of lentils have been demonstrated *in vitro*²⁹ and in mouse models.⁷³⁻⁷⁵ However, human studies examining the impact of lentil consumption on bile acid

metabolism are lacking. It is possible that the lack of modulation to BA metabolism with lentil consumption in our study may be due to an inadequate fiber dose, however, as there is a great paucity of studies examining the impact of acute fiber consumption on postprandial BA responses, we cannot well-assess the effectiveness of our study dose. It is also possible that the postprandial sampling period of four hours was not long enough to identify changes to BA metabolism as other studies have identified significant upregulation in BA synthesis enzymes eight hours after consumption of a fiber-rich meal.¹⁷ BA interactions with meal components other than fiber may also have contributed to the similarity in postprandial BA responses between meal conditions. While less attention has been paid to the interaction between bile acids and plant proteins, studies have reported that dietary soy protein and white rice protein promote the excretion of fecal BAs.⁷⁶⁻⁷⁸ Our control meal utilized white rice to match the carbohydrate content of lentils without adding fiber content in order to isolate the effect of lentil-derived fiber on BAs. Therefore, it is possible that proteins from the rice in control meals also interacted with BAs and promoted a similar effect to lentil-derived fiber. The presence of confounding variables in foods and food matrix effects that can also potentially influence gut function and BA metabolism make it difficult to delineate the physiological impact of fiber when consumed as part of a meal. While the utilization of a whole-food study design best represents the impacts likely to occur in free-living adults with lentil consumption, further study investigating lentils as a single-ingredient are necessary to better define their interaction with BAs and postprandial metabolism in humans.

We further investigated if postprandial plasma BAs correlated with changes in glucose and triglyceride metabolism in response to control versus lentil meals. While no substantial

differences in the postprandial BA, glucose, or triglyceride response between meal conditions were evident, we identified inverse relationships between BAs and triglyceride and glucose responses in lentil versus control meals. There is a paucity of research examining the acute impact of fiber consumption on postprandial BAs and correlation to postprandial lipid and glucose metabolism to compare study findings with as correlation analysis between BA and metabolic markers are most frequently performed on fasting metrics.^{5,23,69,79} The positive correlation of blood TG levels with BAs after consumption of a high-fat meal may be expected as it represents the role of BAs in emulsification of dietary lipids in the proximal intestine. The primary drivers of the positive correlation were CDCA and DCA. Similar to other study findings⁶⁷, CDCA increased in abundance early in our cohort compared to other BAs with large increases thirty minutes after meal consumption. A greater $C_{\max}$ for CDCA was also identified in LEN compared to CON. These factors likely contributed to the positive correlation with TG levels in the LEN. These findings are corroborated by Lamaziere and colleagues who identified strong correlations between CDCA and postprandial TG after consumption of a hypercaloric, high-fat meal.⁶⁷ Though not significant at $\alpha = 0.05$, the abundance of DCA was increased in the LEN compared to CON and had a delayed time to peak abundance compared to CON, 2.4 hours compared to 1.7 hours, likely contributing to a stronger relationship with delayed peak TG levels in the LEN condition. In the control condition, a decrease in TG response was associated with increased abundance of TUDCA which is formed by bacterial epimerization of CDCA to UDCA in the distal intestine and colon followed by reabsorption and transport back to the liver where it is conjugated with taurine.⁸⁰ Considering average oral-cecal transit time, it is unlikely that increases in CDCA and its conjugates in response to meal ingestion reached the large intestine

for bacterial conversion to UDCA followed by enterohepatic transport back to the liver for tertiary BA formation within the first hour of the postprandial period. Indeed, increases in tertiary BAs contributed minimally to plasma BA abundance over the four-hour postprandial period. The negative correlation between tertiary BAs and TG_{AUC} during the control meal could represent declining blood TG levels around the time tertiary BAs became slightly more prevalent.

Consumption of the lentil meal resulted in negative correlations between BAs and blood glucose levels, though, relationships were not significant after correction for multiple comparisons. This relationship was again reversed with the control meal with BAs demonstrating a positive relationship with blood glucose levels. Interestingly, several studies have demonstrated strong, positive correlations between GDCA, GCDCA, and GCA and postprandial glucose levels in response to an oral glucose challenge.⁸¹⁻⁸³ While our meal composition was different, we also identified these three BAs as the primary drivers of strong, positive correlations with postprandial glucose levels. These BAs were also the top three contributors to increased plasma BA abundance in both meal conditions with large increases occurring in the first half hour after meal consumption. Therefore, we hypothesize that, although plasma postprandial glucose responses were not significantly different by meal group at $\alpha=0.05$, the higher glycemic index associated with the control meal contributed to stronger positive correlations between these BAs and postprandial glucose. This interpretation along with prior study results indicate that these BAs may be particularly responsive to carbohydrate intake and glucose homeostasis. In support of this hypothesis, several studies have demonstrated GCA, GDCA, and GCDCA to be associated with increased risk of diabetes.^{84,85}

Strengths of the study include the utilization of a metabolically healthy, overweight and obese cohort as this classification of participants is under-represented in the literature. The crossover design of this study reduces confounding contributions from interindividual variability in BA and glucose and lipid metabolism. The comprehensive measurement of postprandial inflammation, glucose, and lipid measures well-characterizes the metabolic response to acute lentil consumption and allows for assessment of the relationship to postprandial BA responses. We were not able to detect the primary BA, cholic acid, in our LCMS analysis - though its glycine and taurine conjugates were identified in samples and included in analyses. As cholic acid is highly prevalent among BA species, this result indicates an analysis error, not participant biology, and therefore poses a limitation to our BA analysis. In addition, the broad fiber analysis of our study lentils limits our ability to speculate on fiber interactions with postprandial BA and metabolism measurements. Additionally, although participants were asked to maintain their regular dietary and physical activity patterns and match their diet and activity in the 24-hours preceding each blood collection visit, it is possible that failure to comply with these instructions impacted study measures. Study participants were predominantly female (n=22/24) and there is evidence indicating that sex differences are an important variable to consider regarding lipid and glycemic measures⁸⁶ as well as bile acid measures⁸⁷ but we were unable to do so with the limited sample size of male participants. However, our male participants were not detected as outliers in statistical analyses.

Conclusions

In conclusion, our findings provide evidence that acute lentil consumption in individuals with overweight or obesity impacts the relationship between postprandial bile acid responses and

postprandial glucose and triglyceride metabolism. Interestingly, inverse relationships between BAs and glucose and triglyceride responses were present between meal conditions despite a lack of modification to the composition or concentration of these factors from lentil intake. Our findings help address the current limited body of research regarding the impact of acute lentil consumption on postprandial lipid and glucose metabolism and the “same-meal” effect of fiber consumption on the postprandial bile acid response. This information further informs the development of pulse-based strategies to beneficially modify bile acid profiles and lower metabolic disease risk. Further investigation of the impact of acute pulse crop consumption on the postprandial bile acid response is warranted.

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Supplementary Figure 1. Questionnaire assessing satiety during the high-fat meal challenge. Survey completed prior to meal consumption (baseline) and at hourly postprandial timepoints (1, 2, 3, 4 hours).

AFRI Satiety Questionnaire

Directions: Please indicate on each line where your answer falls by marking with a vertical line.

How much do you like your meal provided today?

Not at all (0)

Extremely (10)

How hungry are you?

Not at all (0)

Extremely (10)

How full are you?

Not at all (0)

Extremely (10)

How satisfied are you?

Not at all (0)

Extremely(10)

How strong is your desire to eat?

Very weak (0)

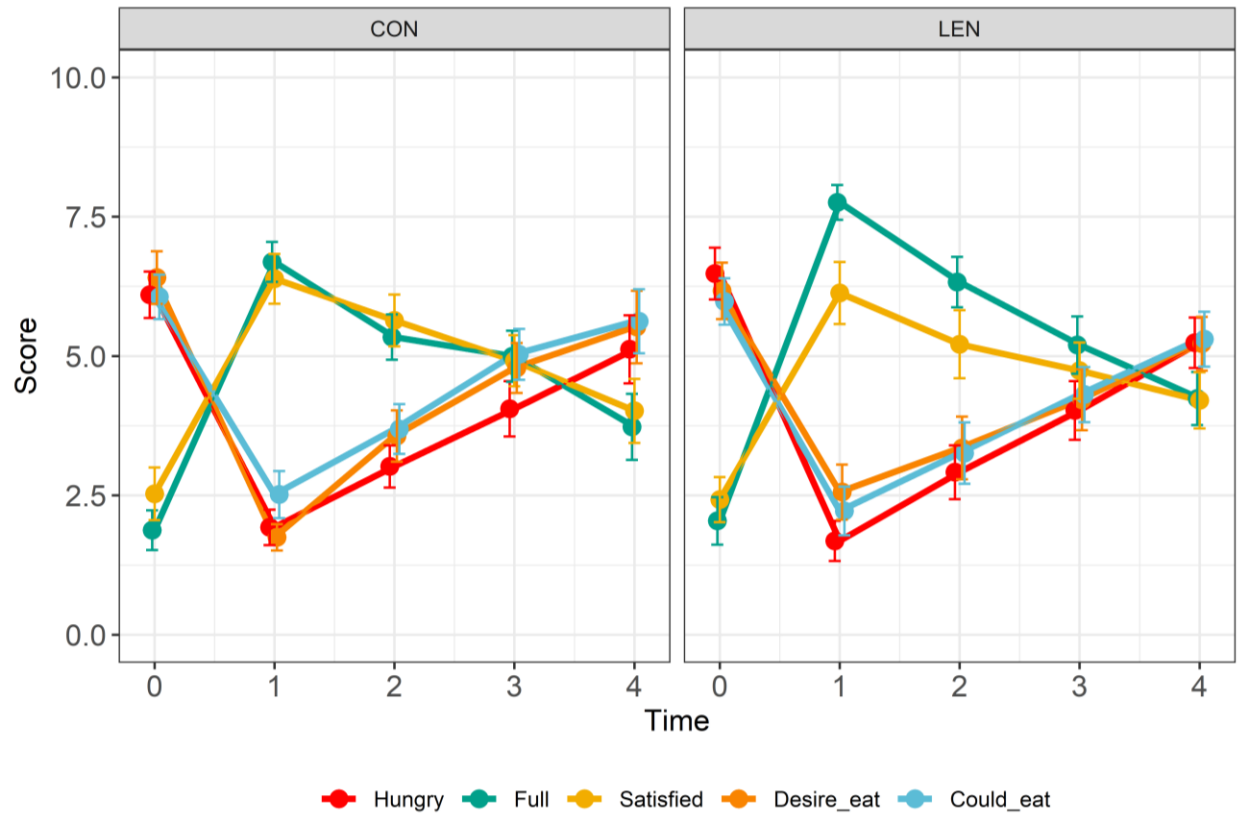
Very Strong(10)

How much do you think you could (or would want to) eat right now?

