DIET-INDUCED ALTERATIONS TO POSTPRANDIAL METABOLISM AND THE GUT MICROBIOTA

by

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DEDICATION

I am a second-generation Filipino American. My mother is from the Pangasinan Province in the Philippines, and my father is from a remote area of Indiana. From Alaska, we eventually grew roots in a predominantly white part of southern Indiana. We lived by very modest means but made the best of what we had. We were often the only Asian Americans in the neighborhood and in our schools. In more ways than one, we tried hard to fit into the community around us. Notably, my siblings and I were never taught Tagalog in the hopes that we would be seen as true Americans. *Walk the walk. Talk the talk.* Yet, we stuck out. My siblings and I have similar stories of being asked about our racial background or receiving flippant comments about our race from our classmates.

"So what are you? Oriental?"
"I heard your mom was a mail order bride."

In high school, I put tremendous effort into becoming the perfect student — quiet and studious, good grades, varsity letters, and academic bowl captain. *After all, how could you say anything bad about someone who was putting in all of this effort*? In college, I continued to work just as hard and tried to find a place to fit in. In graduate school, I reflected deeply on my cultural identity and my place in the upper echelons of academia. I realize I have been afforded many privileges as a second-generation Filipino American that many Filipinos and Filipino Americans do not have. However, in my current space, the lack of Filipino and Filipino American graduate students and faculty is a glaring fact that I think about every day. I did not have family before me who attended college or graduate school who could give me advice nor even other Filipino Americans around me to seek for their wisdom. *But surely, if you work twice as hard, no one will question your place*. I now see that cultural assimilation has robbed me of countless things and has left deep insecurities and marks on my way of thinking and perceiving the world. After many years, I can start to put aside that desperate need to fit in. I embrace my own skin and my culture as some of my greatest strengths. *I am worthy.*

This dissertation is for all current and future Filipino Americans pursuing their graduate education. You may not have peers and faculty that look like you, but remember that you are worthy and you belong.

To Keith – thank you for loving me and pushing me to become a better version of myself. This work frequently pulled me away on many weekends, but you never once batted an eye and always made sure I had breakfast and coffee before heading to the lab. I cannot think of a more meaningful expression of love. Thank you for pulling me out of my dark workaholic spells and encouraging rest and recreation in this beautiful part of the world.

To Shannen – you inspire me to forge new paths in academia so others who look like us and who grew up like us may follow with courage and strength.

To my mom – moving and adapting to a new life in America – Where you changed the spelling of your first name so others could pronounce it, Where people routinely saw you and your family as other – must have been incredibly difficult.

I can be here today because of you and the sacrifices you have made.

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ABSTRACT

Obesity is a key component of a cluster of metabolic risk factors for chronic disease which include dyslipidemia, hyperglycemia, and hypertension. Stark changes in diet and lifestyle contribute to growing metabolic disorder prevalence. Many Americans regularly consume foods low in fiber and rich in fat and sugar, which can negatively influence glucose and lipid metabolism over time. A nutritionally poor diet exerts deleterious effects on the gastrointestinal microbial community which has larger host health implications. As a modifiable risk factor, diet can be part of the solution to counter the rise of chronic disease. However, dietary responses as it pertains to glucose and lipid metabolism display high interindividual variability. This interindividual variability with diet can also be observed at the microbial level in our gastrointestinal system. In metabolically at-risk adults, we examined postprandial responses and the efficacy of a long-term lentil dietary intervention in improving postprandial glycemic responses. We further assessed the effect of an antioxidant-rich juice from the *Aronia melanocarpa* berry and its anti-inflammatory potential against a high fat diet in a gnotobiotic mouse model to evaluate how specific gut microbial communities transferred from metabolically at-risk adults with different inflammatory profiles may impact dietary responses. Postprandial triglyceride responses in adults with overweight and obesity can be partially explained by central adiposity, insulin resistance, and the ability to switch between glucose and fat metabolism to a metabolic stress. A long-term dietary intervention with lentils, a fiber-rich pulse crop, worked in a dose-dependent manner to reduce insulin resistance in adults with increased central adiposity without an increase in gastrointestinal symptoms, a common deterrent to incorporating fiber-foods in the diet. Our gnotobiotic mouse experiments revealed donor-dependent changes in dietary responses. We observed protective effects of *Aronia* juice in mice, particularly in mice from the low inflammation stool donor. Metabolomic changes in phospholipids such as phosphatidylcholine and sphingomyelin were further detected, with changes respectively unique to *Aronia* juice and high-fat diet. Taken together, this dissertation provides an improved understanding of our metabolic responses and microbial alterations to the foods we consume, and how these responses influence the progression of metabolic diseases.

CHAPTER ONE

GENERAL INTRODUCTION

The Start of a Metabolic Cascade

Obesity is a condition defined by having excess adipose deposition and is unfortunately commonplace worldwide.[1] Obesity, and specifically abdominal obesity, is one of the key criteria of metabolic syndrome. Metabolic syndrome is a condition with a cluster of metabolic risk factors, which in addition to abdominal obesity, include dyslipidemia, hypertension, and hyperglycemia.[2] It is believed that abdominal obesity can present without the other metabolic syndrome risk factors at first, but over time, abdominal obesity in conjunction with insulin resistance contributes to the progression of the other risk factors.[3] The presence of metabolic syndrome can ultimately manifest downstream severe complications, which emphasize the importance of strategies to combat the progression of metabolic syndrome in its earliest stages.

Drastic changes in diet and lifestyle over the past century have contributed to the onset and prevalence of metabolic dysfunction and deleterious changes in the gut microbiome. With the aid of industrialization, a growing number of diets include processed foods, displacing raw or minimally processed foods. The collective qualities of processed foods negatively impact glucose and lipid metabolism and promote unhealthy adipose deposition which can exacerbate insulin resistance and drive metabolic syndrome forward. Changes in the diet can also induce gut microbiome composition and functional alterations as they are also recipients of dietary food choices by the host. Microbial changes

subsequently influence host physiology. Specifically, dietary-induced changes in host metabolism may be gut-mediated, introducing a vital but previously overlooked player in the pathogenesis of metabolic disease.

The Rise of Food Industrialization and Obesity

From the raw and minimally processed foods of hunter and gatherer society, humans have overwhelmingly moved to industrial forms of food processing since the 1800s.[4] In the early part of the last century, food shortages, malnutrition, and foodborne illnesses were common during the great economic and political changes.[5] These world pressures encouraged food developments such as nutrient fortification, food preservation, and improved shelf life which in turn, influenced food consumption patterns. One example is refrigeration, a food preservation method, which allowed for households to safely store perishable foods including out of season vegetables and animal-based products like meat and dairy. After the 1890s, it is estimated that refrigeration helped increase dairy and protein consumption respectively by 1.7% and 1.25% annually.[6]

Economic growth after World War II helped to shift the focus from food fortification and preservation to convenience and palatability.[5] Economic and social systems promoted the availability, affordability, and marketing of convenience foods to consumers.[7] As the name suggests, convenience foods are convenient in that they reduce food preparation and cleanup time often at reduced cost. These foods represent a growing global market fueled by consumer demand.[7] Recent estimates suggest that ultra-processed foods make up a large majority of the American diet, representing up to 60% of calories

from 2007-2012.[8] Ultra-processed foods include but are not limited to candies, many cereals, cookies, mass-produced bread, frozen ready-to-eat meals, ice cream, poultry "nuggets" and "fingers" and sugar-sweetened beverages. However, the convenience comes at a nutritional cost. Ultra-processed foods have a poor nutritional profile and can displace raw and minimally processed foods.[9-12] Recent findings indicate suboptimal diet as a leading cause of death globally, with high intake of sodium and low intake of whole grains and fruits as prominent dietary risk factors for mortality and morbidity.[13]

The rise of convenience food mirrors the rise in obesity prevalence in youth and adults.[14,15] Since the first National Health Examination Survey conducted in 1959-1962, the number of adults with obesity has risen each year.[16] In 2017-2018, the prevalence of obesity was 42.4% and the prevalence of severe obesity was 9.2%.[17] The presence of obesity increases the risk of developing comorbidities such as diabetes, cardiovascular disease (CVD), chronic kidney disease, hypertension, high cholesterol, asthma, sleep apnea, and arthritism[18-21] and negatively affects life quality and expectancy.[22,23] The global rise of obesity and comorbidities present an increasing personal, healthcare, and financial burden and warrants substantial scrutinization of the role of diet as a risk factor in obesity and chronic diseases.[24]

The Development of Obesity

Weight gain occurs when caloric intake exceeds energy expenditure, creating a state of positive energy balance over time. Increased energy intake and lower physical activity levels concomitant with increased sedentary activity contribute to positive energy

balance and promote adipose deposition.[25] However, the pathogenesis of obesity is complex. Obesity development is influenced by a multitude of genetic and environmental factors which interact to collectively influence and regulate energy balance.[26]

It is well-established that increased ultra-processed food intake is associated with weight gain and obesity.[27,28] In addition to their poor nutritional profile,[9-12] ultra-processed foods initiate lower feelings of fullness and raise glycemic responses.[29] High glycemic foods undergo rapid breakdown and are absorbed into the blood, elevating blood glucose and insulin responses. Insulin is released from the β-cells of the pancreas in an effort to maintain glucose homeostasis, but it also a regulator of fat oxidation with high insulin promoting lipogenesis and low insulin promoting lipolysis. Consumption of high glycemic foods and the subsequent rise in nonfasting insulin reduce fat oxidation and promote fatty acid storage but over time, regular consumption works to promote weight gain and glucose intolerance.[30] A causative relationship between diet and weight gain was established in a recent randomized controlled trial which found that a provided ultra-processed food diet matched to an unprocessed diet matched for calories, sugar, fat, and fiber increased daily *ad libitum* energy intake and led to weight gain.[31] This novel study makes the case that food processing and its digestibility are drivers of weight gain.

Overnutrition plays a key role in obesity development through promotion of immunometabolic stress leading to ectopic lipid accumulation[32] and adipose tissue dysfunction.[33] Inside the cell, the endoplasmic reticulum constitutes a large network of interconnected sacs and is a major site of lipid synthesis and packaging which can become stressed in periods of excess nutrients and lipid exposure.[34,35] Unresolved endoplasmic

reticulum stress can result in detrimental changes in membrane composition in response to high fatty acid and cholesterol exposure which interferes with the ability of the endoplasmic reticulum to negate lipid accumulation in the cell.[36] Chronic endoplasmic reticulum stress can reduce insulin receptor signaling and progress peripheral insulin resistance.[34] Excess nutrients also promote lipogenesis and deposition of lipids in a healthy or unhealthy expansion pattern.[37] Unhealthy adipose expansion contributes to macrophage infiltration, low-grade inflammation, insulin resistance, and further deposition of lipids from subcutaneous adipose to ectopic.[37] The overproduction of proinflammatory cytokines by expanded unhealthy adipose and further activation of inflammatory pathways by excess fatty acids and glucose helps mediate and perpetuate insulin resistance which can lead to metabolic dysfunction.[38-41]

The Gut Microbiota at the Intersection of Diet and Health

Technological advancements have not only allowed for a radical change in food systems but have also allowed for deep exploration into the vast microbial communities of the human body. The microbiome is a collection of genomes from a microbiota that "indicates the total genetic capacity of the community."[42] Recent estimates have the number of bacterial cells in the human body on the same order of human cells,[43] with the diversity of microbes dependent on body habitat[44] and showing high inter-individual variation.[45]

The human gastrointestinal (GI) tract is approximately 9 meters long and contains within it a diverse microorganism community - including bacteria, archaea, viruses, fungi,

and protozoa. Over 1000 bacterial species were found in fecal samples of a European cohort, with individuals harboring at least 160 bacterial species.[46] The adult gut microbial community is thought to be stable over time, though some taxa experience temporal stability while others fluctuate suggesting GI tract has resident and transient taxa.[45,47,48] The host GI tract provides a relatively stable environment and constant influx of dietary components for commensal bacteria metabolism. In a mutualistic exchange, bacteria produce metabolites, supply vitamins, protect the host from the colonization of pathogenic bacteria, and can also influence the development and physiology of the intestinal epithelial layer and related lymphoid tissues.[49-52] The gut microbiota can be influenced by a variety of intrinsic and extrinsic host factors including delivery method at birth, geography, host genetics, diet, medication including antibiotics, probiotics, age, and stress[53,54] with roughly 20% of the inter-individual microbial taxonomic variability of unrelated individuals explained by environmental variables.[55]

Diet is a major environmental variable which influences the gut microbiota and through its metabolite production, influences the host as well.[56-59] Distinct patterns of microbial communities, or enterotypes, have been identified in healthy individuals and considered to be influenced by long-term dietary habits.[52,60] Modern dietary changes, such as the Western Diet, have been widely examined for their impacts on gut health and relationship to host health. Diets low in microbiota-accessible carbohydrates contribute to the loss of microbial diversity in the GI tract and may lead to microbial extinctions that can compound over generations.[61] Additionally, low-fiber diets contribute to gut barrier dysfunction by alteration in activity of mucus-degrading bacteria and reduced production

of butyrate.[62,63] Elevated animal-based protein consumption may also increase intestinal permeability[64] and elevate production of potentially harmful metabolites such as trimethylamine-*N*-oxide.[65,66] Similarly, high-fat diets alter gut microbial structure,[67] promote gut barrier dysfunction through increased inflammation and permeability,[68-70] and increase absorption and translocation of microbially-produced endotoxins which are potent activators of proinflammatory pathways and are associated with insulin resistance.[71,72] These findings suggest that Western Diet food patterns collectively have detrimental effects on the gut microbiota and may exacerbate insulin resistance through increased proinflammatory activation by gut-derived metabolites.

Much like diet, the gut microbial community has been exposed to new environmental pressures and has been examined for its potential role in obesity and chronic disease development. Obesity and related co-morbidities are linked to alterations in the composition and functional capacity of the gut microbial community.[73-77] Obesity-linked alterations include an Firmicutes to Bacteroidetes ratio,[75,76,78] low bacterial dviersity,[76] and an increased ability to harvest energy from dietary milleau.[75] In contrast, a healthy gut microbiota is generally considered to have high α-diversity, which promotes community resistance to stressors and its ability to return to equilibrium after the stress has ended.[79] Strategies to improve aspects of host metabolism have begun to assess the contributions of the gut microbiota. The seminal study by Zeevi and colleagues demonstrates that the gut microbiome data can accurately predict postprandial glycemic responses, with specific bacterial taxa changing in response to personally tailored diets and associating with

glycemic measures.[80] These findings suggest that the functional pathways of gut bacteria are linked to host metabolism.

Dissertation Aims

Technological, nutritional, and medical advancements have allowed us to eat better and live longer lives. However, these advancements also helped to promote the development and accrual of chronic health conditions over time. Key problems of undernutrition have shifted over the last century to problems of overnutrition enforced by societal and economic systems. Overnutrition has affected our metabolic systems and altered our gut microbial community in a manner placing society at greater risk for development of metabolic diseases (**Figure 1**).

In this unprecedented metabolic world, the treatment of obesity and related chronic disease calls for action on multiple levels. Research on chronic disease prevention strategies which inform and aid behavior change are one necessary action, and research specifically on the early risk factors of metabolic dysregulation are an essential part of the prevention process. Diet has played a role in accelerating the obesity epidemic, but it can also be part of the solution as a modifiable risk factor. Diet, exercise, and behavior modifications can be effective treatments and prevention methods to combat the rise of metabolic syndrome and obesity-related health conditions.[81-83] Dietary approaches which reduce lipid, glycemic, insulin, and inflammatory postprandial responses are methods to target and address early steps in metabolic dysregulation. Food-based challenges such as the oral glucose tolerance test and oral fat tolerance test allow researchers insights into

postprandial responses and thus, the degree of metabolic flexibility. However, high interindividual variability in response to dietary intake and a lack of research in metabolically at-risk individuals can make it difficult to generate recommendations. The reasons behind this variability to foods are poorly understood but may relate in part to host-microbiota interactions. To assess these hypotheses, we sought to 1) better understand the key determinants of the postprandial lipid response to an oral fat tolerance test in metabolically at-risk adults, 2) evaluate whether long-term incorporation of plant-based low-glycemic foods improves glycemic and insulin responses in adults with in adults with increased central adiposity, and 3) evaluate how an antioxidant-rich juice may protect host health from a high-fat diet and its dependency on the gut microbiome by controlling for interindividual variability using a gnotobiotic mouse model.

Chapter 2 is an investigation into the determinants of postprandial lipid metabolism. Dyslipidemia is a characteristic of impaired metabolic health and occurs early in a impaired metabolic cascade. We recruited healthy but metabolically at-risk adults and examined their postprandial lipid response to an oral fat load and sought to understand factors associated with elevated postprandial response. In **Chapter 3**, we assessed the impact of a long-term dietary intervention with fiber-rich foods on glycemic control. Hyperglycemia is another characteristic of impaired metabolic health and often occurs in conjunction with dyslipidemia. Fiber-rich foods have the potential to improve glycemic control but are often not consumed due to perception of increased GI symptoms. We recruited 30 adults with an increased waist circumference, tested glycemic measures before and after a dietary intervention with whole green lentils, and throughout the intervention,

tracked participant perception of GI symptom severity. In **Chapter 4**, we transferred the stool from two human subjects included in the experiment discussed in Chapter 2, who had varying levels of systemic inflammation, into germ-free mice for the creation of a gnotobiotic mouse model. We used the gnotobiotic mouse experiment to control for inter-individual variability and test the impact of an antioxidant-rich juice from the *Aronia melanocarpa* berry during a high-fat diet akin to the modern-day Western Diet. In **Chapter 5**, observations and implications from these collective experiments for clinical and research settings will be reviewed.

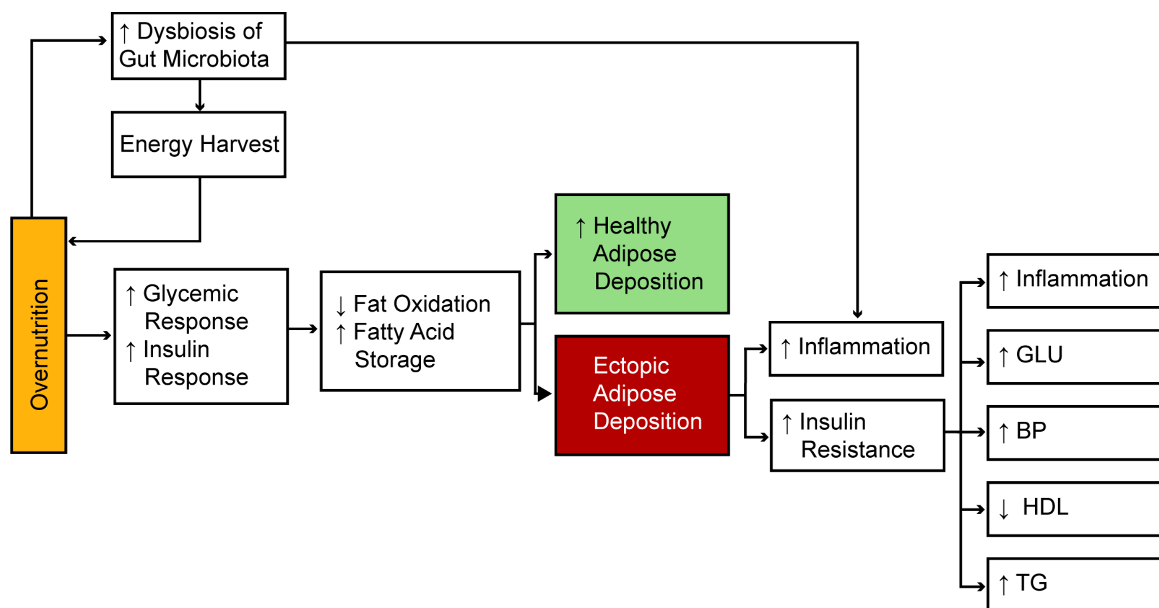

Figure 1 | Schematic displaying the contribution of diet toward the development of metabolic syndrome.

References Cited

1. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017-2018. *NCHS Data Brief.* 2020(360):1-8.

2. Huang PL. A comprehensive definition for metabolic syndrome. *Dis Model Mech.* 2009;2(5-6):231-237.

3. Grundy SM. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. *J Am Coll Cardiol.* 2006;47(6):1093-1100.

4. Huebbe P, Rimbach G. Historical Reflection of Food Processing and the Role of Legumes as Part of a Healthy Balanced Diet. *Foods.* 2020;9(8).

5. Welch RW, Mitchell PC. Food processing: a century of change. *British Medical Bulletin.* 2000;56(1):1-17.

6. Craig LA, Goodwin B, Grennes T. The Effect of Mechanical Refrigeration on Nutrition in the United States. *Social Science History.* 2004;28(2):325-336.

7. Stuckler D, McKee M, Ebrahim S, Basu S. Manufacturing Epidemics: The Role of Global Producers in Increased Consumption of Unhealthy Commodities Including Processed Foods, Alcohol, and Tobacco. *PLOS Medicine.* 2012;9(6):e1001235.

8. Baraldi LG, Martinez Steele E, Canella DS, Monteiro CA. Consumption of ultra-processed foods and associated sociodemographic factors in the USA between 2007 and 2012: evidence from a nationally representative cross-sectional study. *BMJ Open.* 2018;8(3):e020574.

9. Martínez Steele E, Baraldi LG, Louzada MLdC, Moubarac J-C, Mozaffarian D, Monteiro CA. Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. *BMJ Open.* 2016;6(3):e009892.

10. Rauber F, da Costa Louzada ML, Steele EM, Millett C, Monteiro CA, Levy RB. Ultra-Processed Food Consumption and Chronic Non-Communicable Diseases-Related Dietary Nutrient Profile in the UK (2008⁻2014). *Nutrients.* 2018;10(5).

11. Monteiro CA, Cannon G, Levy RB, et al. Ultra-processed foods: what they are and how to identify them. *Public health nutrition.* 2019;22(5):936-941.

12. Elizabeth L, Machado P, Zinöcker M, Baker P, Lawrence M. Ultra-Processed Foods and Health Outcomes: A Narrative Review. *Nutrients.* 2020;12(7).

13. Afshin A, Sur PJ, Fay KA, et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet.* 2019;393(10184):1958-1972.

14. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *Jama.* 2016;315(21):2284-2291.

15. Finucane MM, Stevens GA, Cowan MJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet.* 2011;377(9765):557-567.

16. Harlan WR, Landis JR, Flegal KM, Davis CS, Miller ME. Secular Trends in Body Mass Index in the United States, 1960–1980. *American Journal of Epidemiology.* 1988;128(5):1065-1074.

17. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. [press release]. National Center for Health Statistics: NCHS Data Brief2020.

18. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA-J Am Med Assoc.* 2003;289(1):76-79.

19. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. *JAMA-J Am Med Assoc.* 2014;311(8):806-814.

20. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA-J Am Med Assoc.* 1999;282(16):1523-1529.

21. MacLaughlin HL, Hall WL, Sanders TA, Macdougall IC. Risk for chronic kidney disease increases with obesity: Health Survey for England 2010. *Public health nutrition.* 2015;18(18):3349-3354.

22. Jia H, Lubetkin EI. The impact of obesity on health-related quality-of-life in the general adult US population. *J Public Health (Oxf).* 2005;27(2):156-164.

23. Fontaine KR, Redden DT, Wang CX, Westfall AO, Allison DB. Years of life lost due to obesity. *JAMA-J Am Med Assoc.* 2003;289(2):187-193.

24. Wang YF, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity.* 2008;16(10):2323-2330.

25. Swinburn B, Sacks G, Ravussin E. Increased food energy supply is more than sufficient to explain the US epidemic of obesity. *American Journal of Clinical Nutrition*. 2009;90(6):1453-1456.

26. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. *New England Journal of Medicine*. 2017;376(3):254-266.

27. Machado PP, Steele EM, Levy RB, et al. Ultra-processed food consumption and obesity in the Australian adult population. *Nutrition & Diabetes*. 2020;10(1):39.

28. Cordova R, Kliemann N, Huybrechts I, et al. Consumption of ultra-processed foods associated with weight gain and obesity in adults: A multi-national cohort study. *Clinical Nutrition*. 2021;40(9):5079-5088.

29. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High Glycemic Index Foods, Overeating, and Obesity. *Pediatrics*. 1999;103(3):e26-e26.

30. Dalby MJ, Ross AW, Walker AW, Morgan PJ. Dietary Uncoupling of Gut Microbiota and Energy Harvesting from Obesity and Glucose Tolerance in Mice. *Cell reports*. 2017;21(6):1521-1533.

31. Hall KD, Ayuketah A, Brychta R, et al. Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell metabolism*. 2019;30(1):67-77.e63.

32. Grundy SM. Overnutrition, ectopic lipid and the metabolic syndrome. *Journal of Investigative Medicine*. 2016;64(6):1082.

33. Wiklund P, Zhang X, Pekkala S, et al. Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Scientific Reports*. 2016;6(1):24540.

34. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science*. 2004;306(5695):457-461.

35. Fu S, Yang L, Li P, et al. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature*. 2011;473(7348):528-531.

36. Lemmer IL, Willemsen N, Hilal N, Bartelt A. A guide to understanding endoplasmic reticulum stress in metabolic disorders. *Mol Metab*. 2021;47:101169-101169.

37. Longo M, Zatterale F, Naderi J, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *Int J Mol Sci*. 2019;20(9):2358.

38. Duncan BB, Schmidt MI, Pankow JS, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes.* 2003;52(7):1799-1805.

39. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation.* 2000;102(1):42-47.

40. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor-necrosis-factor-alpha - direct role in obesity linked insulin resistance. *Science.* 1993;259(5091):87-91.

41. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature.* 1997;389(6651):610-614.

42. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature.* 2012;489(7415):242-249.

43. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *Plos Biology.* 2016;14(8).

44. Consortium THMP. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486(7402):207-214.

45. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial Community Variation in Human Body Habitats Across Space and Time. *Science (New York, NY).* 2009;326(5960):1694-1697.

46. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010;464(7285):59-65.

47. Caporaso JG, Lauber CL, Costello EK, et al. Moving pictures of the human microbiome. *Genome Biol.* 2011;12(5).

48. Trosvik P, de Muinck EJ, Stenseth NC. Biotic interactions and temporal dynamics of the human gastrointestinal microbiota. *Isme Journal.* 2015;9(3):533-541.

49. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology.* 2007;19(2):59-69.

50. Magnusdottir S, Ravcheev D, de Crecy-Lagard V, Thiele I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Frontiers in Genetics.* 2015;6.

51. Sommer F, Backhed F. The gut microbiota - masters of host development and physiology. *Nature Reviews Microbiology.* 2013;11(4):227-238.

52. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. *Nature.* 2011;473(7346):174-180.

53. Backhed F, Fraser CM, Ringel Y, et al. Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications. *Cell Host & Microbe.* 2012;12(5):611-622.

54. Schmidt TSB, Raes J, Bork P. The Human Gut Microbiome: From Association to Modulation. *Cell.* 2018;172(6):1198-1215.

55. Rothschild D, Weissbrod O, Barkan E, et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature.* 2018;555(7695):210-+.

56. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334.

57. Carmody RN, Bisanz JE, Bowen BP, et al. Cooking shapes the structure and function of the gut microbiome. *Nat Microbiol.* 2019;4(12):2052-2063.

58. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-+.

59. Albenberg LG, Wu GD. Diet and the Intestinal Microbiome: Associations, Functions, and Implications for Health and Disease. *Gastroenterology.* 2014;146(6):1564-1572.

60. Wu GD, Chen J, Hoffmann C, et al. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science.* 2011;334(6052):105-108.

61. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature.* 2016;529(7585):212-215.

62. Desai MS, Seekatz AM, Koropatkin NM, et al. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell.* 2016;167(5):1339-1353.e1321.

63. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host & Microbe.* 2018;23(6):705-715.

64. Snelson M, Clarke RE, Nguyen TV, et al. Long Term High Protein Diet Feeding Alters the Microbiome and Increases Intestinal Permeability, Systemic

Inflammation and Kidney Injury in Mice. *Mol Nutr Food Res.* 2021;65(8):e2000851.

65. Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res.* 2012;56(1):184-196.

66. Tang WHW, Bäckhed F, Landmesser U, Hazen SL. Intestinal Microbiota in Cardiovascular Health and Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol.* 2019;73(16):2089-2105.

67. Bisanz JE, Upadhyay V, Turnbaugh JA, Ly K, Turnbaugh PJ. Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. *Cell Host & Microbe.* 2019;26(2):265-272.e264.

68. Gulhane M, Murray L, Lourie R, et al. High Fat Diets Induce Colonic Epithelial Cell Stress and Inflammation that is Reversed by IL-22. *Scientific Reports.* 2016;6:17.

69. Cani PD, Bibiloni R, Knauf C, et al. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. *Diabetes.* 2008;57(6):1470-1481.

70. Rohr MW, Narasimhulu CA, Rudeski-Rohr TA, Parthasarathy S. Negative Effects of a High-Fat Diet on Intestinal Permeability: A Review. *Advances in Nutrition.* 2020;11(1):77-91.

71. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *Journal of Lipid Research.* 2009;50(1):90-97.

72. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* 2007;56(7):1761-1772.

73. Brahe LK, Le Chatelier E, Prifti E, et al. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutrition & Diabetes.* 2015;5:7.

74. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature.* 2012;490.

75. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444.

76. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(31):11070-11075.

77. Li J, Zhao FQ, Wang YD, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome.* 2017;5:19.

78. Verdam FJ, Fuentes S, de Jonge C, et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity.* 2013;21(12):E607-E615.

79. Virgin Herbert W, Todd John A. Metagenomics and Personalized Medicine. *Cell.* 2011;147(1):44-56.

80. Zeevi D, Korem T, Zmora N, et al. Personalized Nutrition by Prediction of Glycemic Responses. *Cell.* 2015;163(5):1079-1094.

81. Jakicic JM, Otto AD. Physical activity considerations for the treatment and prevention of obesity. *American Journal of Clinical Nutrition.* 2005;82(1):226S-229S.

82. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine.* 2002;346(6):393-403.

83. Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: the evidence. *Canadian Medical Association Journal.* 2006;174(6):801-809.

CHAPTER TWO

DETERMINANTS OF THE POSTPRANDIAL TRIGLYCERIDE RESPONSE TO A HIGH-FAT MEAL IN HEALTHY OVERWEIGHT AND OBESE ADULTS

Contribution of Authors and Co-Authors

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Determinants of the postprandial triglyceride response to a high-fat meal in healthy overweight and obese adults

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Abstract

Background: Dyslipidemia is a feature of impaired metabolic health in conjunction with impaired glucose metabolism and central obesity. However, the contribution of factors to postprandial lipemia in healthy but metabolically at-risk adults is not well understood. We investigated the collective contribution of several physiologic and lifestyle factors to postprandial triglyceride (TG) response to a high-fat meal in healthy, overweight and obese adults.

Methods: Overweight and obese adults (n = 35) underwent a high-fat meal challenge with blood sampled at fasting and hourly in the 4-hour postprandial period after a breakfast containing 50 g fat. Incremental area under the curve (iAUC) and postprandial magnitude for TG were calculated and data analyzed using a linear model with physiologic and lifestyle characteristics as explanatory variables. Model reduction was used to assess which explanatory variables contributed most to the postprandial TG response.

Results: TG responses to a high-fat meal were variable between individuals, with approximately 57 % of participants exceeded the nonfasting threshold for hypertriglyceridemia. Visceral adiposity was the strongest predictor of TG iAUC (β = 0.53, $p = 0.01$), followed by aerobic exercise frequency (β = 0.31, $p = 0.05$), insulin resistance based on HOMA-IR (β = 0.30, $p = 0.04$), and relative exercise intensity at which substrate utilization crossover occurred (β = 0.05, $p = 0.04$). For postprandial TG magnitude, visceral adiposity was a strong predictor (β = 0.43, $p < 0.001$) followed by aerobic exercise frequency (β = 0.23, $p = 0.01$), and exercise intensity for substrate utilization crossover (β = 0.53, $p = 0.01$).

Conclusions: Postprandial TG responses to a high-fat meal was partially explained by several physiologic and lifestyle characteristics, including visceral adiposity, insulin resistance, aerobic exercise frequency, and relative substrate utilization crossover during exercise.

Trial Registration: ClinicalTrials.gov, NCT04128839, Registered 16 October 2019 – Retrospectively registered.

Keywords: Postprandial lipemia, High-fat meal, Obesity, metabolic syndrome

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Introduction

Obesity is a well-established risk factor for chronic disease [1] with increased adiposity, especially central obesity, present early in the impaired metabolic cascade. Increased fat deposition promotes insulin resistance, dyslipidemia [2–4], and the development of chronic conditions such as diabetes, non-alcoholic fatty liver disease, and coronary artery disease [5]. Lipids and lipoproteins play an essential role in the body, but elevated postprandial lipids are atherogenic [6]. The contribution of postprandial hyperlipidemia to atherosclerosis development is likely through a co-occurrence of endothelial dysfunction, oxidative stress, and inflammation promoted from a single feeding [7]. Epidemiological studies used fasting clinical lipid profiles to predict chronic disease prior to 2009 [8]. As individuals spend the majority of waking hours in a postprandial state, fasting lipid profiles are not reflective of the dynamic nature of lipid metabolism. Evidence now suggests that the postprandial concentration is more predictive of cardiovascular events and disease risk than fasting levels [9–12]. Postprandial triglyceride (TG) in particular has been shown to predict cardiovascular disease, myocardial infarction, ischemic heart disease, and death [13, 14].

Postprandial TG responses to a meal enriched in dietary fat show considerable interindividual variability [15] with high fat meals shown to increase circulating TG at least 50 % from fasting concentrations [4]. Postprandial hypertriglyceridemia is considered present if individuals have TG concentrations greater than 175 mg/dL or 1.98 mmol/L [16]. Prominent epidemiological studies after 2009 measured blood lipids at a single postprandial timepoint which, while more feasible for large cohorts, oversimplifies the postprandial response [11, 14] and lacks the enhanced disease prediction capacity [8]. Summary measures such as total area under the curve and incremental area under the curve (iAUC) are effective summary tools for sampling in the postprandial period. Specific to the TG response after a meal, iAUC better represents TG responses while total AUC more strongly associates with fasting TG concentrations [17]. Furthermore, iAUC serves to normalize baseline interindividual variability which allows for improved assessment of the postprandial TG-rich lipoproteins [17, 18].

Postprandial TG responses are dependent on the amount of dietary fat absorbed and packaged as chylomicrons by the intestine, hepatic clearance triacylglycerol-rich lipoproteins (TRLs), and hepatic production of very low-density lipoprotein (VLDL) [19]. These three aspects of postprandial lipid metabolism are influenced by physiologic and lifestyle factors such as age, central obesity, smoking, alcohol consumption, blood pressure, diet, gender, insulin resistance, and physical activity [3, 19–21]. Central obesity, insulin resistance, and age have been observed to increase the risk of postprandial lipemia while physical activity is one of the few factors observed to attenuate postprandial TG responses [20, 22]. Bouts of physical activity acutely increase lipoprotein lipase (LPL) activity allowing for the peripheral uptake of fatty acids for fat oxidation [23]. These factors and postprandial lipemia are strongly correlated, with regular physical activity leading to a higher oxidation of dietary fat in the postprandial period than with sedentary behavior [22, 24]. The collective impact of multiple physiologic and lifestyle factors in relation to postprandial TG responses can be difficult to ascertain across study methodologies and populations and warrants further investigation.

A better understanding of postprandial TG determinants may increase disease prediction capabilities and allow for more targeted clinical strategies to improve lipid profiles and lower downstream disease risk. In this study, we investigated the postprandial lipemic response to a single high-fat meal in healthy, nondiabetic overweight and obese adults and assessed several factors known to influence the postprandial TG response [20]. We hypothesized that examination of these factors in such a metabolically at-risk cohort would identify early processes of metabolic dysregulation involved in postprandial lipemia.

Methods

Ethics Statement

The protocol was approved by the Institutional Review Board at Montana State University and followed the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their participation. The original study was retrospectively registered October 2019 at ClinicalTrials.gov (NCT04128839).

Study Population

Potential participants were recruited via advertisement between March 2016 to June 2018 for a study which assessed the inflammation-lowering impact of Aronia berries in a humanized mouse model. Our specific analysis focuses on the human cohort recruited for this study and thus, a secondary endpoint of the original study. Inclusion criteria included being between 18 and 55 years old and having a BMI between 27 and 36 kg/m^2. Criteria for exclusion included antibiotics within 90 days of study enrollment, regular use of anti-inflammatory medications, use of estrogen-only contraceptives, wheat and/or dairy allergies or intolerances, were pregnant, or had any musculoskeletal, cardiovascular, gastrointestinal, or immunological condition that could interfere with the study. All potentially eligible

participants were screened over the phone for inclusion and exclusion criteria.

Research Design

The study followed a cross-sectional design. Participants were asked to attend two visits in the Nutrition Research Laboratory at Montana State University. The initial visit involved questionnaires and analysis of body composition and cardiorespiratory fitness. The second visit occurred within two weeks after the first visit and involved blood collection before and after a high-fat meal challenge. Fasting and postprandial lipids, glucose, and insulin were measured for four hours postprandially. Blood pressure, visceral adipose tissue, physical activity frequency, and substrate utilization crossover during a submaximal exercise test were measured.