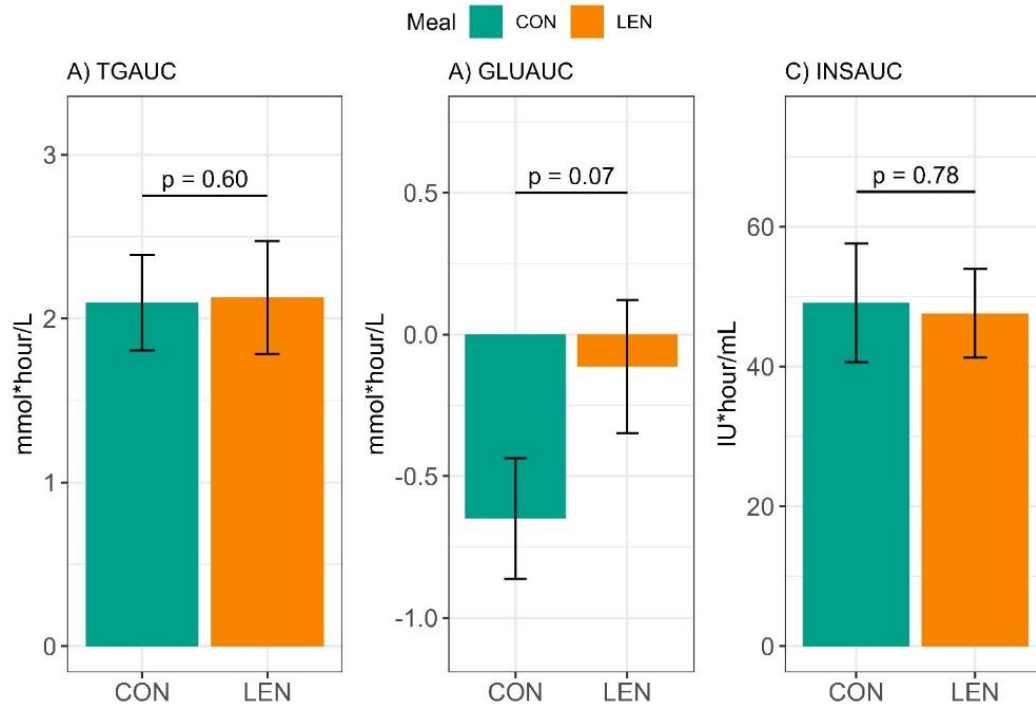
Nothing (0)

A lot (10)

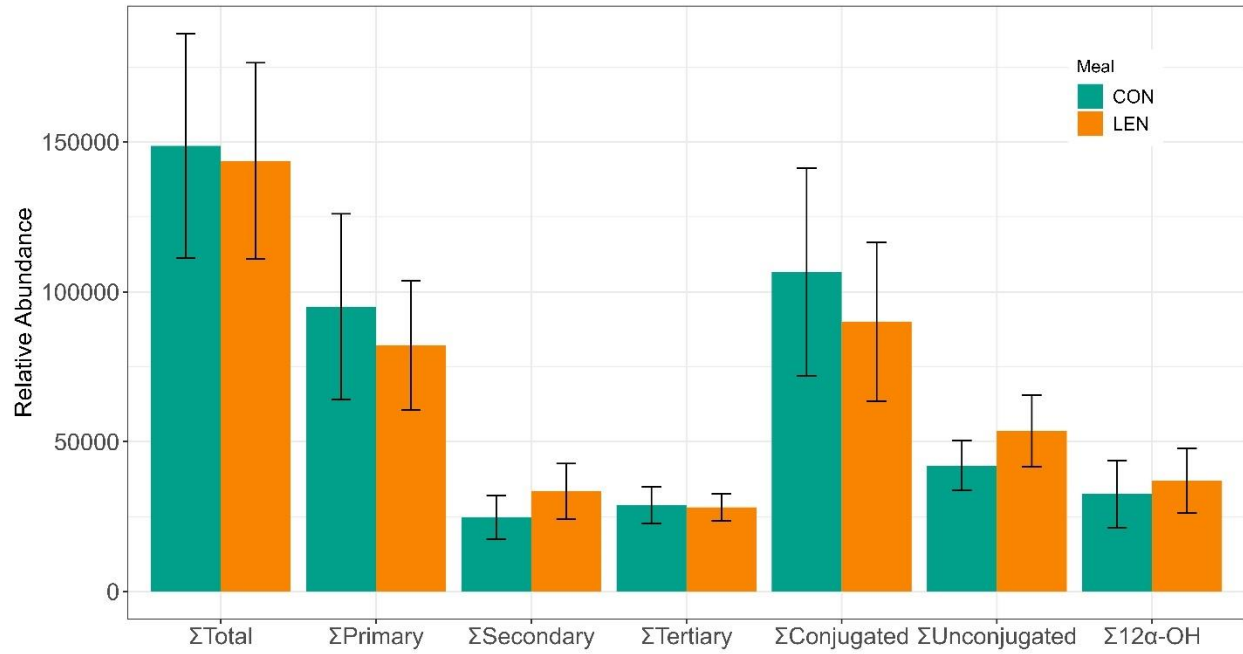
Supplementary Figure 2. Hourly satiety measures over the 4-hour high-fat meal challenge. All measures taken from visual analogue scale with rank notation from 0-10. Points indicate group means, bars represent $\pm$ standard error. CON, control; LEN, lentil.



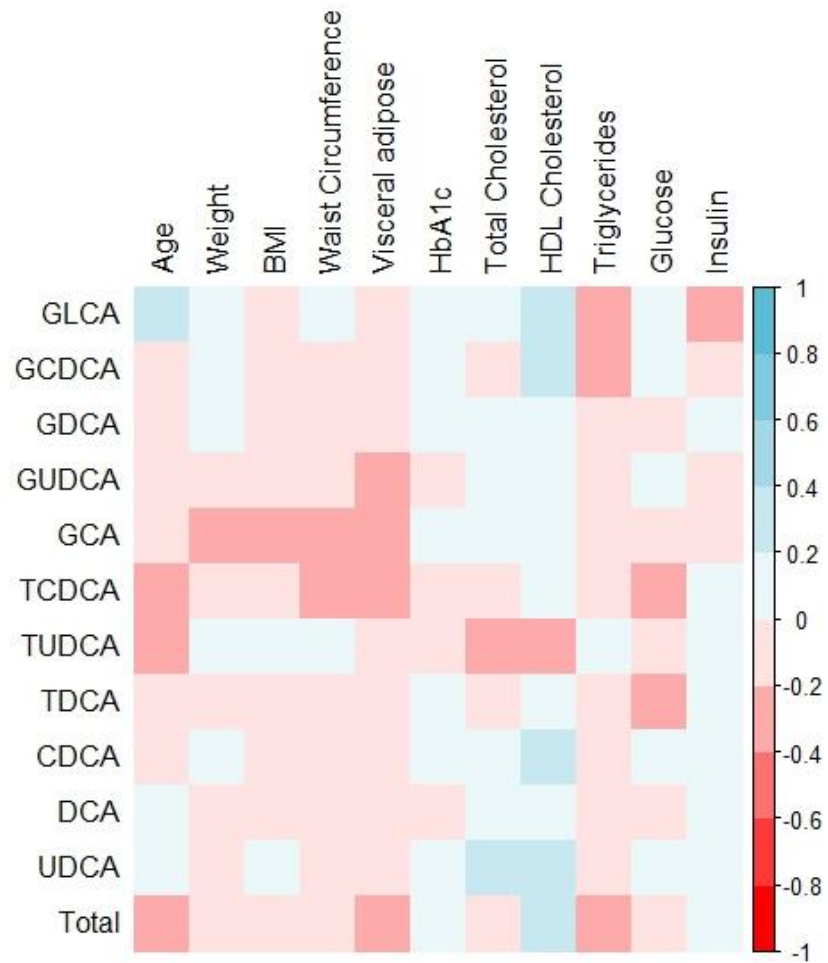
Supplementary Figure 3. Summary plot of A) TG_{AUC} , B) GLU_{AUC} , and C) INS_{AUC} by meal condition. AUC values represent the net sum of values from fasting and hourly timepoints for 4 hours post high-fat meal ingestion. Error bars represent mean $\pm$ standard error. Abbreviations: CON, control; LEN, lentil.



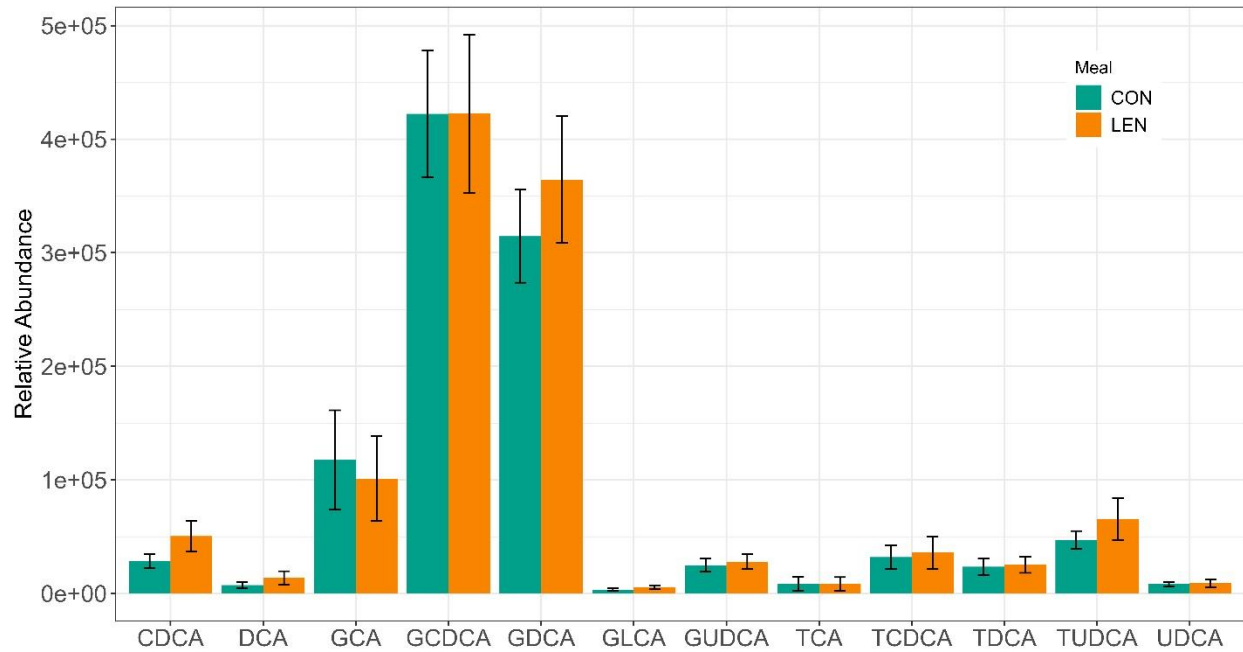
Supplementary Figure 4. Fasting plasma bile acid abundance. Bar height indicates group means, bars represent $\pm$ standard error. Significance at $\alpha=0.05$ indicated with *. CON, control; LEN, lentil.



Supplementary Figure 5. Correlation heat matrix between fasting bile acids and anthropometric and metabolic markers of participants at baseline (n=24). The color of each spot in the heatmap corresponds to the r value of the spearman correlation analysis. Significance at $\alpha=0.05$ indicated with *.



Supplementary Figure 6. Change in plasma bile acid abundance after meal consumption. Change calculated as difference between fasting level and maximum concentration ($C_{\max}$) reached during postprandial time-period. Bar height indicates group means, bars represent $\pm$ standard error. Significance at $\alpha=0.05$ indicated with *. CON, control; LEN, lentil.



Supplementary Table 1. Nutrient and functional analysis of whole green lentils used in the lentil high-fat meal condition.

Analysis	Amount
Nutrient (per 100g)	
Calories (kcal)	362
Moisture (g)	9.5
PRO (g)	23.4
Fat (g)	2.1
Ash (g)	2.7
Total CHO (g)	62.3
Soluble Fiber (%)	0.4
Insoluble Fiber (%)	13.3
Starch (g)	36.27
Functional	
Foam Expansion (%) (5% w/w)	218
Water Holding Capacity (g/g)	1.3
Foam Stability (%)	94
pH (5% w/w)	6.3

Abbreviations: Kcal, kilocalories; PRO, protein; g, grams; CHO, carbohydrate.

Supplementary Table 2. Nutrient profile of lentil and control high-fat meals.

Content	CON	LEN
Kcal	527.4	529.3
PRO g	12.8	13.0
CHO g	28.6	28.1
FAT g	40.2	40.5
Fiber g	0.3	14.5

Abbreviations: CON, control; LEN, lentil.; Kcal, kilocalories; PRO, protein; g, grams; CHO, carbohydrate.

Supplementary Table 3. Habitual dietary intake (n=24) as represented by the 2015 Healthy Eating Index (HEI) total and component scores. HEI component scores were calculated through an online diet history questionnaire (DHQIII) in which participants self-reported the types and frequency of foods consumed.

Component	Maximum points	Score
Adequacy		
Total fruits	5	3.7 ± 1.5
Whole fruits	5	4.4 ± 1.2
Total Vegetables	5	3.9 ± 1.2
Greens and beans	5	4.2 ± 1.3
Whole grains	10	3.3 ± 2.3
Dairy	10	6.6 ± 2.5
Total protein foods	5	4.7 ± 0.7
Seafood and plant proteins	5	4.5 ± 0.9
Fatty acids	10	5.8 ± 3.2
Moderation		
Refined grains	10	8.7 ± 2.0
Sodium	10	4.5 ± 2.9
Added sugars	10	7.9 ± 2.9
Saturated fats	10	5.5 ± 3.2
Total	100	67.9 ± 7.3

Data represent mean ± sd. Abbreviations: DHQ, diet history questionnaire.

Supplementary Table 4. Fasting inflammation levels prior to high-fat meal challenge by meal group. Cytokine measures in pg/dL.