Physical Activity Frequency

Participants were asked to complete a written 3-question questionnaire on their physical activity in the past week. Questions were taken from the FITNESSGRAM Test Administration Manual and in brief, asked "On how many of the past 7 days did you -" perform 30–60 min of aerobic exercise, strengthening activities, and stretching exercise with written examples of each provided for reference [25].

Anthropometrics

Measurements were collected from participants using the validated segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) [26]. Participants were instructed to refrain from eating, drinking, or exercising in the three hours prior to testing. Fat mass percentage and estimated visceral adipose in liters were used for analysis.

Blood Pressure

Systolic and diastolic blood pressure measurements were performed on seated participants after 5–10 min of rest. Two automated measurements were taken with the average of the measurements used for analysis.

Cardiorespiratory Fitness

Participants were asked to complete a modified Bruce protocol on a treadmill for determination of calculated absolute oxygen consumption (VO_2) max at their age-predicted heart rate max. Speed and grade of the treadmill (Woodway GmbH D-79,576, Weil am Rhein, Denmark) were manually changed by the researcher with each progressive three-minute stage until the participant reached 85 % of their age-predicted maximal heart rate. Expired gases were collected and averaged every 15 s for analysis through a metabolic cart system (ParvoMedics, TrueMax 2400 Metabolic System, Sandy, Utah, USA). Heart rate (bpm) and VO_2 (ml/kg/min) data from each participant were input into a simple linear regression model to predict the absolute VO_2 at the age-predicted maximal heart rate based on the equation presented by Tanaka, Monahan, and Seals [27].

Exercise requires metabolically flexibility, the ability to switch between glucose and fat use in response to metabolic demand [28]. Through standard stoichiometric equations and indirect calorimetry methods like the metabolic cart system, utilization of fats and lipids are able to be quantified as kcal during the exercise protocol. The switch or "crossover" point of substrate utilization during exercise reflects the point at which kilocalories per minute of carbohydrate expended exceeded that from fat. The crossover point was determined as the point at which carbohydrate and fat expenditure most rapidly and proportionately differentiated from the other [29]. More information about how the crossover percentage was calculated is available in Additional File 1. Using the VO_2 at the crossover point, we derived the crossover percentage of the calculated absolute VO_2 max.

High-Fat Meal Challenge

The high-fat meal challenge was performed after an overnight fast (10–12 h) during the morning hours. The meal consisted of three pieces of toasted whole wheat bread (Wheat Montana) and approximately 58.3 g of salted butter (Tillamook). 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 early grey black tea (Bigelow) 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.

Blood Sampling

Participants were instructed to avoid alcohol consumption and strenuous physical activity in the 24 h before blood collection and to complete an overnight fast (10–12 h) before blood collection. Venous blood samples were collected through a cannula inserted into the antecubital vein after a 3-mL waste withdrawal, then followed by a sterile saline flush performed by a physician or nurse on the research team. The fasting sample was drawn 30 min after catheter insertion. After meal ingestion, blood was drawn every hour for four hours in the postprandial period, totaling five time points including fasting. This frequency of blood sampling has been

previously shown in healthy populations to accurately describe postprandial lipemia to a high fat meal and are also predictive of 8-hour responses [30, 31]. Blood was collected into 8.5 mL endotoxin-free serum separating and 4.0 mL heparinized vacutainer tubes (BD Vacutainer, Franklin Lakes, New Jersey, USA). The serum tube was allowed to clot for 15 min at room temperature before centrifugation (3000 rpm, 15 min). Serum aliquots were frozen at -80ºC until analysis.

Biochemical Analyses

Blood triglycerides, glucose, and high-density lipoprotein were determined using the Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, USA). Insulin was determined through ELISA (MP Biomedicals, USA) performed according to manufacturer instructions, with the average used for analysis. Mean inter-assay coefficient of variation for samples run in duplicate was 13.3 %.

Insulin Resistance

Fasting blood glucose and insulin were used to determine the homeostatic model of insulin resistance (HOMA-IR) in the original HOMA-IR formula [32]:

$$\frac{Glucose\left(\frac{mmol}{L}\right) * Insulin\left(\frac{mU}{L}\right)}{22.5}$$

Postprandial Lipemic Response

The postprandial lipemic response to the high-fat meal was summarized as iAUC, a calculation method that accurately represents the postprandial TG response to a high-fat meal [17]. The magnitude of the postprandial lipemic response was also calculated by subtracting the fasting TG value from the maximum TG value during the 4-hr postprandial period after the high-fat meal.

Statistical Analysis

Analysis was conducted in RStudio (1.3.1073) running R 4.0.2 [33], and data was visualized using *ggplot2* [34].

To assess which variables most influence TG iAUC and the TG magnitude, initial saturated multivariate linear regression models were created with the following predictor variables: age, sex, relative exercise intensity of substrate utilization crossover, visceral adipose tissue in liters, HOMA-IR, systolic and diastolic blood pressure in mmHg, and aerobic exercise frequency. Model refinement was performed by stepping down one main effect at a time from the initial model. Model reduction was determined through strength of evidence against the null hypothesis using Type III F-tests, in which every test is conditional on every variable in the model. Model refinement stopped when the majority of predictor variables reached their smallest p-value. Final linear models were screened for shared information among predictor variables using variance inflation factors from the *car* package [35], with values > 5 set as the threshold for predictor removal. Further validity conditions were confirmed through residual visualizations. Power was calculated *a posteriori* from the final TG iAUC regression model using the pwr.f2.test in the *pwr* package. Computed power was 95 % at a Type I probability of 0.05.

Results

Of the initial 216 individuals contacted, we assessed the eligibility of 54 individuals through a screening phone call. Forty-three individuals consented to participate in the study (Fig. 1). Two individuals were confirmed to be ineligible based on confirmation of BMI criteria at study onset, and one individual was unable to be reached to complete the full study. Thus, 40 overweight and obese men and women (body mass index, BMI 27–36 kg/m^2) between the ages of 18 and 55 participated in testing of cardiovascular, anthropometric, and metabolic markers and ingestion of a 50 g high-fat meal challenge. Five participants were excluded from analysis due to incomplete data (n = 2) or inability to complete the submaximal treadmill test (n = 3). On average, participants were obese according to BMI and had slightly elevated fasting triglycerides but were otherwise within normal ranges for other metabolic syndrome criteria (Table 1), according to the National Cholesterol Education Program Adult Treatment Panel III definition [36]. Approximately 74 % of our cohort did not have metabolic syndrome (Additional File 2).

Postprandial Lipemic Response to the High-fat Meal

Participants entered the high-fat meal challenge with fasting TG concentrations from normal (< 1.7 mmol/L) to moderately elevated (2.0–5.6 mmol/L) levels but displayed high interindividual variability in blood TG concentrations during the 4-hr postprandial period (Fig. 2 A). During the high-fat meal challenge, 25 participants had peak TG values above the threshold of 1.98 mmol/L (175 mg/dL) for hypertriglyceridemia diagnosis in nonfasting states [16]. The TG response of 35 participants to the high-fat meal challenge was summarized as iAUC and as postprandial magnitude change from fasting concentrations. Most participants had a small-to-modest rise in TG with a median (mean) increase of + 0.77 mmol/L (1.04 mmol/L), but two participants had a TG increase greater than 4 mmol/L (Fig. 2 B). The median (mean) TG iAUC was + 1.70 mmol/L (1.96 mmol/L) and overall, a normalization of interindividual variability was observed (Fig. 2 C).

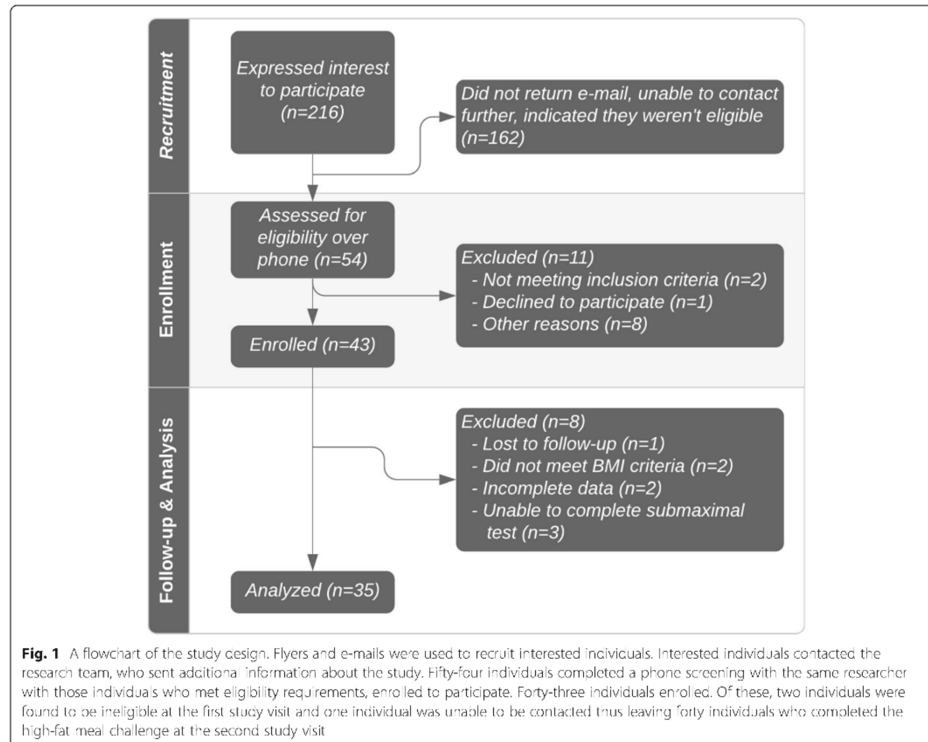

Fig. 1 A flowchart of the study design. Flyers and e-mails were used to recruit interested individuals. Interested individuals contacted the research team, who sent additional information about the study. Fifty-four individuals completed a phone screening with the same researcher with those individuals who met eligibility requirements, enrolled to participate. Forty-three individuals enrolled. Of these, two individuals were found to be ineligible at the first study visit and one individual was unable to be contacted thus leaving forty individuals who completed the high-fat meal challenge at the second study visit

To identify factors that predicted TG iAUC and TG magnitude, model refinement was performed from a saturated multivariate linear model that included factors previously shown to influence postprandial lipid metabolism (Additional File 3, Additional File 4). A summary of the final models can be found in Additional File 5, and the full estimated regression equations can be found in Additional File 6. Both models shared the same predictor variables, with the model for postprandial TG magnitude additionally including systolic blood pressure. The predictor variables explain approximately 28.6 % of the variance in TG iAUC and approximately 30.4 % in TG magnitude according to the adjusted R^2.

Visceral adiposity was the best predictor of TG iAUC followed in unit change by HOMA-IR, aerobic exercise frequency, and relative exercise intensity of substrate utilization crossover. A modest increase was seen with a later shift to carbohydrate metabolism during a submaximal exercise test (Fig. 3 A). Increased visceral adiposity (Fig. 3 B), HOMA-IR (Fig. 3 C), and aerobic exercise frequency (Fig. 3 D) were associated with greater increases in TG iAUC.

Postprandial TG magnitude was also strongly predicted by visceral adiposity. Aerobic exercise and relative exercise intensity of substrate utilization crossover were also determined predictors. As observed with TG iAUC, participants had a modest increase in postprandial TG magnitude with the relative exercise intensity of substrate utilization crossover (Fig. 3 E) and greater changes in postprandial TG magnitude with increased visceral adiposity (Fig. 3 F). Changes in HOMA-IR were associated with a slight increase in magnitude; however, little evidence as indicated by the poor strength of evidence supports this as a predictor (Fig. 3 G). Aerobic exercise frequency was additionally a modest predictor of magnitude (Fig. 3 H). Lastly, systolic blood pressure was the sole predictor not shared between the two models. While systolic blood pressure was the only negative association in the model, there was little evidence to support it as a predictor of postprandial TG magnitude (Fig. 3 I).

Table 1 Participant characteristics (n = 35)

	Mean (SD)
Men/Women	16/19
Age (years)	36.6 (10.2)
BMI (kg/m^2)	30.4 (1.8)
Fat Mass (%)	34.9 (6.6)
Waist Circumference (cm)	95.7 (9.6)
VAT (l)	2.2 (1.4)
VO_2(ml/kg/min)	44.1 (8.6)
HbA1c (%)	5.3 (0.3)
Fasting GLU (mmol/L)	5.4 (0.4)
Fasting TG (mmol/L)	1.7 (1.1)
Fasting CHOL (mmol/L)	4.7 (0.9)
Fasting HDL (mmol/L)	1.3 (0.4)
Systolic BP (mmHg)	114 (13.5)
Diastolic BP (mmHg)	76 (9.5)

Abbreviations: body mass index, *BMI*; visceral adipose tissue, *VAT*; maximal oxygen consumption, VO_2; hemoglobin *A1C*, HbA1c; glucose, *GLU*; triglycerides, *TG*; cholesterol, *CHOL*; high-density lipoprotein, *HDL*; systolic blood pressure, *SBP*; diastolic blood pressure, *DBP*.

Discussion

In the present study, we investigated the acute postprandial effect of a single high-fat meal challenge on blood triglyceride levels in healthy overweight and obese adults. Our meal with butter fat was rich in saturated fatty acids that have been shown to elicit greater postprandial TG responses than meals with mono- or polyunsaturated fatty acids [37]. By analyzing hourly over a 4-hour postprandial period, we were able to quantify the TG response in two succinct ways, (1) iAUC and (2) the magnitude of the postprandial response from relative to fasting TG concentrations. In addition, we were able to determine which biological and lifestyle factors held the greatest predicative power in TG response prediction, especially VAT, insulin resistance, aerobic exercise frequency, and relative exercise intensity of substrate utilization crossover.

On average, our overweight and obese participants had slightly elevated fasting TG but normal blood glucose, blood pressure, waist circumference and high-density lipoprotein (HDL). This finding was unsurprising as approximately 31 % of US adults have elevated fasting TGs above approximately 1.70 mmol/L (150 mg/dL) with increased fasting lipids positively correlated to BMI [4]. Despite participants overall classifying as metabolically healthy, 57 % of our participants had peak postprandial TG above the diagnostic threshold for nonfasting triglyceridemia of 1.98 mmol/L (175 mg/dL), as determined from the Women's Health Study where a single nonfasting time point was used to analyze risk of cardiovascular disease events [16]. In the analysis of the Women's Health Study, cardiovascular event risk was greater (Hazard Ratio, 2.05) with nonfasting TG above 1.98 mmol/L in the 0–4 h since the last meal. The amount of fat present in their meals was not reported, thus limiting direct comparison to the present study. Large cohort studies have found maximal TG changes after normal food intake ranging from + 0.1 to 0.3 mmol/L (+ 8.86 to 26.57 mg/dL) [8], which is notably lower than the mean 1.0 mmol/L (88.57 mg/dL) magnitude we observed in the 4-hour postprandial sampling period after the 50 g high-fat meal. As previously observed in the PREDICT I study, we also observed high interindividual variability in postprandial TG responses which supports use of postprandial response summary measures like iAUC [15].

Of the eight physiologic and lifestyle factors included in our models, visceral adiposity was the strongest predictor of TG iAUC and magnitude following the high-fat meal challenge. Central adiposity, more so than peripheral subcutaneous fat, is strongly linked to numerous metabolic abnormalities such as insulin resistance [38, 39] and chronic inflammation as well as to fasting hypertriglyceridemia [40, 41]. Our finding supports previous work with healthy adults with varying levels of body fat which found a positive correlation between the TG response and visceral adipose tissue [42]. The impact of visceral adiposity superseded the sex effect in both our statistical models, a finding also observed previously [42] and is notable in that gender differences have been found in adipose LPL activity [43]. Adipose tissue is an active endocrine organ that influences glucose and lipid metabolism through various adipokines [38, 44], with visceral adipocytes in particular exhibiting greater lipolytic activity through LPL [44]. Insufficient LPL activity has been previously suggested as an early factor in atherosclerosis [6]. Thus, lifestyle strategies to reduce visceral adipose tissue may have implications for improved postprandial TG response in addition to overall metabolic health.

While hypertriglyceridemia stemming from VAT is generally attributed, at least in part, to insulin resistance, our findings indicate that insulin resistance more closely associated with a sustained elevation in TG than the peak magnitude of the TG response. Participants with a greater degree of insulin resistance displayed a higher postprandial TG response than individuals who were more insulin sensitive, a finding previously observed in studies with healthy and obese individuals [45, 46]. Postprandially, insulin exerts an antilipolytic effect through hormone sensitive lipase, promotes triacylglycerol synthesis, and activates LPL in adipose tissue which is responsible for clearance of triacylglycerol from plasma [47]. In an insulin resistant state, free fatty acids increase as lipolysis is not suppressed and de novo lipogenesis

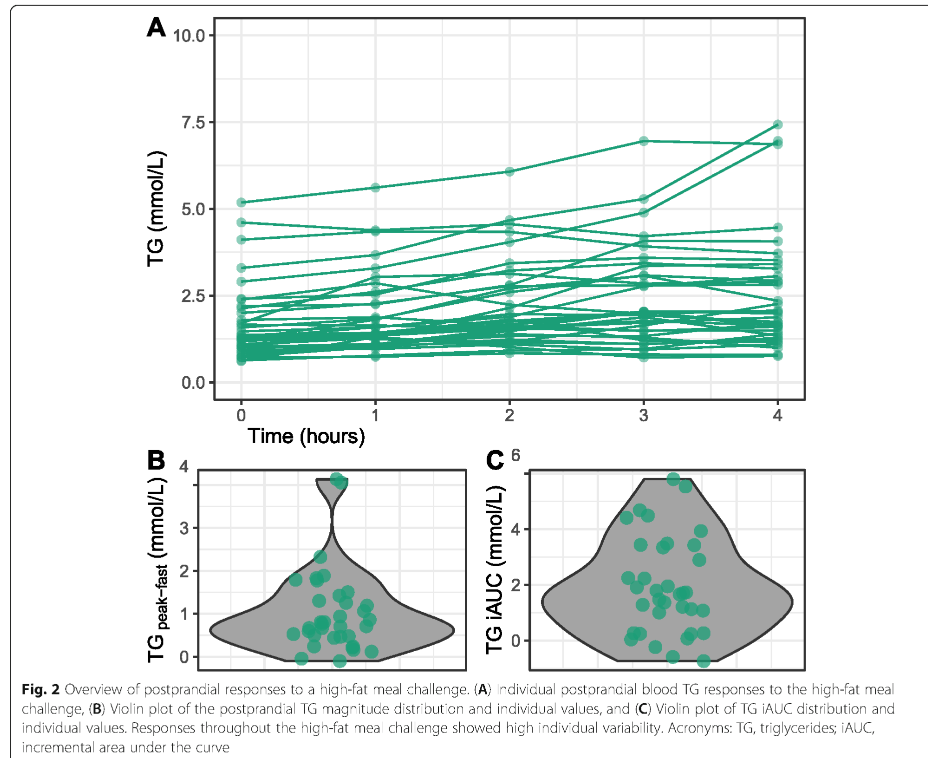

Fig. 2 Overview of postprandial responses to a high-fat meal challenge. (**A**) Individual postprandial blood TG responses to the high-fat meal challenge, (**B**) Violin plot of the postprandial TG magnitude distribution and individual values, and (**C**) Violin plot of TG iAUC distribution and individual values. Responses throughout the high-fat meal challenge showed high individual variability. Acronyms: TG, triglycerides; iAUC, incremental area under the curve

occurs from hyperglycemia which helps in part to explain the sustained elevated TG observed in our study [48]. In turn, the liver secretes more TG-rich VLDL and may exhibit fat accumulation, pushing a feedforward loop advancing insulin resistance. Insulin resistance also promotes increased chylomicron secretion from the intestine and the general postprandial accumulation of TRLs from the liver and intestine [49–51]. Improved insulin sensitivity through physical activity may not only improve glucose transport [52] but may additionally prevent the accumulation of TRLs and reduce the overall TG response in the postprandial period.

Contrary to our expectation that greater frequency of aerobic activity, higher cardiorespiratory fitness, and substrate crossover from fat to carbohydrate at a higher relative exercise intensity would be inversely related to postprandial TG magnitude and TG iAUC, we measured a positive relationship with frequency of aerobic activity and crossover intensity and no relationship with cardiorespiratory fitness. This is a notable finding as habitual physical activity has not been found to be a key determinant of postprandial TG responses [3]. Chronic exercise improves the overall fasting lipid profile through modulation of apolipoprotein A1 expression and increase in HDL by way of increased lecithin-cholesterol acyltransferase activity [53]. Acute exercise has been found to lower the magnitude of the postprandial lipemic response through decreased chylomicron appearance [23], increased oxidation of fatty acids post-exercise [54], decreased VLDL secretion from the liver, and increased LPL activity [23] though LPL activity in particular may be enhanced in men compared to women post-exercise [55]. An energy deficit post-exercise has been suggested as the primary factor in the attenuation of postprandial TG response [56]. Given the positive association of postprandial TG magnitude and TG iAUC with VAT and insulin resistance and no association with cardiorespiratory fitness, it is possible that the self-

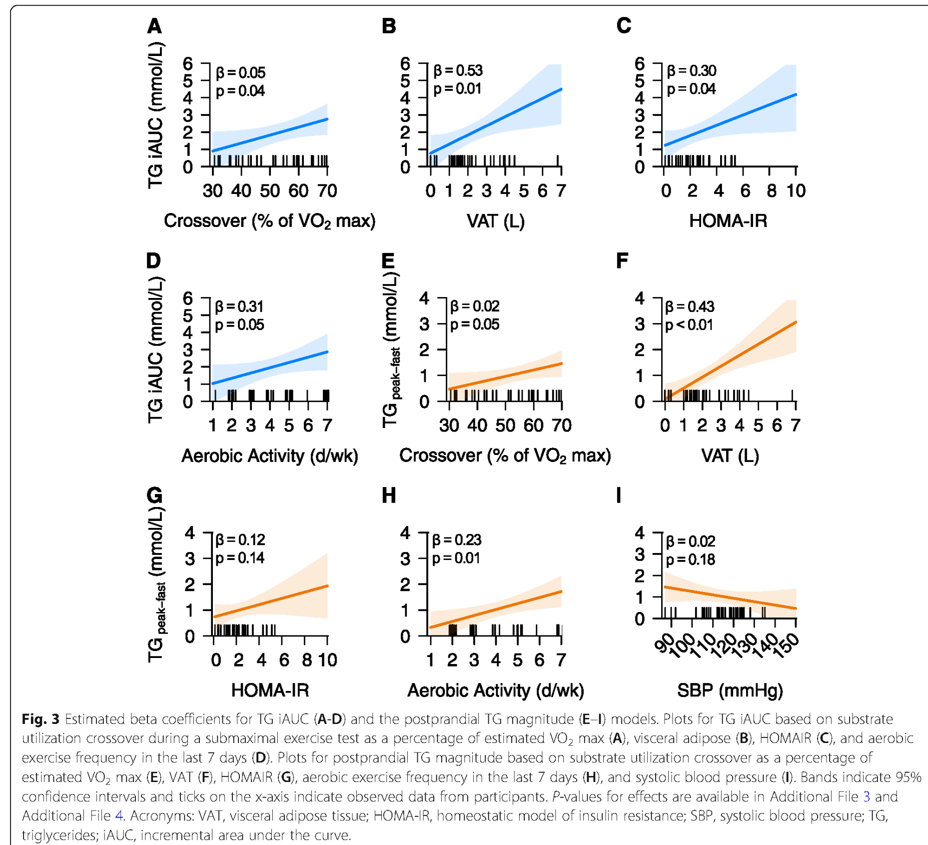

Fig. 3 Estimated beta coefficients for TG iAUC (**A-D**) and the postprandial TG magnitude (**E–I**) models. Plots for TG iAUC based on substrate utilization crossover during a submaximal exercise test as a percentage of estimated VO_2 max (**A**), visceral adipose (**B**), HOMAIR (**C**), and aerobic exercise frequency in the last 7 days (**D**). Plots for postprandial TG magnitude based on substrate utilization crossover as a percentage of estimated VO_2 max (**E**), VAT (**F**), HOMAIR (**G**), aerobic exercise frequency in the last 7 days (**H**), and systolic blood pressure (**I**). Bands indicate 95% confidence intervals and ticks on the x-axis indicate observed data from participants. *P*-values for effects are available in Additional File 3 and Additional File 4. Acronyms: VAT, visceral adipose tissue; HOMA-IR, homeostatic model of insulin resistance; SBP, systolic blood pressure; TG, triglycerides; iAUC, incremental area under the curve.

reported frequency of aerobic activity reflects perception of effort during activities that do not correspond to beneficial impacts on cardiorespiratory fitness. In light of the limited number of studies reporting the relationship between physical activity and postprandial TG, this a notable finding but one that requires further exploration.

The relationship between physical activity and postprandial TG magnitude and TG iAUC was further explored by evaluating relative exercise intensity at which substrate utilization crossover occurred and TG iAUC to the high-fat meal. The percent of VO_2 max at which the rise in carbohydrate oxidation matched fat oxidation and crossed over to begin dominating as fuel was also a predictor of both TG iAUC and postprandial TG magnitude, but one that impacted the response less than visceral adiposity and aerobic exercise frequency. After normalization to estimated VO_2 max, overweight and obese adults with a later shift to carbohydrate utilization during exercise had greater TG iAUC and postprandial TG magnitude to a high-fat meal challenge. To the authors knowledge, this is the first study to examine substrate utilization crossover during submaximal exercise in comparison to postprandial TG responses. Exercise requires an increase in energy expenditure and warrants a shift in energy usage from lipids at low-to-moderate intensity to carbohydrate at high intensity exercise [57]. The inability to effectively and rapidly move between energy systems in response to changing energy requirements and demands is considered metabolic inflexibility [58]. An earlier shift to carbohydrate oxidation during exercise has been noted in sedentary overweight versus

normal weight controls and while the exact mechanisms for reduced fat oxidation are unknown, it is generally attributed to impaired muscle substrate utilization [59]. Submaximal exercise tests can serve as a way to assess the operation status of the body's oxidative machinery, allowing us to detect fat oxidation impairments that precede insulin resistance and possibly influence lipid handling during high lipid availability.

Collectively, increased postprandial TG magnitude and TG iAUC associated positively with VAT, insulin resistance, and relative exercise frequency for substrate utilization crossover while associating negatively with reported frequency of aerobic activity may be a serendipitous finding relevant to exercise training response heterogeneity. This apparent paradox is consistent with the influence of having a high risk for diabetes stemming from familial history or history of gestational diabetes. In one study, relatives of individuals with type 2 diabetes had lower insulin sensitivity and those who did not respond to exercise training with increased insulin sensitivity also did not improve ATP production capacity and increased intrahepatic and intramuscular lipid concentrations [60, 61]. Increased uptake of fatty acids into tissues in individuals with greater VAT and insulin resistance could attenuate postprandial TG responses owing to greater removal of fatty acids from TG in postprandial chylomicrons. Increased abundance of the FAT/CD36 plasma membrane fatty acid transport system is well documented in rats with type 2 diabetes [62, 63]. The possibility of coupling postprandial TG responses with a variable such as substrate crossover point to increase sensitivity of diabetes risk prediction is exciting and worthy of further exploration.

Strengths of the present study include fasting and hourly postprandial lipid measurements in a group of healthy overweight and obese adults with varying levels of body fat percentage and cardiovascular fitness. Our statistical model predictors explain 28–30 % of the variance in the TG iAUC and TG postprandial magnitude. However, our findings are limited to this study meal as varying meal components including fiber and even dairy-based meals matched for fat content affect postprandial responses differently [19, 64]. Additionally, while participants were asked to avoid strenuous exercise and alcohol before blood collection, it is possible they exercised or consumed alcohol the day prior which alter postprandial TG responses. We also recognize factors may be in play that were not measured in the current study. While participants were asked to perform an overnight fast proceeding blood collection, we did not control the fat to carbohydrate ratio in the evening meal which may have influenced the glucose and lipid metabolism the following morning [65]. Furthermore, the inclusion of diet history questionnaires to assess habitual dietary patterns may have potentially informed which metabolic pathways in postprandial lipemia are altered [19]. As we observed a positive relationship between aerobic exercise frequency and TG iAUC, further research in high-fat meal induced postprandial lipemia may benefit by quantifying the frequency, intensity, and duration of physical activity through objective and subjective methods.

In conclusion, our findings support that several physiologic and lifestyle factors including central adipose accumulation, insulin resistance, and exercise are collectively implicated in the metabolic regulation of postprandial lipemia and can be used to predict postprandial TG responses to a high-fat meal. These factors are interrelated and subsequently, must be jointly taken into account in strategies to reduce postprandial lipemia in healthy but at-risk populations. By using appropriate postprandial TG summary measures, we found that healthy, nondiabetic overweight and obese adults with increased visceral adipose tissue and insulin resistance had, on average, greater postprandial TG iAUC to a high fat meal test. We also found that aerobic exercise frequency and the increased ability to use fat during an aerobic exercise test was positively correlated to postprandial TG responses. The present study also highlights the potential value of measuring postprandial TG responses using a standardized challenge for prediction of type 2 diabetes risk and the need to further research in this area.

Abbreviations

BMI: Body mass index; HDL: High density lipoprotein; HOMA-IR: Homeostatic model of insulin resistance; iAUC: Incremental area under the curve; LPL: Lipoprotein lipase; TG: Triglyceride; TRLs: Triacylglycerol rich lipoproteins; VLDL: Very low-density lipoprotein; VO_2: Oxygen consumption

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-021-01543-4.

Additional File 1: Determination of the percentage of VO_2 max at substrate utilization crossover point. Visual provides additional detail using real participant data on how crossover percentage was determined.

Additional File 2: Presence of metabolic syndrome criteria in analyzed study cohort (n = 35). Criteria were based of the National Cholesterol Education Program Adult Treatment Panel III definition of metabolic syndrome [32]. This metabolic syndrome definition includes criteria on central obesity, hypertension, insulin resistance, and dyslipidemia.

Additional File 3: *P*-values decisions during model refinement for the TG iAUC Response. Bolded items indicate the variable that was dropped at each decision (D) step. The final model included variables from D4, with D5 shown to confirm D4 was the final model. No further predictor variables were removed according to variance inflation factor values.

Additional File 4: *P*-values decisions during model refinement for the TG change (peak – fasting) Response. Bolded items indicate the variable that was dropped in the decision (D) step. The final model included the variables from D3, with D4 shown to confirm D3 was the final model. No further predictor variables were removed according to variance inflation factor values.

Additional File 5: Model summaries for TG iAUC response and postprandial TG magnitude. The table is a complement to Fig. 3 and provides additional information on predictor variables as well as F-statistics from statistical output.

Additional File 6: The estimated regression equations for TG iAUC response and postprandial TG magnitude after model refinement. In the equations: i is subject, crossover is the percentage of VO_2 max where carbohydrate became the dominant substrate utilized, VAT is visceral adipose tissue in liters, HOMA-IR is insulin resistance, aerobic is self-reported days with aerobic exercise in last 7 days, SBP is systolic blood pressure in millimeters of mercury, and $\varepsilon_i \sim N(0, \sigma^2_\varepsilon)$.

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Authors' contributions
The authors' responsibilities were as follows — MPM, CJY, and STW: designed the study and acquired funding; SMW, APM, and MPM: conducted the study; SMW curated the data and generated visualizations. SMW: performed statistical analyses; SMW, APM, and MPM: wrote the manuscript; and all authors: read, revised, and approved the final paper.

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Availability of data and materials
Participant data is not publicly available due to them containing information that could compromise research participant privacy, but the minimal data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Study was approved by the Montana State University Institutional Review Board (#MM021116-FC). Written informed consent was obtained from all participants before participation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Burton BT, Foster WR, Hirsch J, Van Itallie TB. Health implications of obesity: an NIH Consensus Development Conference. Int J Obes. 1985;9(3):155–70.
2. Mamo JCL, Watts GF, Barrett PHR, Smith D, James AP, Pal S. Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? American Journal of Physiology-Endocrinology Metabolism. 2001;281(3):E626-E32.
3. Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, et al. Metabolic and Lifestyle Determinants of Postprandial Lipemia Differ From Those of Fasting Triglycerides. Arteriosclerosis, Thrombosis, and Vascular Biology. 2001;21(2):275–81.
4. Miller M, Stone Neil J, Ballantyne C, Bittner V, Criqui Michael H, Ginsberg Henry N, et al. Triglycerides Cardiovascular Disease Circulation. 2011;123(20): 2292–333.
5. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med. 2017;376(3):254–66.
6. Nakajima K, Tokita Y, Tanaka A. Atherogenic postprandial remnant lipoproteins—a causal lipoproteins for the initiation of obesity and atherosclerosis. Journal of Laboratory and Precision Medicine. 2020;5.
7. Zhao Y, Liu L, Yang S, Liu G, Pan L, Gu C, et al. Mechanisms of Atherosclerosis Induced by Postprandial Lipemia. Frontiers in Cardiovascular Medicine. 2021;8(217).
8. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. Pathology. 2019;51(2):131–41.
9. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008;118(20): 2047–56.
10. Jackson KG, Poppitt SD, Minihane AM. Postprandial lipemia and cardiovascular disease risk: Interrelationships between dietary, physiological and genetic determinants. Atherosclerosis. 2012;220(1):22–33.
11. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. Jama. 2007;298(3):309–16.
12. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care. 1999;22(6): 920–4.
13. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama. 2007;298(3):299–308.
14. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118(10):993–1001.
15. Berry S, Valdes A, Davies R, Khatib HA, Delahanty L, Drew D, et al. Large Inter-individual Variation in Postprandial Lipemia Following a Mixed Meal in over 1000 Twins and Singletons from the UK and US: The PREDICT I Study (OR19-06-19). Curr Dev Nutr. 2019;3(Suppl 1):nzz046.OR19-06-19.
16. White KT, Moorthy MV, Akinkuolie AO, Demler O, Ridker PM, Cook NR, et al. Identifying an Optimal Cutpoint for the Diagnosis of Hypertriglyceridemia in the Nonfasting State. Clinical chemistry. 2015;61(9):1156–63.
17. Carstensen M, Thomsen C, Hermansen K. Incremental area under response curve more accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects. Metabolism. 2003;52(8):1034–7.
18. Lairon D, Lopez-Miranda J, Williams C. Methodology for studying postprandial lipid metabolism. Eur J Clin Nutr. 2007;61(10):1145–61.
19. Bozzetto L, Della Pepa G, Vetrani C, Rivellese AA. Dietary Impact on Postprandial Lipemia. Front Endocrinol. 2020;11(337).
20. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr. 2007; 98(3):458–73.
21. Dias CB, Moughan PJ, Wood LG, Singh H, Garg ML. Postprandial lipemia: factoring in lipemic response for ranking foods for their healthiness. Lipids Health Dis. 2017;16(1):178-.
22. Bergouignan A, Momken I, Lefai E, Antoun E, Schoeller DA, Platat C, et al. Activity energy expenditure is a major determinant of dietary fat oxidation and trafficking, but the deleterious effect of detraining is more marked than the beneficial effect of training at current recommendations. The American Journal of Clinical Nutrition. 2013;98(3):648–58.
23. Teeman CS, Kurti SP, Cull BJ, Emerson SR, Haub MD, Rosenkranz SK. Postprandial lipemic and inflammatory responses to high-fat meals: a review of the roles of acute and chronic exercise. Nutr Metab. 2016;13(1):80.
24. Wulan SN, Schrauwen-Hinderling VB, Westerterp KR, Plasqui G. Substrate utilization and metabolic profile in response to overfeeding with a high-fat diet in South Asian and white men: a sedentary lifestyle study. International Journal of Obesity. 2020;44(1):136–46.
25. Research CIfA. FITNESSGRAM Test Administration Manual. 2nd ed. Champaign: Human Kinetics; 1999.
26. Bosy-Westphal A, Schautz B, Later W, Kehayias JJ, Gallagher D, Müller MJ. What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. European Journal Of Clinical Nutrition. 2013;67:14.

27. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. J Am Coll Cardiol. 2001;37(1):153–6.
28. Goodpaster BH, Sparks LM. Metabolic Flexibility in Health and Disease. Cell Metabol. 2017;25(5):1027–36.
29. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J Appl Physiol. 1994;76(6):2253–61.
30. Maraki M, Aggelopoulou N, Christodoulou N, Katsarou C, Anapliotis P, Kavouras SA, et al. Validity of abbreviated oral fat tolerance tests for assessing postprandial lipemia. Clin Nutr. 2011;30(6):852–7.
31. Weiss EP, Fields DA, Mittendorfer B, Haverkort MA, Klein S. Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test. Metabolism. 2008;57(10):1479–85.
32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
33. Team RC. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2019.
34. Wickham H. Elegant graphics for data analysis. New York: Springer-Verlag; 2009.
35. Fox J, Weisberg S. An {R} companion to applied regression. 3rd ed. ed. Thousand Oaks: Sage; 2019.
36. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009;2(5–6):231–7.
37. Monfort-Pires M, Delgado-Lista J, Gomez-Delgado F, Lopez-Miranda J, Perez-Martinez P, Ferreira SRG. Impact of the Content of Fatty Acids of Oral Fat Tolerance Tests on Postprandial Triglyceridemia: Systematic Review and Meta-Analysis. Nutrients. 2016;8(9):580.
38. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med. 2008;14(11–12):741–51.
39. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? Curr Opin Endocrinol Diabetes Obes. 2012;19(2):81–7.
40. Cibičková Ľ, Langová K, Vaverková H, Lukeš J, Cibiček N, Karásek D. Superior Role of Waist Circumference to Body-Mass Index in the Prediction of Cardiometabolic Risk in Dyslipidemic Patients. Physiol Res. 2019;68(6):931–8.
41. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39–48.
42. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, et al. Gender Difference in Postprandial Lipemia. Arteriosclerosis, Thrombosis, and Vascular Biology. 1999;19(10):2448-55.
43. Pedersen SB, Jønler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. The Journal of Clinical Endocrinology Metabolism. 1994;78(6):1354–9.
44. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001;414(6865):799–806.
45. Jeppesen J, Hollenbeck Clarie B, Zhou MY, Coulston Ann M, Jones C, Chen YDI,. Arteriosclerosis. et al. Relation Between Insulin Resistance, Hyperinsulinemia, Postheparin Plasma Lipoprotein Lipase Activity, and Postprandial Lipemia. Thrombosis Vascular Biology. 1995;15(3):320–4.
46. Blaak EE, Hul G, Verdich C, Stich V, Martinez A, Petersen M, et al. Fat Oxidation before and after a High Fat Load in the Obese Insulin-Resistant State. The Journal of Clinical Endocrinology Metabolism. 2006;91(4):1462–9.
47. Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. J Clin Investig. 1982;69(5):1119–25.
48. Finck BN. Targeting Metabolism, Insulin Resistance, and Diabetes to Treat Nonalcoholic Steatohepatitis. Diabetes. 2018;67(12):2485.
49. Higgins V, Adeli K. Postprandial dyslipidemia in insulin resistant states in adolescent populations. J Biomed Res. 2020;34(5):328–42.
50. Harbis A, Perdreau S, Vincent-Baudry S, Charbonnier M, Bernard M-C, Raccah D, et al. Glycemic and insulinemic meal responses modulate postprandial hepatic and intestinal lipoprotein accumulation in obese, insulin-resistant subjects. The American Journal of Clinical Nutrition. 2004;80(4):896–902.
51. Annuzzi G, Giacco R, Patti L, Di Marino L, De Natale C, Costabile G, et al. Postprandial chylomicrons and adipose tissue lipoprotein lipase are altered in type 2 diabetes independently of obesity and whole-body insulin resistance. Nutr Metab Cardiovasc Dis. 2008;18(8):531–8.
52. Borghouts LB, Keizer HA. Exercise and insulin sensitivity: A review. Int J Sports Med. 2000;21(1):1–12.
53. Muscella A, Stefàno E, Marsigliante S. The effects of exercise training on lipid metabolism and coronary heart disease. Am J Physiol Heart Circ Physiol. 2020;319(1):H76–88.
54. Freese EC, Gist NH, Cureton KJ. Effect of prior exercise on postprandial lipemia: an updated quantitative review. J Appl Physiol. 2013;116.
55. Perreault L, Lavely JM, Kittelson JM, Horton TJ. Gender differences in lipoprotein lipase activity after acute exercise. Obes Res. 2004;12(2):241–9.
56. Burton FL, Malkova D, Caslake MJ, Gill JMR. Energy replacement attenuates the effects of prior moderate exercise on postprandial metabolism in overweight/obese men. Int J Obes. 2008;32.
57. Brooks GA. Importance of the 'crossover' concept in exercise metabolism. Clin Exp Pharmacol Physiol. 1997;24(11):889–95.
58. Olson KA, Schell JC, Rutter J. Pyruvate and Metabolic Flexibility: Illuminating a Path Toward Selective Cancer Therapies. Trends Biochem Sci. 2016;41(3): 219–30.
59. Pérez-Martin A, Dumortier M, Raynaud E, Brun JF, Fédou C, Bringer J, et al. Balance of substrate oxidation during submaximal exercise in lean and obese people. Diabetes Metab. 2001;27(4 Pt 1):466–74.
60. Kacerovsky-Bielesz G, Chmelik M, Ling C, Pokan R, Szendroedi J, Farukuoye M, et al. Short-Term Exercise Training Does Not Stimulate Skeletal Muscle ATP Synthesis in Relatives of Humans With Type 2 Diabetes. Diabetes. 2009; 58(6):1333.
61. Sparks LM. Exercise training response heterogeneity: physiological and molecular insights. Diabetologia. 2017;60(12):2329–36.
62. Chabowski A, Chatham JC, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, et al. Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats. Am J Physiol Endocrinol Metab. 2006; 291(3):E675-82.
63. Luiken JJ, Arumugam Y, Dyck DJ, Bell RC, Pelsers MM, Turcotte LP, et al. Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. J Biol Chem. 2001;276(44):40567–73.
64. Hansson P, Holven KB, Øyri LKL, Brekke HK, Biong AS, Gjevestad GO, et al. Meals with Similar Fat Content from Different Dairy Products Induce Different Postprandial Triglyceride Responses in Healthy Adults: A Randomized Controlled Cross-Over Trial. J Nutr. 2019;149(3):422–31.
65. Robertson MD, Henderson RA, Vist GE, Rumsey RDE. Extended effects of evening meal carbohydrate-to-fat ratio on fasting and postprandial substrate metabolism. The American Journal of Clinical Nutrition. 2002;75(3): 505–10.

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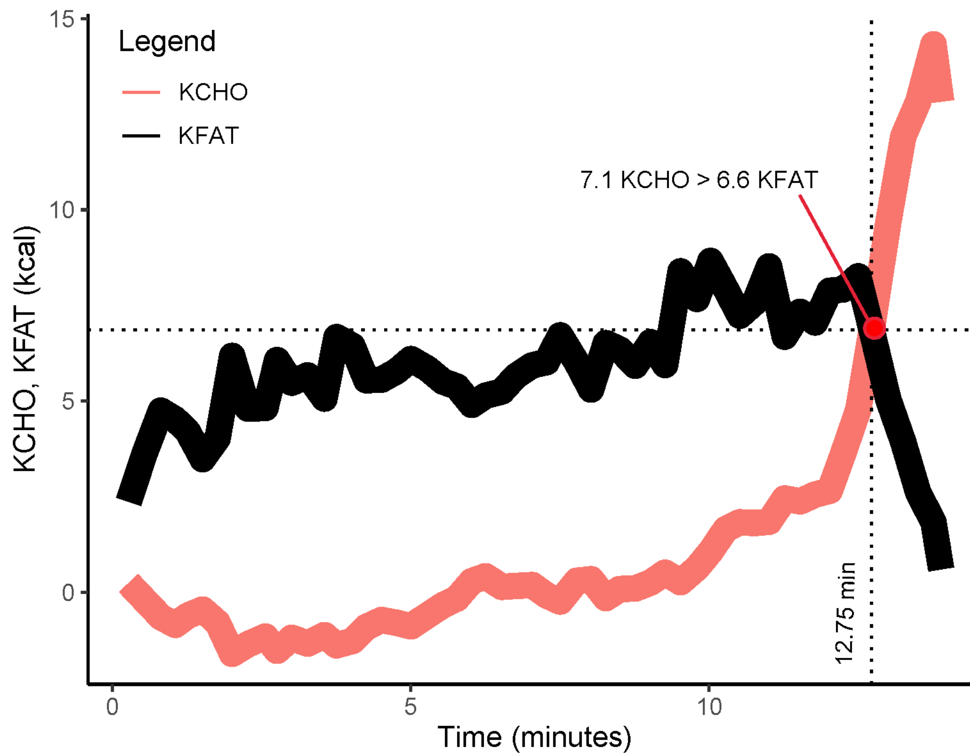
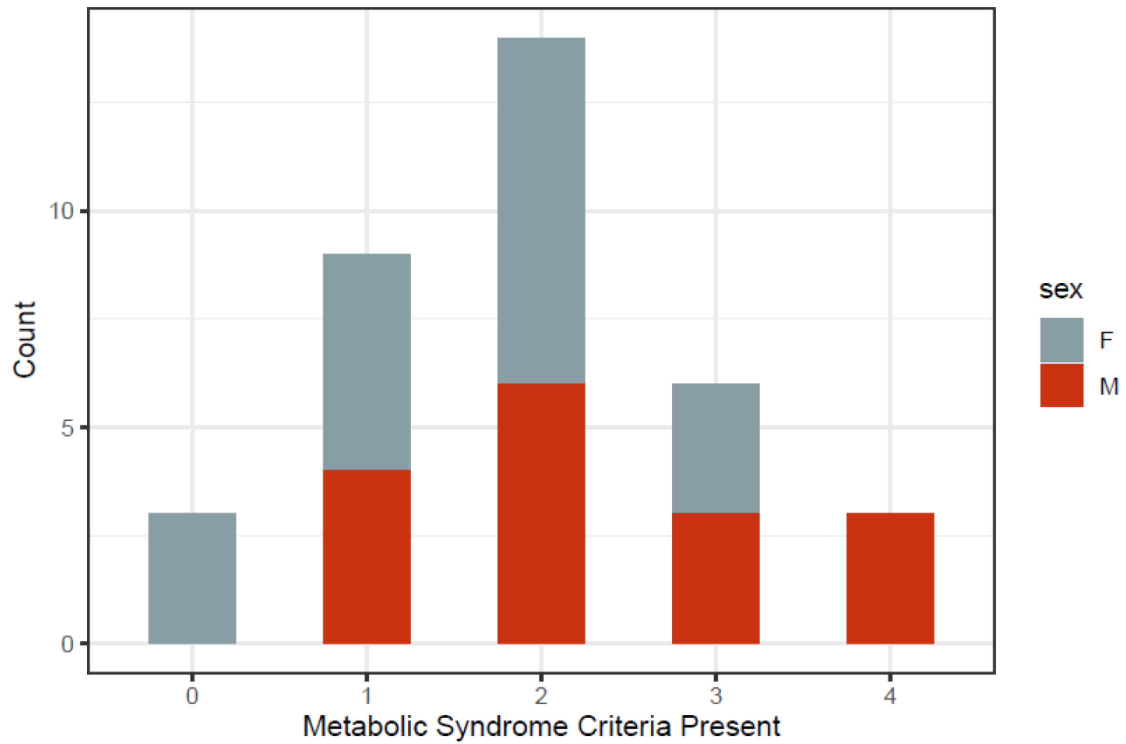


Example determination of Crossover Percentage
Data in this example is from one female participant.

Time at which KCHO>KFAT = 12.75 minutes
VO_2/kg at 12.75 minutes = 35.54 ml/kg/min

Crossover VO_2 / Calculated absolute VO_2 max = % of VO_2 max at crossover (ie, Crossover %)
35.54 ml/kg/min / 50.72 ml/kg/min = **68.1%**

Additional File 1 | Determination of the percentage of VO_2 max at substrate utilization crossover point. Visual provides additional detail using real participant data on how crossover percentage was determined.



Additional File 2 | Presence of metabolic syndrome criteria in analyzed study cohort (n = 35). Criteria were based of the National Cholesterol Education Program Adult Treatment Panel III definition of metabolic syndrome [32]. This metabolic syndrome definition includes criteria on central obesity, hypertension, insulin resistance, and dyslipidemia.

	Age	Sex	Crossover	VAT	HOMAIR	Aerobic	SBP	DBP	R^2_{adj}
Full	0.671	0.797	0.004	0.117	0.043	0.058	0.555	**0.807**	0.195
D1	0.704	**0.843**	0.042	0.101	0.041	0.052	0.474	NA	0.223
D2	**0.727**	NA	0.033	0.015	0.037	0.048	0.478	NA	0.249
D3	NA	NA	0.032	0.011	0.032	0.046	**0.518**	NA	0.272
D4*	NA	NA	0.036	0.013	0.037	**0.054**	NA	NA	0.286
D5	NA	NA	0.090	0.050	0.083	NA	NA	NA	0.217

*Final model. Abbreviations: DBP, diastolic blood pressure; HOMAIR, homeostatic model of insulin resistance; SBP, systolic blood pressure; VAT, visceral adipose tissue.

Additional File 3 | *P*-values decisions during model refinement for the TG iAUC Response. Bolded items indicate the variable that was dropped at each decision (D) step. The final model included variables from D4, with D5 shown to confirm D4 was the final model. No further predictor variables were removed according to variance inflation factor values.

	Age	Sex	Crossover	VAT	HOMAIR	Aerobic	SBP	DBP	R^2_{adj}
Full	**0.820**	0.524	0.046	0.052	0.150	0.019	0.546	0.809	0.242
D1	NA	0.543	0.042	0.034	0.132	0.017	0.569	**0.751**	0.269
D2	NA	**0.475**	0.037	0.032	0.123	0.011	0.139	NA	0.292
D3*	NA	NA	0.046	<0.001	0.141	0.012	**0.182**	NA	0.304
D4	NA	NA	0.075	0.002	**0.279**	0.023	NA	NA	0.283

*Final model. Abbreviations: DBP, diastolic blood pressure; HOMAIR, homeostatic model of insulin resistance; SBP, systolic blood pressure; VAT, visceral adipose tissue.

Additional File 4 | *P*-values decisions during model refinement for the TG change (peak – fasting) Response. Bolded items indicate the variable that was dropped in the decision (D) step. The final model included the variables from D3, with D4 shown to confirm D3 was the final model. No further predictor variables were removed according to variance inflation factor values.

Model	Coefficient	F-statistic	p-value
$\widehat{TG}_{iAUC}$			
Crossover[1] (%)	0.05	4.80	0.04
VAT (L)	0.53	7.05	0.01
HOMA-IR	0.30	4.78	0.04
Aerobic Frequency[2] (d/wk)	0.31	4.00	0.05
$\widehat{TG}_{(peak-fast)}$			
Crossover (%)	0.02	4.36	0.05
VAT (L)	0.43	14.0	<0.01
HOMA-IR	0.12	2.30	0.14
Aerobic Frequency (d/wk)	0.23	7.15	0.01
SBP (mmHg)	0.02	1.87	0.18

[1]Crossover indicates the percentage of VO_2 max during a submaximal exercise test where carbohydrate (versus fat) became the dominant substrate utilized.
[2]Aerobic frequency indicates the self-reported number of days where participants completed aerobic exercise in last 7 days.
Abbreviations: maximal oxygen consumption, VO_2; visceral adipose tissue, VAT; homeostatic model of insulin resistance, HOMA-IR; systolic blood pressure, SBP.

Additional File 5 | Model summaries for TG iAUC response and postprandial TG magnitude. The table is a complement to Fig. 3 and provides additional information on predictor variables as well as F-statistics from statistical output.

$$\widehat{TG}_{iAUC,i} = -3.67 + 0.05*X_{Crossover,i} + 0.53*X_{VAT,i} + 0.30*X_{HOMAIR,i} + 0.31*X_{Aerobic,i} + \varepsilon_i$$

$$\widehat{TG}_{(peak-fast),i} = -0.66 + 0.02*X_{Crossover,i} + 0.43*X_{VAT,i} + 0.12*X_{HOMAIR,i} + 0.23*X_{Aerobic,i} - 0.02*X_{SBP,i} + \varepsilon_i$$

Additional File 6 | The estimated regression equations for TG iAUC response and postprandial TG magnitude after model refinement. In the equations: i is subject, crossover is the percentage of VO_2 max where carbohydrate became the dominant substrate utilized, VAT is visceral adipose tissue in liters, HOMA-IR is insulin resistance, aerobic is self-reported days with aerobic exercise in last 7 days, SBP is systolic blood pressure in millimeters of mercury, and $\varepsilon_i \sim N(0, \sigma^2_\varepsilon)$.

CHAPTER THREE

EIGHT WEEKS OF LENTIL CONSUMPTION ATTENUATES INSULIN RESISTANCE PROGRESSION WITHOUT INCREASED GASTROINTESTINAL SYMPTOM SEVERITY – A RANDOMIZED CLINICAL TRIAL

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Software, Investigation, Writing – Review & Editing, Project Administration

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Contributions: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Validation, Funding Acquisition.

Manuscript Information

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Title: *Eight weeks of lentil consumption attenuates insulin resistance progression without increased gastrointestinal symptom severity – A Randomized Clinical Trial*

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Abbreviations

BMI; body mass index
CHOL; cholesterol
CON; Control
DHQ III; diet history questionnaire III
FM; fat mass
GI; gastrointestinal
GLU; glucose
HbA1c; Glycated Hemoglobin
HDL; high-density lipoprotein
HEI; healthy eating index
HI; High
HOMA-IR; Homeostatic Model of Insulin Resistance
iAUC; incremental area under the curve
INS; insulin
$ISI_{0,120}$; Insulin sensitivity index 0, 120
LDL; low-density lipoprotein
MOD; Moderate
OGTT; oral glucose tolerance test
PPGR; postprandial glycemic response
T2DM; Type 2 Diabetes Mellitus
TG; triglyceride
VAT; visceral adipose tissue
WC; waist circumference

Abstract

Lentils lower acute glycemic responses and promote satiety, benefits that may aid in chronic disease prevention. However, perceived gastrointestinal impacts may deter inclusion of dietary pulses in the diet. We hypothesized that 8-weeks of meals with lentils would improve glycemic control and improve satiety in metabolically at-risk, non-diabetic adults. As gastrointestinal symptoms are rarely reported, we also explored the temporal effects of symptom severity. Adults with an increased waist circumference (male>40", female>35") participated in an 8-week dietary intervention that included 5 prepared mid-day meals each week that were isocaloric but varied in cooked green lentil dosage: 0g (CON), 300g (MOD), or 600g (HI). Assessments included glucose and insulin integrated area under the curve measured during a 75g oral glucose tolerance test, hepatic insulin resistance (HOMA-IR) and peripheral insulin resistance. On one randomized day each week, satiety was assessed at 4:00 pm and gastrointestinal symptoms at 8:00 pm. Linear model assessed intervention impacts on glycemic measures. Thirty adults [mean±SD, age: 41.6±11.7, BMI: 35.1±6.3] completed the intervention. After 8 weeks, HOMA-IR increased in CON (+1.2 units) and decreased in a dose-dependent manner in MOD (0.3 units, p=0.03) and HI (-0.4 units, p<0.01). Most participants (87.4%) reported no to mild gastrointestinal symptoms. Of symptoms, flatulence was mild on average with bloating, abdominal discomfort, and cramping severity 0.3, 0.5, and 0.5 units lower (p<0.001). We observed a dose-dependent reduction on rising hepatic insulin resistance and low gastrointestinal symptom severity with long-term lentil consumption in metabolically at-risk adults.

Keywords (MeSH Terms): Lentils; insulin resistance; hyperglycemia, postprandial; prediabetes; satiety responses; clinical trial, randomized

1. Introduction

The Centers for Disease Control and Prevention estimates that 13% of US adults have diabetes and that 10.5% of adults have prediabetes based on their fasting glucose and glycated hemoglobin levels (HbA1c) [1]. Type 2 diabetes mellitus (T2DM) is a progressive disorder characterized by chronic elevated blood glucose and altered glucose, lipid, and protein metabolism from impaired insulin delivery and action [2]. There are genetic, behavioral, and metabolic risk factors associated with greater risk of T2DM development that in part include excessive body fat, particularly around the abdomen, and increased insulin resistance in the early phase of T2DM progression [3]. Lifestyle interventions such as healthy dietary changes can be used to prevent T2DM [4] with plant-based diets rich in high-quality plant food groups such as whole grains, fruits, vegetables, nuts, and legumes shown to reduce T2DM risk by 20% [5].

Repeated reduction of the postprandial glycemic response (PPGR) plays a vital role in the prevention and management of metabolic syndrome and diabetes [6, 7]. Regular incorporation of pulses offers a practical dietary means to improve glycemic control, blood lipids, body weight, and reduce disease risk in metabolically at-risk populations [8, 9]. Pulses are considered legumes and include chickpeas, lentils, and dry peas which are nutritionally dense offering bioactive compounds and fiber while relatively low in fat and sodium. The high fiber content in pulses contributes to delayed gastric emptying and helps give them a low glycemic index score, offering acute benefits to fasting blood glucose and insulin as well as a reduction to PPGR to the next subsequent meal that has been coined as “the second meal effect” [10-12]. Individuals that regularly consume

foods lower in the glycemic index scale and higher in fiber may accumulate the benefit of repeated second meal effects and have improved glucose tolerance and a decreased risk for insulin resistance and developing type 2 diabetes [13].

Despite evidence supporting multiple health benefits, legume consumption among US adults is low with 15% of the total population estimated to eat the amount of legumes (≥ 87.5 g/day) recommended to achieve disease prevention benefits [14]. Consumer incorporation may be difficult due to real and perceived barriers including abdominal discomfort, long preparation time, lack of knowledge on preparation, and lack of familiarity due to familial and cultural food habits [15]. Consumers commonly report gastrointestinal symptoms as a barrier [16]; yet, GI symptoms are rarely reported in pulse interventions [9]. Increased transparency of reported gastrointestinal symptoms in pulse interventions and addressing other barriers can help encourage and increase pulse consumption among consumers so that they may secure health benefits.

There are limited randomized clinical trials evaluating long-term pulse consumption on metabolic health, especially trials examining specifically lentil consumption at current USDA pulse recommendations which is approximately 1.5 cups of cooked pulses for adults per week with a caloric need of 2000 calories per day [9, 17]. This is of note as glycemic benefits vary by pulse variety, how pulses are added to the diet (i.e., whole vs ground), pulse dose, and duration of pulse inclusion in the diet [10]. We hypothesized that regular midday lentil consumption in individuals who are at greater risk of metabolic

disease would improve glycemic control and satiety, with more pronounced effects at a higher lentil dosage. This study sought to determine how eight weeks of 0, 300 (1.5 cups), or 600 (3 cups) grams of lentils per week affect glycemic control and anthropometric measures. We strategically offered midday meals to adults with increased central adiposity to allow the second meal effect to occur during the evening meal.

2. Methods and Materials

2.1. Ethics Statement. The protocol was approved by the Institutional Review Board at Montana State University (MM100218). Written informed consent was obtained from all participants prior to participation. The study was registered at ClinicalTrials.gov (NCT04448067).

2.2. Study Population. Potential participants were recruited by advertisement through flyers, email mailing lists, word of mouth, and snowballing recruitment methods. Eligible individuals were 18-70 years and had waist circumferences greater than or equal to 35 inches for females and 40 inches for males. Eligible participants exceeded the metabolic syndrome criteria for waist circumference [18], a criterion meant to recruit individuals with greater risk of T2DM development [3]. Exclusion criteria included diabetes, wheat allergies, blood sugar medications, lipid or cholesterol-lowering medications, anti-inflammatory medications, and pregnancy.

2.3. Research Design. The study was a single-blind randomized clinical trial with 0, 300, or 600 g of whole lentils five days a week for eight weeks. Randomization was performed by drawing out of a hat in blocks of six. Participants were assigned meal groups before the dietary intervention. Participants were asked to come to the Nutrition Research Laboratory at Montana State University on three occasions (**Figure 1**). After an initial screening visit with anthropometric measurements, an oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast before and after the 8-week dietary intervention. Weekly online survey questionnaires were delivered during the intervention to assess satiety and gastrointestinal (GI) issues. Metabolic health, satiety, and gastrointestinal symptom measures were primary outcomes of interest.

2.4. Dietary Intervention. Participants underwent an 8-week dietary intervention, which included 5 prepared mid-day meals each week that were isocaloric but varied in dose of cooked whole green lentils (provided by AGT Foods Canada): 0 g (CON), 300 g (MOD), and 600 g (HI) per week. Throughout the 8 weeks, participants were asked to consume the same five provided meals each week but were otherwise asked to maintain their normal dietary and physical activity patterns. Provided meals were prepared by a registered dietician and professional chef on the research team. Meals included were soup, curry with basmati rice, street tacos, loaf with mashed potatoes and cooked zucchini, and pasta with Bolognese sauce. A detailed weekly diet menu and nutrient profile is listed in **Table 1.** Control meals were matched in total energy to MOD and HI lentil meals and included ground turkey or chicken instead of green lentils. The intent of

this study was to evaluate equivalent meals with meat (CON), a mixture of meat and lentils (MOD), and strictly lentils (HI) as the protein sources in the meals. The strength of this approach is that it maximizes the translational value on the impact of incorporating lentils into the diet as a protein source. A limitation of this approach is that the macronutrient composition of the meals was not identical.

2.5. Lentil Sampling and Nutritional Analysis. Medium green lentils (CDC Richlea cultivar) used in the diet were sent courtesy of AGT Foods Canada to Medallion Labs (Minneapolis, MN) for analytical testing with the standalone nutrient breakdown and functional properties are provided in **Supplementary File 1**. Dietary fiber was determined through the analytical method AOAC 991.43.

2.6. Oral-Glucose Tolerance Test. The test meal contained white bread (Franz Premium White BIG) totaling 75 g carbohydrate intake. Total energy content of the test meal was 400 kcal, with a macronutrient composition of 5 g fat, 75 g carbohydrate, and 10 g protein. Meals were provided in the study laboratory. Water was provided ad libitum with the meal. Participants were instructed to consume the meal within 15 minutes with the postprandial period timing based on when the meal began. During the two hours of the OGTT, participants were asked to remain seated and complete a written 24-hour dietary recall and an online survey on their habitual diet.

2.7. Blood Sampling. Participants were instructed to avoid alcohol consumption and strenuous physical activity in the 24 hours before their visit and to complete an overnight fast (≥ 10 hours) before blood collection. Fasting blood samples were collected by a trained phlebotomist via venipuncture of the antecubital vein in the morning before ingestion of OGTT. Venous blood at fasting was collected into 8.5 mL endotoxin-free serum separating and 4.0 mL heparinized vacutainer tubes (BD Vacutainer, Franklin Lakes, New Jersey, USA). Blood was obtained by fingerstick at 30, 60, 90, and 120 minutes in the 2-hour postprandial period and collected with microcapillary serum separating 200 uL tubes (RAM Scientific Inc., Nashville, Tennessee). Blood samples were allowed to clot for 15 minutes before centrifugation at 1200 RPM for 15 minutes with resulting serum aliquoted and stored at -80ºC until analysis.

2.8. Biochemical Analysis. Fasting blood lipid profiles were determined from whole blood run on Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, USA). Components derived from lipid panels include cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG). HbA1x was determined using the DCA Vantage Analyzer (Siemens Medical Solutions Diagnostics, Cergy-Pontoise, France) performed according to manufacturer instructions. For blood glucose (GLU) determination, whole blood was collected into a microcuvette and run immediately on the HemoCue Hb 201+ System (HemoCue AB, Angelholm, Sweden). For insulin (INS) determination, fingerstick blood samples were collected and centrifuged at 1200 RPM for 15 minutes with resulting serum aliquoted and stored at -

80ºC. INS concentrations from fasting and postprandial samples were determined through a high-sensitivity INS ELISA kit according to manufacturer instructions (ALPCO, Salem, NH, United States) with a mean inter-assay coefficient of variation of 5.2%. Blood glucose and insulin responses to the OGTT were each summarized as incremental area under the curve method (iAUC) in an effort to normalize high interpersonal variability in PPGRs to the same meal [19, 20].

2.9. Insulin Resistance. Fasting blood GLU and INS were used to determine the homeostatic model of insulin resistance (HOMA-IR), a measure of hepatic insulin resistance at fasting calculated with the original HOMA-IR formula [21]. Body weight and GLU and INS concentrations at 0 and 120 minutes of the OGTT were used to calculate the insulin sensitivity index ($ISI_{0,120}$), a validated measure of peripheral insulin resistance [22].

2.10. Habitual Dietary Habit Survey. A validated diet history questionnaire (DHQ III) was self-assessed through a web-based survey offered by the National Cancer Institute. Participants were provided a laptop and asked to complete the survey at the baseline blood collection visit. The DHQ III consists of 135 food and beverage and 26 supplement questions to assess dietary frequency and portion sizes over the past 12 months. Through built-in programming 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 2015-2020 U.S. Dietary Guidelines for Americans. 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) [23, 24].

2.11. Anthropometrics. Measurements were collected from participants using the validated segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) [25]. Participants were instructed to refrain from eating, drinking, or exercising in the three hours prior to testing. Fat mass (%), estimated visceral adipose (liters), and skeletal muscle mass (%) were used for analysis.

2.12. Satiety and Gastrointestinal Surveys. Two brief surveys addressing participant hedonic response, satiety, and GI issues were delivered through email using the online platform Qualtrics on the same day, once a week, sequentially delivered on rotation for each weekday, for each week of the 8-week dietary intervention. At 4:00 pm, participants were asked how much they enjoyed their mid-day meal followed by their levels of hunger, fullness, satisfaction, and desire to eat in relation to the study meal they consumed mid-day. At 8:00 pm, participants were asked how they felt overall that day regarding common GI symptoms. The latter included four sub questions asking them to rank the severity (None, Mild, Moderate, Severe) of their flatulence, bloating, cramping, and abdominal discomfort. Participants were able to view definitions of each GI issue

during completion of each survey. Survey questions and response scales are outlined in **Supplementary File 2**.

2.13. Statistical Analyses. Analysis and visualizations were conducted in RStudio (1.4.1106) running R 4.0.5, with data visualized using *ggplot2[26]* and *effects* [27] packages.

Descriptive summary statistics were performed for participant physical and biological characteristics with general linear models used to determine if participants on average differed between meal groups before the intervention began. Habitual dietary patterns, determined through HEI scores, at baseline were also analyzed through general linear models with three less individuals included in the analysis due to survey incompletion. Variables that demonstrated group differences were further analyzed using post-hoc t-tests.

General linear models were used to assess the impact of the dietary intervention on the following categories of variables: anthropometric measures – weight, fat mass, skeletal muscle mass, and waist circumference; blood lipid measures – CHOL, LDL, HDL, TG; glycemic measures – GLU iAUC, INS iAUC, HOMA-IR, and $ISI_{0,120}$. 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 – meal group; blood lipid measures – meal group,

visceral adipose tissue; glycemic measures – meal group, change in body mass index (BMI), and HbA1c. Predictor variables for glycemic measures were chosen based on previous findings by Zeevi and colleagues [20]. Validity conditions were confirmed through visualizations of the response and model residuals.

Linear mixed effects models were used to assess the impact of a week and meal interaction on satiety measures, accounting for meal enjoyment. GI symptom severity was analyzed through a non-linear mixed effects model with the predictors as a week and meal interaction and symptom type, with week on a quadratic polynomial. Subject was the random effect for models assessing satiety and GI symptom severity.

3. Results

3.1. General characteristics of Participants. Five participants were lost to follow-up or withdrew from the study for non-intervention related reasons, leaving 30 healthy overweight and obese adults [mean ± SD, age: 41.6 ± 11.7, BMI: 35.1 ± 6.3] who completed the 8-week intervention (**Figure 2**).

The proportion of males to females was different between meal groups with the greatest proportion of males observed in MOD, X^2 ((3, n = 30) = 14.1, $p < 0.01$). Between meal groups at baseline, participants had similar anthropometric and metabolic characteristics (**Table 2**). Participants classified as obese according to BMI. Among metabolic characteristics, fasting TG, CHOL, HDL and GLU were normal on average across meal

groups at baseline. One-third of participants (2 CON, 5 MOD, 3 HI) were considered prediabetic based on American Diabetes Association 2021 classification guidelines [28].

3.2. Dietary Habits at Baseline. Self-reported habitual dietary patterns assessed through the online DHQ III were scored to US 2015 dietary recommendations through built-in HEI scoring. Three participants, one from each meal group, did not complete the survey and were unable to be scored and were not included in dietary pattern analyses.

Total HEI scores, which indicate overall dietary quality, were not different between meal groups at baseline (**Table 3**). Sixty-two percent of participants were above the average national HEI score (58.3) for adults, with a group average score of 62.3. With regard to foods to consume in adequacy, self-reported intake of total vegetables and greens and beans did vary between groups. Total vegetable intake in MOD was 0.97 points greater than CON ($t = 2.1$, $p = 0.04$) and 1.52 points greater than HI ($t = -3.2$, $p < 0.01$). Additionally, intake of greens and beans was 1.50 points greater in MOD than HI than MOD ($t = -2.8$, $p < 0.01$). Meal groups were similar at baseline among components to consume in moderation.

3.3. Blood Lipids. No meal group differences were detected in the change (post – pre) total CHOL ($F = 1.0$, $p = 0.36$), LDL ($F = 0.94$, $p = 0.40$), HDL ($F = 0.62$, $p = 0.55$), or TG ($F = 0.15$, $p = 0.86$) after accounting for change in VAT. However, we did observe a

positive relationship ($\beta = 0.29$) of visceral adipose tissue on fasting blood TG ($F = 4.5$, $p = 0.04$).

3.4. Anthropometrics. Meal group differences were not detected in the change of body weight ($F = 2.0$, $p = 0.15$), fat mass percentage ($F = 0.98$, $p = 0.39$), skeletal muscle mass percentage ($F = 0.66$, $p = 0.52$), or VAT ($F = 0.35$, $p = 0.71$).

3.5. Insulin Resistance. After accounting for BMI and HbA1c, MOD and HI had lower hepatic insulin resistance than CON as measured through HOMA-IR. One MOD participant was identified as an influential po/int based on a Cook's Distance > 1 and removed from HOMA-IR analysis. Participants who consumed meat-based CON meals, on average, increased in HOMA-IR after 8-weeks (**Figure 3**). Participants in MOD also had an increase in HOMA-IR, but this increase after 8-weeks was 0.9 units lower than CON ($t = -2.2$, $p = 0.03$). Participants in HI had a reduction in HOMA-IR, with the change in HOMA-IR on average 1.5 units lower than CON ($t = -3.5$, $p = 0.002$). BMI and HbA1c were both predictors to the change in HOMA-IR. Interestingly, participants who increased in BMI had on average a reduction in HOMA-IR ($t = -4.0$, $p < 0.001$). HbA1c was a positive predictor of the change in HOMA-IR, with greater HbA1c related to a rise in HOMA-IR after 8-weeks ($t = 3.4$, $p < 0.01$). Variance explained by our model was approximately 49.6% according to adjusted R_2.

Eight weeks of MOD ($t = 1.3$, $p = 0.21$) or HI ($t = -0.31$, $p = 0.67$) lentil consumption did not improve peripheral insulin resistance as measured by $ISI_{0,120}$, compared to CON. Further, HbA1c ($t = -0.91$, $p = 0.37$) and BMI ($t = 1.5$, $p = 0.15$) were not predictors of $ISI_{0,120}$.

3.6. Postprandial glucose and insulin. The addition of lentils over 8-weeks did not impact GLU iAUC ($F = 0.41$, $p = 0.67$), accounting for BMI ($F = 1.9$, $p = 0.18$) and HbA1c ($F < 0.01$, $p = 0.95$). Similarly, INS iAUC was not impacted by the lentil addition for 8-weeks ($F = 0.84$, $p = 0.44$), accounting for BMI ($F = 0.03$, $p = 0.86$) and HbA1c ($F = 0.23$, $p = 0.64$). The same participant in MOD who was removed as an influential point in HOMA-IR analysis was also removed from INS iAUC analysis based on a Cook's Distance > 1. Our male participants were not detected as outliers in our modelling of glycemic responses and were consequently included in all analyses. Average GLU and INS concentrations during the OGTT before and after the intervention are displayed in **Supplementary File 3.**

3.7. Satiety During the Intervention. The overall response rate to the 4:00 pm survey on satiety was 95.8%. The mean response rates were 95%, 95.5%, and 97.2% for CON, MOD, and HI, respectively. A decline in meal enjoyment was experienced by CON and HI over 8 weeks, while meal enjoyment was maintained in MOD over 8 weeks **Supplementary File 4**. Satiety measures were not different by meal group over 8 weeks but were largely dependent on meal enjoyment with the exception of self-reported

fullness (**Table 4**). An inverse relationship was observed between meal enjoyment and self-reported hunger ($\beta = -0.16$), desire to eat ($\beta = -0.51$), and amount that they could eat ($\beta = -0.38$) while a positive relationship was observed between meal enjoyment and satisfaction ($\beta = 0.73$). Only in desire to eat was a meal main effect observed.

3.8. Gastrointestinal Issues During the Intervention. The overall mean response rate to the 8:00 pm survey on GI issues was 95.4%. Mean response rates for the GI survey were 93.8%, 95.5%, and 97.2% respectively for CON, MOD, and HI. Regarding symptom severity responses throughout the entire intervention, 87.4% were none to mild, 11.3% were moderate, and 1.8% were severe.

Reported flatulence was mild on average throughout the duration of the study and was the GI issue with the highest severity ($p < 0.001$). Flatulence was followed sequentially in symptom severity by bloating, abdominal discomfort, and cramping (**Figure 4A**). The frequency of reported bloating, abdominal discomfort, and cramping fluctuated over eight weeks but only in the MOD group did the frequency of average GI symptoms increase then decline after 5 weeks (**Figure 4B,** $t = -2.9$, $p < 0.01$). Mild flatulence was reported by the majority of participants in HI, with an increasing trend seen over time. Weekly fluctuations in symptom severity are visualized in **Supplementary File 5**.