Cytokine	CON (n=24)	LEN (n=24)	p-value
IL-6	11.92 ± 16.46	11.88 ± 16.76	0.96
IL-1 β	13.60 ± 11.46	13.07 ± 12.12	0.38
IL-17	70.19 ± 43.15	68.65 ± 41.75	0.63
IL-23	1274.90 ± 954.67	1188.41 ± 1043.37	0.18
IL-10	113.99 ± 190.95	113.87 ± 201.90	0.98
TNF- α	27.38 ± 14.26	26.05 ± 12.42	0.57
GMCSF	97.66 ± 146.94	96.94 ± 151.34	0.79
IFN- γ	246.92 ± 187.86	234.90 ± 161.20	0.47

Data represent mean $\pm$ standard deviation. Linear mixed effect modeling used to determine differences ($p < 0.05$) between group means. Abbreviations: CON, control; LEN, lentil.; IL, interleukin; TNF- α , tumor necrosis factor alpha; GM-CSF, granulocyte macrophage colony stimulating factor, IFN- γ , interferon-gamma.

Supplementary Table 5. Postprandial inflammation by intervention group. Postprandial values for each inflammation marker summarized as net area under the curve (AUC) using fasting and 1-, 2-, 3-, and 4- hour postprandial measurements. Cytokine measures in pg*hr/dL.

Cytokine	CON (n=24)	LEN (n=24)	p-value
IL-6	-4.57 (17.47)	-3.16 (22.66)	0.51
IL-1 β	0.71 (9.98)	-1.06 (10.97)	0.50
IL-17	5.48 (39.01)	6.96 (56.50)	0.89
IL-23	-185.76 (481.4)	-154.47 (406.97)	0.74
IL-10	-38.97 (136.31)	21.17 (268.21)	0.21
TNF- α	0.64 (14.02)	1.10 (39.19)	0.95
GMCSF	-14.50 (51.02)	-22.02 (58.32)	0.55
IFN- γ	-31.90 (213.72)	49.26 (255.25)	0.20

Data represent mean $\pm$ standard deviation. Linear mixed effect modeling used to determine differences ($p < 0.05$) between group means. Abbreviations: CON, control; LEN, lentil.; IL, interleukin; TNF- α , tumor necrosis factor alpha; GM-CSF, granulocyte macrophage colony stimulating factor, IFN- γ , interferon-gamma.

CHAPTER FOUR

POLYPHENOL-RICH ARONIA INTERVENTION ALTERS
GLUCOSE AND LIPID METABOLISM AND INFLUENCES
MICROBIAL DIVERSITY: A RANDOMIZED CLINICAL
TRIAL

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

Author: M.L. Chamberlin

Contributions: Conducted the study; curated the data; generated visualizations; performed statistical analyses; wrote the manuscript; read, revised, and approved final manuscript.

Co-Author: S.M.G. Wilson

Contributions: Curated the data; generated visualizations; performed statistical analyses; wrote the manuscript; read, revised, and approved final manuscript.

Co-Author: M.P. Miles

Contributions: Study conceptualization; funding acquisition; methodology development; study validation; read, revised, and approved final manuscript.

Manuscript Information

M.L. Chamberlin, S.M.G Wilson, and M.P. Miles

Foods

Status of Manuscript: [Put an x in one of the options below, then delete instruction in brackets]

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-reviewed journal
- ☐ Accepted by a peer-reviewed journal
- ☐ Published in a peer-reviewed journal

MDPI

Abstract

Polyphenol-rich Aronia fruits have great potential as a functional food with established anti-inflammatory, hypolipidemic, and hypoglycemic biologic activities. However, clinical intervention trials investigating the impact of Aronia fruit consumption on human health are limited and conflicting. To characterize the impact of Aronia on metabolic health, a randomized, controlled, double-blinded, parallel intervention trial was conducted using 13 human subjects who ingested either 0 mL (PLA) or 100 mL (ARO) of Aronia juice daily for 30 days. Anthropometric measurements, fasting and postprandial measures of glucose and lipid metabolism and inflammation, and 16S rRNA gut microbial composition data were collected before and after the intervention period. Daily supplementation with Aronia prevented a rise in total cholesterol levels ($\beta = -0.50$, $p = 0.03$) and reduced postprandial glucose ($\beta = -3.03$, $p < 0.01$). We did not observe a condition difference in microbial community structure at any taxonomic level but did identify a decrease ($\beta = -18.2$, $p = 0.04$) in microbial richness with Aronia supplementation. Our clinical trial further informs the development of polyphenol-based dietary strategies to lower metabolic disease risk.

Introduction

Non-communicable, chronic diseases including cardiovascular disease and type 2 diabetes are among the highest contributors to global morbidity and mortality rates.¹ Low-grade inflammation² and cellular damage from oxidative stress^{3,4} contribute to the pathogenesis of these diseases. Polyphenols are dietary bioactive compounds found in a wide variety of foods that exhibit anti-inflammatory and antioxidant activity by preventing activation of pro-

inflammatory cellular mediators and cellular oxidative stress from reactive oxygen and nitrogen species.⁵⁻⁸ Polyphenols can also regulate antioxidant enzyme concentrations further contributing to cellular redox homeostasis.⁵ In addition, polyphenols have documented immune-stimulating and vasodilatory properties⁹⁻¹¹ which have led to considerable interest in polyphenol-rich food consumption as a dietary strategy to combat chronic disease progression.

Aronia melanocarpa, or chokeberry, is a hardy, cold weather shrub which produces fruit rich in dietary polyphenols. Aronia fruits are the richest fruit source of polyphenols, primarily derived from anthocyanins, procyanidins, and hydroxycinnamic acids, with 1756 mg/100 g as determined by chromatography methods.¹² The high polyphenol content of Aronia fruit confers high antioxidative potential – ranked the second highest of fruits after elderberries with 1752 mg/100 g.¹² The health promoting potential of polyphenol-rich Aronia fruits defines them as a functional food. This recognition has led to the widespread commercial production of Aronia-based products including supplements, juices, preserves, and teas. However, there are limited human studies examining the metabolic benefits of Aronia fruit consumption, and results are conflicting with some studies citing improvement to measures of glucose and lipid metabolism and inflammation and other studies reporting no effect.^{13,14} Conflicting results may be due to dose, duration, or supplement delivery method. In addition, differences in metabolic health status of the study population are also likely to impact study results.

Variability in gut microbial compositions may also contribute to conflicting study results. Polyphenol bioavailability is influenced, in part, by the physicochemical properties of the compound while polyphenol bioefficacy is likely mediated by the gut microbiome.¹⁵⁻¹⁸ While some polyphenols are absorbed in the small intestine, the majority of polyphenols pass to the

large intestine for microbial enzymatic action. Gut bacteria can metabolize polyphenols into phenolic metabolites.^{19,20} The bioavailability and bioefficacy of microbial-produced phenolic derivatives frequently exceeds the parent compound.²⁰⁻²³ Furthermore, evidence suggests that polyphenol intake can alter microbiome composition in an antimicrobial and prebiotic-like manner²⁴ which has the potential to influence host postprandial responses.²⁵ However, there is a paucity of research investigating the influence of Aronia consumption on the gut microbiome in adults.

Furthermore, there is high heterogeneity in health biomarker coverage of polyphenol interventions in randomized controlled trials. While the importance of postprandial metabolism in relation to metabolic health and disease risk is well-established²⁶⁻³⁰, these measures are not consistently analyzed in non-acute supplementation studies. Clinical trials investigating how polyphenol-rich foods influence postprandial metabolism and the gut microbial community are needed to accurately assess their efficacy to beneficially impact health. We therefore completed a 30-day clinical intervention trial in healthy adults to assess the impact of Aronia fruit juice consumption on metabolism and gut microbial composition. In this exploratory investigation, we assessed both fasting and postprandial lipidemic, glycemic, and inflammatory responses to long-term Aronia consumption as well as changes to microbial taxonomy through 16S rRNA sequencing. These findings provide valuable insights to propel development of polyphenol-based dietary strategies to improve health and reduce chronic disease risk.

Materials and Methods

Ethics Statement

The protocol was approved by the Institutional Review Board at Montana State

University (MC010819). The informed consent was reviewed verbally in-person, allowing time for questions and clarifications. Written informed consent was obtained from all participants prior to their participation. The study was retrospectively registered February 2022 at ClinicalTrials.gov (NCT05255718).

Study Population

Potential participants were recruited on a rolling basis in Bozeman, Montana from April 2019 through September 2019 via flyer advertisements, word of mouth, email lists, and snowball recruitment methods. Interested individuals were screened for study eligibility by email or phone. Potential participants were 18-55 years of age and were excluded for chronic disease diagnosis, regular use of anti-inflammatory medications, use of blood pressure or lipid-lowering medications, use of antibiotics in the prior 90-days, allergies to wheat, chokeberry, and/or dairy, or were pregnant.

Research Design

The study was a double-blind, randomized, placebo-controlled intervention trial with 0 or 100 milliliters (mL) of Aronia juice per day for 30 days. Testing took place in the Nutrition Research Laboratory at Montana State University. Participants reported to the lab for data collection over three visits. Subject consent and anthropometric measurements were completed at the first visit. Visit 2 occurred after a one-week diet washout period during which participants were asked to omit foods with high polyphenol content from their diet. A high-fat meal challenge was performed at Visits 2 and 3 with a 30-day intervention period in between. Participants brought a self-collected stool sample to these visits for gut microbial analysis. A two-day timeframe was incorporated into the intervention period, allowing for a potential intervention

duration ranging from 28 to 32 days. Participants consumed their last juice dose the day prior to their final visit. Participants were instructed to omit foods with high polyphenolic content from their diet for the duration of the study.

Anthropometrics

All anthropometric measurements were collected from participants at baseline and after the intervention. Subjects were instructed to refrain from eating, drinking, or exercising in the 3 hours prior to testing. A validated³¹ segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) was utilized to assess BMI, fat mass (%), and visceral adipose (L). Blood pressure was taken in the morning of Visits 2 and 3 prior to the high-fat meal challenge after subjects had been seated for at least 15 minutes. Automated measurement was taken in duplicate with the mean used for analysis.

Randomization

Block randomization was performed using the *blockrand* function in the R *blockrand* package.³² Three blocks with two equally numbered levels were created, with the seed for each block individually set based on the last five integers returned by the *sys.time* function. Participants were randomized to Aronia (ARO) or placebo (PLA) group after completion of the first visit. Randomization and juice preparation was performed by a designated research team member who did not interact with participants. Investigators performing research visits were not aware of treatment allocation. After study completion, the juice condition was unblinded for data analysis.

Supplement Intervention

Participants were asked to drink 100 mL daily of ARO or PLA juice during the 30-day intervention period. This intervention dose was based on prior studies utilizing a similar or smaller anthocyanin dose demonstrating decreased postprandial lipid and inflammation response to acute intake³³ and decreased inflammation markers with chronic intake (~30 days) in healthy humans.³⁴ Timing of juice intake was not enforced but participants were encouraged to consume juice at roughly the same time every day to improve compliance. Raw juice was heat pasteurized before study use. The PLA juice was flavor, color, and carbohydrate-matched to ARO juice and consisted of 28.8 grams (g) black cherry Koolaid© mix (no sugar added), 128.5 g sorbitol, 74.5 g glucose, 77.9 g fructose, 4 oz lemon juice, 16 drops of blue food coloring, and enough water to create 1 liter (L) of solution. Participants returned to the laboratory approximately three times during the intervention to receive a fresh 1 L supply of juice provided in a half gallon plastic jug. Plastic jugs were placed into labeled paper bags and refrigerated until participant pickup.

During the intervention period, participants were instructed to consume their standard diet with the exception of omitting foods with high polyphenolic content. Participants were provided with a list of foods to avoid at the first visit (**Supplementary Table 1**). They were instructed to follow this diet for a one-week washout period prior to their second visit as well as the subsequent intervention period for a total of approximately five weeks. Adherence to diet guidelines was verified with a self-reported diet adherence questionnaire completed during the one-week washout period as well as the intervention period.

Aronia Juice LCMS and NMR Analysis

Aronia juice was a blend of Mackenzie, Viking, and Autumn Magic cultivars grown at

the Western Agricultural Research Center in Corvallis, Montana. Aronia juice was analyzed to determine both phenolic and carbohydrate composition as previously described.¹⁸ In brief, polyphenol content was determined using a targeted liquid chromatography, mass spectrometry (LCMS) method developed for use on an Agilent 6,538 quadrupole-time of flight (Q-TOF) mass spectrometer (Agilent, CA, United States) and an Agilent 1290 ultrahigh performance liquid chromatography system (Agilent, CA, United States). Separation was achieved using an Acquity HSST-3 UPLC reverse phase column, 1.8 μ M, 100mm/2.1mm (Waters, MA, United States). Juice carbohydrate content was determined by nuclear magnetic resonance (NMR) analysis using a Bruker 600MHz Avance III NMR spectrometer (Bruker, MA, United States) with a 600MHz TCI (H-C/N-D05Z) LT Probe. Determined carbohydrate concentrations were used to develop matching placebo juice. Concentrations of phenolic and carbohydrate concentrations in the final Aronia juice are provided in **Table 1**.

Table 1. Carbohydrate and polyphenol content of Aronia juice.

Compound	Concentration
Carbohydrate (mM)	
Fructose	433.40
D-glucose	435.89
Sorbitol	672.74
Polyphenol (μM)	
<u>Phenolic Acids</u>	
Chlorogenic acid	8598.77
Neochlorogenic acid	8987.59
<u>Anthocyanins</u>	
Cyanidin 3-xyloside	42.57
Cyanidin 3-glucoside	66.92
Cyanidin 3-galactoside	589.40
Cyanidin 3-arabinoside	1224.29
<u>Flavonols</u>	
Quercitin 3-galactoside	19.36
Quercitin 3-rutinoside	33.47
Quercitin 3-glucoside	127.45

Habitual Diet Assessment

Habitual dietary intake was collected using the validated diet history questionnaire (DHQ III), a web-based survey offered by the National Cancer Institute. While seated during the high-fat meal challenge at visit 2, participants were provided a laptop and asked to complete a 30-day recall. Participants were encouraged to give their best estimates of habitual intake during the recall with the acknowledgment that exact recollection of portion sizes for all food and beverage intake is unlikely. The DHQ III consists of 135 food and beverage and 26 supplement questions to assess dietary frequency and portion sizes. Through built-in analysis of DHQ III surveys, we obtained a calculated Healthy Eating Index (HEI, 2015) scores as an indicator of diet quality in compliance with the 2015-2020 US Dietary Guidelines for Americans.^{35,36} Total HEI score is the sum of nine components based on adequacy (total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids) and four components based on moderation (refined grains, sodium, added sugars, saturated fats).

High-Fat Meal Challenge

The meal consisted of three pieces of toasted whole wheat bread (Wheat Montana, Three Forks, MT, USA) and approximately 58.3 g of salted butter (Tillamook, OR, USA). Total energy content of the high-fat meal challenge was 714 kcal with a macronutrient breakdown of 50 g fat, 54 g carbohydrate, and 12 g protein. Approximately 43.1% of the caloric content was from fat and saturated fats making up approximately 57% of the total fat load. The meal contained approximately 9 g of dietary fiber. Water was provided with the meal and caffeinated earl grey black tea (Bigelow, Fairfield, CT, USA) was provided for participants who identified as habitual

coffee consumers. Participants were asked to consume the meal in 15 minutes, and the postprandial period timing began when participants started the meal.

Blood Sampling

Participants were instructed to avoid alcohol consumption and strenuous physical activity in the 24 hours prior to their second and third visit and to complete an overnight fast (10 - 12 hours) before blood collection. All blood samples were collected by a certified phlebotomist via venipuncture. After fasting blood collection, participants consumed the high-fat meal followed by blood collection at hours 1, 2, 4, and 6 after meal ingestion. Blood was collected into serum separating, EDTA additive, and heparinized additive vacutainer tubes (BD Vacutainer, Franklin Lakes, New Jersey, USA). The serum tube was allowed to clot for 15 minutes at room temperature before centrifugation (1200 RPM, 15 min). Serum aliquots were frozen at -80°C until analysis. Whole blood from the heparinized tube was immediately utilized for blood marker analysis.

Analysis of blood markers

Blood lipid markers were determined from whole blood collected in a sodium heparin tube run on Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, USA) which included total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG) and glucose (GLU). Glycated hemoglobin (HbA1c) was determined using the Affinion2 analyzer (Abbott, Princeton, NJ, USA) from whole blood collected in an EDTA additive tube.

Analysis of Inflammation Biomarkers

Cytokine measurement was performed using high-sensitivity multiplexing technology (Bio-Rad Bio-Plex® 200 HTS, Hercules, CA, USA) following procedures by Millipore (EMD Millipore Corporation, Billerica, MA, USA). Selected cytokines included granulocyte macrophage colony stimulating factor (GM-CSF), interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and five interleukins (IL) including IL-1 β , IL-6, IL-10, IL-17, IL-23. Serum samples at each time point were run in duplicate and the mean used for analysis. Sample values that were under the detectable limit were replaced with $\frac{1}{2}$ the minimum detectable concentration for the respective cytokine for analysis.³⁷

Stool Collection

Participants were asked to collect two fecal samples in total for determination of the gut microbiome composition. Self-collection kits were provided, and participants were asked to follow printed instructions to self-collect a stool sample within 24 hours of their baseline and final visit. After initial collection into a sterile disposable commode, a small portion of the sample was transferred into a sterile 50-mL Eppendorf tube and kept refrigerated until transportation to researchers. Upon arrival for their study visit, samples were processed in an anaerobic chamber with pre-reduced phosphate buffer saline and aliquoted into cryogenic vials at -80°C until DNA extraction.

Genomic DNA Extraction and Microbial Analysis

Extraction of bacterial DNA from human fecal samples was performed using Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Inc.) and bead beating. Extracted DNA was stored at -80°C until analysis. DNA was shipped overnight to the University of Michigan, Michigan

Microbiome Project for Illumina MiSeq amplicon sequencing of the 16S rRNA V4 region. After DNA quantification, V4 amplicon libraries were generated with dual-index barcoded primers, then by library purification, pooling, and MiSeq paired-end sequencing. Sequencing reads were processed and curated with MOTHUR software (Version 1.35.1) (mothur.org) following MOTHUR standard operating procedures for the MiSeq platform.³⁸ In brief, paired-end reads were assembled into contiguous sequences and screened for length and quality. Remaining contigs were aligned to the SILVA ribosomal RNA database (Release 132) to improve alignment of rRNA sequences. Potentially chimeric sequences were identified and removed using the UCHIME algorithm in MOTHUR. Taxonomic classifications were assigned using the Bayesian classifier of the Ribosomal Database Project. Non-target reads were removed, and operational taxonomic units (OTU) were assigned using VSEARCH distance-based clustering at the 97% similarity threshold.

Statistical Methods

Analysis and visualizations were conducted in RStudio (2023.06.1) running R 4.3.1, with data visualized using ggplot2³⁹ and effects⁴⁰ packages. Power was calculated a posteriori from the linear regression model for fasting total cholesterol using the pwr.f2.test in the pwr package. Computed power was 97% at a Type 1 Probability of 0.05.

Anthropometric, Blood Marker, and Inflammation Biomarker Analysis. Descriptive summary statistics were performed for participant physical and biological characteristics and general linear models used to determine if characteristics differed between intervention group at baseline. General linear models were used to assess the impact of the intervention on the following categories of variables: anthropometric measures (weight, fat mass percent and

visceral adipose); fasting blood lipid measures (CHOL, LDL, HDL, TG); fasting glycemic measures (GLU, HbA1c); and fasting inflammation markers (GM-CSF, IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-17, IL-23). Inflammation markers were normalized using R package `bestNormalize`⁴¹ prior to statistical analysis. Dependent variables were calculated as the change in values from baseline (post-intervention – pre-intervention). Predictor variables were shared within each category of variables and are as follows: anthropometric measures –intervention group; blood lipid measures –intervention group, visceral adipose tissue; glycemic measures –intervention group; inflammation measures –intervention group, visceral adipose tissue. Habitual dietary patterns, represented by DHQ-III HEI scores, were analyzed through general linear models to detect dietary differences between intervention groups at baseline.

Analysis of postprandial metabolic (TG, GLU) and inflammatory (GM-CSF, IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-17, and IL-23) measures were conducted by summarizing each markers measurements at timepoints 0 (fasting) and 1, 2, 4, and 6 hours postprandially as net area under the curve (AUC) using the `auctime`⁴² R package. Each dependent variable was then summarized as the change in AUC (post-intervention – pre-intervention). AUC values for inflammation markers were normalized using R package `bestNormalize` prior to statistical analysis. General linear models were used to assess the impact of intervention group on each dependent variable. Predictor variables were identical to variables utilized in corresponding fasting models.

Gut Microbial Statistical Analysis. A total of 738,952 raw reads were obtained across all samples. To aid unbiased diversity matrices due to sequencing depth, data was randomly subsampled to the minimum number of sequences across samples. Subsampling resulted in a total of 168,028 high quality reads.

We performed ecological analyses and visualizations with RStudio (Version 2023.12.0.369) running base R 4.3.2 unless specified differently. Alpha diversity was calculated at the OTU level using (Version 1.35.1) (mothur.org) and vegan (Version 2.6-4). Beta-diversity analyses were performed on subsampled data with filtering of OTUs less than 3 counts in at least 20% of the samples. Permutational multivariate analysis of distance matrices, or PERMANOVA, was performed with 999 permutations using adonis2 in vegan (Version 2.6-4). Fixed effects included an interaction between time and juice condition, underlying main effects in the interaction, and subject. We examined community structure at each taxonomic level (phylum - genus) in addition to taxa selected by hierarchical feature engineering using taxaHFE without the permuted random forest.⁴³ Beta-diversity was visualized using non-metric dimensional scaling from phyloseq.⁴⁴

We also performed a differential abundance analysis to identify possible changes in genera from four weeks of juice supplementation. We utilized MaAsLin2⁴⁵ with subject as the random effect and an interaction between time and juice condition as our fixed effects. Default MaAsLin2 parameters were utilized. We then extracted interaction output and re-calculated q-values for multiple testing adjustment with the Benjamini-Hochberg method.

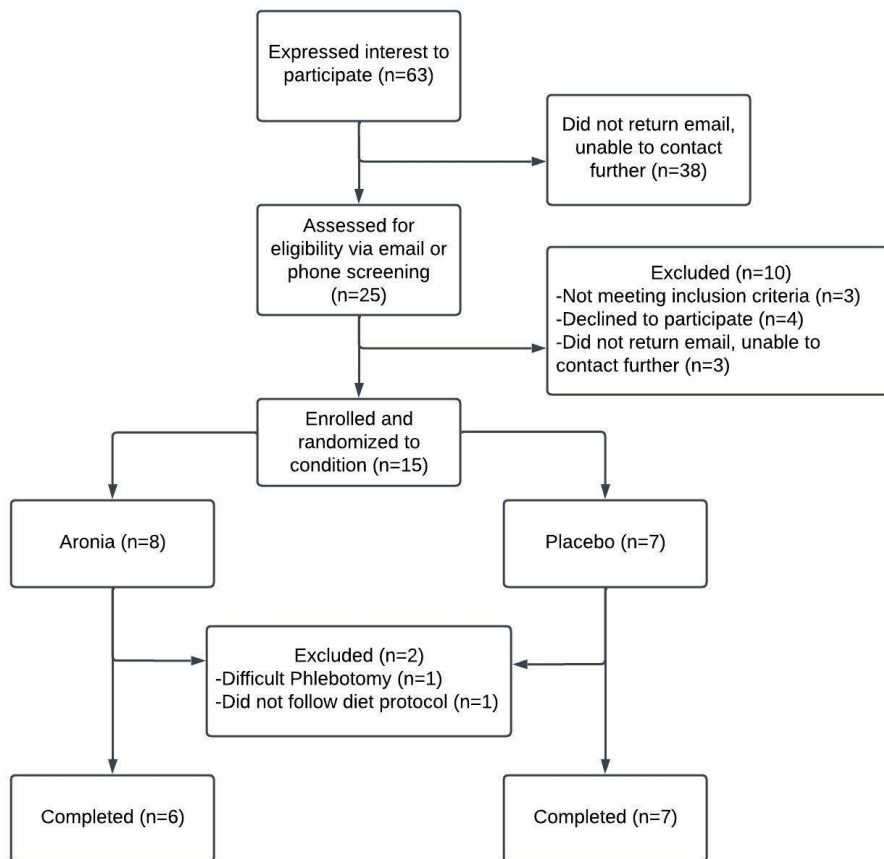
Results

General Characteristics of Participants

A total of 25 participants were screened for study participation of which 10 were excluded for not meeting inclusion criteria, lost to follow-up or withdrew for non-intervention related reasons. Fifteen individuals were randomized to an intervention group with two individuals lost for different reasons (**Figure 1**). One participant was excluded from the study

due to phlebotomy difficulties. Another participant was excluded from blood analyses due to acute alcohol consumption prior to last blood collection visit but was included in microbial composition analysis (n=14). A total of 13 adults (mean $\pm$ SD); age (32.87 ± 6.98 , years); BMI (25.75 ± 4.66 kg/m²) were included in statistical analysis for blood marker analysis.