4. Discussion

The key finding of this study was identification of dose dependent benefits of dietary lentils for the attenuation of the progression of insulin resistance in non-diabetic adults with overweight and obesity, which is line with our hypothesis. To our knowledge, this is the first study that has examined the impact of long-term lentil consumption on glycemic responses without the introduction of physical activity or energy restriction. We specifically looked to assess the impact of weekly lentils meeting and surpassing USDA weekly pulse recommendations in a manner that incrementally utilized lentils instead of meat. In an effort to understand how participants responded to the intervention, we also surveyed participants about their satiety and GI symptoms. Contrary to our hypothesis that satiety would increase in a dose dependent manner with lentil consumption, we did not observe changes in satiety with lentil consumption compared to meat consumption. Our weekly survey strategy provided novel insights on temporal changes in satiety and GI discomfort with dietary strategies incorporating an increased dietary fiber load.

Our main finding was that lentil consumption helped to prevent a rise in HOMA-IR with an incremental decrease in HOMA-IR with increasing dietary lentils amounts. HOMA-IR is a measure of fasting insulin resistance and is reflective of hepatic insulin resistance. In a meta-analysis of 12 randomized clinical trials in adults without T2DM, only one trial found a decrease in HOMA-IR after 16-weeks of pulse consumption [29, 30]. The rise of blood glucose and insulin after a meal stimulates glucose disposal in the periphery, predominantly into skeletal muscle. Thus, dynamic postprandial conditions are reflective of peripheral insulin resistance. Diet-related changes to dynamic postprandial responses

such as INS iAUC and GLU iAUC were not observed which mirrors the lack of change also observed in $ISI_{0,120}$, our measure of peripheral insulin resistance. Increased insulin production during the postprandial phase normally signals the liver to cease glucose production, increase glucose consumption, and store consumed glucose as glycogen or lipids. In insulin resistant individuals, insulin is unable to effectively regulate glucose metabolism resulting in increased glucose production with de novo lipogenesis. The beneficial effect of fiber on fasting glycemic control was previously suggested to be proportionate to the amount of soluble fiber in foods [31]. Low soluble fiber content is of note as high viscosity soluble fibers help slow the passage of the food matrix through the small intestine, which contributes to slowed degradation and absorption of carbohydrate. The whole green lentils in our study meals provided intact fiber, contained approximately 13 g (per 100 g uncooked) of fiber with the majority as insoluble fiber. Insoluble fiber may also combat the rise in insulin resistance [32] by the potential to disrupt the absorption of protein in the small intestine [33]. Increased plasma amino acid concentrations through amino acid infusions were found to increase insulin resistance [34]; thus, it is possible that the consumption of animal-based products contributed to the rise of insulin resistance observed in our study.

Hepatic insulin resistance is a key contributor to fasting [35] and postprandial lipemia [36]. We did not measure postprandial lipemia in the current study, but with regards to fasting blood lipids, we did not observe changes after eight weeks of dietary modification. These findings are in contrast to improved serum total and LDL cholesterol

previously reported in a metanalysis of 10 clinical trials with at least three weeks of legume consumption [37]. Diets high in fiber can contribute to impaired lipid emulsification, decreased chylomicron production, increased clearance of triglyceride-rich lipoproteins, and decreased very-low density lipoprotein production [38]. The cholesterol-lowering impact of lentils may be attributed in part to the ability of bile acids to bind to soluble fiber in the intestinal tract, which helps to prevent reabsorption in the distal ileum and increase its excretion in feces [39]. To maintain the bile acid pool, reduced bile acid absorption triggers increased bile acid production mediated through increased clearance of LDL to uptake cholesterol necessary for bile acid synthesis. It is unclear whether bile acid production was impacted through incorporation of whole green lentils. Further investigation of acute and long-term effects of pulses on bile acid synthesis and secretion is warranted as bile acid metabolites were also recently shown to predict postprandial glucose and insulin responses [40].

A unique finding of the present study with high relevance to dietary strategies for weight management was that meal enjoyment was a key factor in self-reported satiety measures with higher meal enjoyment related to less hunger and desire to eat in the afternoon. Individuals in MOD maintained their meal enjoyment on average through the study compared to CON and HI, who showed a gradual decline in meal enjoyment. An acute benefit of increased fiber consumption is higher satiety, or a perceived feeling of fullness after a meal [41]. Soluble fiber delays gastric emptying through an increase in digesta viscosity, which helps individuals feel fuller longer after a meal [31]. Individuals who

have reduced feelings of hunger may be compelled to eat less at their second meal, promoting a reduction in caloric intake. Satiety in this current study did not vary by meal group during the intervention, and average satiety did not translate to changes in weight, body fat or skeletal muscle percentage. Obese adults have previously reported greater satiety benefit with fiber compared to studies who examined lower BMI participants [41], an effect that may not have been observed in this study given the low soluble fiber content of our study lentils. However, we note that the dietary intervention was not designed as a weight loss intervention but to simulate a realistic change in dietary behavior in free-living adults. As increased energy intake has been identified as the key driver of the United States obesity epidemic [42], it becomes imperative to regularly incorporate meals which promote satiety and reduce caloric intake to aid weight management.

Another important assessment relevant to dietary strategies to improve metabolic health assessed in the present study was GI symptom frequency and temporal changes in symptoms during the 8-week dietary intervention. GI symptoms are infrequently reported in interventions, with only three publications in a recent systematic review of 20 pulse intervention reporting GI symptoms, none of which were exclusively lentils [9]. In our study, participants with moderate lentil consumption reported a decline in frequency of GI symptoms after 5 weeks. Severe symptoms were reported infrequently with no participants withdrawing from the study due to GI symptom severity. Of specific symptoms, flatulence had the highest severity but was mild on average. GI discomfort

largely arises from gas production during bacterial fermentation of increased soluble fiber and resistant starches which cannot be readily digested by intestinal enzymes. Intestinal gas production was previously reported to subside in healthy individuals as colonic bacteria adapt to the dietary fiber and resistant starches with long-term consumption of fiber-rich food [43]. It is possible that participants in MOD may have had this adaptation occur sooner as participants were already consuming greater vegetables in their habitual diet than CON and HI as assessed by HEI component scores. Collectively, these findings may aid the effort to counter consumer perception of GI symptom severity as a barrier to lentil inclusion.

We recognize that there are additional factors that may have influenced changes to insulin resistance and PPGR that we were not measured in the current study. First, while participants were asked to maintain their regular dietary and physical activity patterns, it is possible participants incorporated additional healthy lifestyle patterns that would improve glycemic control. Second, it is possible that antioxidants present in whole lentils may have impacted oxidative stress which is implicated in the development of insulin resistance [44]. Third, this study did not assess the impact of the gut microbial community on PPGR. The human gut microbial community is in part influenced by diet [45]. Specific microbial taxa are related to plant-based foods [46] and PPGR [20] which help highlight an ongoing interaction between diet, microbiota, and host responses. Soluble fiber and resistant starch in lentils can undergo bacterial fermentation to produce short chain fatty acids such as butyrate which has been shown to have anti-inflammatory

and insulin-sensitizing effects [47, 48]. As the gut microbiota can metabolize dietary fiber as well as bile acids [49], it is imperative to understand how temporal effects of lentil consumption on the gut microbial community may modulate host postprandial responses as it relates to chronic disease development. In addition, we do recognize that future dietary interventions with pulses would be improved by a more even proportion of males to females to better account for gender-specific differences in insulin sensitivity [50].

In conclusion, the findings of the present study provide evidence that long-term lentil consumption above USDA legume recommendations (1.5 cup/week) in heavier individuals at greater risk of impaired glucose tolerance can reduce an upward drift in hepatic insulin resistance, a key target in the early phase in progression of T2DM. Additionally, our findings add to the limited body of research regarding satiety and GI symptoms with legume consumption with specific findings including the importance of meal enjoyment in satiety and temporal changes in GI symptom severity. Further investigation of the long-term impact of insoluble and fermentable fibers on glycemic control, bile acids, and potential gut-mediated pathways, is warranted in at-risk populations.

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Author Contributions: The authors' responsibilities were as follows: MPM and WK., Conceptualization; MPM, MEG, and W.K., Methodology; SMGW and EJP, Software; SMGW, Formal Analysis; EJP and SMW, Investigation; MEG and MPM, Resources; SMGW, Data Curation; SMGW, Writing – Original Draft Preparation; SMGW, EJP, MEG, WK, and MPM, Writing – Review & Editing; SMGW, Visualization; EJP, Project Administration; MPM, Supervision; MPM, Validation; WK and MPM, Funding Acquisition.

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References

[1] CDC. National diabetes statistics report, 2020. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services. 2020:12-5.

[2] Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014;11:1185-200. doi: 10.7150/ijms.10001

[3] Ley SH, Schulze MB, Hivert MF, Meigs JB, Hu FB. Risk factors for type 2 diabetes. In: Cowie CC, Casagrande SS, Menke A, Cissell MA, Eberhardt MS, Meigs JB, et al., editors. Diabetes in america. Bethesda (MD) of interest.: National Institute of Diabetes and Digestive and Kidney Diseases (US); 2018.

[4] Uusitupa M, Khan TA, Viguiliouk E, Kahleova H, Rivellese AA, Hermansen K, et al. Prevention of type 2 diabetes by lifestyle changes: A systematic review and meta-analysis. Nutrients. 2019;11. doi: 10.3390/nu11112611

[5] Satija A, Bhupathiraju SN, Rimm EB, Spiegelman D, Chiuve SE, Borgi L, et al. Plant-based dietary patterns and incidence of type 2 diabetes in us men and women: Results from three prospective cohort studies. PLOS Medicine. 2016;13:e1002039. doi: 10.1371/journal.pmed.1002039

[6] Grundy Scott M, Stone Neil J, Bailey Alison L, Beam C, Birtcher Kim K, Blumenthal Roger S, et al. 2018 aha/acc/aacvpr/aapa/abc/acpm/ada/ags/apha/aspc/nla/pcna guideline on the management of blood cholesterol. Journal of the American College of Cardiology. 2019;73:e285-e350. doi: 10.1016/j.jacc.2018.11.003

[7] American Diabetes Association. 3. Prevention or delay of type 2 diabetes: Standards of medical care in diabetes—2020. Diabetes Care. 2020;43:S32. doi: 10.2337/dc20-S003

[8] Jenkins DJA, Kendall CWC, Augustin LSA, Mitchell S, Sahye-Pudaruth S, Blanco Mejia S, et al. Effect of legumes as part of a low glycemic index diet on glycemic control and cardiovascular risk factors in type 2 diabetes mellitus: A randomized controlled trial. Archives of Internal Medicine. 2012;172:1653-60. doi: 10.1001/2013.jamainternmed.70

[9] Ferreira H, Vasconcelos M, Gil AM, Pinto E. Benefits of pulse consumption on metabolism and health: A systematic review of randomized controlled trials. Critical Reviews in Food Science and Nutrition. 2021;61:85-96. doi: 10.1080/10408398.2020.1716680

[10] Sievenpiper JL, Kendall CWC, Esfahani A, Wong JMW, Carleton AJ, Jiang HY, et al. Effect of non-oil-seed pulses on glycaemic control: A systematic review and meta-

analysis of randomised controlled experimental trials in people with and without diabetes. Diabetologia. 2009;52:1479. doi: 10.1007/s00125-009-1395-7

[11] Higgins JA. Whole grains, legumes, and the subsequent meal effect: Implications for blood glucose control and the role of fermentation. J Nutr Metab. 2012;2012:829238-. doi: 10.1155/2012/829238

[12] Hafiz MS, Campbell MD, O'Mahoney LL, Holmes M, Orfila C, Boesch C. Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: A systematic review and meta-analysis of acute and long-term randomized controlled trials. Eur J Nutr. 2021. doi: 10.1007/s00394-021-02685-y

[13] Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: Dietary components and nutritional strategies. Lancet. 2014;383:1999-2007. doi: 10.1016/s0140-6736(14)60613-9

[14] Perera T, Russo C, Takata Y, Bobe G. Legume consumption patterns in us adults: National health and nutrition examination survey (nhanes) 2011-2014 and beans, lentils, peas (blp) 2017 survey. Nutrients. 2020;12. doi: 10.3390/nu12051237

[15] Murty CM, Pittaway JK, Ball MJ. Chickpea supplementation in an australian diet affects food choice, satiety and bowel health. Appetite. 2010;54:282-8. doi: 10.1016/j.appet.2009.11.012

[16] Henn K, Goddyn H, Olsen SB, Bredie WLP. Identifying behavioral and attitudinal barriers and drivers to promote consumption of pulses: A quantitative survey across five european countries. Food Quality and Preference. 2022;98:104455. doi: https://doi.org/10.1016/j.foodqual.2021.104455

[17] USDHHS. Dietary guidelines for americans, 2020-2025. 9th ed2020.
[18] Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009;2:231-7. doi: 10.1242/dmm.001180

[19] Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, et al. Glycemic index methodology. Nutr Res Rev. 2005;18:145-71. doi: 10.1079/NRR2005100

[20] Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. Cell. 2015;163:1079-94. doi: 10.1016/j.cell.2015.11.001

[21] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9. doi: 10.1007/BF00280883

[22] Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (isi(0,120)): Comparison with other measures. Diabetes Res Clin Pract. 2000;47:177-84. doi: 10.1016/s0168-8227(99)00116-3

[23] Reedy J, Lerman JL, Krebs-Smith SM, Kirkpatrick SI, Pannucci TE, Wilson MM, et al. Evaluation of the healthy eating index-2015. J Acad Nutr Diet. 2018;118:1622-33. doi: 10.1016/j.jand.2018.05.019

[24] Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, et al. Update of the healthy eating index: Hei-2015. J Acad Nutr Diet. 2018;118:1591-602. doi: 10.1016/j.jand.2018.05.021

[25] Bosy-Westphal A, Schautz B, Later W, Kehayias JJ, Gallagher D, Müller MJ. What makes a bia equation unique? Validity of eight-electrode multifrequency bia to estimate body composition in a healthy adult population. European Journal Of Clinical Nutrition. 2013;67:S14. doi: 10.1038/ejcn.2012.160

[26] Wickham H. Elegant graphics for data analysis. New York: Springer-Verlag; 2009.
[27] Fox J. Effect displays in r for generalised linear models. Journal of Statistical Software. 2003;8:1 - 27. doi: 10.18637/jss.v008.i15

[28] American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes—2021. Diabetes Care. 2021;44:S15. doi: 10.2337/dc21-S002

[29] Bielefeld D, Grafenauer S, Rangan A. The effects of legume consumption on markers of glycaemic control in individuals with and without diabetes mellitus: A systematic literature review of randomised controlled trials. Nutrients. 2020;12:2123. doi: 10.3390/nu12072123

[30] Kazemi M, McBreairty LE, Chizen DR, Pierson RA, Chilibeck PD, Zello GA. A comparison of a pulse-based diet and the therapeutic lifestyle changes diet in combination with exercise and health counselling on the cardio-metabolic risk profile in women with polycystic ovary syndrome: A randomized controlled trial. Nutrients. 2018;10:1387. doi: 10.3390/nu10101387

[31] McRorie JW, Jr., McKeown NM. Understanding the physics of functional fibers in the gastrointestinal tract: An evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. J Acad Nutr Diet. 2017;117:251-64. doi: 10.1016/j.jand.2016.09.021

[32] McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome

in the framingham offspring cohort. Diabetes Care. 2004;27:538-46. doi: 10.2337/diacare.27.2.538

[33] Weickert MO, Roden M, Isken F, Hoffmann D, Nowotny P, Osterhoff M, et al. Effects of supplemented isoenergetic diets differing in cereal fiber and protein content on insulin sensitivity in overweight humans. Am J Clin Nutr. 2011;94:459-71. doi: 10.3945/ajcn.110.004374

[34] Krebs M, Krssak M, Bernroider E, Anderwald C, Brehm A, Meyerspeer M, et al. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes. 2002;51:599-605. doi: 10.2337/diabetes.51.3.599

[35] Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, et al. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides. Arteriosclerosis, Thrombosis, and Vascular Biology. 2001;21:275-81. doi: 10.1161/01.ATV.21.2.275

[36] Wilson SM, Maes AP, Yeoman CJ, Walk ST, Miles MP. Determinants of the postprandial triglyceride response to a high-fat meal in healthy overweight and obese adults. Lipids in Health and Disease. 2021;20:107. doi: 10.1186/s12944-021-01543-4

[37] Bazzano LA, Thompson AM, Tees MT, Nguyen CH, Winham DM. Non-soy legume consumption lowers cholesterol levels: A meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis. 2011;21:94-103. doi: 10.1016/j.numecd.2009.08.012

[38] Bozzetto L, Della Pepa G, Vetrani C, Rivellese AA. Dietary impact on postprandial lipemia. Front Endocrinol. 2020;11. doi: 10.3389/fendo.2020.00337

[39] Khatoon N, Prakash J. Nutritional quality of microwave-cooked and pressure-cooked legumes. International Journal of Food Sciences and Nutrition. 2004;55:441-8. doi: 10.1080/09637480400009102

[40] Henninger J, Eliasson B, Smith U, Rawshani A. Identification of markers that distinguish adipose tissue and glucose and insulin metabolism using a multi-modal machine learning approach. Scientific Reports. 2021;11:17050. doi: 10.1038/s41598-021-95688-y

[41] Clark MJ, Slavin JL. The effect of fiber on satiety and food intake: A systematic review. Journal of the American College of Nutrition. 2013;32:200-11. doi: 10.1080/07315724.2013.791194

[42] Swinburn B, Sacks G, Ravussin E. Increased food energy supply is more than sufficient to explain the us epidemic of obesity. American Journal of Clinical Nutrition. 2009;90:1453-6. doi: 10.3945/ajcn.2009.28595

[43] Mego M, Manichanh C, Accarino A, Campos D, Pozuelo M, Varela E, et al. Metabolic adaptation of colonic microbiota to galactooligosaccharides: A proof-of-concept-study. Aliment Pharmacol Ther. 2017;45:670-80. doi: 10.1111/apt.13931

[44] Ganesan K, Xu B. Polyphenol-rich lentils and their health promoting effects. Int J Mol Sci. 2017;18:2390. doi: 10.3390/ijms18112390

[45] Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. Cell Host & Microbe. 2012;12:611-22. doi: 10.1016/j.chom.2012.10.012

[46] Asnicar F, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, et al. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nature Medicine. 2021;27:321-32. doi: 10.1038/s41591-020-01183-8

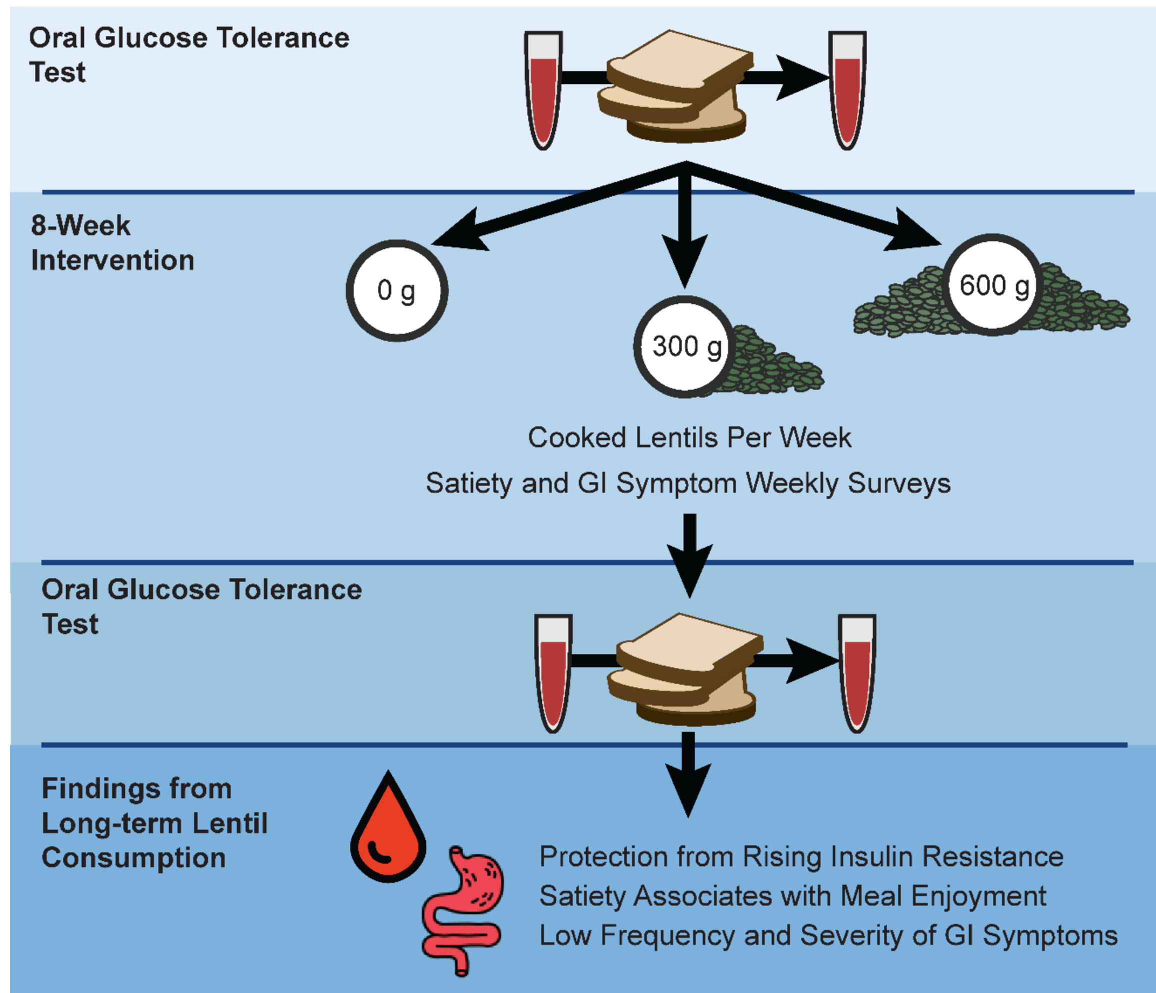
[47] Zhai S, Qin S, Li L, Zhu L, Zou Z, Wang L. Dietary butyrate suppresses inflammation through modulating gut microbiota in high-fat diet-fed mice. FEMS Microbiol Lett. 2019;366. doi: 10.1093/femsle/fnz153

[48] Gao Z, Yin J, Zhang J, Ward R. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 2009;58. doi: 10.2337/db08-1637

[49] Guzior DV, Quinn RA. Review: Microbial transformations of human bile acids. Microbiome. 2021;9:140. doi: 10.1186/s40168-021-01101-1

[50] Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. Gender Medicine. 2009;6:60-75. doi: 10.1016/j.genm.2009.02.002.

Graphical Abstract Legend



Graphical Abstract | An oral glucose tolerance test was conducted before and after an 8-week dietary intervention containing 0, 300, or 600g weekly of cooked green lentils. During the intervention, participants were also asked to complete satiety and gastrointestinal symptom surveys once a week. We found that with lentil consumption, meal enjoyment is associated with multiple measures of satiety, reported, gastrointestinal symptoms were low in frequency and severity, and prevents a rise in insulin resistance.

Figure Captions

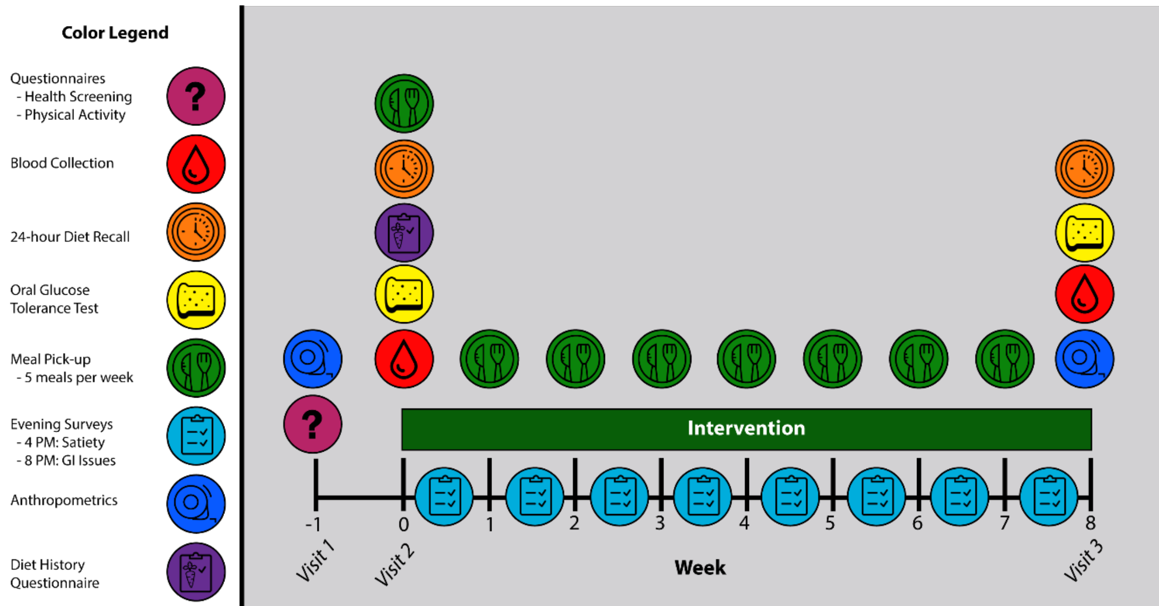


Figure 1 | **Time course of the eight-week dietary intervention.** Timeline of study events and how frequently events occurred during the course of study participation.

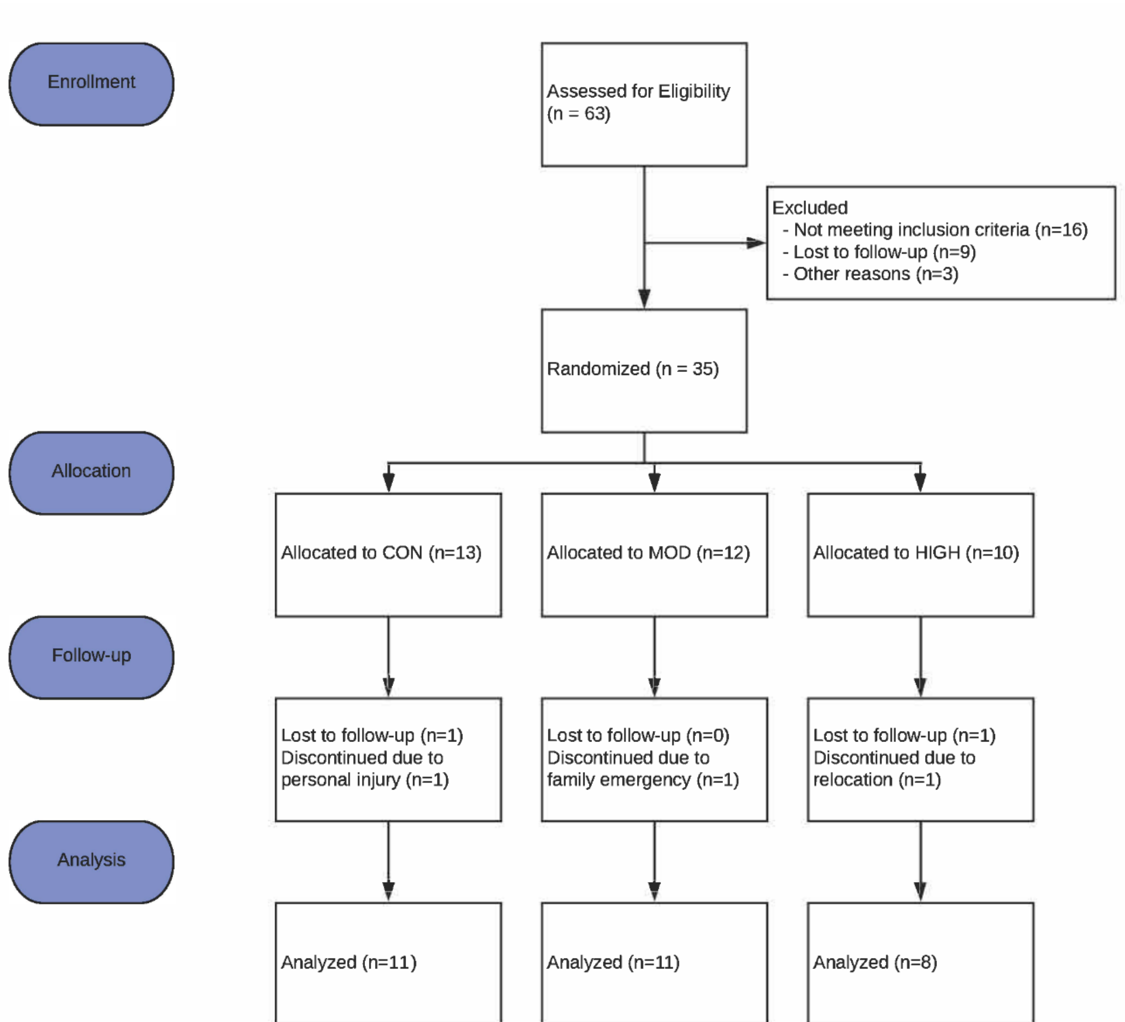


Figure 2 | **A CONSORT flowchart of the study design and enrollment.** Flyers and e-mails were used to recruit interested individuals. Interested individuals contacted the research team, who sent additional information about the study. Sixty-three individuals completed a phone screening with the research team with those individuals who met eligibility requirements, enrolled to participate. Participants were randomly allocated to one of three experimental groups with varying weekly doses of lentils: CON, control (0 g/week); MOD, moderate (300 g/week); HI, high (600 g/week).

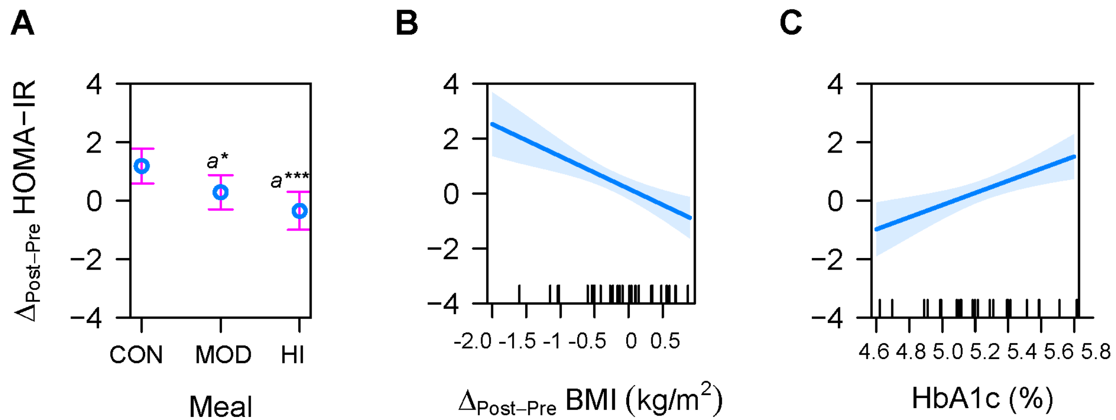


Figure 3 | **Plots for estimated beta coefficients of predictor variables for the change in HOMA-IR.** Bands and bars indicate 95% confidence intervals and the ticks on the x-axis in B) and C) indicate the observed values. Blue points in A) and the blue line in B) and C) indicate the average change in HOMA-IR as determined by the general linear model. One MOD participant was identified as an influential point and removed from HOMA-IR analysis. The symbol *a* indicates difference from CON. P-values are at a level of * 0.05, ** 0.01, and *** 0.001. Acronyms: BMI, body mass index; Hba1c, glycated hemoglobin; HOMA-IR, homeostatic model of insulin resistance; CON, control (0 g/week); MOD (300 g/week), moderate; HI, high (600 g/week).

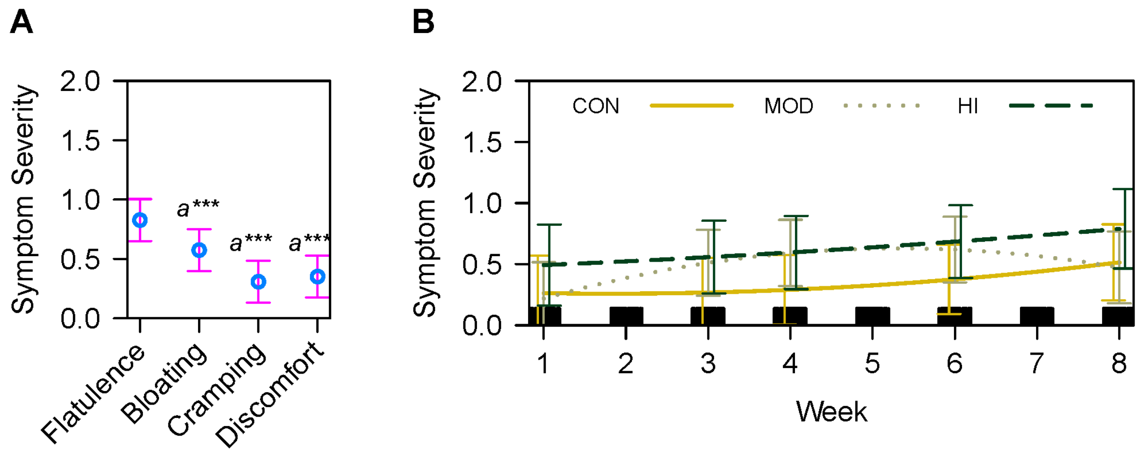


Figure 4 | **Gastrointestinal issue symptom severity with A) Symptom Main Effect and B) Meal and Time Interaction.** Symptom severity was scored as follows: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe. Points indicate group means, bars indicate 95% confidence intervals, and ticks on the x-axis indicate observed data frequency. The symbol *a* indicates difference from flatulence. P-values are at a level of * 0.05, ** 0.01, and *** 0.001. Acronyms: CON, control (0 g/week); MOD (300 g/week), moderate; HI, high (600 g/week).

Tables

Table 1 | **Sample weekly menu from the 8-week dietary intervention and weekly total nutrient profile.** Macronutrient totals were rounded to nearest whole number. Calorie total reflects unrounded amount calculation.

	CON – 0 g	MOD – 300 g	HI – 600 g
Bolognese	Turkey Bolognese + ½ cup cooked rotini pasta	Lentil & Turkey Bolognese + ½ cup cooked rotini pasta	Lentil Bolognese + ½ cup cooked rotini pasta
Curry	Chicken curry + 1/3 cup cooked basmati rice	Lentil & 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 & 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 & 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	Lentil + Chicken Soup	Lentil Soup
Nutrient Total	1658 kcal, 114 g PRO, 143 g CHO, 13 g Dietary Fiber, 72 g Total Fat, 27 g Saturated Fat	1714 kcal, 99 g PRO, 212 g CHO, 49 g Dietary Fiber, 55 g Total Fat, 22 g Saturated Fat	1774 kcal, 88 g PRO, 270 g CHO, 79 g Dietary Fiber, 42 g Total Fat, 19 g Saturated Fat

Abbreviations: control, CON moderate lentil, MOD; high lentil, HI; Kcal, kilocalories; PRO, protein; g, grams; CHO, carbohydrate.

Table 2 | **Participant characteristics at baseline, grouped by assigned dietary intervention (n=30).** Blood profile determined from venous blood sample at fasting. Data represent mean and standard deviation. Sex p-value determined through 3-sample test for given proportions; all other p-values determined by ANOVA.

	CON (n=10)	**MOD** (n=11)	**HI** (n=9)	***p*-value**
Males/Females	1/9	3/8	1/8	**<0.01**
Age (years)	38.8 ± 10.9	43.1 ± 12.5	43.0 ± 12.3	0.66
BMI (kg/m^2)	35.5 ± 5.2	34.2 ± 6.8	35.9 ± 7.3	0.82
FM (%)	45.3 ± 5.4	41.7 ± 8.4	47.0 ± 7.1	0.25
WC (cm)	109.7 ± 13.9	108.0 ± 12.1	107.1 ± 11.2	0.90
VAT (l)	3.5 ± 1.8	3.5 ± 1.6	3.3 ± 1.3	0.94
GLU (mmol/L)	4.9 ± 0.7	5.2 ± 0.6	5.2 ± 0.4	0.21
TG (mmol/L)	1.4 ± 0.7	1.3 ± 0.5	1.5 ± 0.5	0.70
CHOL (mmol/L)	4.9 ± 1.0	4.6 ± 0.9	4.8 ± 0.7	0.76
HDL (mmol/L)	1.4 ± 0.4	1.3 ± 0.3	1.5 ± 0.6	0.39
HbA1c (%)	5.2 ± 0.3	5.2 ± 0.3	5.4 ± 0.3	0.10

Abbreviations: control, CON moderate lentil, MOD; high lentil, HI; body mass index, BMI; fat mass, FM; visceral adipose tissue, WC, waist circumference; VAT; hemoglobin A1c, HbA1c; glucose, GLU; triglycerides, TG; cholesterol, CHOL; high density lipoprotein, HDL.

Table 3 | **HEI component scores at baseline, grouped by assigned meal group (n=27)**. HEI component scores were calculated through an online DHQ III in which participants self-reported the types and frequency of foods consumed in the last 12 months. Three participants (one from each meal group) did not fully complete the DHQ III and thus, HEI scores were not available. Information about the standards for scoring each HEI component can be found in an article by Krebs-Smith and colleagues [24]. Data represents mean and standard deviation. Meal effect p-values determined by Type II F-test.