Figure 1. A CONSORT flowchart of the study design and enrollment. Flyers and emails were used to recruit interested individuals. Interested individuals contacted the research team, who sent additional information about the study. Twenty-five individuals completed an initial eligibility screening with the research team. Fifteen individuals met study requirements and were randomly allocated to Aronia (100 mL/day) or placebo (0 mL/day).



The participant cohort generally exhibited good metabolic health (**Table 2**). On average, participants in both intervention groups were mildly overweight as classified by BMI and had

normal blood pressure as defined by the American Heart Association guidelines.⁴⁶ Fasting blood GLU and HbA1c were normal on average. Fasting total CHOL, LDL cholesterol, and TG were normal and HDL was intermediate in both intervention groups as compared to standard reference. No differences in anthropometric or blood measures were detected between groups at baseline.

Table 2. Participant characteristics at baseline, grouped by assigned dietary intervention (n=13). Blood profile determined from fasting venous blood sample.

	Placebo (n=7)	Aronia (n=6)	p-value
Sex (Males/Females)	3/3	4/3	0.92
Age (Years)	32.4 ± 7.0	35.0 ± 7.8	0.55
BMI (kg/m ²)	25.5 ± 4.0	26.4 ± 6.2	0.77
Fat Mass (%)	25.5 ± 13.4	29.5 ± 10.7	0.57
Visceral adipose (L)	0.84 ± 0.93	1.77 ± 2.1	0.34
Blood Pressure (mmHg)			
Systolic	111.00 ± 20.78	108.88 ± 15.71	0.84
Diastolic	67.83 ± 18.23	67.00 ± 10.61	0.92
HbA1c (%)	4.87 ± 0.34	5.05 ± 0.35	0.37
Fasting Glucose (mmol/L)	5.33 ± 0.31	5.11 ± 0.46	0.34
Triglycerides (mmol/L)	1.03 ± 0.41	1.41 ± 1.46	0.52
Total Cholesterol (mmol/L)	4.29 ± 0.62	4.46 ± 0.88	0.70
LDL Cholesterol (mmol/L)	2.81 ± 0.72	2.69 ± 0.42	0.71
HDL Cholesterol (mmol/L)	1.40 ± 0.38	1.36 ± 0.55	0.90

Data represent mean ± standard deviation. P-value for Sex determined by 2-sample test for given proportions; all other p-values determined by a two-sample t-test. Abbreviations: body mass index, BMI; fat mass, hemoglobin A1C, HbA1c; high-density lipoprotein, HDL.

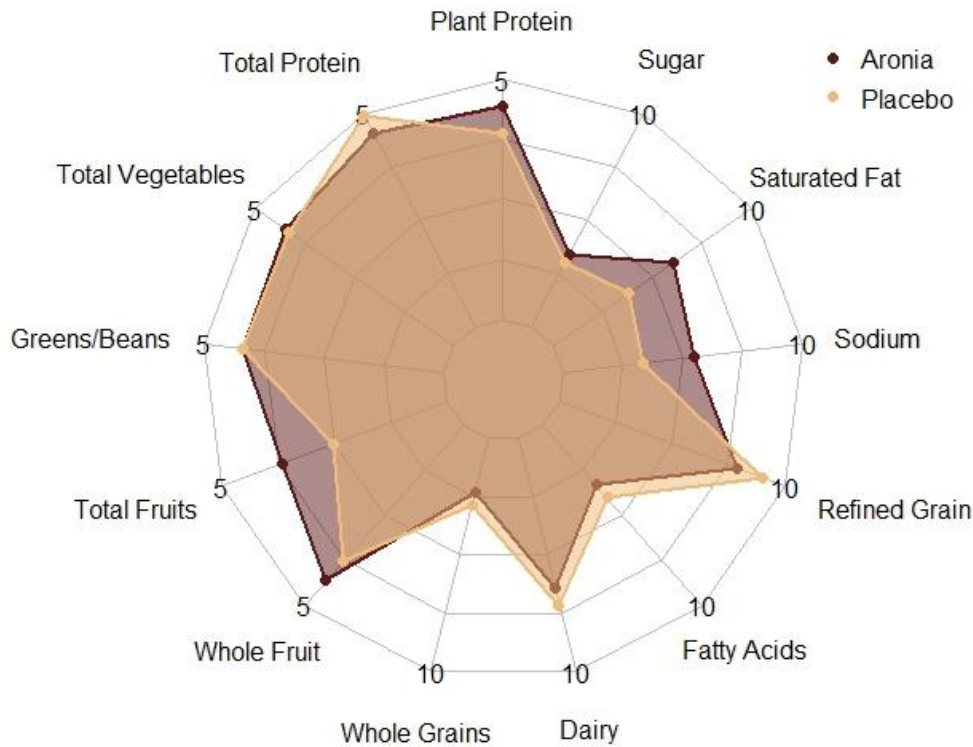
Anthropometric Measures

No differences were observed between ARO and PLA in the change of fat mass percentage ($F = 1.02$, $p = 0.34$), body weight ($F = 0.09$, $p = 0.77$), or visceral adipose tissue ($F = 1.23$, $p = 0.29$) over the 30-day intervention.

Habitual Diet Analysis

Participants in ARO and PLA had similar habitual diets at baseline as determined by comparisons of HEI total and component scores from the self-reported DHQ-III questionnaire (**Figure 2**). HEI component and total HEI scores were not different by intervention group with the exception of saturated fat intake which was lower in the ARO group ($t = -2.54$, $p = 0.03$) (**Supplementary Table 2**). Importantly, HEI component scores for categories associated with high polyphenol content including total vegetables ($F = 0.03$, $p = 0.87$), greens and beans ($F = 0.25$, $p = 0.62$), total fruit ($F = 1.88$, $p = 0.20$), and whole fruit ($F = 0.46$, $p = 0.51$) were not different between intervention groups. Along with fruits and vegetables, caffeinated beverages such as coffee and tea are major dietary sources of polyphenol intake.¹² Participants were not instructed to avoid these beverages during the intervention, but caffeine intake (mg/day) was not different between groups ($F = 0.01$, $p = 0.95$) at baseline. Although the DHQ-III questionnaire does not estimate polyphenol intake directly, the congruence in component HEI component scores and caffeine intake between intervention groups suggests a comparable habitual intake of polyphenols.

Figure 2. Radar plot depicting habitual dietary intake of participants by intervention group at baseline as described by average HEI component scores. HEI component scores were calculated through an online DHQ III in which participants self-reported the types and frequency of foods consumed. Scores of adequacy include total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins and fatty acids. Scores of moderation include refined grains, sodium, added sugars and saturated fats. Points indicate group means. Total score possible for each category listed on chart.

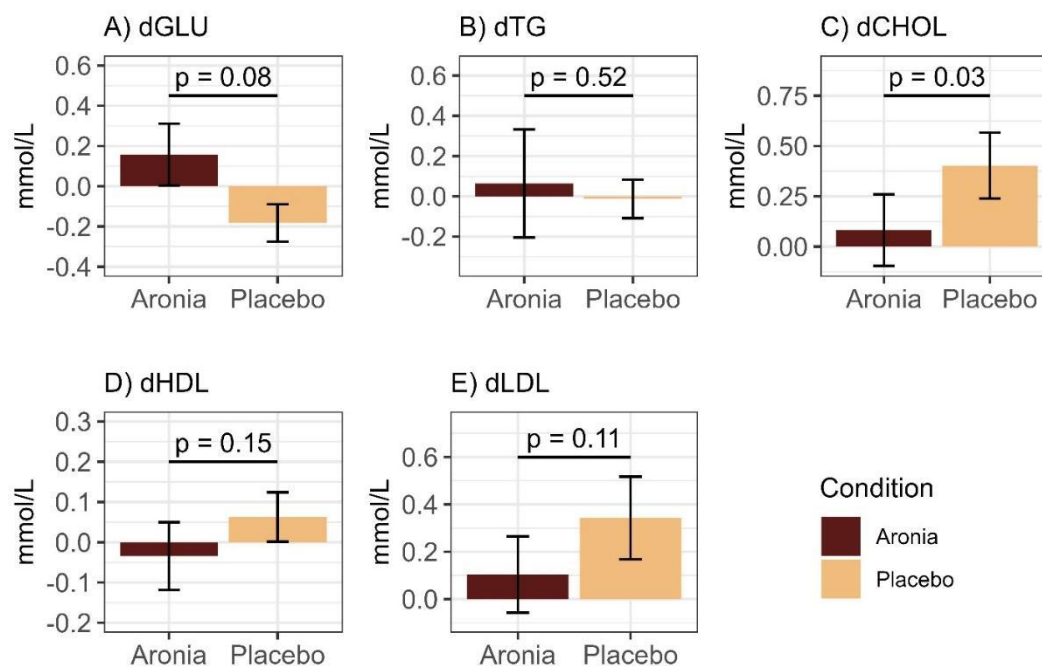


Fasting and Postprandial Lipid and Glycemic Measures

Fasting blood lipid panels were completed pre- and post- intervention to determine if markers were modulated by Aronia intervention. We found that the Aronia supplementation prevented increases in total CHOL ($\beta = -0.50$, $p = 0.03$) over the 30-day supplementation period compared to the placebo group (**Figure 3**). ARO had a small increase of 0.08 mmol/L representative of maintained baseline levels whereas PLA averaged an increase of 0.40 mmol/L.

No difference was detected between intervention groups in fasting GLU ($F = 3.82$, $p = 0.08$), TG ($F = 0.44$, $p = 0.52$), HDL ($F = 2.39$, $p = 0.15$), or LDL ($F = 3.13$, $p = 0.11$).

Figure 3. Summary plots of changes (post-intervention – pre-intervention) in A) fasting glucose (dGLU), B) fasting triglyceride (dTG), C) fasting total cholesterol (dCHOL), D) fasting HDL cholesterol (dHDL), and E) fasting LDL cholesterol (dLDL). Delta values calculated as (post-intervention – pre-intervention). Differences between intervention groups determined by ANOVA.

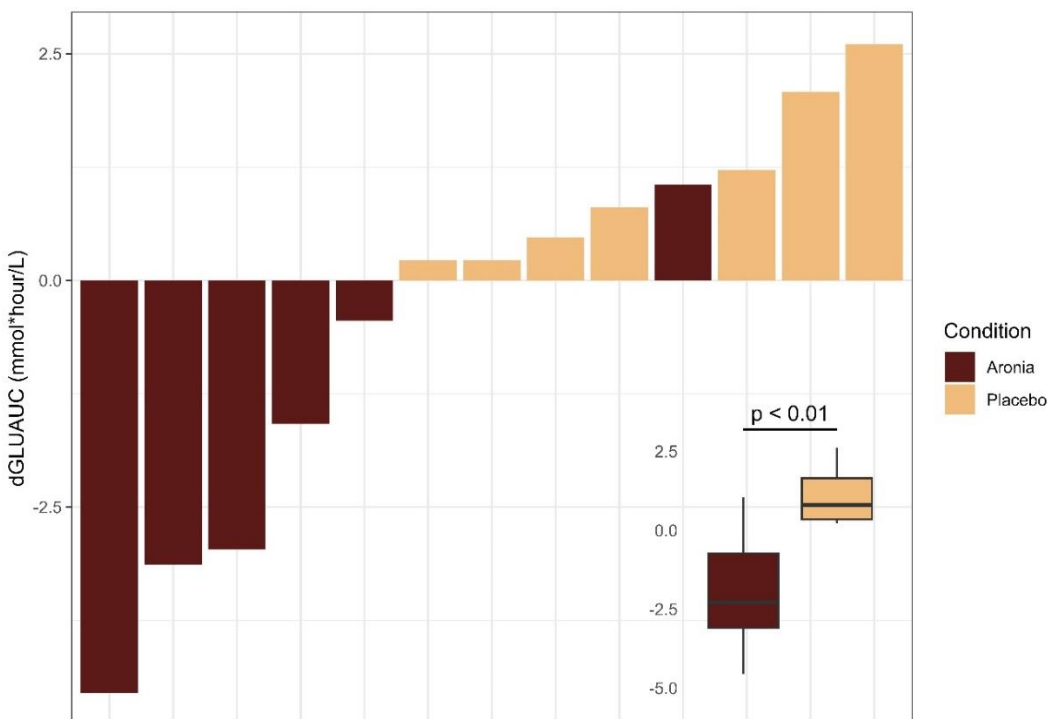


To better understand the impact of Aronia consumption on postprandial metabolism, we examined postprandial triglyceride and glucose responses to a high-fat meal challenge. Participants entered the meal challenge with fasting TG concentrations considered normal (<1.7 mmol/L) with an average of 1.44 mmol/L and 1.02 mmol/L in the ARO and PLA groups, respectively. There was no effect of Aronia supplementation ($F = 1.19$, $p = 0.30$) on the change in TG AUC (dTGAUC) with an average dTGAUC of 1.27 mmol/L in ARO and 0.10 mmol/L in PLA (Supplementary Figure 1). There was substantial variation between individuals in the dTGAUC

in both intervention groups with responses ranging from a 0.91 mmol/L decrease to a 4.97 mmol/L increase from pre- to post- intervention. A summary of the postprandial triglyceride response by intervention group is provided in **Supplementary Figure 2**.

Aronia supplementation lowered postprandial blood glucose responses ($\beta = -3.03$, $p < 0.01$) with an average decrease of 1.94 mmol/L compared to a 1.09 mmol/L average increase in the PLA group (**Figure 4**). All participants maintained blood glucose levels under 7.8 mmol/L at the 2-hour postprandial timepoint indicating a normal blood glucose response to a mixed meal. A summary of the postprandial blood glucose response before and after the intervention is provided in **Supplementary Figure 3**.

Figure 4. Summary plot of individual changes (post-intervention – pre-intervention) in glucose area under the curve (dGLU_{AUC}). dGLU_{AUC} values represent the change in the sum of values from fasting and hourly timepoints for 6 h post high-fat meal ingestion. Each bar is representative of the value for one participant (n = 13). Difference in dGLU_{AUC} between meal groups determined with ANOVA and displayed in inset.



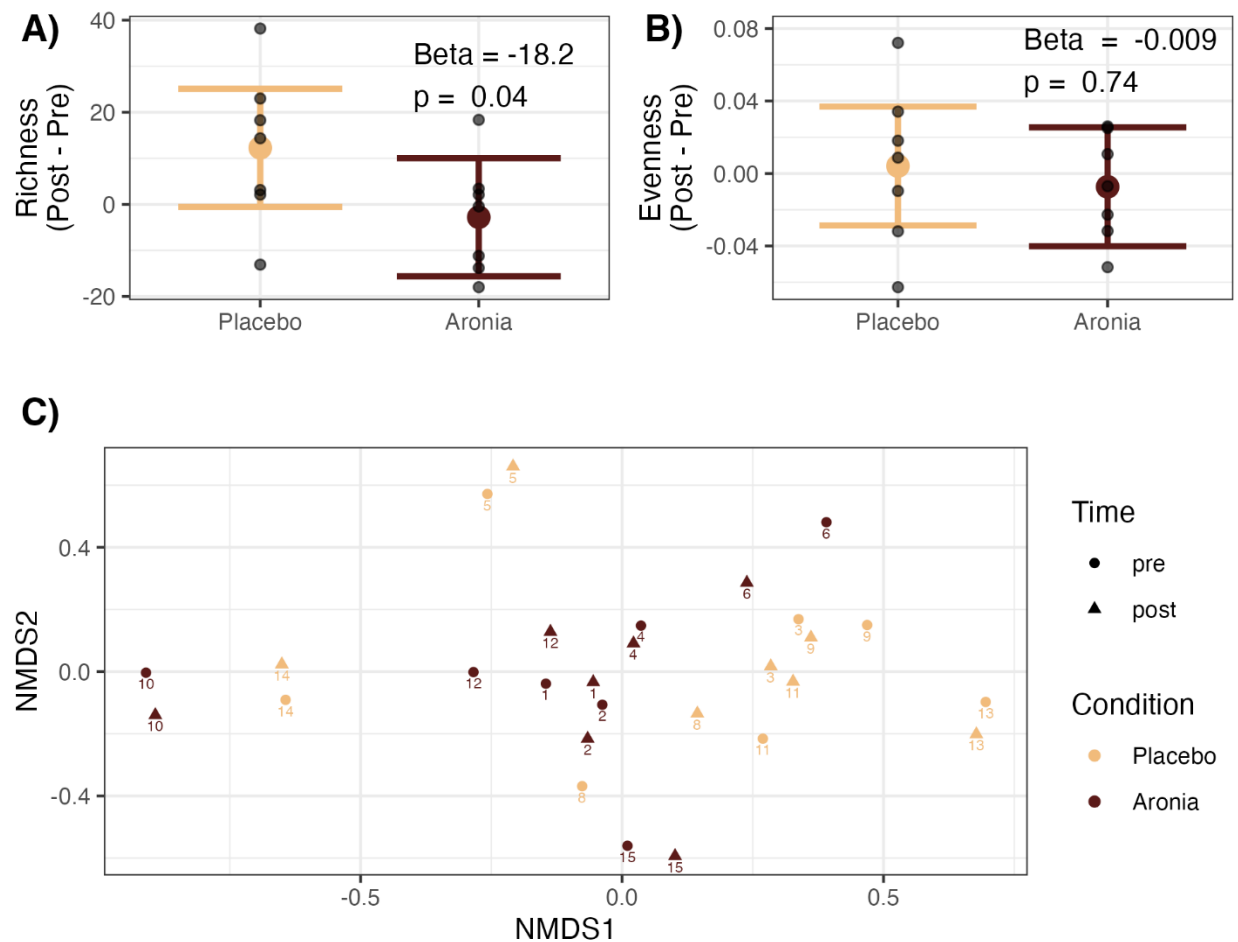
Fasting and Postprandial Inflammation

As high-fat meals can induce an inflammatory response⁴⁷, we examined the impact of 30-days of Aronia supplementation on postprandial inflammation in addition to fasting levels. We did not detect an effect of intervention group on changes in fasting or postprandial inflammation markers from pre- to post- intervention (**Supplementary Table 3**).

Alpha and Beta Diversity

We examined the impact of four weeks of juice consumption on microbial richness and evenness (alpha-diversity) and community composition (beta-diversity). We found that the gut microbial community of individuals who consumed Aronia juice had an average decrease in microbial richness ($\beta = -18.2$, $p = 0.04$, **Figure 5A**) accounting for BMI ($\beta = -0.31$, $p = 0.73$). We did not detect a difference in microbial evenness as measured by Pielou's evenness between Aronia and Placebo groups ($p = 0.74$, **Figure 5B**). We also did not observe a difference in the microbial community structure at any taxonomic level or with microbial features selected through hierarchical feature engineering after the intervention (**Supplementary Table 4**). Unconstrained ordination at the genus-level is provided in **Figure 5C**.

Figure 5. Alpha and Beta-Diversity of the Gut Microbiome. **A)** Microbial richness, **B)** Microbial Evenness, and **C)** Microbial Community Structure. Richness and evenness beta coefficients were derived from non-parametric rank-based estimation methods. Beta-diversity at the genus-level was visualized using non-metric dimensional scaling (NMDS). Each point represents a single sample with numbers indicating the specific subject.



Differential Microbial Taxa

With the decrease in microbial richness, we also performed a differential abundance analysis using MaAsLin to examine what microbial genera may have changed in abundance after four weeks. Without multiple testing correction, we detected a decrease in the abundance of *Holdemania* and *Barnesiella* with Aronia consumption and increased abundance of three unidentified genera within *Oxalobacteraceae*, *Prevotellaceae*, and *Pasturellaceae*.

(**Supplementary Table 5**). These five associations were not maintained after multiple testing correction.

Discussion

High polyphenolic content Aronia fruits have great potential as a functional food with established anti-inflammatory, hypolipidemic, and hypoglycemic biologic activities.⁴⁸ The beneficial impact of polyphenolics on metabolism are likely mitigated by gut microbial metabolism of polyphenols. We conducted a 30-day clinical trial to characterize the effects of Aronia intervention in human participants by analyzing fasting and postprandial inflammation, lipid, and glucose metabolism biomarkers as well as gut microbial composition. The main findings of this study include decreased postprandial glucose, attenuated fasting cholesterol, and a reduction in microbial richness with Aronia juice supplementation.

We found that 30-days of Aronia juice supplementation prevented increases in fasting total cholesterol levels compared to a placebo-matched control. Improvements to blood lipid levels with polyphenolic intake are well-demonstrated in the literature.⁹ Several potential mechanisms for their cholesterol-lowering effect have been identified in murine models including inhibition of intestinal lipid absorption⁴⁹ and regulation of genes related to glycerophospholipid metabolism.⁵⁰ Fewer controlled trials have focused on Aronia-based interventions, however two meta-analyses of randomized controlled trials investigating the impact of Aronia on cholesterol levels identified improved measures with supplementation.^{13,51} It is possible that the significant difference in CHOL levels between Aronia and placebo groups is reflective of the low-polyphenolic diet followed during the study with the lack of polyphenolic intake in the placebo group contributing to increased CHOL levels. No other changes to blood

lipid measures including HDL, LDL, or TG were identified in our study, which is supported by results from a randomized crossover trial examining Aronia supplementation in a healthy participant cohort.⁵² We are not aware of any other studies examining changes to the postprandial triglyceride response with long-term Aronia supplementation to provide context for our study measure indicating no improvement. However, this finding may be reasonably expected given the metabolically healthy status of our participants and the high inter-individual variability in their responses both before and after the intervention.

Aronia supplementation lowered postprandial glucose responses to the high-fat meal challenge. In a similar study, 100 mL of Aronia juice was found to lower postprandial blood glucose in healthy subjects.⁵³ This decrease was attributed to inhibited enzymatic activity of dipeptidyl peptidase IV, α -glucosidase, and angiotensin-converting enzyme by Aronia juice. Inhibition of these enzymes reduces blood glucose levels through different mechanisms including incretin regulation and epithelial glucose transport.⁵⁴⁻⁵⁶ However, blood glucose measurements were made in response to acute Aronia juice ingestion which differs from our study as no juice was consumed the day of high-fat meal testing. Therefore, it is possible that a separate mechanism is responsible for the lowered blood glucose responses in our study. Several factors impact postprandial glycemic responses include baseline glycemic status and insulin secretion. We did not measure fasting or postprandial insulin responses, which prevents us from speculating on the relationship to insulin secretion in our study. However, the baseline glycemic status of our participants was optimal as measured by fasting blood glucose and HbA1c and no changes to these metrics were identified with Aronia supplementation. More studies examining

postprandial glucose responses to long-term Aronia supplementation are needed to better define the beneficial impacts of Aronia on glycemic control.

Contrary to our hypothesis, fasting and postprandial inflammation levels were not improved with 30 days of Aronia juice consumption. Randomized controlled trials have reported lowered inflammation^{52,57} with Aronia supplementation, though some found no change.⁵⁸ These studies were conducted in metabolically at-risk populations and had intervention periods lasting 6-12 weeks. It is possible that fasting inflammation markers were unchanged in our study due to a much shorter intervention period and the use of metabolically healthy participants as the study cohort. Improvements to postprandial inflammation markers were also not observed. This is likely because maximal serum concentrations of polyphenols were not aligned to the timing of the high-fat meal, the inflammatory stimulus used in this study, for greatest anti-inflammatory benefit. Hydroxycinnamic acids and anthocyanins from Aronia are partially absorbed in the small intestine and reach their maximal serum concentrations within three hours of consumption.^{59,60} However, 90-95% of polyphenols pass to the large intestine and are subject to digestive metabolism by gastrointestinal microbes. These microbial derivatives of the parent polyphenols have a delayed entry into circulation. Consumption of isotope labelled cyanidin-3-glucoside in healthy adults revealed that derivatives ferulic and hippuric acid respectively took an average of 8.2 and 15.7 hours to reach maximal plasma concentrations.⁶¹ Furthermore, some anthocyanin metabolites have a half-life of up to 96 hours.⁶¹ Therefore, it is possible that some bioactive derivatives were in circulation during challenge meal testing. However, plasma concentrations were likely a small fraction of peak concentrations after acute ingestion. The authors hypothesize that timing of juice consumption and challenge meal testing may also have

contributed to the lack of change to postprandial triglyceride responses with Aronia consumption.

We found that Aronia supplementation reduced microbial richness, an aspect of alpha diversity, without affecting the beta diversity of the gut microbiome. It is believed that a high alpha diversity in the context of the gut provides functional redundancy to help ensure continued specialized metabolic functions.⁶² A reduction in richness could be indicative of an antimicrobial effect from high polarity phenolic derivatives, though this effect seems less likely as we did not observe differentially abundant bacteria after multiple testing correction. It is also possible that refraining from polyphenol-rich foods during the intervention contributed to reduced richness. Since polyphenols co-occur with other nutrients such as fiber, omitting polyphenol-rich foods may have limited the habitual dietary substrate pool for growth of the established microbial community.⁶³ It is unlikely that the introduction of Aronia juice as a supplement to generally healthy adults with a good diet would shift the gut microbial community. However, we have previously seen that in times of large microbial disturbances, such as with a high-fat diet, that Aronia juice may prevent further declines in alpha-diversity.¹⁸ Taken all together, the lack of community wide changes and specific microbial signals suggests that Aronia polyphenols are metabolized by a range of microbial taxa in the gut microbiome of healthy adults. Further study is needed to assess the metabolic capacity of the human gut microbiome to metabolize anthocyanins and how resulting phenolic derivatives may influence postprandial outcomes.