		Score			
Component	**Maximum Points**	**CON** (n=9)	**MOD** (n=10)	**HI** (n=8)	***p*-value**
Adequacy					
Total Fruits	5	2.6 ± 1.2	3.9 ± 1.5	3.2 ± 1.4	0.15
Whole Fruits	5	3.6 ± 1.6	4.4 ± 1.5	3.8 ± 1.2	0.48
Total Vegetables	5	3.7 ± 1.1	4.7 ± 0.5	3.1 ± 1.3	**0.01**
Greens and Beans	5	4.4 ± 1.1	4.9 ± 0.2	3.4 ± 1.6	**0.03**
Whole Grains	10	2 ± 1.1	2.6 ± 1.6	3.6 ± 2	0.13
Dairy	10	8.1 ± 2	6.3 ± 1.8	7.8 ± 1.7	0.09
Total Protein Foods	5	4.8 ± 0.4	4.9 ± 0.3	4.7 ± 0.6	0.66
Seafood & Plant Proteins	5	4.5 ± 0.8	4.3 ± 1	4.4 ± 1.1	0.90
Fatty Acids	10	2.6 ± 2.1	2.9 ± 3.2	4.8 ± 2.8	0.19
Moderation					
Refined Grains	10	8.4 ± 1.5	8 ± 3.3	9.4 ± 1	0.40
Sodium	10	3 ± 1.8	4.1 ± 3.2	5.5 ± 2.6	0.17
Added Sugars	10	7.6 ± 1.2	7.8 ± 2.1	6.3 ± 3	0.30
Saturated Fats	10	2.9 ± 2.2	5.6 ± 2.8	4.1 ± 3.1	0.13
Total		58.2 ± 6.7	66.1 ± 10.3	62.3 ± 13.2	0.26

Abbreviations: control, CON; moderate, MOD; high, HI.

Table 4 | **P-values of the β-coefficients derived from mixed effects models on satiety survey measures.** Satiety survey questions and response levels are provided in **Supplementary File 2**. Meal*Week indicates an interaction between meal group (CON, MOD, HI) and time (week).

Question	**Meal**	**Week**	**Meal Enjoyment**	**Meal*Week**
Meal enjoyment	0.43	**0.05**	NA	**0.03**
Hunger	0.53	0.44	**0.02**	0.24
Fullness	0.93	0.39	0.07	0.87
Satisfaction	0.83	0.78	**<0.001**	0.20
Desire to eat	**<0.01**	**0.02**	**<0.001**	0.99
Amount could eat	**0.01**	0.25	**<0.001**	0.57

Supplementary File 1 | **Nutrient and functional analysis of whole green lentils used in the intervention.**

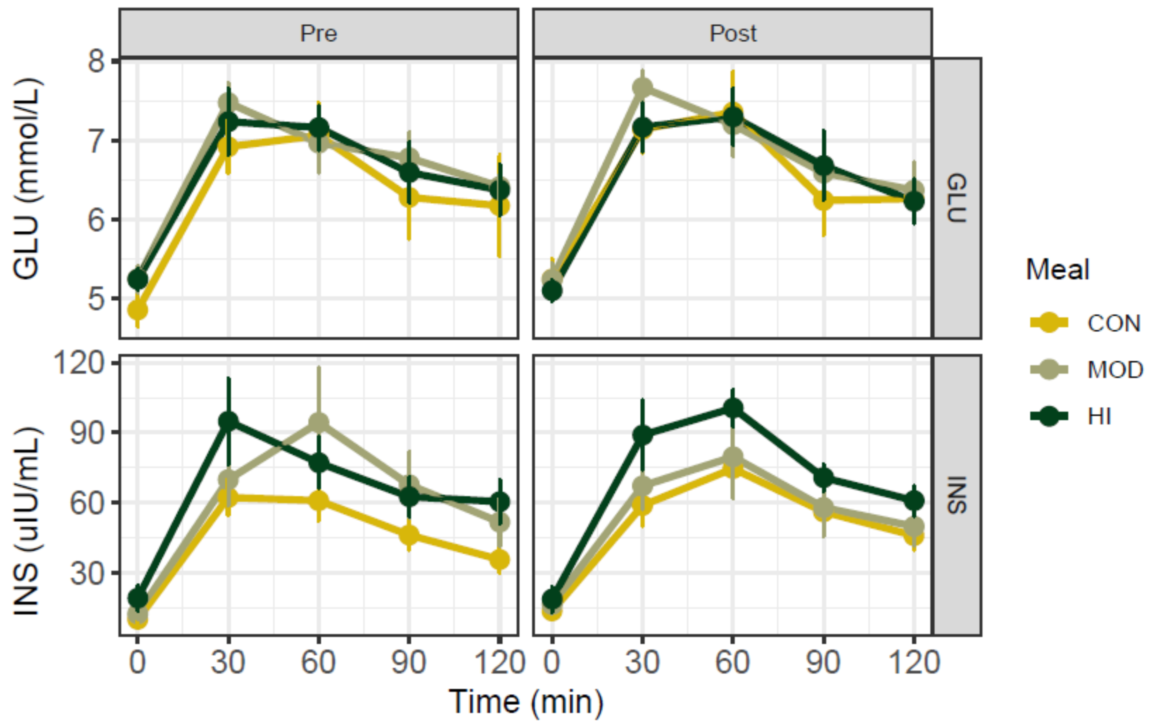
Analysis	Amount
Nutrient (per 100g)	
Calories (kcals)	369
Moisture (g)	8.2
PRO (g)	23.5
Fat (g)	2.3
Ash (g)	2.4
Total CHO (g)	63.6
Soluble Fiber (%)	0.7
Insoluble Fiber (%)	12.5
Starch (g)	43.4
Functional	
Foam Expansion (%) (5% w/w)	226
Water Holding Capacity (g/g)	1.3
Foam Stability (%)	96
pH (5% w/w)	6.3

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

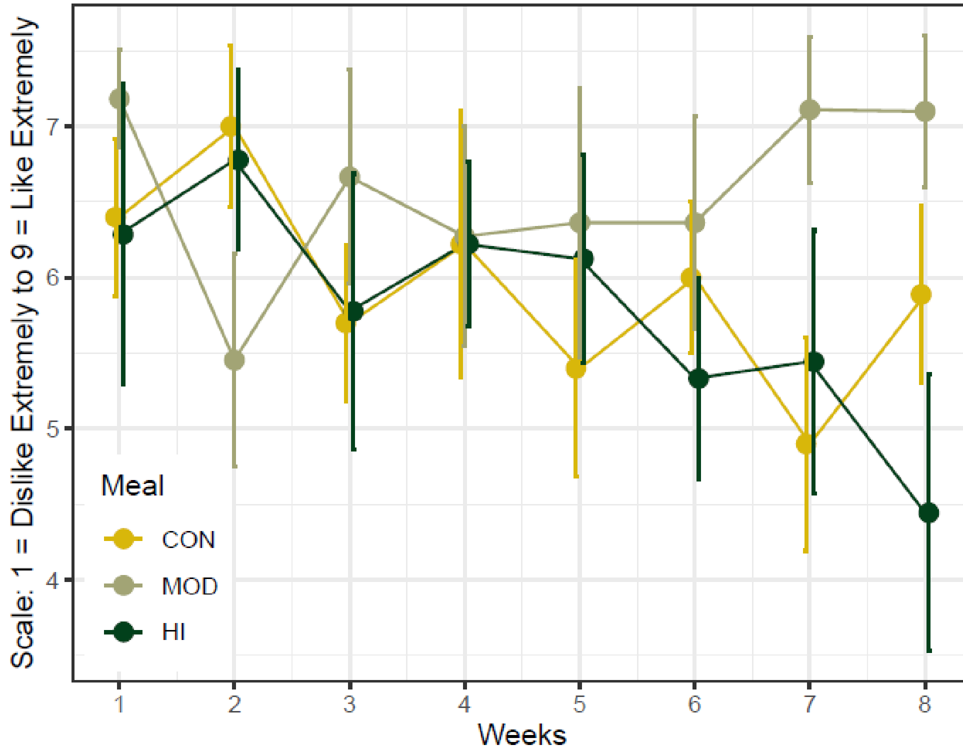
Supplementary File 2 | **Survey questions assessing satiety and gastrointestinal issues**. Surveys were delivered once a week each week of the 8-week dietary intervention through an online survey. Symptom severity was scored as follows 0=None, 1=Mild, 2=Moderate, 3=Severe.

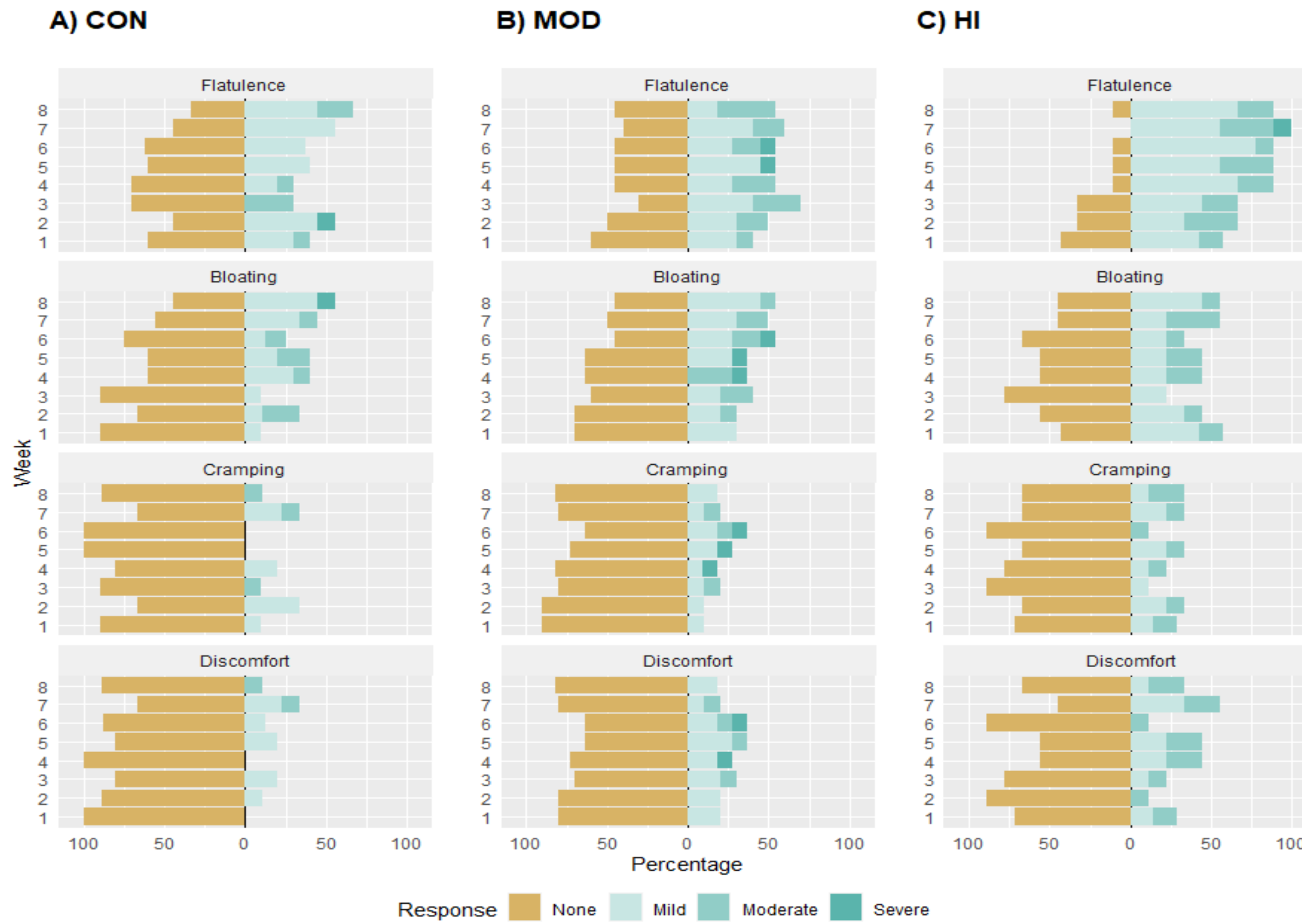
Question	Response
4:00 PM Satiety	Ordinal
How much do you like your meal provided today?	1 = dislike extremely, 5= neither like or dislike, 9 = like extremely
How hungry are you?	0 = not at all, 10 = extremely
How full are you?	0 = not at all, 10 = extremely
How satisfied do you feel?	0 = not at all, 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 File 3 | **Averages of glucose (GLU) and insulin (INS) concentrations during the OGTT before and after the dietary intervention**. Points represent averages per time point by group with bars indicated standard deviation of the mean. Acronyms: CON, control (0 g/week); MOD (300 g/week), moderate; HI, high (600 g/week).



Supplementary File 4. **Meal enjoyment over the course of an 8-week dietary intervention with varying weekly doses of lentils**. Points represent weekly means with bars depicting standard deviation. Acronyms: CON, control (0 g/week); MOD (300 g/week), moderate; HI, high (600 g/week).





Supplementary File 5. **Frequency of self-reported gastrointestinal symptom severity over the 8-week dietary intervention.** Percentages reflect the frequency of the severity level each week by meal group. CON, control (0 g/week); MOD (300 g/week), moderate; HI, high (600 g/week).

CHAPTER FOUR

INFLAMMATORY STIMULUS IN HUMANIZED MOUSE MODELS REVEALS THE ANTIOXIDANT EFFECTS OF ARONIA SUPPLEMENTATION

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Cell Metabolism

Title: INFLAMMATORY STIMULUS IN HUMANIZED MOUSE MODELS REVEALS THE ANTIOXIDANT EFFECTS OF ARONIA SUPPLEMENTATION

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Summary

Aronia melanocarpa products are an emerging health-food product based on the high antioxidant potential and high phenolic content of Aronia berries. Antioxidant-rich foods, such as Aronia, have been shown to counter inflammatory stimuli and positively modulate the gut microbiome. We therefore sought to investigate microbial and metabolomic profiles through the lens of inflammation and Aronia supplementation. This was accomplished through the humanization of gnotobiotic mice with human donors of different inflammation status and an 8-week study where humanized mice were supplemented with either Aronia juice or a matched carbohydrate beverage. Stool and blood samples were collected throughout the study and were examined via next-generation sequencing and mass spectrometry analysis, respectively. Combining multiple analytical tools allowed for a robust view of the impact of Aronia on the gut microbiome, metabolism and inflammation.

Keywords (MeSH Terms): gastrointestinal microbiome; diet, high fat; obesity; inflammation; functional food; metabolomics

Introduction

Chronic low-grade inflammation is a pathological characteristic of obesity-related conditions such as metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease and is noted by increased and sustained systemic levels of proinflammatory cytokines with no resolution (Duncan et al., 2003; Hotamisligil, 2006; Volpato et al., 2001). The accumulation and pro-inflammatory activation of macrophages in expanding adipose tissue, particularly around the abdomen, is considered a major contributor to chronic low-grade inflammation and promotes a detrimental shift in glucose and lipid metabolism (Tall and Yvan-Charvet, 2015; Weisberg et al., 2003; Wiklund et al., 2016). In addition to central adiposity, altered glucose metabolism, lipid metabolism, and hypertension are key components of metabolic syndrome, which proceeds and underlies major chronic diseases (Huang, 2009). As an early driver of metabolic syndrome and chronic disease progression, intervention strategies are needed to counter inflammatory stimuli and chronic low-grade inflammation in metabolically at-risk populations.

Of growing clinical interest are interactions between the gut microbiota, a large group of diverse bacteria in the human gastrointestinal tract, and altered metabolic states in obesity and related conditions (Ley et al., 2005; Li et al., 2017; Qin et al., 2012; Turnbaugh et al., 2006). A strong body of evidence in human and animal models supports the contribution of the gut microbiota to obesity development through increased energy harvest (Ley et al., 2005; Musso et al., 2011; Perry et al., 2016; Qin et al., 2012; Turnbaugh et al., 2006; Verdam et al., 2013). The composition and functionality of the gut microbiota, and ability

to influence the host, is largely dependent on genetic and environmental factors (Heintz-Buschart and Wilmes, 2018; Rothschild et al., 2018). Host diet, as a modifiable environmental factor, has been shown to influence gut microbial composition (Arumugam et al., 2011; David et al., 2014; Rothschild et al., 2018; Turnbaugh et al., 2009). A high-fat diet (HFD) is often utilized in animal models to promote obesity and has been shown to concomitantly promote inflammation in the colon, adipose tissue, skeletal muscle, and the liver (Lam et al., 2012; Lee et al., 2011; Serino et al., 2012). The link between the gut microbiota and host-inflammation is suspected to be increased gut barrier permeability, which is affected collectively by alterations in gut bacteria, mucus bilayer composition, intestinal epithelial cells held together by tight junction proteins, and immune cells (Cani et al., 2008; Rohr et al., 2020; Stremmel et al., 2012). Chronic exposure to dietary fat may negatively affect tight junction proteins and mucosal layer integrity, promoting increased translocation of bacterial constituents into the lamina propria where they can induce an inflammatory response and initiate a positive feedback loop (Cani et al., 2008; Cani et al., 2009; Rohr et al., 2020). Thus, frequent perturbations to barrier function can have profound clinical implications in diseases with localized inflammation and may partially explain a rise in systemic inflammation.

To combat inflammation, incorporation of functional foods may serve as a beneficial dietary strategy for metabolically at-risk populations to reduce the onset and progression of chronic disease (Comalada et al., 2005; Hertog et al., 1993). Functional foods exert various health benefits, with phenolic-rich plants in particular countering inflammation by

neutralizing oxidative damage (Halliwell, 2007). Phenolic plant compounds are natural antioxidants which can mediate cellular damage from free radical scavenging and cellular signaling affecting inflammatory gene expression (Soobrattee et al., 2005). The amount of anthocyanin absorbed from the stomach and small intestine differs between anthocyanins, but the anthocyanins that reach the large intestine are subject to bacterial degradation into phenolic metabolites (Fang, 2014). In the distal gut, anthocyanins may positively impact gut barrier integrity and prevent further translocation of bacterial constituents, reducing stimuli for downstream proinflammatory processes (Gil-Cardoso et al., 2016). In addition to a potential localized effect, anthocyanins which enter the blood have the potential to exert anti-inflammatory properties systemically. However, the ability of phytochemicals to affect the host is partly dependent on gut bacterial metabolism (Fernandes et al., 2014; Laparra and Sanz, 2010).

The chokeberry, or *Aronia*, is of particular interest as an antioxidant-rich food. This hardy shrub produces berries with the highest known antioxidant capacity for fresh fruit as measured by oxygen radical absorbance capacity (Nutrient Data, 2010). Commercial varieties are derived from a cross between *Aronia melanocarpa*, which is native to the United States, and European Mountain Ash (*Sorbus aucuparia*) sometimes classified as *Aronia mitschurinii* (Leonard et al., 2013). Aronia berries have almost four times the anthocyanin content of blueberries, a lauded natural antioxidant-rich food (Kulling and Rawel, 2008). The antioxidant capacity of Aronia berries stems from two main groups of phenolic compounds: anthocyanins and procyanidins. Aronia anthocyanins have been

previously shown to reduce inflammatory stimuli (Banjari et al., 2017; Bräunlich et al., 2013; Martin et al., 2014; Oszmianski and Sapis Jean, 1988; Taira et al., 2015), the expression and concentration of pro-inflammatory cytokines (Bräunlich et al., 2013; Kim et al., 2015; Martin et al., 2014; Palócz et al., 2016), and influence the colonic environment (Taira et al., 2015).

Dietary strategies, such as increased antioxidant availability through consuming phenolic-rich foods, present possible targeted therapeutic avenues for early chronic disease prevention. Dietary patterns can positively or negatively modulate gut microbiota composition and function, both important in mediating host responses through production of bioactive compounds. Therefore, compounds in host systemic circulation may provide key insights into the dynamic interplay between the gut microbiome and the host. In this study, we recruited healthy but metabolically at-risk human subjects and assessed their metabolic and systemic inflammatory profile. We created a humanized mouse model by transplanting stool from a human donor with high or low systemic inflammation into germ-free mice to account for variability in host genetics and host gut microbiota. We then introduced Aronia or control juice to their offspring and added a HFD as an inflammatory stimulus. We investigated whether ingestion of polyphenolic rich Aronia juice could offer protective effects against HFD, and if that protection is dependent on gut microbial alterations including the inflammation phenotype of the human donor.

Results

Human subjects were physically similar but differed in inflammation, insulin resistance, and metabolic syndrome. Of the 54 individuals screened to participate, forty subjects (women, n=24; men, n=16), completed the full study (**Supplemental Figure 1**). Subjects were 21 to 55 years of age with a mean (SD) age of 36.4 (10.1) years and BMI of 30.5 (2.0) $kg/m.^2$. This cohort was metabolically at-risk based on BMI criteria and did not present with metabolic disease. Subjects completed an oral fat challenge to which the postprandial lipemic and inflammatory responses have been explained previously (Peach et al., 2021; Wilson et al., 2021).

We used high-sensitivity bead-based multiplexing on fasting serum samples from all 40 subjects to determine concentrations of six proinflammatory cytokines. Subjects were grouped as low or high inflammation based on their six baseline cytokine makers. Nineteen subjects classified as low (LO), and sixteen classified as with high (HI) inflammation phenotype. Five subjects were not classified as having LO or HI inflammation (**Supplemental Figure 2**). A k-means analysis of postprandial inflammatory responses grouped 17 participants as LO, 14 as mid (MID) and 9 as HI. Physical and fasting metabolic characteristics between fasting inflammation groupings were largely similar, except for HOMA-IR and proportion of individuals with metabolic syndrome (**Table 1**). In the context of established metabolic syndrome criteria, HI subjects had a greater proportion of individuals presenting with metabolic syndrome than LO (45.5% vs 26.7%). Average HOMA-IR in HI subjects was 1.6 HOMA-IR units greater than LO ($p = 0.04$).

The proportion of individuals with metabolic syndrome was also different by inflammation response groupings ($p = 0.02$), with a larger proportion of HI inflammation responders presenting with metabolic syndrome. We also detected a difference in total cholesterol ($p = 0.02$), with LO responders having higher cholesterol than MID and HI responders.

Stool donors were metabolically similar but differed in inflammation and alpha diversity. Two subjects were selected in total from the human cohort based on their fasting and postprandial inflammatory profile to be stool donors (**Figure 1).** Selected subjects were female, without metabolic syndrome, and differed in their inflammation profile. The LO donor was LO in fasting inflammation and their postprandial response to an oral fat load. Similarly, the HI donor was HI in fasting and their postprandial inflammatory response. In addition, the LO stool donor had a higher OTU richness (377 vs 338) and Shannon Index (4.1 vs 3.7) than HI. LO and HI donors also differed in β-diversity (**Figure 2A**).

Microbial community of stool donor was effectively transferred to second-generation pups by fecal microbiota transplant to germ-free dams. We compared the gut microbial composition of pups from dams inoculated with different fecal microbiota transplant (FMT) human donors. Beta-diversity analysis using Bray-Curtis PCoA plots demonstrates that second-generation mice with direct exposure from their inoculated GF mice parent resemble their respective human stool donor (**Figure 2B**). Moreover, the microbial community was distinct by donor, a distinctness detectable throughout the duration of the mouse experiment ($R = 0.343$, $p < 0.001$, $ANOSIM_{donor}$).

Aronia juice increased α-diversity and offered a protective effect against HFD-induced changes in a donor-dependent manner. At baseline, second-generation HI INF mice had a lower Shannon index, a measure of α-diversity, on average compared to mice exposed to LO INF microbiome (β = -0.37, p = 0.001). Second-generation mice began a two-week acclimatization period with CON and ARO juice. A donor main effect for ARO on α-diversity was observed with an average increase in the Shannon index of 0.28 (p = 0.045) after two weeks (**Figure 3A**). We did not detect a three-way interaction between juice treatment, time, and donor (p = 0.28).

After the juice acclimation, we presented a six-week HFD to induce obesity and as an inflammatory challenge in the presence of juice. With the introduction of a HFD, α-diversity declined with CON juice irrespective of FMT donor microbiome exposure (**Figure 3B**). ARO juice provided protection from HFD-induced loss of α-diversity but only in second-generation mice exposed to LO INF microbiome (p = 0.02). HI mice which received ARO juice displayed the same decline in α-diversity as CON groups.

We performed a canonical correspondence analysis (CCA) to understand the response of the microbial community to the introduction of juice and eventually the joint impact of juice and HFD. In **Figure 4A**, a distinct separation in the second-generation microbial community based on the original human FMT donor is observed (F = 34.0, p = 0.001). Additionally, the introduction of juice promoted a similar shift in β-diversity that was

donor-independent (F = 4.5, p = 0.001). However, mice which received ARO had a decreased shift in β-diversity over two weeks compared to mice on CON (F = 4.2, p=0.001). A shift in β-diversity was seen after only two weeks on an HFD (F = 9.0, p = 0.001) and was less pronounced in LO mice (F = 6.7, p = 0.001) (**Figure 4B**). A modest Aronia effect on β-diversity was observed across LO and HI and was in juxtaposition with the HFD (F = 2.7, p = 0.004).

With the addition of Aronia and control juice, eight and twelve bacterial genera were altered and unique to LO and HI second-generation mice respectively (**Figure 5A**). Twelve shared bacterial genera were altered with juice supplementation in LO and HI. Of the twelve genera that were shared, the abundance of *Alistipes*, *Bacteroides*, *Barnesiella*, *Butyricmonas*, *Odoribacter*, *Paraprevotella*, and *Phocaelcola* decreased in response to juice supplementation with the decline shared across LO and HI INF mice. *Blautia* and *Collinsella* were among the genera that increased with juice supplementation in both groups with greater increases seen in LO. A large increase in Bifidobacterium with supplementation was detected in LO but not HI mice. Treatment-specific alterations relative to CON juice include increased *Eisenbergiella, Faecaelbacillus,* and *Ruminococcus* in HI and increased *Turibacter* in LO. LO INF mice had elevated *Butyricimonas* and *Coprobacter* at the onset of the experiment compared to HI INF mice with the concentrations of both genera decreasing with the introduction of sugar-rich juice.

A HFD introduced widespread microbial changes in second-generation mice that were donor-independent and donor-dependent (**Figure 5B**). Both donor groups of second-generation mice had fourteen genera each which were altered with HFD. Among bacterial genera which were shared between LO and HI second-generation mice, we found increases in eleven bacterial genera with a HFD that were dependent on donor. LO INF mice were enriched in *Bilophila* and *Alistipes* from HFD compared to HI INF mice. HI INF mice had greater *Collinsella* and *Escherichia-Shigella* and decreased *Howardella* with HFD compared to LO INF mice. Additional impacts of Aronia supplementation were observed with *Akkermansia, Bilophila, Bifidobacterium, Barnesiella*, *Lactococcus, Ruminococcus,* and *Ruthenbacterium.* Aronia supplementation increased *Akkermansia, Lactococcus, Ruminococcus,* and *Ruthenbacterium* in both LO and HI INF mice. *Bifidobacterium* increased with CON juice but only in HI INF mice. Of the shared bacterial genera between LO and HI mice, the following genera decreased in abundance with HFD: *Bacteroides, Eisenbergiella, Neglecta, Paraprevotella, Negativibacillus,* and *Turibacter*. Aronia juice prevented HFD-induced losses in *Neglecta* and *Negativibacillus*, while other genera were similarly impacted despite the juice provided.

<u>Phenolic and carbohydrate analysis of several varieties of Aronia juice aided in treatment juice variety selection.</u> LCMS and NMR analyses were selected and completed to determine strain specific compound compositions of the Aronia juices. Three different varieties and two harvests of chokeberry were tested for phenolic and carbohydrate content (**Table 2**). From this data and juice availability, the Aronia blend was selected as the

treatment juice. Carbohydrate concentrations were also quantified in the Aronia blend and matched in the control juice **(Table 2).**

Metabolomics analysis of the mice before juice treatment indicated disparate metabolic profiles by donor. Our investigation into metabolomic profiling began with an analysis of serum samples from the first week, prior to juice treatment. From this analysis, different metabolomic profiles were observed in second-generation mice based on stool donor (**Figure 6A**). While the differences were global, a focused examination of the metabolites that best differentiated the groups was also performed. Mice transfected with the low-inflammation microbiome showed upregulation of diacylglycerides and other lipids relative to the mice transfected with the high-inflammation microbiome (**Figure 6B**). LO mice also demonstrated an increase in indolepyruvate, a metabolite associated with specific microbes found in human gut microbiomes.

An investigation into metabolomics data in week two showed the influence of juice treatment. Although the metabolite profile of initial serum samples grouped by donor; the effect of juice treatment is clearly seen by week two (**Figure 7A**). Week two samples begin to show definitive grouping based on treatment first, followed by donor (**Figure 7B**). A closer inspection of discriminative metabolites between treatments at week two indicate altered lipid metabolism in mice receiving Aronia supplementation relative to mice receiving matched carbohydrate juice. Specifically, phosphatidylcholines and sphingolipids were shown to be upregulated in mice receiving Aronia juice.

The introduction of a high-fat diet dramatically altered metabolomic profiles. After samples were collected in week two, the diet was changed to a high-fat diet. This alteration in diet led to dramatic changes in metabolic profiles that superseded the effects of both the microbiome and the juice treatment. A heatmap of discriminating metabolites in weeks two and week four demonstrates the impact of the high-fat diet. Grouping now occurs first by time, then by treatment and finally by donor (**Figure 8**). Upregulation of sphingolipids was found to discriminate pre- and post-HFD metabolomic profiles.

After 8 weeks of juice treatment, both treatment and donor had a significant influence on metabolomic profiles. Although the effect of treatment and donor were intertwined in their effects as shown by PCAs where the samples are grouped by treatment or donor, looking at treatments by donor or donors by treatment reveals the individual impacts of each variable **(Figure 9A-C)**. A closer examination of the discriminating metabolites between groups show that Aronia juice supplementation again results in an upregulation of specific PCs (**Figure 9D**).

TMAO levels were downregulated with Aronia supplementation. Due to the continued differences seen in PC concentrations between treatment groups, trimethylamine-N-oxide (TMAO) concentrations were investigated. TMAO concentrations were found to be significantly lower ($p < 0.001$) in the Aronia supplemented mice at the conclusion of the study (**Figure 10**).

Discussion

Using gnotobiotic mice, we were able to elucidate the impact of Aronia juice supplementation on the gut microbiome and the serum metabolome before and during a high-fat diet. Success in this study relied heavily on our ability to humanize mice by transferring microbiomes from human stool donors who were metabolically similar but had a different inflammatory profile and the ability to transfer the donor microbiome to second-generation pups. Additionally, we were able to generate unique metabolomic profiles in each donor specific mouse population. By exploring the beta diversity of the microbiomes along with the grouping of the global metabolomes and specific microbial populations and metabolites, our analysis indicated that two distinct humanized mouse populations were created. This is a significant observation that could impact the way in which inflammation is studied in animal models.

Each second-generation mouse population had a unique microbial profile that closely resembled that of the original human stool donor. Our human stool donors were metabolically similar and were chosen based on their distinct low and high systemic inflammatory profile. A higher microbial diversity was observed in the selected human LO microbiome profile versus HI microbiome profile, a trait that was also detected in the second-generation mice and helped separate the microbial communities into two distinct groups at baseline. The transfer of microbiomes from inoculated germ-free mice to the second-generation without declines in diversity has been previously observed (Turnbaugh et al., 2009). Additionally, our findings echo previous research which found that low gut

bacterial diversity in humans correlated with a pronounced inflammatory phenotype (Le Chatelier et al., 2013). High bacterial diversity is an accepted indicator of a healthy gut and promotes resistance in microbial communities to ecological perturbations and the ability to return to equilibrium (Ley et al., 2005). Along with differing MBs, metabolomic analysis indicated discrete global profiles from each donor population. Metabolomic profiles grouped based on donor and indicated the presence of discriminating metabolites. One of discriminating metabolites, indolepyruvate, can only be produced by specific *Clostridium* species. Indolepyruvate was upregulated in the LO mice, which also showed a greater abundance of *Clostridium* relative to the HI mice.

After two weeks of juice supplementation and normal diet, a shift was detected in the gut microbiota of second-generation mice. Juice rich in glucose, fructose, and sorbitol in general initiated a pronounced shift in β-diversity after two weeks across both LO and HI mice. Regardless of donor, juice elicited similar effects across genera. The impact of polyphenolic compounds in Aronia on β-diversity was minimal though we did detect an Aronia main effect for increased α-diversity in second-generation mice. Bacterial changes specific to Aronia supplementation were donor-specific and included increased *Eisenbergiella, Faecaelbacillus,* and *Ruminococcus* in HI and increased *Turicibacter* in LO. *Eisenbergiella* is negatively correlated with inflammatory gene expression (Li et al., 2020), and *Faecalbacillus* was found to positively associate with hypertension (Louca et al., 2021), with the metabolic capacity of either genus still largely unknown. *Ruminococc*i spp., is strongly host-associated and requires fermentable carbohydrates for growth (La

Reau et al., 2016; La Reau and Suen, 2018). Aronia and the control juice were matched for sugars which make the Aronia-driven increase with *Ruminococcus* surprising. Increased *Turicibacter* is consistent with decreased inflammation in obesity (Jiao et al., 2018) with anti-inflammatory effects potentially mediated in part by butyrate production (Zhong et al., 2015).

Along with donor-specific changes in microbes, two weeks was sufficient to see a notable change in metabolic activity wherein metabolomic profiles grouped by juice treatment. This was a shift from the initial clustering where profiles were grouped by donor. This metabolic switch was characterized by an increase in PCs in Aronia treated mice. Increased PC composition has been indicated in a variety of positive health benefits. In addition to their role as structural components of cellular membranes, PCs can counter LPS-induced inflammation by inhibiting TNF expression as well as limiting inflammatory responses by suppressing pro-inflammatory MAPK and CAM pathway signaling (Chen et al., 2018; Pyun et al., 2020; Tan et al., 2020). These positive benefits are augmented by the ability of PCs to impact membrane composition and fluidity. Barriers in the intestinal mucus are ameliorated with higher PC composition and a decrease in PC composition is seen in inflammatory diseases like IBD and UC (Stremmel et al., 2021; Treede et al., 2009; Treede et al., 2007). This is likely due to PC-specific modulation of membrane composition and a PC-induced increase in membrane fluidity, which has been shown to promote normal cell function. Reduction in PCs has been indicated in rigid membranes and an increase in aging processes (Dai et al., 2021; Dawaliby et al., 2016; Fajardo et al., 2011). Further, the

increased serum PCs seen in Aronia treated mice may be due to complexation of the polyphenolic compounds in Aronia with phosphatidylcholines. Polyphenolics are larger molecules which do not pass easily through the intestinal barrier by diffusion, thereby limiting the functional antioxidant capacity. PC-polyphenolic complexation improves miscibility and absorption from the gut for host bioavailability (Gupta and Dixit, 2011; Sharma et al., 2010). Given we did not observe phosphatidylcholine increases in CON mice receiving the same base diet, we propose that the increase in phosphatidylcholine with Aronia supplementation was facilitated through the complexation process of polyphenolic compounds present in Aronia juice.

To induce obesity and elicit an inflammatory response, the normal diet (13.3% fat) was switched to a high-fat diet (41.7% fat), reflective of a Western Diet, after two weeks on Aronia or sugar-matched control treatment. This allowed for a comparison of Aronia supplementation with and without an inflammatory stimulus as well as providing a model to determine the impacts associated with a HFD. Seminal work in germ-free mouse models has shown that obesity development with a HFD is dependent on the gut microbiota (Bäckhed et al., 2007). Further, HFD-induce reproducible shifts in the gut microbiome and these changes are independent of fluctuations in body weight (Bisanz et al., 2019; Xiao et al., 2017) and stimulate inflammation systemically in a microbiota dependent manner (Cani et al., 2008; Cani et al., 2009; Duan et al., 2018).. We observed a large HFD- induced shift in the microbial community across donor groups. We detected elevated *Lactococcus* with HFD, a finding which may be attributed to dietary contaminants in experimental HFD diet

(Bisanz et al., 2019). It was further observed that *Collinsella* was enriched with HFD, with greater concentrations observed in HI INF mice. *Collinsella* is positively associated with a fiber-poor diet, serum cholesterol, triglyceride, and insulin levels (Astbury et al., 2020; Gomez-Arango et al., 2018; Lahti et al., 2013) which supports dysregulated lipid metabolism consistent with obesity development. Of the donor-independent reductions we detected with HFD, reduction in *Eisenbergiella* and *Turicibacte*r may reflect an increased proinflammatory environment (Jiao et al., 2018; Li et al., 2020). HFDs promote increased gut permeability and allow for increased chylomicron transport and translocation of lipopolysaccharide (LPS), a potent immunological stimulator (Cani et al., 2008; Cani et al., 2009; Ghoshal et al., 2009). While we did not measure LPS in the study, LPS has been previously shown to increase 2.7-fold with a HFD compared to standard diet (Cani et al., 2007). When LPS translocates from the gut into the blood through paracellular diffusion or incorporation through chylomicrons, it is recognized by the LPS-binding protein and transported to the membrane bound CD14/TLR4 complex on monocytes and macrophages (Cani et al., 2007; Ghoshal et al., 2009). The outcome of LPS signaling is the upregulation of pro-inflammatory cytokines such as TNF-α (Copeland et al., 2005) and TLR4 in the liver and adipose tissue (Fei and Zhao, 2013; Manco et al., 2010).