While our study demonstrated several important impacts of Aronia juice supplementation in humans, we also acknowledge study limitations. Our experiment was set up as a pilot study to explore the impacts of Aronia supplementation on measures of metabolic health in humans. The

size of our participant cohort is a result of this study design and we acknowledge that a greater sample size is needed to confirm and further characterize metabolic responses to supplementation. The study cohort was also characterized as metabolically healthy at baseline which likely indicates a smaller effect size for study measures in our participants compared to individuals with metabolic risk factors. In addition, although participants were asked to maintain their regular physical activity patterns for the duration of the study, it is possible that alterations to exercise patterns prior to bloodwork collection visits impacted study measures. Non-compliance with dietary foods to avoid during the intervention could also impact study results. While participants were asked to record instances when they consumed a food item from the high-polyphenolic food item list provided, they were not excluded from the study for doing so. Additionally, while caffeinated beverages such as tea and coffee are major dietary sources of polyphenols,¹² participants were not instructed to limit intake of these items during the intervention period. This decision was purposefully made to improve study feasibility and participant recruitment. For these reasons, it is possible that polyphenolic intake from diet during the intervention was different between groups and impacted study results.

Our study uniquely characterizes Aronia-induced metabolic impacts in humans. Although the benefits of polyphenol supplementation are known to be mitigated, at least in part, by the gut microbiome, studies examining changes in taxonomy in response to Aronia supplementation in humans are limited. In addition to fasting markers of metabolic health, we measured postprandial lipid, glucose, and inflammation responses to a high-fat meal challenge. While the importance of postprandial metabolism in relation to metabolic health and disease risk is well-established²⁶⁻³⁰, these measures are not consistently analyzed in non-acute supplementation studies. The

completion of these measures in our study more comprehensively characterizes the impact of our Aronia dose on metabolic health. Other study strengths include the use of a whole-foods based intervention in free-living adults which best characterizes dietary changes likely to be made by individuals seeking to improve metabolic health.

Conclusion

Thirty days of supplementation with Aronia juice prevented increases in total cholesterol and improved postprandial glucose responses. We did not observe changes in other measures of glucose and lipid metabolism or inflammation. A decrease in microbial alpha-diversity as measured by microbial richness was observed which was likely a side-effect of dietary restrictions during the intervention to isolate the impact of Aronia-derived polyphenols. Further clinical trials investigating Aronia-mediated health impacts are warranted and may consider a larger supplementation dose or longer intervention period to further identify potential metabolic benefits of supplementation. This information further informs study design for future clinical trials and provides novel insight needed for the development of polyphenolic-based dietary strategies to improve health.

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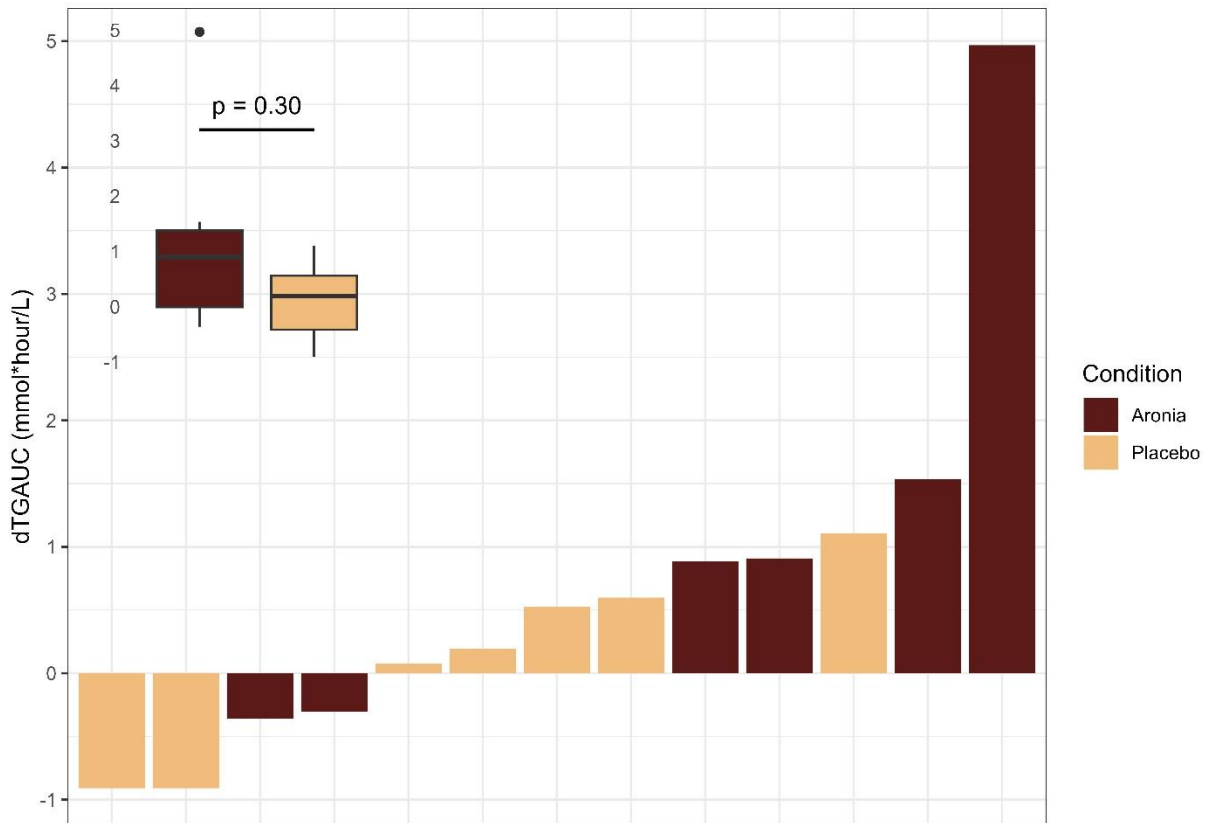
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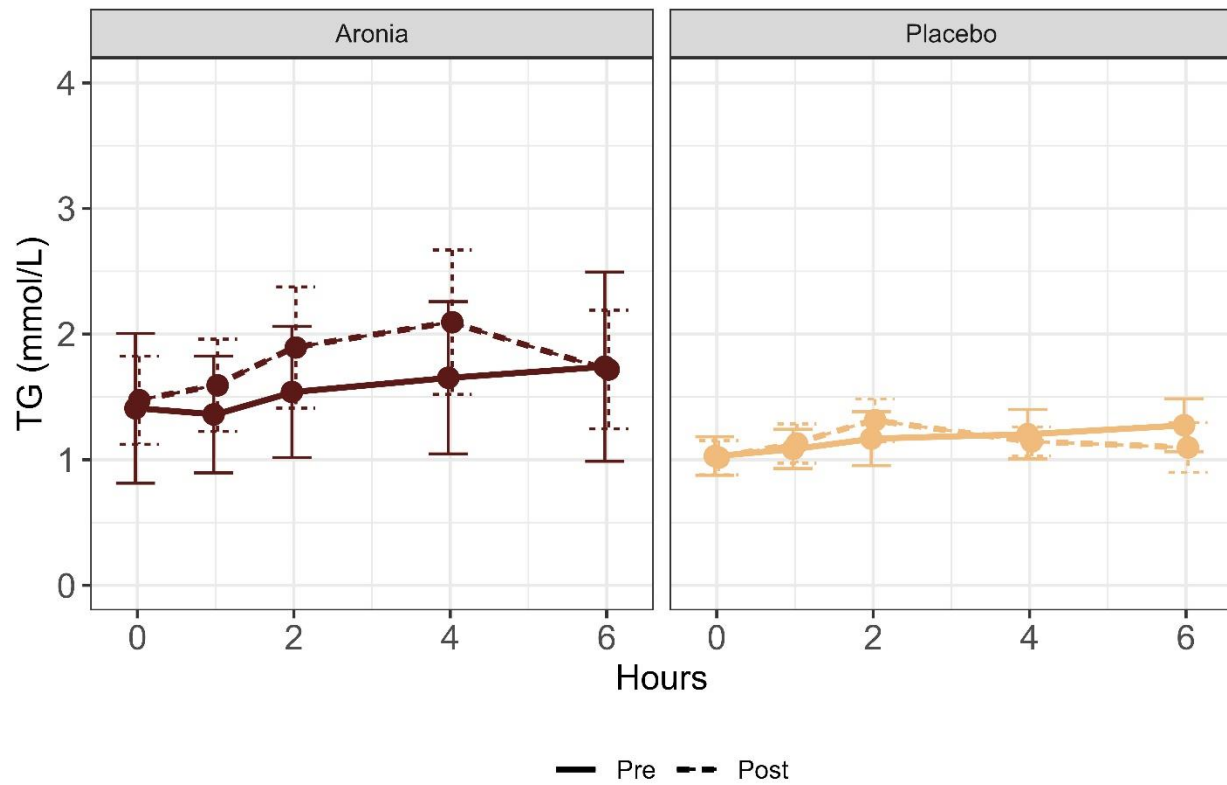
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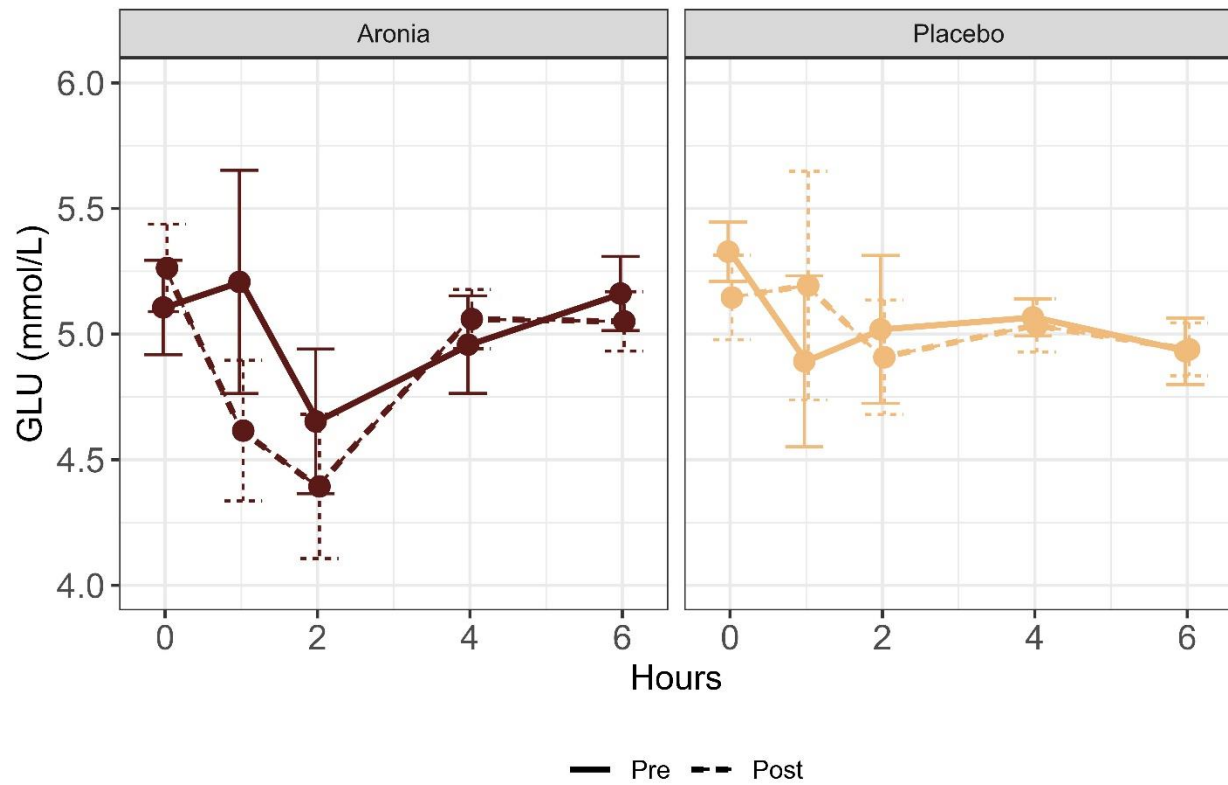
Supplementary Figure 1. Summary plot of individual changes (post-intervention–pre-intervention) in triglyceride area under the curve (dTG_{AUC}). dTG_{AUC} values represent the change in the sum of values from fasting and hourly timepoints for 6 h post high-fat meal ingestion. Each bar is representative of the value for one participant (n = 13). Difference in dTG_{AUC} between meal groups determined with ANOVA and displayed in inset.