The influence of the HFD was also seen in the metabolomics data where metabolomic profiles from week two (pre-HFD) and week four (two-weeks after starting HFD) separated by week and not by donor or juice treatments. A comparison of the top discriminating features through hierarchical clustering between weeks two and four

showed strong grouping pre- and post-HFD introduction. Significant changes were observed indicating a large-scale shift in metabolic activity due to the HFD and the associated inflammatory stimulus. A closer look at specific discriminating metabolites revealed that sphingomyelin was upregulated after the HFD introduction. Sphingolipids are important components of cellular membranes and are signaling molecules important in the regulation of inflammatory pathways (Brown et al., 2019; Nixon, 2009). Although definitive clarity of the effects of sphingolipids on inflammation requires further study, initial results have demonstrated TNF dependent increases in sphingomyelin (Spiegel and Milstien, 2011). Upregulation of sphingomyelin induces production of sphingomyelin-1-phosphate (S1P) through the action of the kinases Sphk1 and Sphk2 in the sphingolipid-to-glycerolipid pathway (Dobrosotskaya et al., 2002). S1P increases have been associated with immune recruitment, lymphocyte activity, inflammation and progression of inflammatory diseases and cancers (Nagahashi et al., 2018). In this study, we also observed a HFD-induced decline in *Bacteroides,* a Bacteroidetes genus implicated in the production of sphingolipids (Johnson et al., 2020). A lack of *Bacteroides*-derived sphingolipids was previously shown to increase intestinal inflammation in irritable bowel disease subjects though interestingly, host-derived sphingolipids were increased systemically (Brown et al., 2019). Therefore, it is likely that the upregulation of sphingomyelin we detected was an early stress response to increased dietary fat load, mediated by elevated LPS-stimulation of TLR4 and proinflammatory activation.

The mice continued the experimental juice treatment for an additional six weeks concomitant with a HFD. After six weeks, additional changes were seen in both the microbiota and the metabolomic profiles of each juice treatment group. A key finding in this study was the donor-dependent protective effect of Aronia supplementation from a HFD-induced decline in α-diversity. Further, HFD facilitated a prominent shift in the β-diversity of the microbiota to which the LO INF mice were more resistant. The impact of Aronia supplementation on the global microbial community composition during HFD was modest. However, a clear set of microbial genera were enriched with HFD. Aronia supplementation prevented a rise in *Barnesiella,* and *Escheria-Shigella* and promoted greater *Bilophila, Ruminococcus,* and *Ruthenbacterium* during the HFD. Alterations were donor dependent with Aronia supplementation affecting taxa in LO INF mice more prominently than HI. Higher numbers of the genera *Barnesiella* and *Escheria-Shigella* have been observed with HFD (Le Roy et al., 2013; Singh et al., 2020). *Bilophila* is a bile-tolerant microorganism that is associated with greater fat intake (David et al., 2014). *Bilophila* members are also unique in that they can use taurine as a terminal electron receptor and produce hydrogen sulfide, known for its antioxidant potential. Drastic decreases in *Ruminococcus* are linked to increased intestinal permeability which may have larger implications in TLR4 driven inflammatory activation (Leclercq et al., 2014). Limited information is available on *Ruthenbacterium*, a relatively new genus (Shkoporov et al., 2016). *Ruthenbacterium* produces acetic and succinic acid from glucose fermentation, which is consistent with juice consumption, but its relationship to polyphenolics is unclear. Taken together, these microbial changes support the anti-inflammatory role of Aronia.

After the HFD introduction, metabolomic profiling indicated the continued influence of Aronia supplementation. However, the donor impact was stronger with high-fat diet than with normal diet and more donor-specific effects were discovered. Due to multiple strong effectors, examination of the metabolomic profiles show little separation by donor or by treatment. Yet, an analysis of metabolomic profiles for each donor indicated different characteristics based on juice treatment. This provides evidence that different donor microbiota interacts differently with the antioxidant rich Aronia juice leading to disparate metabolomic profiles. A focused analysis at 8-weeks was also completed and showed donor and treatment specific metabolites upregulated in the mouse models with Aronia supplementation again resulting in an increase in PCs. To determine if the PC increase was influenced solely by complexion of PCs with polyphenolic compounds or if there was a microbial influence, TMAO was isolated and quantified. TMAO has been shown to be a biomarker for increased risk of cardiovascular disease and other chronic inflammatory diseases and is formed in the liver through the oxidation of TMA by FMO3. TMA can only be generated by a subset of gut microbiota that possess cutC, a glycyl radical enzyme. The cleavage of choline by cutC forms TMA which can then be transported across the lumen. Our data indicates that at week 8, Aronia treated mice had a significantly lower TMAO concentration than control mice. Further analysis of the Aronia treated mice revealed that TMAO concentrations decreased more dramatically in the HI mice than in the LO mice showing a shift in microbial makeup presumably resulting in a decrease in microbes possessing cutC.

In conclusion, we were able to successfully humanize the second-generation of germ-free mice inoculated with stool from human donors with different systemic inflammatory profiles, the results of which were reflected in disparate microbiota and metabolomic profiles. This allowed for a comparison of antioxidant treatment, consisting of *Aronia melanocarpa* juice between two distinct second-generation mouse populations. Using next-generation sequencing and mass spectrometry analysis, donor specific microbial communities and metabolites demonstrated distinct responses to juice treatment and the subsequent introduction of an inflammatory stimulus through a HFD introduction. Aronia juice offered protective effects against a HFD that were microbiome dependent. Metabolomic responses were centered around an increase in the phospholipids phosphatidylcholine with juice supplementation and sphingomyelin with the introduction of a HFD. Increases were particularly striking in mice with high-inflammation microbiomes and indicated a change towards a low-inflammation state.

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Author Contributions: The authors' responsibilities were as follows: Conceptualization, CJY, STW, ZJM, BB, and MPM; Methodology, MPM, CJY, STW, BB, JTP; Software, SMGW and JTP; Formal Analysis, SMGW, JTP; Investigation, SMGW, JTP, HF, LG, SAR; Resources, STW, ZJM, MPM; Data Curation, SMGW, JTP; Writing – Original Draft Preparation; SMGW, JTP; Writing – Review & Editing, SMGW, JTP, CJY, STW, BB and MPM; Visualization, SMGW, JTP; Project Administration, SMGW; Supervision – STW, MPM; Validation – NA; Funding Acquisition, CJY, STW, ZJM, MPM.

Declaration of Interests: The authors declare no competing interests.

Inclusion and diversity. One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. One or more of the authors of this paper self-

identifies as a member of the LGBTQ + community. We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects.

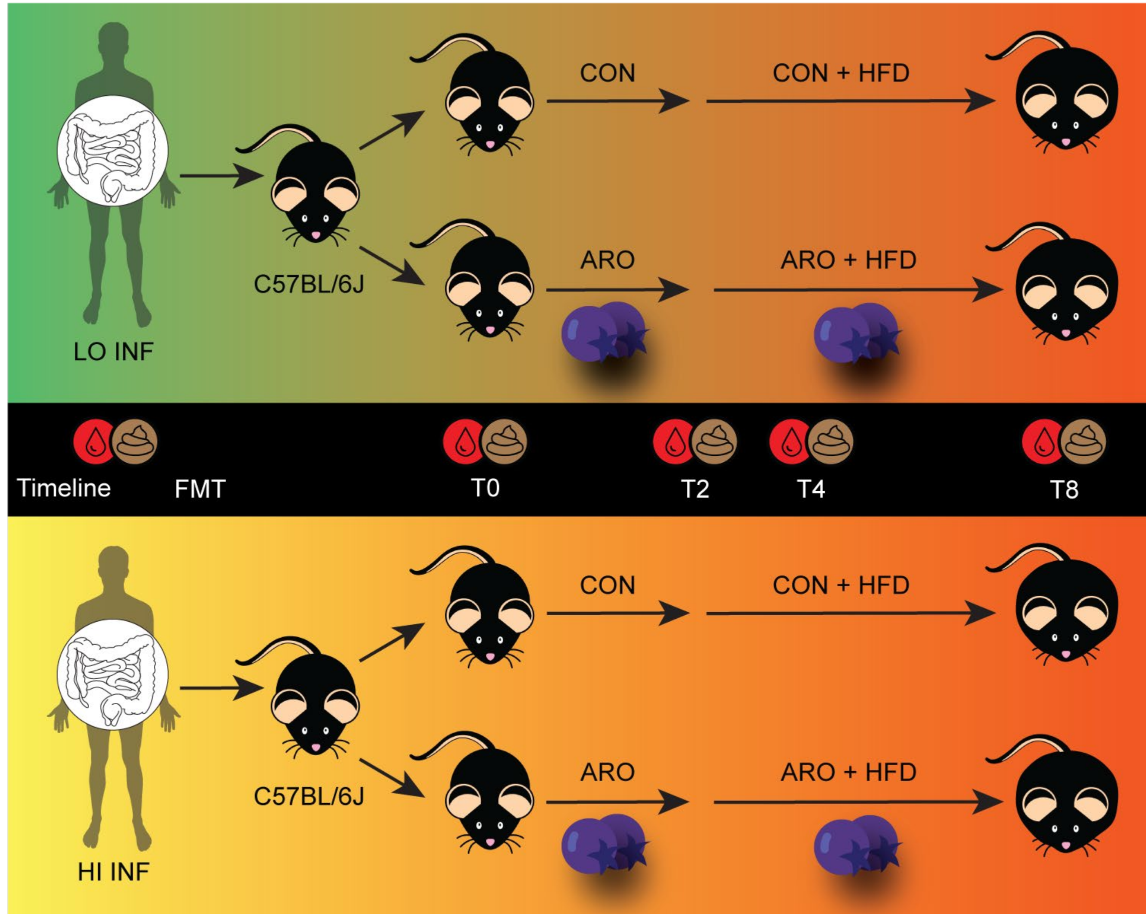


Figure 1. **Outline of Key Events and Sample Collection.** Adults (n=40) with a BMI 27-36 kg/m^2 self-collected stool samples and underwent testing for anthropometric, metabolic, and proinflammatory measures. Germ-free female C57BL/6J mice were inoculated with stool from 1 of 2 human donors with low or high chronic low-grade inflammation. Second generation mice received Aronia (ARO) juice (ARO_{LO}, = 3, ARO_{HI}, n=5), or a sugar-matched control (CON) for 2 weeks (CON_{LO}, n=3, CON_{HI}, n = 3). After a 2-week juice acclimation period, mice switched from standard chow to a high-fat diet (HFD) for 6 weeks but resumed their original juice treatment. Blood and fecal samples were assessed at baseline, two weeks (T2), four weeks (T4), and eight weeks (T8) for gut microbial composition (by Illumina MiSeq amplicon sequencing) and serum metabolites (by LCMS).

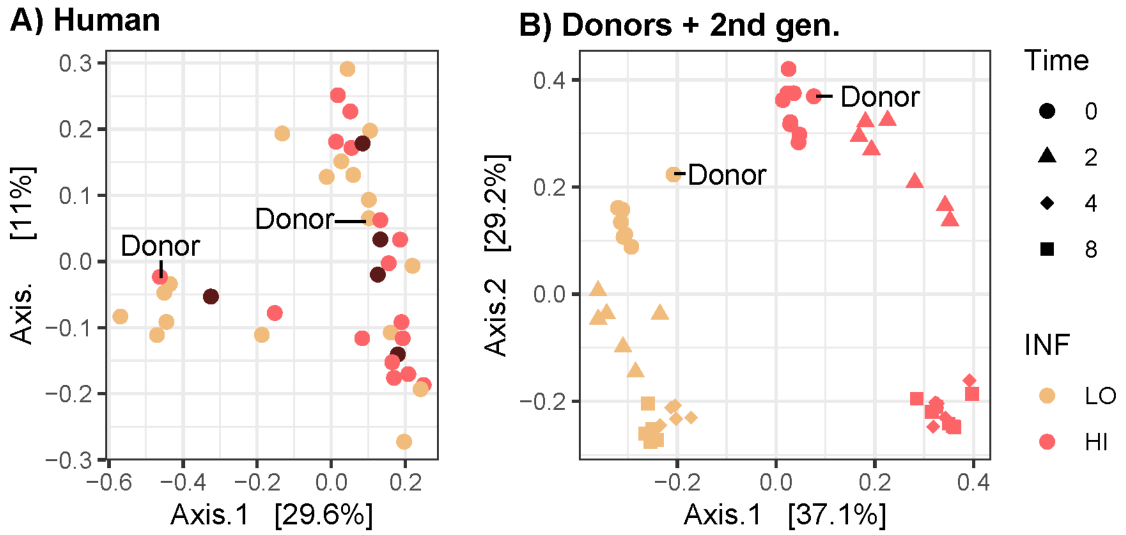


Figure 2. **Bray-Curtis Principal Coordinate Analysis (PCoA) of the gut microbial community for the A) human cohort (n=40), B) human donor compared to 2nd generation mice (n=13)**. Our LO and HI INF donors are highlighted in A) and B). PCoA results were plotted according to the first two components. Axes explain the percentage of variance in the gut microbial composition at the genera level between samples. Abbreviations: ARO, Aronia juice; LO, low inflammation; HI, high inflammation; INF, inflammation.

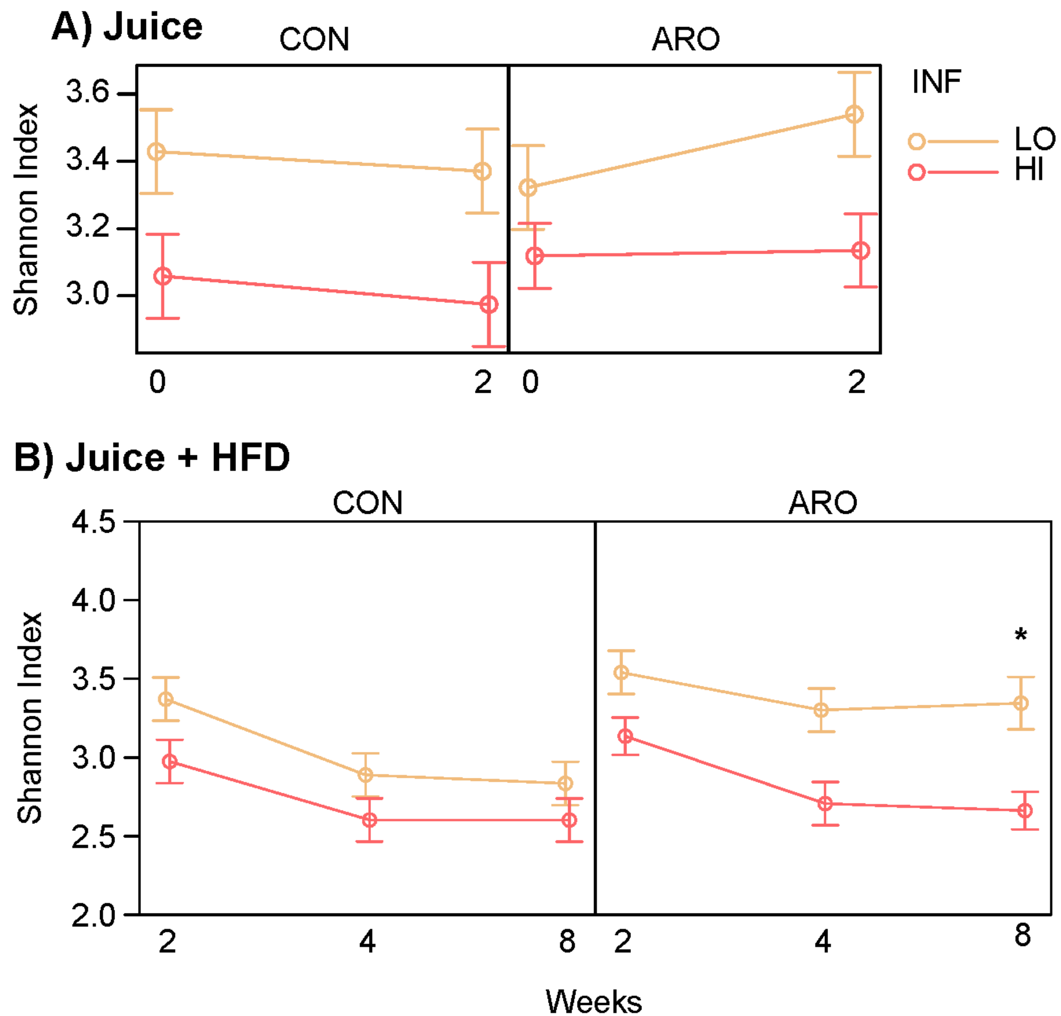


Figure 3. **Shannon Index, an alpha diversity measure, of the gut microbial community in second-generation mice in response to A) 2-Week juice treatment and B) juice treatment and HFD.** *Indicates a difference in means at the end of the HFD (Time 8) by donor and juice treatment. Points indicate group average and bars represent 95% confidence intervals. Abbreviations: ARO, Aronia juice; CON, control juice; LO, low inflammation; HI, high inflammation; INF, inflammation; HFD, high-fat diet.

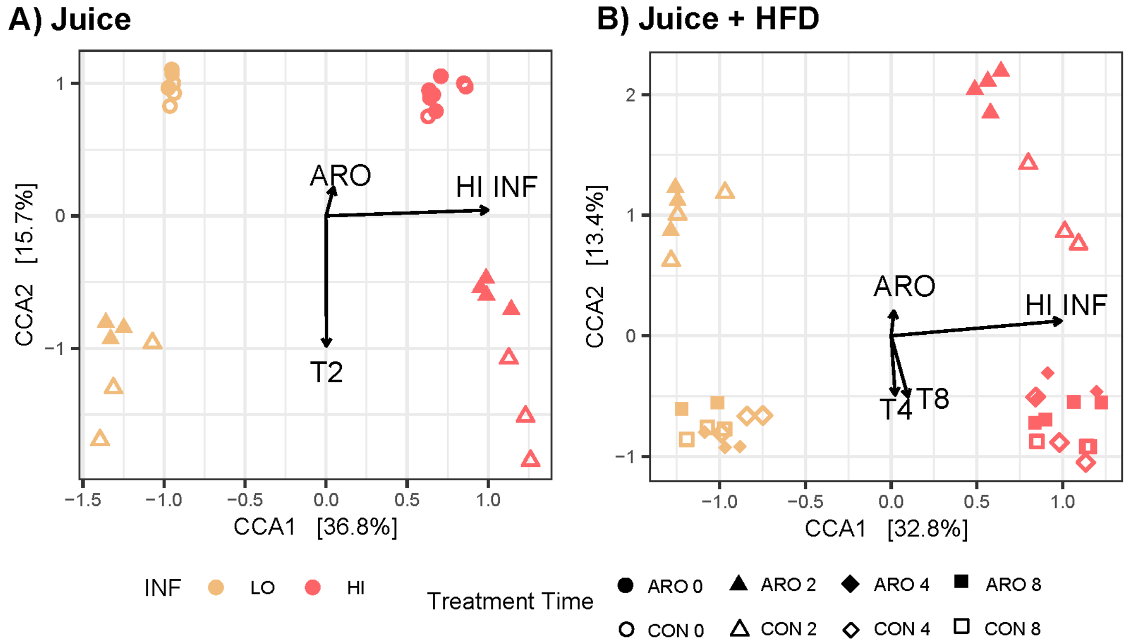


Figure 4. **Canonical Correspondence Analysis in 2nd generation mice with treatment, time, and inflammation as constraining variables for the A) first two weeks with juice and B) six-weeks with juice and HFD**. CCA results were plotted according to the first two components. Axes explain the percentage of variance in the gut microbial composition at the genera level between samples. Abbreviations: ARO, Aronia juice; LO, low inflammation; HI, high inflammation; INF, inflammation; T, time in weeks.

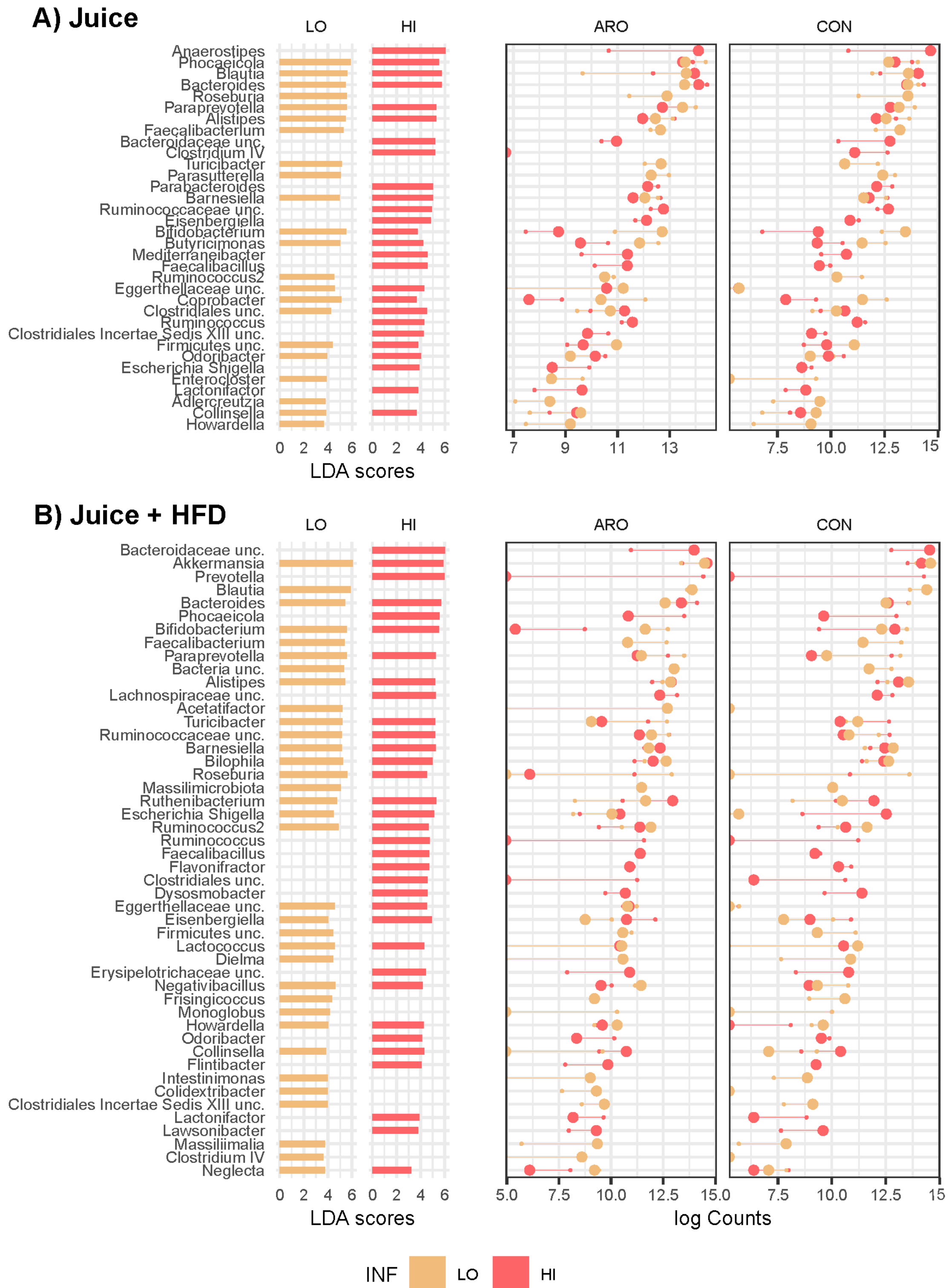


Figure 5. **Discriminating bacterial genera by donor, then by treatment conditions.** The larger point in each dumbbell plot indicates the later time point. LefSe uses the Kruskal-

Wallis test ($p < 0.05$) followed by linear discriminant analysis (LDA) score > 2.0. Abbreviations: ARO, Aronia juice; CON, control juice; LO, low inflammation; HI, high inflammation; unc., unclassified.

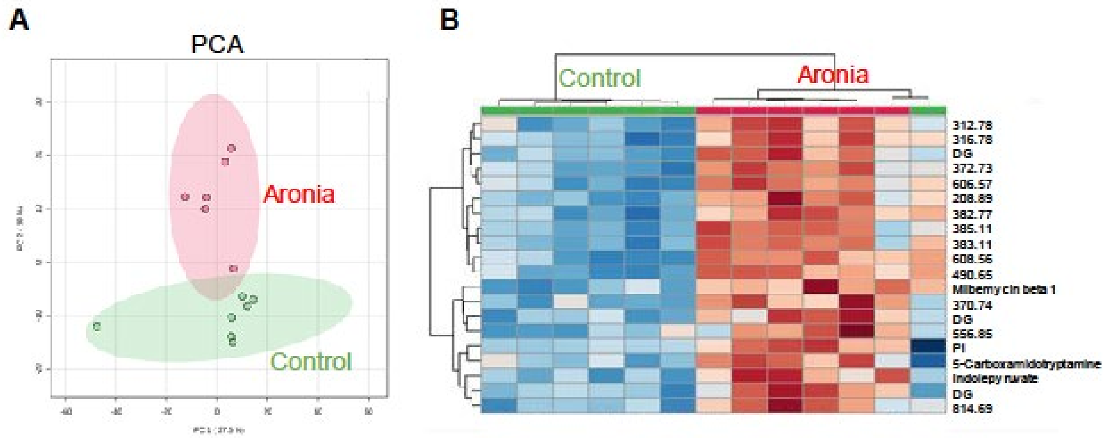


Figure 6. **Profiling of serum metabolites in second generation mice at T0**. A) A principal component analysis (PCA) by juice treatment. B) Heatmap for T0 indicating top 20 metabolites. Red – upregulation; blue, downregulation.

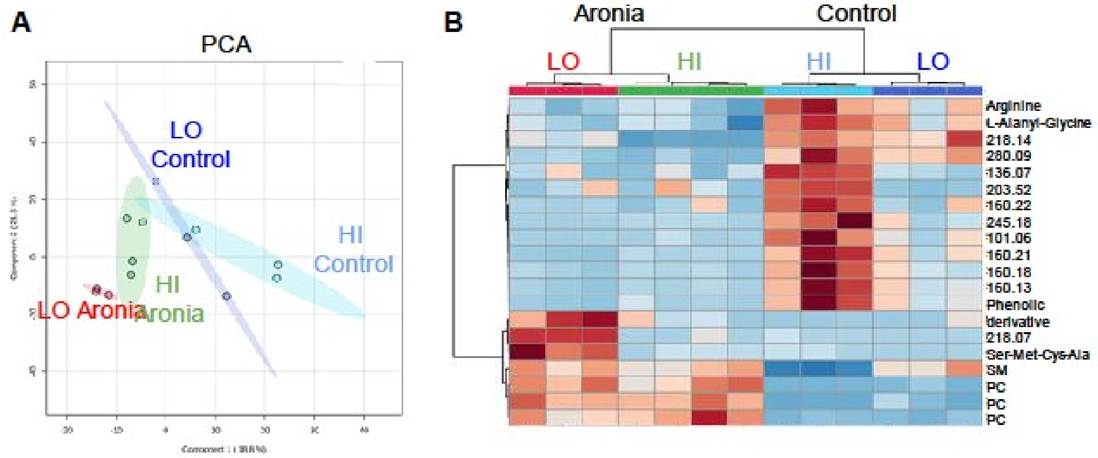


Figure 7. **Profiling of serum metabolites in second generation mice at T2**. A) A principal component analysis (PCA) by donor and juice treatment. B) Heatmap for T2 indicating top 20 metabolites. Dendrogram indicating separation by juice treatment followed by donor type. Red – upregulation; blue, downregulation. Abbreviations: ARO, Aronia juice; CON, control juice; LO, low inflammation; HI, high inflammation.

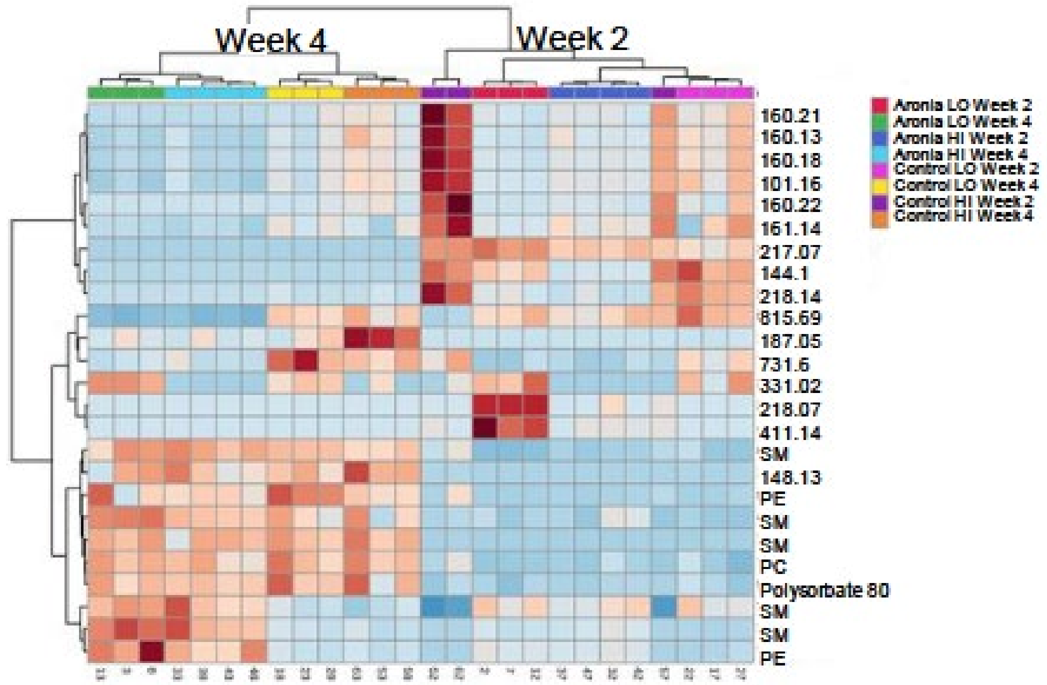


Figure 8. **Heatmap showing top 25 discriminating features at T2 and T4**. The dendrogram at the top shows grouping first by time, then juice treatment, followed by donor group. Red – upregulation; blue, downregulation. Abbreviations: LO, low inflammation; HI, high inflammation.

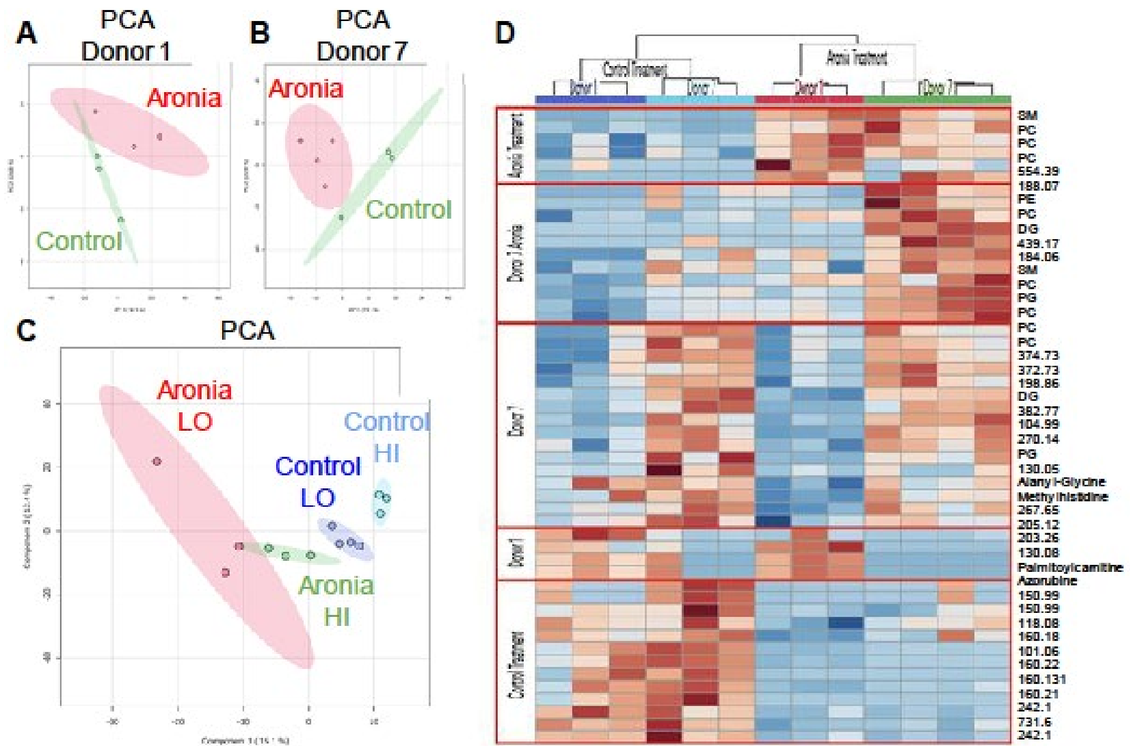


Figure 9. **Principal component Analysis (PCA) of mouse metabolites at T8.** A) Metabolite PCA of Juice Treatment in LO donor mice, B) Metabolite PCA of Juice Treatment in HI donor mice, C) Metabolite PCA of Juice treatment by juice treatment and donor group, and D) Heatmap showing top 45 discriminating features. The dendrogram at the top shows grouping first by juice treatment followed by donor group. Red – upregulation; blue, downregulation.

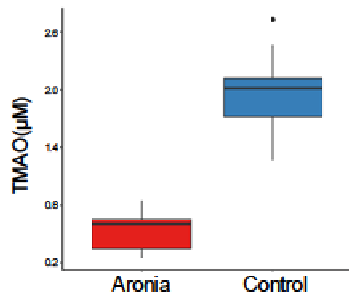


Figure 10. **Serum trimethylamine-N-oxide (TMAO) concentrations by juice treatment at T8**. * indicates p-value less than 0.05.

Tables with Titles and Legends

Table 1 | **Anthropometric and Metabolic Characteristics of Inflammation Groupings and of Selected Human Stool Donors.** Metabolic syndrome criteria are based on the National Cholesterol Education Program Adult Treatment Panel III definition.

	Baseline Inflammation			*Inflammation Response*				*Donors (n=2)*	
	LO (n=19)	**HI** (n=16)	***p*-value**	**LO (n=17)**	**MID (n=14)**	**HI (n=9)**	**p-value**	***LO***	***HI***
Women/Men	12/7	9/7	0.63	8/9	5/9	3/6	0.53	1/0	1/0
Age (years)	36.4 ± 10.5	35.3 ± 10.3	0.72	39.2 ± 9.6	34.8 ± 10.7	33.4 ± 9.8	0.3	52	34
BMI (kg/m^2)	30.3 ± 1.9	30.6 ± 2.0	0.91	30.8 ± 2.6	30.1 ± 1.3	30.2 ± 1.6	0.67	27.7	35.9
FM (%)	35.0 ± 5.9	36.7 ± 7.8	0.73	35.7 ± 7.6	35.8 ± 6.7	36.8 ± 6.6	0.92	35.1	48.1
Insulin Resistance									
HbA1c (%)	5.28 ± 0.25	5.22 ± 0.32	0.74	5.4 ± 0.2	5.2 ± 0.2	5.2 ± 0.4	0.06	5.1	5.4
HOMA-IR	1.7 ± 1.3	3.3 ± 2.5	**0.04**	2.2 ± 1.2	3.3 ± 2.6	1.6 ± 1.4	0.21	1.3	2.4
Metabolic Syndrome									
Presence/Absence	4/15	5/11	**0.03**	4/13	3/11	3/6	**0.02**	0/1	0/1
Fasting GLU (mmol/L)	5.3 ± 0.3	5.5 ± 0.4	0.53	5.4 ± 0.3	5.4 ± 0.5	5.5 ± 0.3	0.88	5.4	5.2
Fasting TG (mmol/L)	1.5 ± 1.0	1.7 ± 0.9	0.33	1.9 ± 1.2	1.3 ± 0.7	1.7 ± 1.2	0.34	2.4	2.6
Fasting CHOL (mmol/L)	4.7 ± 0.8	4.6 ± 0.8	0.65	5.1 ± 1.0	4.4 ± 0.6	4.4 ± 0.7	**0.02**	5.9	5.5
Fasting HDL (mmol/L)	1.4 ± 0.4	1.3 ± 0	0.27	1.4 ± 0.3	1.4 ± 0.5	1.3 ± 0.5	0.89	1.6	2.0
Waist Circumference (cm)	94.4 ± 7.6	96.2 ± 9.8	0.8	95.6 ± 11.2	95.2 ± 9.4	95.4 ± 5.3	0.99	92.3	90.25
SBP (mmHg)	117 ± 14	111 ± 11	0.27	116 ± 9	112.4 ± 16	110 ± 15	0.55	128	114
DBP (mmHg)	78 ± 10	74 ± 7	0.33	78 ± 9	75 ± 11	72 ± 8	0.41	78	83

Data represents mean and standard deviation. Difference in sex and metabolic syndrome presence proportion were determined by a 2-sample test (baseline inflammation) or 3-sample test (Inflammation Response) for given proportions. All other p-values were determined by ANOVA.

Abbreviations: low inflammation, LO; high inflammation, HI; body mass index, BMI; fat mass, FM; hemoglobin A1C, HbA1c; HOMA-IR, homeostatic model of insulin resistance; glucose, GLU; triglycerides, TG; cholesterol, CHOL, high-density lipoprotein, HDL; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 | **Phenolic and carbohydrate content within different blends of unpasteurized *Aronia melanocarpa* juice.** The blend on the far left was utilized for the gnotobiotic mouse experiments. Glucitol is also more commonly known as sorbitol.

Compound	Blend	Autumn Magic Blend	Autumn Magic 1st Harvest	Autumn Magic 2nd Harvest	MacKenzie Blend	MacKenzie 1st Harvest	MacKenzie 2nd Harvest	Vikiing Blend	Viking 1st Harvest	Viking 2nd Harvest
Rutin (uM)	33.47	55.25	26.16	111.34	26.80	14.90	36.68	25.89	21.95	23.91
Neochlorogenic Acid (uM)	8987.59	18712.04	19689.92	20084.80	4553.87	4606.17	4271.11	4992.04	7593.83	3180.72
Chlorogenic Acid (uM)	8598.77	10198.03	11133.28	10836.42	6813.11	7551.76	6231.16	7575.53	10949.01	4408.06
cyanidin-3-galactoside (uM)	589.40	721.99	428.14	1466.07	489.68	444.64	504.47	457.91	709.69	341.58
cyanidin-3-glucoside (uM)	66.92	58.12	19.71	151.95	54.31	42.28	50.48	53.05	85.73	45.41
cyanidin-3-arabinoside (uM)	1224.29	1460.81	896.36	3014.61	1086.11	976.62	1139.28	1032.85	1496.99	776.40
cyanidin-3-xyloside (uM)	42.57	51.77	87.01	40.43	57.79	61.02	67.56	56.03	82.22	39.30
quercetin-3-galactoside (uM)	19.36	37.92	12.32	85.99	26.92	78.21	18.32	6.57	5.31	8.61
quercetin-3-glucoside (uM)	127.45	128.47	82.87	255.13	42.85	27.38	60.10	35.14	37.52	33.44
D-Glucose (mM)	135.07	135.07	163.46	135.07	135.66	135.07	135.07	135.07	111.16	135.07
Fructose (mM)	483.65	351.61	483.65	360.01	439.60	452.80	433.08	458.44	360.64	484.50
Glucitol (mM)	712.72	856.96	712.72	712.72	712.72	666.36	685.90	712.72	497.05	679.68

Methods

Ethics Statement. The human subjects and IACUC were approved by the Institutional Review Board at Montana State University. For human subjects, written informed consent was obtained prior to participation. The study was retrospectively registered October 2019 at ClinicalTrials.gov (NCT04128839).

Human Cohort. Potential subjects were recruited via advertisement between March 2016 to June 2018 and screened over the phone for eligibility. Inclusion criteria included being between 18 and 55 years old and having a body mass index between 27 and 36 kg/m^2. Criteria for exclusion included antibiotics within 90 days of study enrollment, regular use of anti-inflammatory medications, use of estrogen-only contraceptives, wheat and/or dairy allergies or intolerances, were pregnant, or had any musculoskeletal, cardiovascular, gastrointestinal, or immunological condition that could interfere with the study. All potentially eligible subjects were screened over the phone for inclusion and exclusion criteria. Forty overweight and obese men and women participated in testing of anthropometric and changes in serum metabolic and inflammatory markers during a high-fat meal challenge with a 50 g oral fat load.

Anthropometrics. Measurements were collected from subjects using the validated segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Germany) (Bosy-Westphal et al., 2013). Subjects were instructed to refrain from eating, drinking, or exercising in the three hours prior to testing. Fat mass (%) and estimated visceral adipose (liters) were used for descriptive analysis.

Blood Sampling. Subjects were instructed to avoid alcohol consumption and strenuous physical activity in the 24 hours before their visit and to complete an overnight fast (10 - 12 hours) before blood collection. Participant fasting blood samples were collected from the antecubital vein by a certified nurse or physician in the morning (6:30 – 8:30 AM). Blood was collected into 8.5 mL endotoxin-free serum separating tubes and allowed to clot for 15 minutes at room temperature before centrifugation (3000 rpm, 15 min). Serum aliquots were frozen at -80ºC until analysis.

Metabolic Syndrome Markers. According to the National Cholesterol Education Program Adult Treatment Panel III definition, metabolic syndrome is the co-occurrence of insulin resistance, excess central adiposity, dyslipidemia, and hypertension (Huang, 2009). Blood markers of metabolic syndrome were determined from whole blood run on Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, CA, USA) and included high density lipoprotein (HDL), triglyceride (TG), and glucose (GLU). Blood pressure was taken in the morning after subjects had been seated for at least fifteen minutes. Waist circumference, as an indicator of central adiposity, was taken in conjunction with anthropometric testing.

Insulin Resistance. Elevated glycated hemoglobin (HbA1c) and the homeostatic model of insulin resistance (HOMA-IR) are early indicators of insulin resistance. HbA1c determined using the DCA Vantage Analyzer (Siemens Medical Solutions Diagnostics, Cergy-Pontoise, France) performed according to manufacturer instructions. Insulin was

determined through ELISA (MP Biomedicals, USA) performed according to manufacturer instructions, with the average used for analysis. Fasting blood glucose and insulin were used to determine HOMA-IR according to the original HOMA-IR formula (Matthews et al., 1985).

Inflammation Phenotyping. A multi-cytokine approach was used for determination of fasting systemic inflammatory profile. Cytokine measurement was performed on serum samples using high-sensitivity multiplexing technology (Bio-Rad Bio-Plex® 200 HTS) following procedures by Millipore (EMD Millipore Corporation, MA, USA). Classic systemic pro-inflammatory cytokines included granulocyte macrophage colony stimulating factor (GM-CSF), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α. Interleukin-17 and IL-23, both of which serve a pro-inflammatory and regulatory role in the gut mucosa, were also measured. Human serum samples at each time point during the high-fat meal challenge were run in duplicate with the mean used for analysis.

Subjects were categorized as having high fasting inflammation (HI) if they were above the group median at baseline in at least 4 of the 6 cytokines. Subjects were considered to have low fasting inflammation (LO) in they were above the group median in two or less cytokines. Individuals above the group median in 3 cytokines were considered neither HI nor LO. Fasting phenotype separation was confirmed by a two-sample t-test of each cytokine measure. Using a k-means analysis, the 40 participants were also grouped based on postprandial cytokine concentrations in response to an oral fat tolerance test (Peach

2021). This analysis determined that the cohort contained three distinct inflammation groups based on cytokine levels. Groups were labeled as low-responders (LO), mid-responders (MID) and high-responders (HI).

Stool Sample Collection. Collection kits were provided, and subjects were asked to follow printed instructions to self-collect a stool sample in the 24 hours before their blood collection visit. After initial collection into a sterile disposable commode, a small portion of the sample was transferred into a sterile 50-mL conical vial and refrigerated until transportation to researchers. Samples were processed in an anaerobic chamber (Coy) with pre-reduced phosphate buffer saline and aliquoted into cryogenic vials at -80ºC until analysis.

Gut microbiota transplant and colonization. Two stool donors were selected based on their inflammation profile which occurred prior to gut microbial community profiling. Female germ-free (GF) C57BL/6J mice, originally purchased from the Jackson Laboratory (Bar Harbor, ME) were housed and bred at the American Association for the Accreditation of Laboratory Animal Care-accredited Animal Resource Center at Montana State University. Mice were held in individually ventilated cages with sterile bedding before and after fecal transplantation from selected human stool donors. Two female mice received an inoculation with fecal material from a human donor categorized as having low or high systemic inflammation based on serum levels of six proinflammatory cytokines. Human donor stool slurry aliquots were administered to GF mice through oral gavage. Sexually

mature male GF C57BL/6J mice were added to each cage approximately one week after transplantation. Male mice removed prior to birth of pups. Pups from the inoculated dams were co-housed by sex (3 – 5 mice/cage) with different microbial inoculations placed in separate isolators. Mice from each microbial inoculation were assigned to one of two juice groups: Aronia (ARO_{LO}, = 3, ARO_{HI}, n=5), or a sugar-matched juice (CON_{LO}, n=3, CON_{HI}, n = 3).

Aronia juice analysis. Aronia juice was a blend of Mackenzie, Viking, and Autumn Magic cultivars grown at the Western Agricultural Research Center in Corvallis, Montana. Processed Aronia juice was examined to determine both the phenolic and carbohydrate composition. For quantifying the phenolic content, an LCMS method was developed. The selected method was developed for use on an Agilent 6538 quadrupole-time of flight (Q-TOF) mass spectrometer (Agilent, CA, USA) and an Agilent 1290 ultrahigh performance liquid chromatography system (Agilent, CA, USA) located at the Montana State University Proteomics, Metabolomics and Mass Spectrometry Facility. Separation was achieved using an Acquity HSST-3 UPLC reverse phase column, 1.8µM, 100mm/2.1mm (Waters, MA, USA). The novel method was 18 minutes in length and consisted of HPLC grade water and acetonitrile (Fisher, MA, USA), both with 0.1% formic acid (Fisher, MA, USA), as mobile phases A and B, respectively. Analysis began with a flow rate of 0.3mL/minute and 95% A. At two minutes, the mobile phase composition changed linearly to 5% A at 15 minutes. A was held at 5% for 1 minute and then a wash with 95% A was completed for the final

two minutes of the run. The column compartment was kept at a constant 30°C throughout the analysis.

This method was used to analyze two different harvest times for three different varieties (genotypes) of Aronia. Standards were initially used to determine the retention time and peak area of nine phenolic standards at five different concentrations. Standards included anthocyanins and procyanidins previously found in Aronia (Bräunlich et al., 2013). The collected data was then used to generate standard curves. Juice samples from different blends were diluted with HPLC grade water, 1:50 juice:water, and placed in mass spectrometry vials. Juice blends were analyzed via the same LCMS method as the phenolic standards and the concentration of specific phenolics were determined in each juice sample.

Carbohydrate composition was also explored using nuclear magnetic resonance (NMR) analysis. Juice was diluted with sodium trimethylsilylpropanesulfonate (DSS) and placed in an NMR tube. Analysis was performed using a Bruker 600MHz Avance III NMR spectrometer (Bruker, MA, USA) with a 600MHz TCI (H-C/N-D05Z) LT Probe located at the Montana State University Nuclear Magnetic Resonance Core Facility. Based on this analysis, carbohydrate standards were purchased and added to the placebo juice for the control mice. Placebo juice was analyzed via NMR and was confirmed to have identical carbohydrate concentrations as the Aronia juice.

Juice and Diet Administration. At baseline (T0), regular drinking water was replaced with ARO or CON juice to begin a two-week familiarization period with the juice. ARO mice received an unpasteurized blend of Aronia juice, and CON mice received a sugar-matched beverage containing water, sorbitol, glucose, and fructose. The mice were housed in cages with free access to their respective juice. During the familiarization period, all mice received standard chow (LabDiet 5013).

After the 2-week familiarization period (T2), mice began a 6-week high-fat diet, delivered ad libitum concomitant with juice consumption. The HFD (Teklad TD.96132) was chosen to induce obesity and present an inflammatory stimulus (Duan et al., 2018). The HFD mimics a Western style diet and consisted of 40.6% fat, 40.7% carbohydrate, and 18.7% protein and was particularly rich in sugars and trans-fatty acids. All chow provided was sterilized via autoclaving or irradiation. A total of 150 mL of juice was provided per cage each week. Juice was refilled three times each week.

Murine Sample Collection. Weekly measurements of body weight and food and fluid intake were recorded. Fecal pellets were collected at baseline (T0), at HFD start (T2), after 2-weeks of HFD (T4), and at the end of the 6-week HFD (T8). Stool samples were frozen at -80ºC until bulk DNA extraction. Blood samples were collected at the same interval into serum separating tubes. Whole blood was allowed to clot for 15 minutes before centrifugation at 1200 RPM for 15 minutes with resulting serum aliquoted and stored at -

80ºC until analysis. After T8 sample collection, mice were euthanized via rapid CO_2 asphyxiation.

One mouse in the second-generation had substantial weight loss (≥ 20% starting body weight) in the first week of the experiment and was euthanized according to IACUC protocol, leaving ARO_{HI} with four mice in total. All other groups were steady throughout the experiment.

Genomic DNA Extraction 16S rRNA gene sequencing. Extraction of bulk bacterial DNA from fecal samples was performed using Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Inc.) and bead beating. Extracted DNA was stored at -80ºC 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. Raw sequencing reads were processed and curated using MOTHUR software (Version 1.35.1) following the MOTHUR standard operating procedure for the MiSeq platform (Schloss et al., 2009). In short, paired-end reads were assembled into contiguous sequences and screened for length and quality. The remaining contigs were aligned to the SILVA ribosomal RNA database (Release 132), a comprehensive collection of aligned 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.

A total of 2 732 246 raw reads were obtained across all samples. To aid unbiased diversity matrices due to sequencing depth, data was randomly subsampled at the minimum number of sequences across samples. Subsampling resulted in a total of 2 082 652 high quality reads. Alpha diversity was calculated using the *phyloseq* 1.38.0 (R). Beta-diversity analyses and microbial biomarker analyses through LefSe were performed on subsampled data with filtering of OTUs less than 3 counts in at least 20% of the samples. Permutational analysis of distance matrices with stratification by cage and 999 permutations was performed using the *adonis* function in the vegan package 2.5-6 (R). Canonical correspondence analysis (CCA) was used to assess the impact of time, donor, and juice treatment on the microbial community using *phyloseq* 1.38.0 (R). The contribution of variables in CCA was assessed through a permutation test with 999 permutations with cage stratification. Separate LefSe analyses were performed in MicrobiomeAnalyst by donor to assess taxonomic changes in response to the juice and to the HFD (Dhariwal et al., 2017).

<u>LCMS metabolomics analysis.</u> Serum samples were removed from -80°C storage and allowed to thaw. 20μL of thawed serum was removed and placed in a clean vial after which 80μL of ice-cold acetone was added to precipitate protein. Samples were then stored at -

80°C overnight to aid precipitation. The next day, samples were spun in a centrifuge for 10 minutes at 20,000xg. 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 (Eppendrof, Hamburg, Germany) to dryness. Dried samples were stored at -80°C until ready for LCMS analysis, at which time the samples were reconstituted with 40µL of MeOH:H_20 (50:50) and placed in a clean mass spectrometry vial.

LCMS analysis was completed on the same system as the phenolic analysis, an Agilent 6538 MS coupled to an Agilent 1290 UHPLC. Initial sample separation was accomplished on an Acquity BEH-HILIC column, 1.7µm, 2.1mm/100mm (Waters, MA, USA). A 15-minute method was employed using water and acetonitrile, both with 0.1% formic acid, as mobile phases A and B, respectively. 45% A was held for the first two minutes, after which a linear gradient increased A until 11 minutes to 70%. 70% was held for two additional minutes until switching to 0% A at 13 minutes. The column compartment was kept at 40°C and the flow rate was 0.2mL/min. MSMS, or tandem MS, was completed on the same system with the same LCMS settings to identify metabolites. Collision energies of 10, 20 30 and 40V were used to fragment analytes. This method yielded over 1,000 metabolites from each sample.

A second targeted LCMS analysis was also undertaken, specifically to determine trimethylamine-N-oxide concentrations in the mouse serum. Analysis was completed on

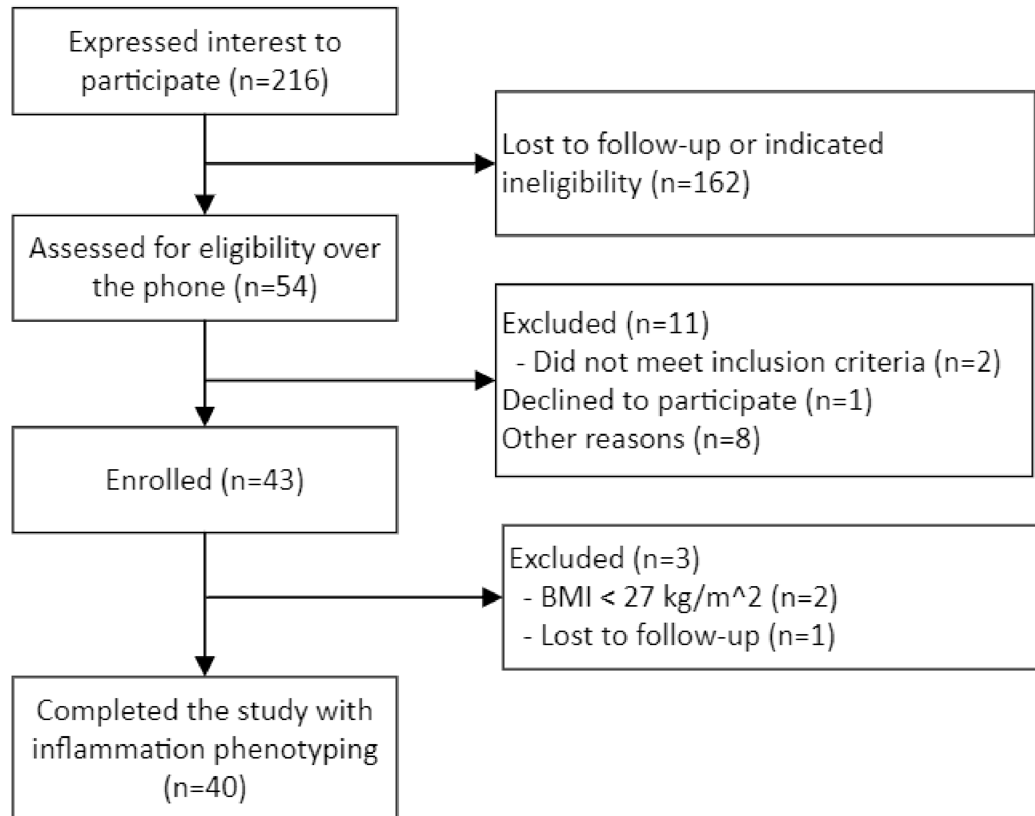
the same instrument as previously described but with a different method. A 6-minute targeted method was used with acetonitrile and 10mmol/L ammonium formate as mobile phases A and B, respectively. A 10-40% A gradient was used over 6 minutes with a compartment temperature of 30°C (Awwad et al., 2016). TMAO retention time and a standard curve was generated by analyzing authentic standards. After serum sample analysis, TMAO relative concentrations were integrated, and the concentrations were determined using MassHunter.

LCMS and NMR data analysis. After MS analysis, data was converted to either a .mzML format for MS data or a .mgf format for MSMS data using MSConvert (Chambers et al., 2012). MS data was then interpreted using mzMine and statistical analysis was completed using MetaboAnalyst (Chong et al., 2019; Pluskal et al., 2010). MSMS data was examined to identify metabolites using SIRIUS software by searching all biological databases using a 15ppm error window (Dührkop et al., 2019). NMR results were examined using Chenomix NMR Suite software.

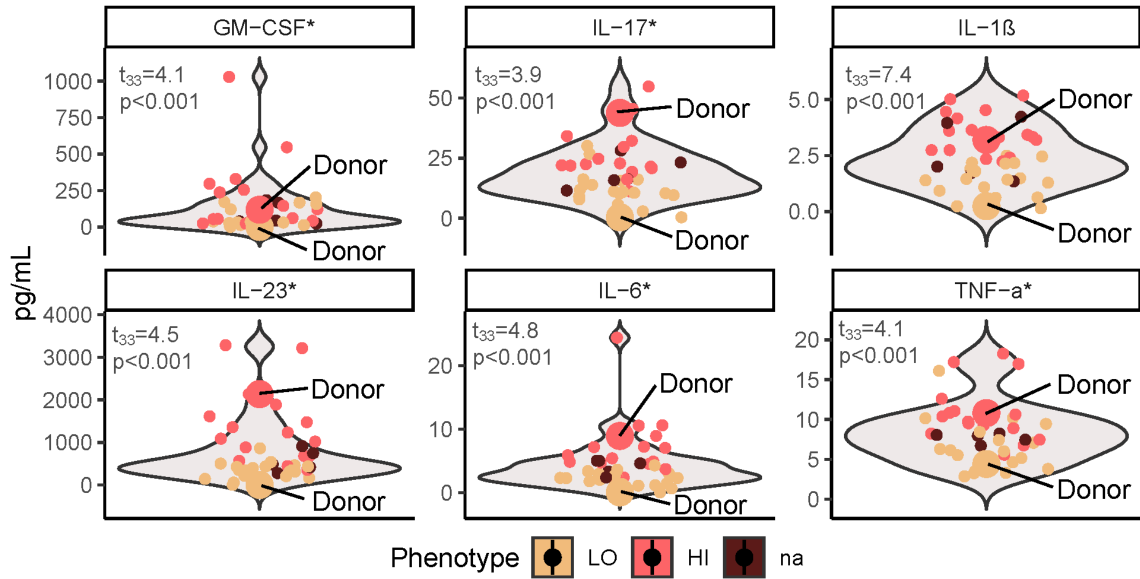
Availability of data and materials. Sequence data from the full human cohort (n=40) have been deposited in the Sequence Read Archive (SRA) with the accession code PRJNA596000. Other participant data is not publicly available due to them containing information that could compromise research participant privacy. Sequence data from the gnotobiotic mice experiment have been deposited in the Sequence Read Archive (SRA).

Statistical Analysis. Microbial ecological analyses and visualizations performed in RStudio (V. 1.4.1106) running base R 4.1.2. Descriptive statistics of the participant physical characteristics and metabolic profile by inflammation phenotype were performed by ANOVA.

Supplemental Information Titles and Legends



Supplemental File 1. **Human consort diagram.** Flyers and e-mails were used to recruit interested individuals. Over two hundred interested individuals contacted the research team, who sent them additional information about the study in return. Fifty-four individuals completed a phone screening with the same researcher. Forty-three individuals met the requirements and were enrolled to participate. Of these, two individuals were found to be ineligible on the first visit and one individual was unable to be contacted. Forty individuals completed the remaining blood work portion of the study and were able to have their serum inflammatory profile phenotyped.



Supplemental File 2. **Fasting proinflammatory profile grouped by LO and HI inflammation phenotype.** Test statistics and p-values were determined by two sample t-test with phenotype (excluding NA n=5) as the grouping variable. *Indicates the cytokine values were logged to meet normality assumption. Abbreviations: low inflammation, LO; high inflammation, HI; granulocyte macrophage colony-stimulating factor, GM-CSF; interleukin, IL; tumor necrosis factor, TNF.

References

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., et al. (2011). Enterotypes of the human gut microbiome. Nature 473, 174-180.

Astbury, S., Atallah, E., Vijay, A., Aithal, G.P., Grove, J.I., and Valdes, A.M. (2020). Lower gut microbiome diversity and higher abundance of proinflammatory genus Collinsella are associated with biopsy-proven nonalcoholic steatohepatitis. Gut Microbes 11, 569-580.

Awwad, H.M., Geisel, J., and Obeid, R. (2016). Determination of trimethylamine, trimethylamine N-oxide, and taurine in human plasma and urine by UHPLC–MS/MS technique. Journal of Chromatography B 1038, 12-18.

Bäckhed, F., Manchester, J.K., Semenkovich, C.F., and Gordon, J.I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proceedings of the National Academy of Sciences 104, 979.

Banjari, I., Misir, A., Savikin, K., Jokic, S., Molnar, M., De Zoysa, H.K.S., and Waisundara, V.Y. (2017). Antidiabetic effects of Aronia melanocarpa and its Other Therapeutic Properties. Front. Nutr. 4, 6.

Bisanz, J.E., Upadhyay, V., Turnbaugh, J.A., Ly, K., and Turnbaugh, P.J. (2019). Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host & Microbe 26, 265-272.e264.

Bosy-Westphal, A., Schautz, B., Later, W., Kehayias, J.J., Gallagher, D., and Müller, M.J. (2013). What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. European Journal Of Clinical Nutrition 67, S14.

Bräunlich, M., Slimestad, R., Wangensteen, H., Brede, C., Malterud, K.E., and Barsett, H. (2013). Extracts, Anthocyanins and Procyanidins from Aronia melanocarpa as Radical Scavengers and Enzyme Inhibitors. Nutrients 5, 663-678.

Brown, E.M., Ke, X., Hitchcock, D., Jeanfavre, S., Avila-Pacheco, J., Nakata, T., Arthur, T.D., Fornelos, N., Heim, C., Franzosa, E.A., et al. (2019). Bacteroides-Derived Sphingolipids Are Critical for Maintaining Intestinal Homeostasis and Symbiosis. Cell Host Microbe 25, 668-680.e667.

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761-1772.

Cani, P.D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A.M., Delzenne, N.M., and Burcelin, R. (2008). Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. Diabetes 57, 1470-1481.

Cani, P.D., Possemiers, S., Wiele, T., Guiot, Y., Everard, A., and Rottier, O. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58.

Chambers, M.C., Maclean, B., Burke, R., Amodei, D., Ruderman, D.L., Neumann, S., Gatto, L., Fischer, B., Pratt, B., Egertson, J., et al. (2012). A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology 30, 918-920.

Chen, M., Pan, H., Dai, Y., Zhang, J., Tong, Y., Huang, Y., Wang, M., and Huang, H. (2018). Phosphatidylcholine regulates NF-κB activation in attenuation of LPS-induced inflammation: evidence from in vitro study. Animal Cells and Systems 22, 7-14.

Chong, J., Wishart, D.S., and Xia, J. (2019). Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. Current Protocols in Bioinformatics 68, e86.

Comalada, M., Camuesco, D., Sierra, S., Ballester, I., Xaus, J., Gálvez, J., and Zarzuelo, A. (2005). In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. European Journal of Immunology 35, 584-592.

Copeland, S., Warren, H.S., Lowry, S.F., Calvano, S.E., Remick, D., the, I., and the Host Response to Injury, I. (2005). Acute Inflammatory Response to Endotoxin in Mice and Humans. Clinical and Diagnostic Laboratory Immunology 12, 60-67.

Dai, Y., Tang, H., and Pang, S. (2021). The Crucial Roles of Phospholipids in Aging and Lifespan Regulation. Front Physiol 12, 775648.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-+.

Dawaliby, R., Trubbia, C., Delporte, C., Noyon, C., Ruysschaert, J.M., Van Antwerpen, P., and Govaerts, C. (2016). Phosphatidylethanolamine Is a Key Regulator of Membrane Fluidity in Eukaryotic Cells. The Journal of biological chemistry 291, 3658-3667.

Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., and Xia, J. (2017). MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research 45, W180-W188.

Dobrosotskaya, I.Y., Seegmiller, A.C., Brown, M.S., Goldstein, J.L., and Rawson, R.B. (2002). Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296, 879-883.

Duan, Y., Zeng, L., Zheng, C., Song, B., Li, F., Kong, X., and Xu, K. (2018). Inflammatory Links Between High Fat Diets and Diseases. Frontiers in immunology 9, 2649-2649.

Dührkop, K., Fleischauer, M., Ludwig, M., Aksenov, A.A., Melnik, A.V., Meusel, M., Dorrestein, P.C., Rousu, J., and Böcker, S. (2019). SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nature methods 16, 299-302.

Duncan, B.B., Schmidt, M.I., Pankow, J.S., Ballantyne, C.M., Couper, D., Vigo, A., Hoogeveen, R., Folsom, A.R., Heiss, G., and Atherosclerosis Risk in Communities, S. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 52, 1799-1805.

Fajardo, V.A., McMeekin, L., and LeBlanc, P.J. (2011). Influence of phospholipid species on membrane fluidity: a meta-analysis for a novel phospholipid fluidity index. J Membr Biol 244, 97-103.

Fang, J. (2014). Bioavailability of anthocyanins. Drug Metab Rev 46, 508-520.

Fei, N., and Zhao, L.P. (2013). An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. Isme Journal 7, 880-884.

Fernandes, I., Faria, A., Calhau, C., de Freitas, V., and Mateus, N. (2014). Bioavailability of anthocyanins and derivatives. J. Funct. Food. 7, 54-66.

Ghoshal, S., Witta, J., Zhong, J., de Villiers, W., and Eckhardt, E. (2009). Chylomicrons promote intestinal absorption of lipopolysaccharides. Journal of Lipid Research 50, 90-97.

Gil-Cardoso, K., Gines, I., Pinent, M., Ardevol, A., Blay, M., and Terra, X. (2016). Effects of flavonoids on intestinal inflammation, barrier integrity and changes in gut microbiota during diet-induced obesity. Nutr. Res. Rev. 29, 234-248.

Gomez-Arango, L.F., Barrett, H.L., Wilkinson, S.A., Callaway, L.K., McIntyre, H.D., Morrison, M., and Dekker Nitert, M. (2018). Low dietary fiber intake increases

<i>Collinsella</i> abundance in the gut microbiota of overweight and obese pregnant women. Gut Microbes 9, 189-201.

Gupta, N.K., and Dixit, V.K. (2011). Absorption enhancement of grape seed polyphenols by complexation with phosphatidyl choline. Drug Delivery 18, 312-319.

Halliwell, B. (2007). Biochemistry of oxidative stress. Biochem Soc Trans 35, 1147-1150.

Heintz-Buschart, A., and Wilmes, P. (2018). Human Gut Microbiome: Function Matters. Trends in microbiology 26, 563-574.

Hertog, M.G.L., Feskens, E.J.M., Kromhout, D., Hertog, M.G.L., Hollman, P.C.H., Hertog, M.G.L., and Katan, M.B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet 342, 1007-1011.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. Nature 444, 860-867.

Huang, P.L. (2009). A comprehensive definition for metabolic syndrome. Dis Model Mech 2, 231-237.

Jiao, N., Baker, S.S., Nugent, C.A., Tsompana, M., Cai, L., Wang, Y., Buck, M.J., Genco, R.J., Baker, R.D., Zhu, R., et al. (2018). Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol. Genomics 50, 244-254.

Johnson, E.L., Heaver, S.L., Waters, J.L., Kim, B.I., Bretin, A., Goodman, A.L., Gewirtz, A.T., Worgall, T.S., and Ley, R.E. (2020). Sphingolipids produced by gut bacteria enter host metabolic pathways impacting ceramide levels. Nature Communications 11, 2471.

Kim, M., Choi, S.Y., Lee, P., and Hur, J. (2015). Neochlorogenic Acid Inhibits Lipopolysaccharide-Induced Activation and Pro-inflammatory Responses in BV2 Microglial Cells. Neurochemical research 40, 1792-1798.

Kulling, S.E., and Rawel, H.M. (2008). Chokeberry (Aronia melanocarpa) - A review on the characteristic components and potential health effects. Planta Med 74, 1625-1634.

La Reau, A.J., Meier-Kolthoff, J.P., and Suen, G. (2016). Sequence-based analysis of the genus Ruminococcus resolves its phylogeny and reveals strong host association. Microb Genom 2, e000099-e000099.

La Reau, A.J., and Suen, G. (2018). The Ruminococci: key symbionts of the gut ecosystem. Journal of Microbiology 56, 199-208.

Lahti, L., Salonen, A., Kekkonen, R.A., Salojärvi, J., Jalanka-Tuovinen, J., Palva, A., Orešič, M., and de Vos, W.M. (2013). Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data. PeerJ 1, e32-e32.

Lam, Y.Y., Ha, C.W.Y., Campbell, C.R., Mitchell, A.J., Dinudom, A., Oscarsson, J., Cook, D.I., Hunt, N.H., Caterson, I.D., Holmes, A.J., et al. (2012). Increased Gut Permeability and Microbiota Change Associate with Mesenteric Fat Inflammation and Metabolic Dysfunction in Diet-Induced Obese Mice. Plos One 7, e34233.

Laparra, J.M., and Sanz, Y. (2010). Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacological Research 61, 219-225.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.M., Kennedy, S., et al. (2013). Richness of human gut microbiome correlates with metabolic markers. Nature 500, 541-546.

Le Roy, T., Llopis, M., Lepage, P., Bruneau, A., Rabot, S., Bevilacqua, C., Martin, P., Philippe, C., Walker, F., Bado, A., et al. (2013). Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut 62, 1787-1794.

Leclercq, S., Matamoros, S., Cani, P.D., Neyrinck, A.M., Jamar, F., Stärkel, P., Windey, K., Tremaroli, V., Bäckhed, F., Verbeke, K., et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A 111, E4485-4493.

Lee, Y.S., Li, P.P., Huh, J.Y., Hwang, I.J., Lu, M., Kim, J.I., Ham, M., Talukdar, S., Chen, A., Lu, W.J., et al. (2011). Inflammation Is Necessary for Long-Term but Not Short-Term High-Fat Diet Induced Insulin Resistance. Diabetes 60, 2474-2483.

Leonard, P.J., Brand, M.H., Connolly, B.A., and Obae, S.G. (2013). Investigation of the Origin of Aronia mitschurinii using Amplified Fragment Length Polymorphism Analysis. HortScience horts 48, 520-524.

Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. (2005). Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America 102, 11070-11075.

Li, H.-Y., Zhang, H.-L., Zhao, F.-J., Wang, S.-Q., Wang, Z.-X., and Wei, Z.-Y. (2020). Modulation of Gut Microbiota, Short-Chain Fatty Acid Production, and Inflammatory Cytokine Expression in the Cecum of Porcine Deltacoronavirus-Infected Chicks. Front. Microbiol. 11, 897-897.

Li, J., Zhao, F.Q., Wang, Y.D., Chen, J.R., Tao, J.E., Tian, G., Wu, S.L., Liu, W.B., Cui, Q.H., Geng, B., et al. (2017). Gut microbiota dysbiosis contributes to the development of hypertension. Microbiome 5, 19.

Louca, P., Nogal, A., Wells, P.M., Asnicar, F., Wolf, J., Steves, C.J., Spector, T.D., Segata, N., Berry, S.E., Valdes, A.M., et al. (2021). Gut microbiome diversity and composition is associated with hypertension in women. Journal of Hypertension 39.

Manco, M., Putignani, L., and Bottazzo, G.F. (2010). Gut Microbiota, Lipopolysaccharides, and Innate Immunity in the Pathogenesis of Obesity and Cardiovascular Risk. Endocrine Reviews 31, 817-844.

Martin, D.A., Taheri, R., Brand, M.H., Draghi, A., Sylvester, F.A., and Bolling, B.W. (2014). Anti-inflammatory activity of aronia berry extracts in murine splenocytes. J. Funct. Food. 8, 68-75.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412-419.

Musso, G., Gambino, R., and Cassader, M. (2011). Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med 62, 361-380.

Nagahashi, M., Yamada, A., Katsuta, E., Aoyagi, T., Huang, W.C., Terracina, K.P., Hait, N.C., Allegood, J.C., Tsuchida, J., Yuza, K., et al. (2018). Targeting the SphK1/S1P/S1PR1 Axis That Links Obesity, Chronic Inflammation, and Breast Cancer Metastasis. Cancer Res 78, 1713-1725.

Nixon, G.F. (2009). Sphingolipids in inflammation: pathological implications and potential therapeutic targets. Br J Pharmacol 158, 982-993.

Nutrient Data Laboratory. (2010). USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods. USDA.

Oszmianski, J.A.N., and Sapis Jean, C. (1988). Anthocyanins in Fruits of Aronia Melanocarpa (Chokeberry). Journal of Food Science 53, 1241-1242.

Palócz, O., Pászti-Gere, E., Gálfi, P., and Farkas, O. (2016). Chlorogenic Acid Combined with Lactobacillus plantarum 2142 Reduced LPS-Induced Intestinal Inflammation and Oxidative Stress in IPEC-J2 Cells. Plos One 11, e0166642.

Peach, J.T., Wilson, S.M., Gunderson, L.D., Frothingham, L., Tran, T., Walk, S.T., Yeoman, C.J., Bothner, B., and Miles, M.P. (2021). Temporal metabolic response yields a dynamic biosignature of inflammation. iScience 24, 102817.

Perry, R.J., Peng, L., Barry, N.A., Cline, G.W., Zhang, D.Y., Cardone, R.L., Petersen, K.F., Kibbey, R.G., Goodman, A.L., and Shulman, G.I. (2016). Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. Nature 534, 213-+.

Pluskal, T., Castillo, S., Villar-Briones, A., and Orešič, M. (2010). MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 11, 395.

Pyun, C.-W., Seo, T.-S., Kim, D.-J., Kim, T.-W., and Bae, J.-S. (2020). Protective Effects of Ligularia fischeri and Aronia melanocarpa Extracts on Alcoholic Liver Disease (In Vitro and In Vivo Study). BioMed research international 2020, 9720387-9720387.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., and Zhang, F. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 490.

Rohr, M.W., Narasimhulu, C.A., Rudeski-Rohr, T.A., and Parthasarathy, S. (2020). Negative Effects of a High-Fat Diet on Intestinal Permeability: A Review. Advances in Nutrition 11, 77-91.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P.I., Godneva, A., Kalka, I.N., Bar, N., et al. (2018). Environment dominates over host genetics in shaping human gut microbiota. Nature 555, 210-+.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75, 7537-7541.

Serino, M., Luche, E., Gres, S., Baylac, A., Berge, M., Cenac, C., Waget, A., Klopp, P., Iacovoni, J., Klopp, C., et al. (2012). Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 61, 543-553.

Sharma, A., Gupta, N.K., and Dixit, V.K. (2010). Complexation with phosphatidyl choline as a strategy for absorption enhancement of boswellic acid. Drug Delivery 17, 587-595.