Supplementary Figure 2. Postprandial triglyceride (TG) response to a 50-gram, high-fat meal challenge by meal group before and after the 30-day intervention. Points indicate group means, bars represent $\pm$ standard error.



Supplementary Figure 3. Postprandial glucose (GLU) response to a 50-gram, high-fat meal challenge by meal group before and after the 30-day intervention. Points indicate group means, bars represent $\pm$ standard error.



Supplementary Table 1: List of Foods to Avoid. List of foods provided to participants at first visit to avoid eating for one-week dietary washout period before second visit and for duration of the 30-day intervention period.

Berries	Fruit	Vegetables	Beans	Nuts	Other
Blueberries	Apples	Kale	Pinto	Walnut	Dark chocolate
Blackberries	Black Plum	Broccoli	Black	Pecan	Black rice
Strawberries	Prune	Beets	Red		
Cranberries		Artichoke	Kidney		
Cherries					
Raspberries					
Goji					

Supplementary Table 2. Habitual dietary intake as represented by HEI total and component scores by intervention group. HEI component scores were calculated through an online DHQ III in which participants self-reported the types and frequency of foods consumed. Bold p-values indicate strong evidence of a difference between meal groups.

Component	Maximum points	Score		p-value
		ARO (n=6)	PLA (n=7)	
Adequacy				
Total fruits	5	3.7 ± 1.6	2.5 ± 1.4	0.20
Whole fruits	5	4.3 ± 1.3	3.8 ± 1.6	0.51
Total Vegetables	5	4.3 ± 1.4	4.2 ± 1.0	0.87
Greens and beans	5	4.2 ± 1.9	4.6 ± 0.9	0.52
Whole grains	10	2.4 ± 1.0	2.8 ± 1.6	0.52
Dairy	10	6.4 ± 1.1	7.2 ± 1.9	0.39
Total protein foods	5	4.5 ± 0.7	5.0 ± 0.1	0.15
Seafood and plant proteins	5	4.5 ± 1.1	3.9 ± 1.4	0.44
Fatty acids	10	3.3 ± 2.4	4.0 ± 3.4	0.68
Moderation				
Refined grains	10	7.9 ± 2.6	9.0 ± 1.3	0.35
Sodium	10	5.5 ± 3.6	3.4 ± 1.3	0.17
Added sugars	10	5.7 ± 3.4	4.5 ± 3.0	0.53
Saturated fats	10	6.1 ± 1.6	3.8 ± 1.7	0.03
Total	100	62.8 ± 7.0	58.7 ± 8.5	0.38

Data represent mean ± sd. Significance between group means determined with t-test.

Abbreviations: ARO, aronia; PLA, Placebo; DHQ, diet history questionnaire.

Supplementary Table 3. Change in fasting and postprandial inflammation by intervention group. Fasting values represent timepoint 0 (fasting) collected prior to 50 gram, high-fat meal challenge. Postprandial values summarized as net area under the curve (AUC) using fasting and 1-, 2-, 4-, and 6- hour postprandial measurements. Delta values calculated as (post-intervention – pre-intervention) and expressed by Δ Baseline to represent changes in fasting inflammation and Δ AUC to represent changes in postprandial inflammation.

Cytokine	ARO (n=6)		PLA (n=7)		p-value	
	Δ Baseline	Δ AUC	Δ Baseline	Δ AUC	Δ Baseline	Δ AUC
IL-6	0.84 ± 1.15	11.49 ± 34.40	-0.14 ± 1.85	-3.42 ± 10.33	0.34	0.53
IL-1 β	0.19 ± 0.49	-0.48 ± 3.15	-0.33 ± 0.60	1.50 ± 2.70	0.13	0.17
IL-17	1.13 ± 1.38	-1.22 ± 14.39	-0.17 ± 4.81	-16.56 ± 58.54	0.53	0.58
IL-23	27.20 ± 75.03	-114.03 ± 520.99	14.20 ± 88.89	73.04 ± 781.89	0.09	0.27
IL-10	2.98 ± 3.97	-13.94 ± 21.15	-3.40 ± 9.13	-10.83 ± 75.56	0.17	0.66
TNF- α	0.96 ± 1.38	-3.30 ± 8.73	-0.98 ± 3.01	6.95 ± 15.05	0.24	0.14
GM-CSF	1.84 ± 5.55	9.41 ± 41.23	-1.03 ± 20.44	-13.07 ± 98.92	0.78	0.68
IFN- γ	9.24 ± 21.76	-63.90 ± 136.44	4.56 ± 135.64	-542.55 ± 1678.36	0.90	0.43

Data represent mean $\pm$ standard error. Linear mixed modeling used to determine differences ($p < 0.05$) between group means.

Abbreviations: ARO, control; PLA, placebo; IL, interleukin; TNF- α , tumor necrosis factor alpha; GM-CSF, granulocyte macrophage colony stimulating factor, IFN- γ , interferon-gamma; AUC, area under the curve.

Supplementary Table 4. PERMANOVA results at each taxonomic level for the time and juice interaction. P-values and R^2 values reflect the interaction between juice condition and time.

Taxon	R²	Pr(>F)	Features
Phylum	0.002	0.95	10
Class	0.004	0.97	22
Order	0.009	0.95	32
Family	0.004	0.99	55
Genus	0.009	0.99	160
HFE selected	0.011	0.947	89

Abbreviations: HFE, hierarchical feature engineering; OTU, operational taxonomic unit.

Supplementary Table 5. Microbial taxa associated with Aronia juice consumption before multiple testing correction. Taxa were identified using MaAsLin2.

Genus	Coefficient	Std. Error	p-value	q-value
<i>Holdemania</i>	-0.674	0.205	0.0064	0.82
<i>Oxalobacteraceae</i> unclassified	0.353	0.152	0.0387	0.82
<i>Barnesiella</i>	-0.312	0.141	0.0469	0.82
<i>Prevotellaceae</i> unclassified	0.546	0.263	0.0487	0.82
<i>Pasteurellaceae</i> unclassified	0.316	0.146	0.0508	0.82

CHAPTER FIVE

CONCLUSION

Diet is a modifiable risk factor contributing to overnutrition, central adiposity, and metabolic dysregulation.¹ In addition to improvements in anthropometric indicators like weight and visceral adiposity, dietary alterations can improve metabolic health by decreasing glycemic, lipidemic, and inflammatory responses to food consumption.²⁻⁴ Therefore, healthy dietary changes represent a critical strategy to prevent and reduce the burden of metabolic disease. Assessing changes to metabolism and improvements to metabolic risk in response to diet is critical to the formation of dietary interventions. However, variability in individual responses to diet presents a challenge to accurate metabolic health assessment. Understanding contributing factors to interindividual variability is still an active area of research, but gut microbial composition, inflammation phenotype, and metabolomic profiles help establish a more accurate picture of metabolic health and variable response to diet. In addition, postprandial measures of commonly utilized fasting metabolic health biomarkers such as triglyceride and glucose levels provide insight into acute responses to food and are more accurately correlated to metabolic disease risk.⁵⁻⁸

In **Chapter 2**, the impact of chronic lentil consumption on fasting and postprandial measures of inflammation, glucose, and lipid metabolism to an oral fat challenge were assessed in adults with elevated metabolic risk. Twelve weeks of daily lentil consumption lowered low-density lipoprotein and total cholesterol levels. While fasting measures of glycemic control and inflammation were unchanged, postprandial glucose, IL-17, and IL-1 β responses to the oral fat challenge were decreased with lentil supplementation. These findings support the use of fasting

and dynamic measures of glucose and lipid metabolism as well as inflammation to better define metabolic response to diet. In addition, the measurement of multiple inflammatory markers provides physiological insight into inflammation status. As a colonic inflammation marker, lowered IL-17 may be mediated by changes to the gut microbiota and subsequent production of microbial metabolites from habitual lentil consumption. Lowered postprandial IL-1 β may reflect the decreased postprandial glucose response in the lentil group as IL-1 β secretion by peritoneal macrophages occurs in a glucose dependent manner.⁹ Importantly, lentil consumption was not associated with gastrointestinal symptoms – a known barrier to pulse-consumption. These results implicate daily consumption of lentils as a safe and effective dietary strategy to improve metabolic health and further inform the development of pulse-based dietary strategies to lower disease risk and to slow or reverse metabolic disease progression in at-risk populations.

In **Chapter 3**, the potential of acute lentil intake to favorably alter the postprandial bile acid, glucose, lipid, and inflammatory responses to an oral fat challenge is examined with a randomized crossover trial in overweight or obese adults. While both meals elicited significant postprandial responses, the 14-gram fiber difference between the lentil and control meals was not enough to significantly alter measures between meal conditions. Further, the composition and concentration of serum bile acid responses to both meals were similar with no difference in total, primary, secondary, tertiary, or 12- α hydroxylated serum bile acids. However, differential relationships between individual bile acids and the postprandial glucose and lipid responses were identified between meal conditions. This finding may indicate a two-way interaction between meal components and bile acids on postprandial metabolism. This study provides novel information regarding the postprandial bile acid response to lentil intake which is a critical gap in

the literature and supports research indicating that the metabolic health benefits associated with fiber are dose and fiber-type specific.

In **Chapter 4**, the metabolic impact of Aronia-derived polyphenolics were examined in a randomized, controlled, double-blind, parallel clinical trial. Similar to the chronic lentil study, fasting and postprandial measures of inflammation were assessed as well as changes to gut microbial composition. Consumption of 100 mL of Aronia juice per day for 30 days attenuated total cholesterol and reduced postprandial glucose. No changes to gut microbial taxa were detected, but a decrease in microbial alpha diversity as measured by richness with Aronia supplementation was identified. The study dose of 100 mL/ day is one of the smaller doses utilized in trials investigating polyphenolic intake. The positive metabolic effects seen with this dose are therefore informative to the development of polyphenolic recommendations.

This body of work provides insight into metabolic response to diet by assessing postprandial lipid, glucose, and inflammatory responses, as well as alterations to gut microbial taxonomy and metabolomic profiles to acute and chronic dietary interventions. Study results highlight the importance of these measures in assessing metabolic health as well as their interrelatedness. Across all three studies, a high amount of interindividual variability was identified in postprandial lipid, glucose, and inflammatory responses to oral fat challenges. This finding aligns with large cohort studies identifying significant variations in these measures even with identical food intake. Differences in interindividual response to diet are attributed to a myriad of factors including gut microbial composition and genetics.¹⁰ Clear evidence for the importance of these factors in determining metabolic health and response to diet provides a strong case for the use of personalized nutrition in the generation of dietary recommendations.

Personalized nutrition stands at the forefront of dietary science, offering tailored dietary recommendations based on individual genetic makeup, lifestyle factors, and health goals. As a registered dietitian, while I recognize its immense potential to optimize health outcomes, I also emphasize the need for caution. The integration of personalized nutrition into clinical practice requires a solid foundation of scientific evidence to ensure efficacy and safety. Premature adoption without sufficient validation risks misleading individuals and potentially causing harm. Therefore, it's crucial to prioritize rigorous research and clinical validation in the development and implementation of personalized nutrition strategies. By advocating for evidence-based practice guidelines, we can harness the transformative power of personalized nutrition while safeguarding against misinformation and promoting optimal health for all.

A major hurdle to the adoption of personalized nutrition in practice lies in the sheer volume and complexity of data generated when utilizing omics approaches to measure factors like the metabolome and microbial genome. The vast amount of complex data generated challenges not only data management and processing machinery, but also the extraction of meaningful biological insights and interpretation. In addition, omics studies currently face limitations in reproducibility and standardization across different laboratories and platforms.¹¹ The development of advanced computational tools and machine learning algorithms are expanding to keep pace with data generation and collaborative efforts towards standardized protocol generation, shared data repositories, and quality control measures are a developing focus of omics research.

The profound relationship between food and health has been recognized for centuries. This longstanding recognition has propelled the evolution of nutrition research from its origins in

single-nutrient discoveries to a more comprehensive examination of foods, dietary patterns, and their interaction with the individual consuming them. The evolution of nutrition research has culminated in the development of personalized nutrition, which provides a new lens through which to examine diet-induced changes to metabolic health.

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