Shkoporov, A.N., Chaplin, A.V., Shcherbakova, V.A., Suzina, N.E., Kafarskaia, L.I., Bozhenko, V.K., and Efimov, B.A. (2016). Ruthenibacterium lactatiformans gen. nov., sp. nov., an anaerobic, lactate-producing member of the family Ruminococcaceae isolated from human faeces. International Journal of Systematic and Evolutionary Microbiology 66, 3041-3049.

Singh, R.P., Halaka, D.A., Hayouka, Z., and Tirosh, O. (2020). High-Fat Diet Induced Alteration of Mice Microbiota and the Functional Ability to Utilize Fructooligosaccharide for Ethanol Production. Frontiers in Cellular and Infection Microbiology 10.

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I., and Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 579, 200-213.

Spiegel, S., and Milstien, S. (2011). The outs and the ins of sphingosine-1-phosphate in immunity. Nature Reviews Immunology 11, 403-415.

Stremmel, W., Ehehalt, R., Staffer, S., Stoffels, S., Mohr, A., Karner, M., and Braun, A. (2012). Mucosal protection by phosphatidylcholine. Dig Dis 30 Suppl 3, 85-91.

Stremmel, W., Vural, H., Evliyaoglu, O., and Weiskirchen, R. (2021). Delayed-Release Phosphatidylcholine Is Effective for Treatment of Ulcerative Colitis: A Meta-Analysis. Dig Dis 39, 508-515.

Taira, T., Yamaguchi, S., Takahashi, A., Okazaki, Y., Yamaguchi, A., Sakaguchi, H., and Chiji, H. (2015). Dietary polyphenols increase fecal mucin and immunoglobulin A and ameliorate the disturbance in gut microbiota caused by a high fat diet. Journal of clinical biochemistry and nutrition 57, 212-216.

Tall, A.R., and Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. Nature Reviews Immunology 15, 104.

Tan, W., Zhang, Q., Dong, Z., Yan, Y., Fu, Y., Liu, X., Zhao, B., and Duan, X. (2020). Phosphatidylcholine Ameliorates LPS-Induced Systemic Inflammation and Cognitive Impairments via Mediating the Gut–Brain Axis Balance. Journal of agricultural and food chemistry 68, 14884-14895.

Treede, I., Braun, A., Jeliaskova, P., Giese, T., Füllekrug, J., Griffiths, G., Stremmel, W., and Ehehalt, R. (2009). TNF-alpha-induced up-regulation of pro-inflammatory cytokines is reduced by phosphatidylcholine in intestinal epithelial cells. BMC Gastroenterol 9, 53.

Treede, I., Braun, A., Sparla, R., Kühnel, M., Giese, T., Turner, J.R., Anes, E., Kulaksiz, H., Füllekrug, J., Stremmel, W., et al. (2007). Anti-inflammatory effects of phosphatidylcholine. The Journal of biological chemistry 282, 27155-27164.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444.

Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., and Gordon, J.I. (2009). The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. Sci. Transl. Med. 1, 6ra14.

Verdam, F.J., Fuentes, S., de Jonge, C., Zoetendal, E.G., Erbil, R., Greve, J.W., Buurman, W.A., de Vos, W.M., and Rensen, S.S. (2013). Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity 21, E607-E615.

Volpato, S., Guralnik, J.M., Ferrucci, L., Balfour, J., Chaves, P., Fried, L.P., and Harris, T.B. (2001). Cardiovascular disease, interlenkin-6, and risk of mortality in older women - The women's health and aging study. Circulation 103, 947-953.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation 112, 1796-1808.

Wiklund, P., Zhang, X., Pekkala, S., Autio, R., Kong, L., Yang, Y., Keinänen-Kiukaanniemi, S., Alen, M., and Cheng, S. (2016). Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Scientific Reports 6, 24540.

Wilson, S.M., Maes, A.P., Yeoman, C.J., Walk, S.T., and Miles, M.P. (2021). Determinants of the postprandial triglyceride response to a high-fat meal in healthy overweight and obese adults. Lipids in Health and Disease 20, 107.

Xiao, L., Sonne, S.B., Feng, Q., Chen, N., Xia, Z., Li, X., Fang, Z., Zhang, D., Fjære, E., Midtbø, L.K., et al. (2017). High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome 5, 43.

Zhong, Y., Nyman, M., and Fåk, F. (2015). Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. Molecular Nutrition & Food Research 59, 2066-2076.

CHAPTER FIVE

CONCLUSION

Overnutrition is the leading driver of obesity.[1] Overnutrition contributes to metabolic stress, gut microbiota dysbiosis, and promotes both lipid accumulation, unhealthy lipid deposition, and adipose dysfunction.[2,3] Excess adipose accumulation promotes dysregulated carbohydrate and lipid metabolism facilitated through increased insulin resistance, affect inflammatory processes,[4] and work together to cascade into more deleterious metabolic diseases if left chronically unchecked (**Figure 1**).[5,6] For this reason, emphasizing chronic disease prevention in its earliest stages of altered metabolism is critical. Adults with excess adipose and with central obesity, but who do not present with disease, are the ideal population to assess early alterations in energy regulation. Acute food challenges and dietary interventions provide a “stress” to test the metabolic machinery and assess the responsiveness, or lack thereof, in the system. The studies in this dissertation examine early alterations in metabolic dysfunction such as lipid metabolism, glycemic control, and inflammation through a food-driven perspective.

In **Chapter 2**, I examined the postprandial triglyceride response to a high-fat meal in adults with overweight and obesity in relation to a compendium of lifestyle and physiologic factors. Using a postprandial summary measure and multivariate analysis, I found that individuals with the highest postprandial triglyceride response to an oral fat load had higher central adiposity, insulin resistance, and interestingly, increased ability to oxidize fat during an aerobic exercise test. Elevated postprandial lipids contribute to

oxidative stress, inflammation, and endothelial dysfunction to promote a collective atherogenic effect. The factors I observed to predict postprandial triglycerides are interrelated and add new information in healthy, but at-risk, adults to inform chronic disease risk prediction.

In **Chapter 3**, I tested the efficacy of a long-term lentil consumption on the glycemic control of free-living adults with increased central adiposity. Similar in principle to the high-fat meal challenge, I tested glycemic control through the delivery of an oral glucose tolerance test before and after eight weeks of consuming meals containing varying amounts of whole green lentils. Consumption of lentils, a fiber-rich and plant-based protein, promoted a dose-dependent decrease in insulin resistance compared to consumption of an animal-based protein, but did not alter postprandial glucose and insulin responses. Further, I added novel findings on lentil consumption in relation to gastrointestinal symptoms. Lentil incorporation and related health benefits were obtained without an increase in gastrointestinal symptom severity, which may serve to counter consumer perceptions of increased gastrointestinal complaints with pulse crops.

In **Chapter 4**, our interdisciplinary research team created a gnotobiotic mouse model through a fecal microbial transplantation from two human stool donors with different inflammatory profiles chosen from the study described in Chapter 2. Through this mouse model, we were able to control for the variability in gastrointestinal microbes and diet normally present in free-living adults, and specifically test the impact of an antioxidant rich juice from the *Aronia melanocarpa* berry in response to a high-fat diet (HFD), a dietary stimulus for inflammation and obesity. Using next-generation

sequencing and mass spectrometry analysis, we observed distinct microbial and metabolomic profiles by donor and pronounced shifts in each with the introduction of a HFD. In the microbial community, we detected donor-specific and juice-specific changes. Notably, LO donor mice receiving Aronia juice were protected from a HFD-induced decline in alpha-diversity. Alterations in specific taxa *Barnesiella, Escheria-Shigella, Bilophilia, Ruminococcus,* and *Ruthenbacterium* in LO donor mice support an anti-inflammatory role of Aronia juice. Host serum concentrations of phospholipids, phosphatidylcholine and sphingomyelin, were respectively altered with the introduction of Aronia juice and HFD.

The metabolic responses to the same food display high variability between individuals which encourage a more personalized approach to nutrition. Future research geared toward nutritional interventions must carefully consider the interindividual variation in metabolic responses as being influenced by a compendium of biological and lifestyle influences including the gut microbiota and gut-mediated metabolic compounds.

These data herein provide insight into how metabolically at-risk individuals, not yet with disease, respond to a dietary challenge and through gnotobiotic mouse models, how the gut microbiota and metabolic profiles are altered with a large dietary and metabolic stress. These data highlight the interrelationship between energy regulation and immune function, to which the function of one is dependent on the function of the other, and host health.

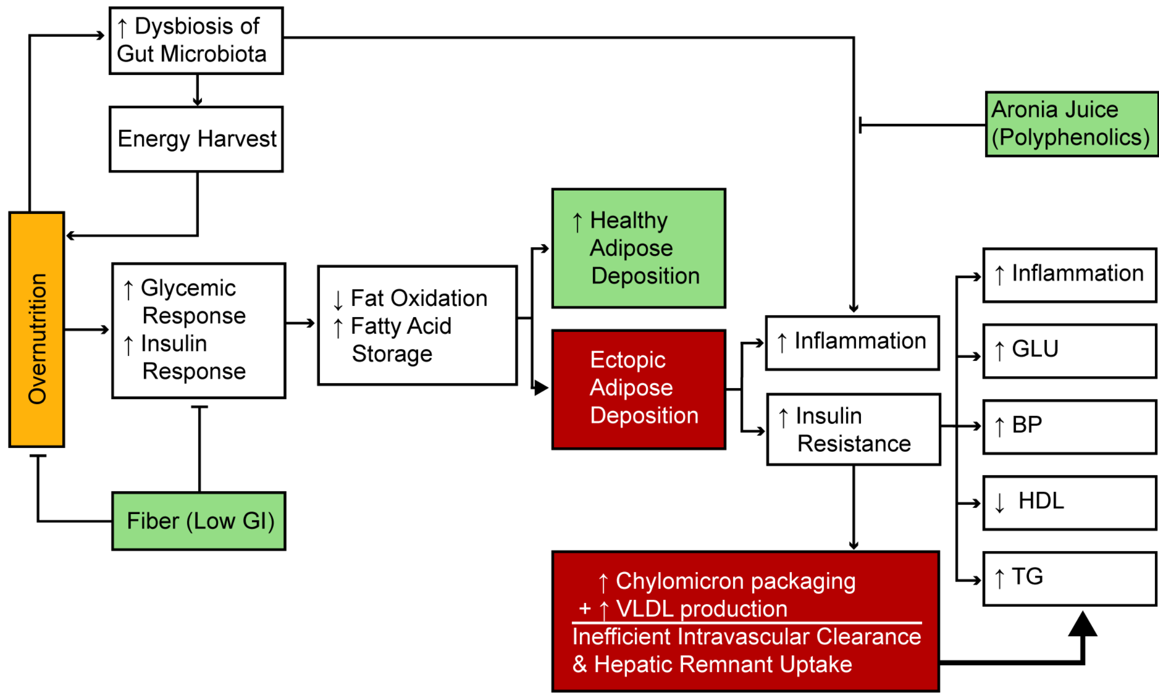


Figure 1 | Schematic displaying the contribution of diet toward the development of metabolic syndrome - revisited. Research from this dissertation are highlighted and include work on postprandial lipemia (Chapter 2), dietary fiber consumption and glycemic responses (Chapter 3), and the influence of a polyphenolic rich juice on high-fat diet-induced outcomes in a humanized mouse model (Chapter 4).

References

1. Swinburn B, Sacks G, Ravussin E. Increased food energy supply is more than sufficient to explain the US epidemic of obesity. American Journal of Clinical Nutrition. 2009;90(6):1453-1456.

2. Longo M, Zatterale F, Naderi J, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci. 2019;20(9):2358.

3. Xiao L, Sonne SB, Feng Q, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome. 2017;5(1):43.

4. Tripathy D, Mohanty P, Dhindsa S, et al. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes. 2003;52(12):2882-2887.

5. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation. 2000;102(1):42-47.

6. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-867.

CUMULATIVE REFERENCES CITED

Afshin, A., Sur, P.J., Fay, K.A., Cornaby, L., Ferrara, G., Salama, J.S., Mullany, E.C., Abate, K.H., Abbafati, C., Abebe, Z., et al. (2019). Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 393, 1958-1972.

Albenberg, L.G., and Wu, G.D. (2014). Diet and the Intestinal Microbiome: Associations, Functions, and Implications for Health and Disease. Gastroenterology 146, 1564-1572.

American-Diabetes-Association (2020). 3. Prevention or Delay of Type 2 Diabetes: Standards of Medical Care in Diabetes—2020. Diabetes Care 43, S32.

American-Diabetes-Association (2021). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care 44, S15.

Annuzzi, G., Giacco, R., Patti, L., Di Marino, L., De Natale, C., Costabile, G., Marra, M., Santangelo, C., Masella, R., and Rivellese, A.A. (2008). Postprandial chylomicrons and adipose tissue lipoprotein lipase are altered in type 2 diabetes independently of obesity and whole-body insulin resistance. Nutr Metab Cardiovasc Dis 18, 531-538.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., et al. (2011). Enterotypes of the human gut microbiome. Nature 473, 174-180.

Asnicar, F., Berry, S.E., Valdes, A.M., Nguyen, L.H., Piccinno, G., Drew, D.A., Leeming, E., Gibson, R., Le Roy, C., Khatib, H.A., et al. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nature Medicine 27, 321-332.

Astbury, S., Atallah, E., Vijay, A., Aithal, G.P., Grove, J.I., and Valdes, A.M. (2020). Lower gut microbiome diversity and higher abundance of proinflammatory genus Collinsella are associated with biopsy-proven nonalcoholic steatohepatitis. Gut Microbes 11, 569-580.

Awwad, H.M., Geisel, J., and Obeid, R. (2016). Determination of trimethylamine, trimethylamine N-oxide, and taurine in human plasma and urine by UHPLC–MS/MS technique. Journal of Chromatography B 1038, 12-18.

Backhed, F., Fraser, C.M., Ringel, Y., Sanders, M.E., Sartor, R.B., Sherman, P.M., Versalovic, J., Young, V., and Finlay, B.B. (2012). Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications. Cell Host & Microbe 12, 611-622.

Bäckhed, F., Manchester, J.K., Semenkovich, C.F., and Gordon, J.I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proceedings of the National Academy of Sciences 104, 979.

Banjari, I., Misir, A., Savikin, K., Jokic, S., Molnar, M., De Zoysa, H.K.S., and Waisundara, V.Y. (2017). Antidiabetic effects of Aronia melanocarpa and its Other Therapeutic Properties. Front. Nutr. 4, 6.

Bansal, S., Buring, J.E., Rifai, N., Mora, S., Sacks, F.M., and Ridker, P.M. (2007). Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. Jama 298, 309-316.

Baraldi, L.G., Martinez Steele, E., Canella, D.S., and Monteiro, C.A. (2018). Consumption of ultra-processed foods and associated sociodemographic factors in the USA between 2007 and 2012: evidence from a nationally representative cross-sectional study. BMJ Open 8, e020574.

Bazzano, L.A., Thompson, A.M., Tees, M.T., Nguyen, C.H., and Winham, D.M. (2011). Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis 21, 94-103.

Bergouignan, A., Momken, I., Lefai, E., Antoun, E., Schoeller, D.A., Platat, C., Chery, I., Zahariev, A., Vidal, H., Gabert, L., et al. (2013). Activity energy expenditure is a major determinant of dietary fat oxidation and trafficking, but the deleterious effect of detraining is more marked than the beneficial effect of training at current recommendations. The American Journal of Clinical Nutrition 98, 648-658.

Berry, S., Valdes, A., Davies, R., Khatib, H.A., Delahanty, L., Drew, D., Chan, A.T., Segata, N., Franks, P., and Spector, T. (2019). Large Inter-individual Variation in Postprandial Lipemia Following a Mixed Meal in over 1000 Twins and Singletons from the UK and US: The PREDICT I Study (OR19-06-19). Curr Dev Nutr 3, nzz046.OR019-006-019.

Bielefeld, D., Grafenauer, S., and Rangan, A. (2020). The Effects of Legume Consumption on Markers of Glycaemic Control in Individuals with and without Diabetes Mellitus: A Systematic Literature Review of Randomised Controlled Trials. Nutrients 12, 2123.

Bisanz, J.E., Upadhyay, V., Turnbaugh, J.A., Ly, K., and Turnbaugh, P.J. (2019). Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host & Microbe 26, 265-272.e264.

Blaak, E.E., Hul, G., Verdich, C., Stich, V., Martinez, A., Petersen, M., Feskens, E.F.M., Patel, K., Oppert, J.M., Barbe, P., et al. (2006). Fat Oxidation before and after a High Fat

Load in the Obese Insulin-Resistant State. The Journal of Clinical Endocrinology & Metabolism 91, 1462-1469.

Borghouts, L.B., and Keizer, H.A. (2000). Exercise and insulin sensitivity: A review. International Journal of Sports Medicine 21, 1-12.

Bosy-Westphal, A., Schautz, B., Later, W., Kehayias, J.J., Gallagher, D., and Müller, M.J. (2013). What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. European Journal Of Clinical Nutrition 67, S14.

Bozzetto, L., Della Pepa, G., Vetrani, C., and Rivellese, A.A. (2020). Dietary Impact on Postprandial Lipemia. Front. Endocrinol. 11.

Brahe, L.K., Le Chatelier, E., Prifti, E., Pons, N., Kennedy, S., Hansen, T., Pedersen, O., Astrup, A., Ehrlich, S.D., and Larsen, L.H. (2015). Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. Nutrition & Diabetes 5, 7.

Bräunlich, M., Slimestad, R., Wangensteen, H., Brede, C., Malterud, K.E., and Barsett, H. (2013). Extracts, Anthocyanins and Procyanidins from Aronia melanocarpa as Radical Scavengers and Enzyme Inhibitors. Nutrients 5, 663-678.

Brooks, G.A. (1997). Importance of the 'crossover' concept in exercise metabolism. Clin Exp Pharmacol Physiol 24, 889-895.

Brooks, G.A., and Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J Appl Physiol 76, 2253-2261.

Brouns, F., Bjorck, I., Frayn, K.N., Gibbs, A.L., Lang, V., Slama, G., and Wolever, T.M.S. (2005). Glycemic index methodology. Nutr. Res. Rev. 18, 145-171.

Brown, E.M., Ke, X., Hitchcock, D., Jeanfavre, S., Avila-Pacheco, J., Nakata, T., Arthur, T.D., Fornelos, N., Heim, C., Franzosa, E.A., et al. (2019). Bacteroides-Derived Sphingolipids Are Critical for Maintaining Intestinal Homeostasis and Symbiosis. Cell Host Microbe 25, 668-680.e667.

Burton, B.T., Foster, W.R., Hirsch, J., and Van Itallie, T.B. (1985). Health implications of obesity: an NIH Consensus Development Conference. Int J Obes 9, 155-170.

Burton, F.L., Malkova, D., Caslake, M.J., and Gill, J.M.R. (2008). Energy replacement attenuates the effects of prior moderate exercise on postprandial metabolism in overweight/obese men. Int J Obes 32.

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761-1772.

Cani, P.D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A.M., Delzenne, N.M., and Burcelin, R. (2008). Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. Diabetes 57, 1470-1481.

Cani, P.D., Possemiers, S., Wiele, T., Guiot, Y., Everard, A., and Rottier, O. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58.

Caporaso, J.G., Lauber, C.L., Costello, E.K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J., Knights, D., Gajer, P., Ravel, J., Fierer, N., et al. (2011). Moving pictures of the human microbiome. Genome Biol. 12.

Carmody, R.N., Bisanz, J.E., Bowen, B.P., Maurice, C.F., Lyalina, S., Louie, K.B., Treen, D., Chadaideh, K.S., Maini Rekdal, V., Bess, E.N., et al. (2019). Cooking shapes the structure and function of the gut microbiome. Nat. Microbiol 4, 2052-2063.

Carstensen, M., Thomsen, C., and Hermansen, K. (2003). Incremental area under response curve more accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects. Metabolism 52, 1034-1037.

CDC (2020). National diabetes statistics report, 2020. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services, 12-15.

Chabowski, A., Chatham, J.C., Tandon, N.N., Calles-Escandon, J., Glatz, J.F., Luiken, J.J., and Bonen, A. (2006). Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats. Am J Physiol Endocrinol Metab 291, E675-682.

Chambers, M.C., Maclean, B., Burke, R., Amodei, D., Ruderman, D.L., Neumann, S., Gatto, L., Fischer, B., Pratt, B., Egertson, J., et al. (2012). A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology 30, 918-920.

Chen, M., Pan, H., Dai, Y., Zhang, J., Tong, Y., Huang, Y., Wang, M., and Huang, H. (2018). Phosphatidylcholine regulates NF-κB activation in attenuation of LPS-induced inflammation: evidence from in vitro study. Animal Cells and Systems 22, 7-14.

Chong, J., Wishart, D.S., and Xia, J. (2019). Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. Current Protocols in Bioinformatics 68, e86.

Cibičková, Ľ., Langová, K., Vaverková, H., Lukeš, J., Cibiček, N., and Karásek, D. (2019). Superior Role of Waist Circumference to Body-Mass Index in the Prediction of Cardiometabolic Risk in Dyslipidemic Patients. Physiol Res 68, 931-938.

Clark, M.J., and Slavin, J.L. (2013). The effect of fiber on satiety and food intake: a systematic review. Journal of the American College of Nutrition 32, 200-211.

Comalada, M., Camuesco, D., Sierra, S., Ballester, I., Xaus, J., Gálvez, J., and Zarzuelo, A. (2005). In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. European Journal of Immunology 35, 584-592.

Consortium, T.H.M.P. (2012). Structure, function and diversity of the healthy human microbiome. Nature 486, 207-214.

Copeland, S., Warren, H.S., Lowry, S.F., Calvano, S.E., Remick, D., the, I., and the Host Response to Injury, I. (2005). Acute Inflammatory Response to Endotoxin in Mice and Humans. Clinical and Diagnostic Laboratory Immunology 12, 60-67.

Cordova, R., Kliemann, N., Huybrechts, I., Rauber, F., Vamos, E.P., Levy, R.B., Wagner, K.-H., Viallon, V., Casagrande, C., Nicolas, G., et al. (2021). Consumption of ultra-processed foods associated with weight gain and obesity in adults: A multi-national cohort study. Clinical Nutrition 40, 5079-5088.

Costello, E.K., Lauber, C.L., Hamady, M., Fierer, N., Gordon, J.I., and Knight, R. (2009). Bacterial Community Variation in Human Body Habitats Across Space and Time. Science (New York, N.Y.) 326, 1694-1697.

Couillard, C., Bergeron, N., Prud'homme, D., Bergeron, J., Tremblay, A., Bouchard, C., Mauriège, P., and Després, J.-P. (1999). Gender Difference in Postprandial Lipemia. Arteriosclerosis, Thrombosis, and Vascular Biology 19, 2448-2455.

Craig, L.A., Goodwin, B., and Grennes, T. (2004). The Effect of Mechanical Refrigeration on Nutrition in the United States. Social Science History 28, 325-336.

Dai, Y., Tang, H., and Pang, S. (2021). The Crucial Roles of Phospholipids in Aging and Lifespan Regulation. Front Physiol 12, 775648.

Dalby, M.J., Ross, A.W., Walker, A.W., and Morgan, P.J. (2017). Dietary Uncoupling of Gut Microbiota and Energy Harvesting from Obesity and Glucose Tolerance in Mice. Cell reports 21, 1521-1533.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-+.

Dawaliby, R., Trubbia, C., Delporte, C., Noyon, C., Ruysschaert, J.M., Van Antwerpen, P., and Govaerts, C. (2016). Phosphatidylethanolamine Is a Key Regulator of Membrane Fluidity in Eukaryotic Cells. The Journal of biological chemistry 291, 3658-3667.

Desai, M.S., Seekatz, A.M., Koropatkin, N.M., Kamada, N., Hickey, C.A., Wolter, M., Pudlo, N.A., Kitamoto, S., Terrapon, N., Muller, A., et al. (2016). A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. Cell 167, 1339-1353.e1321.

Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., and Xia, J. (2017). MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research 45, W180-W188.

Dias, C.B., Moughan, P.J., Wood, L.G., Singh, H., and Garg, M.L. (2017). Postprandial lipemia: factoring in lipemic response for ranking foods for their healthiness. Lipids in health and disease 16, 178-178.

Dobrosotskaya, I.Y., Seegmiller, A.C., Brown, M.S., Goldstein, J.L., and Rawson, R.B. (2002). Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296, 879-883.

Duan, Y., Zeng, L., Zheng, C., Song, B., Li, F., Kong, X., and Xu, K. (2018). Inflammatory Links Between High Fat Diets and Diseases. Frontiers in immunology 9, 2649-2649.

Dührkop, K., Fleischauer, M., Ludwig, M., Aksenov, A.A., Melnik, A.V., Meusel, M., Dorrestein, P.C., Rousu, J., and Böcker, S. (2019). SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nature methods 16, 299-302.

Duncan, B.B., Schmidt, M.I., Pankow, J.S., Ballantyne, C.M., Couper, D., Vigo, A., Hoogeveen, R., Folsom, A.R., Heiss, G., and Atherosclerosis Risk in Communities, S. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 52, 1799-1805.

Elizabeth, L., Machado, P., Zinöcker, M., Baker, P., and Lawrence, M. (2020). Ultra-Processed Foods and Health Outcomes: A Narrative Review. Nutrients 12.

Fajardo, V.A., McMeekin, L., and LeBlanc, P.J. (2011). Influence of phospholipid species on membrane fluidity: a meta-analysis for a novel phospholipid fluidity index. J Membr Biol 244, 97-103.

Fang, J. (2014). Bioavailability of anthocyanins. Drug Metab Rev 46, 508-520.

Fei, N., and Zhao, L.P. (2013). An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. Isme Journal 7, 880-884.

Fernandes, I., Faria, A., Calhau, C., de Freitas, V., and Mateus, N. (2014). Bioavailability of anthocyanins and derivatives. J. Funct. Food. 7, 54-66.

Ferreira, H., Vasconcelos, M., Gil, A.M., and Pinto, E. (2021). Benefits of pulse consumption on metabolism and health: A systematic review of randomized controlled trials. Critical Reviews in Food Science and Nutrition 61, 85-96.

Festa, A., D'Agostino, R., Jr., Howard, G., Mykkanen, L., Tracy, R.P., and Haffner, S.M. (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 102, 42-47.

Finck, B.N. (2018). Targeting Metabolism, Insulin Resistance, and Diabetes to Treat Nonalcoholic Steatohepatitis. Diabetes 67, 2485.

Finucane, M.M., Stevens, G.A., Cowan, M.J., Danaei, G., Lin, J.K., Paciorek, C.J., Singh, G.M., Gutierrez, H.R., Lu, Y.A., Bahalim, A.N., et al. (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet 377, 557-567.

Flegal, K.M., Kruszon-Moran, D., Carroll, M.D., Fryar, C.D., and Ogden, C.L. (2016). Trends in Obesity Among Adults in the United States, 2005 to 2014. Jama 315, 2284-2291.

Fontaine, K.R., Redden, D.T., Wang, C.X., Westfall, A.O., and Allison, D.B. (2003). Years of life lost due to obesity. JAMA-J. Am. Med. Assoc. 289, 187-193.

Fox, C.S., Massaro, J.M., Hoffmann, U., Pou, K.M., Maurovich-Horvat, P., Liu, C.Y., Vasan, R.S., Murabito, J.M., Meigs, J.B., Cupples, L.A., et al. (2007). Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 116, 39-48.

Fox, J. (2003). Effect Displays in R for Generalised Linear Models. Journal of Statistical Software 8, 1 - 27.

Fox, J., and Weisberg, S. (2019). An {R} companion to applied regression. (Thousand Oaks, CA: Sage).

Freese, E.C., Gist, N.H., and Cureton, K.J. (2013). Effect of prior exercise on postprandial lipemia: an updated quantitative review. J Appl Physiol 116.

Fu, S., Yang, L., Li, P., Hofmann, O., Dicker, L., Hide, W., Lin, X., Watkins, S.M., Ivanov, A.R., and Hotamisligil, G.S. (2011). Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. Nature 473, 528-531.

Ganesan, K., and Xu, B. (2017). Polyphenol-Rich Lentils and Their Health Promoting Effects. Int J Mol Sci 18, 2390.

Gao, Z., Yin, J., Zhang, J., and Ward, R. (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 58.

Geer, E.B., and Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. Gender Medicine 6, 60-75.

Ghoshal, S., Witta, J., Zhong, J., de Villiers, W., and Eckhardt, E. (2009). Chylomicrons promote intestinal absorption of lipopolysaccharides. Journal of Lipid Research 50, 90-97.

Gil-Cardoso, K., Gines, I., Pinent, M., Ardevol, A., Blay, M., and Terra, X. (2016). Effects of flavonoids on intestinal inflammation, barrier integrity and changes in gut microbiota during diet-induced obesity. Nutr. Res. Rev. 29, 234-248.

Gomez-Arango, L.F., Barrett, H.L., Wilkinson, S.A., Callaway, L.K., McIntyre, H.D., Morrison, M., and Dekker Nitert, M. (2018). Low dietary fiber intake increases <i>Collinsella</i> abundance in the gut microbiota of overweight and obese pregnant women. Gut Microbes 9, 189-201.

Goodpaster, B.H., and Sparks, L.M. (2017). Metabolic Flexibility in Health and Disease. Cell metabolism 25, 1027-1036.

Grundy Scott, M., Stone Neil, J., Bailey Alison, L., Beam, C., Birtcher Kim, K., Blumenthal Roger, S., Braun Lynne, T., de Ferranti, S., Faiella-Tommasino, J., Forman Daniel, E., et al. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. Journal of the American College of Cardiology 73, e285-e350.

Grundy, S.M. (2006). Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. J Am Coll Cardiol 47, 1093-1100.

Grundy, S.M. (2016). Overnutrition, ectopic lipid and the metabolic syndrome. Journal of Investigative Medicine 64, 1082.

Gulhane, M., Murray, L., Lourie, R., Tong, H., Sheng, Y.H., Wang, R., Kang, A., Schreiber, V., Wong, K.Y., Magor, G., et al. (2016). High Fat Diets Induce Colonic Epithelial Cell Stress and Inflammation that is Reversed by IL-22. Scientific Reports 6, 17.

Gupta, N.K., and Dixit, V.K. (2011). Absorption enhancement of grape seed polyphenols by complexation with phosphatidyl choline. Drug Delivery 18, 312-319.

Gutt, M., Davis, C.L., Spitzer, S.B., Llabre, M.M., Kumar, M., Czarnecki, E.M., Schneiderman, N., Skyler, J.S., and Marks, J.B. (2000). Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract 47, 177-184.

Guzior, D.V., and Quinn, R.A. (2021). Review: microbial transformations of human bile acids. Microbiome 9, 140.

Hafiz, M.S., Campbell, M.D., O'Mahoney, L.L., Holmes, M., Orfila, C., and Boesch, C. (2021). Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: a systematic review and meta-analysis of acute and long-term randomized controlled trials. Eur J Nutr.

Hales, C.M., Carroll, M.D., Fryar, C.D., and Ogden, C.L. (2020a). Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017-2018. NCHS Data Brief, 1-8.

Hales, C.M., Carroll, M.D., Fryar, C.D., and Ogden, C.L. (2020b). Prevalence of obesity and severe obesity among adults: United States, 2017–2018. (National Center for Health Statistics: NCHS Data Brief).

Hall, K.D., Ayuketah, A., Brychta, R., Cai, H., Cassimatis, T., Chen, K.Y., Chung, S.T., Costa, E., Courville, A., Darcey, V., et al. (2019). Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. Cell metabolism 30, 67-77.e63.

Halliwell, B. (2007). Biochemistry of oxidative stress. Biochem Soc Trans 35, 1147-1150.

Hansson, P., Holven, K.B., Øyri, L.K.L., Brekke, H.K., Biong, A.S., Gjevestad, G.O., Raza, G.S., Herzig, K.-H., Thoresen, M., and Ulven, S.M. (2019). Meals with Similar Fat Content from Different Dairy Products Induce Different Postprandial Triglyceride Responses in Healthy Adults: A Randomized Controlled Cross-Over Trial. The Journal of nutrition 149, 422-431.

Harbis, A., Perdreau, S., Vincent-Baudry, S., Charbonnier, M., Bernard, M.-C., Raccah, D., Senft, M., Lorec, A.-M., Defoort, C., Portugal, H., et al. (2004). Glycemic and insulinemic meal responses modulate postprandial hepatic and intestinal lipoprotein accumulation in obese, insulin-resistant subjects. The American Journal of Clinical Nutrition 80, 896-902.

Hardy, O.T., Czech, M.P., and Corvera, S. (2012). What causes the insulin resistance underlying obesity? Curr Opin Endocrinol Diabetes Obes 19, 81-87.

Harlan, W.R., Landis, J.R., Flegal, K.M., Davis, C.S., and Miller, M.E. (1988). Secular Trends in Body Mass Index in the United States, 1960–1980. American Journal of Epidemiology 128, 1065-1074.

Heintz-Buschart, A., and Wilmes, P. (2018). Human Gut Microbiome: Function Matters. Trends in microbiology 26, 563-574.

Henn, K., Goddyn, H., Olsen, S.B., and Bredie, W.L.P. (2022). Identifying behavioral and attitudinal barriers and drivers to promote consumption of pulses: A quantitative survey across five European countries. Food Quality and Preference 98, 104455.

Henninger, J., Eliasson, B., Smith, U., and Rawshani, A. (2021). Identification of markers that distinguish adipose tissue and glucose and insulin metabolism using a multi-modal machine learning approach. Scientific Reports 11, 17050.

Hertog, M.G.L., Feskens, E.J.M., Kromhout, D., Hertog, M.G.L., Hollman, P.C.H., Hertog, M.G.L., and Katan, M.B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet 342, 1007-1011.

Heymsfield, S.B., and Wadden, T.A. (2017). Mechanisms, Pathophysiology, and Management of Obesity. New England Journal of Medicine 376, 254-266.

Higgins, J.A. (2012). Whole grains, legumes, and the subsequent meal effect: implications for blood glucose control and the role of fermentation. J Nutr Metab 2012, 829238-829238.

Higgins, V., and Adeli, K. (2020). Postprandial dyslipidemia in insulin resistant states in adolescent populations. J Biomed Res 34, 328-342.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. Nature 444, 860-867.

Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993). Adipose expression of tumor-necrosis-factor-alpha - direct role in obesity linked insulin resistance. Science 259, 87-91.

Huang, P.L. (2009). A comprehensive definition for metabolic syndrome. Dis Model Mech 2, 231-237.

Huebbe, P., and Rimbach, G. (2020). Historical Reflection of Food Processing and the Role of Legumes as Part of a Healthy Balanced Diet. Foods 9.

Jackson, K.G., Poppitt, S.D., and Minihane, A.M. (2012). Postprandial lipemia and cardiovascular disease risk: Interrelationships between dietary, physiological and genetic determinants. Atherosclerosis 220, 22-33.

Jakicic, J.M., and Otto, A.D. (2005). Physical activity considerations for the treatment and prevention of obesity. American Journal of Clinical Nutrition 82, 226S-229S.

Jenkins, D.J.A., Kendall, C.W.C., Augustin, L.S.A., Mitchell, S., Sahye-Pudaruth, S., Blanco Mejia, S., Chiavaroli, L., Mirrahimi, A., Ireland, C., Bashyam, B., et al. (2012). Effect of Legumes as Part of a Low Glycemic Index Diet on Glycemic Control and Cardiovascular Risk Factors in Type 2 Diabetes Mellitus: A Randomized Controlled Trial. Archives of Internal Medicine 172, 1653-1660.

Jeppesen, J., Hollenbeck Clarie, B., Zhou, M.Y., Coulston Ann, M., Jones, C., Chen, Y.D.I., and Reaven Gerald, M. (1995). Relation Between Insulin Resistance, Hyperinsulinemia, Postheparin Plasma Lipoprotein Lipase Activity, and Postprandial Lipemia. Arteriosclerosis, Thrombosis, and Vascular Biology 15, 320-324.

Jia, H., and Lubetkin, E.I. (2005). The impact of obesity on health-related quality-of-life in the general adult US population. J Public Health (Oxf) 27, 156-164.

Jiao, N., Baker, S.S., Nugent, C.A., Tsompana, M., Cai, L., Wang, Y., Buck, M.J., Genco, R.J., Baker, R.D., Zhu, R., et al. (2018). Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol. Genomics 50, 244-254.

Johnson, E.L., Heaver, S.L., Waters, J.L., Kim, B.I., Bretin, A., Goodman, A.L., Gewirtz, A.T., Worgall, T.S., and Ley, R.E. (2020). Sphingolipids produced by gut bacteria enter host metabolic pathways impacting ceramide levels. Nature Communications 11, 2471.

Kacerovsky-Bielesz, G., Chmelik, M., Ling, C., Pokan, R., Szendroedi, J., Farukuoye, M., Kacerovsky, M., Schmid, A.I., Gruber, S., Wolzt, M., et al. (2009). Short-Term Exercise Training Does Not Stimulate Skeletal Muscle ATP Synthesis in Relatives of Humans With Type 2 Diabetes. Diabetes 58, 1333.

Kazemi, M., McBreairty, L.E., Chizen, D.R., Pierson, R.A., Chilibeck, P.D., and Zello, G.A. (2018). A Comparison of a Pulse-Based Diet and the Therapeutic Lifestyle Changes Diet in Combination with Exercise and Health Counselling on the Cardio-Metabolic Risk

Profile in Women with Polycystic Ovary Syndrome: A Randomized Controlled Trial. Nutrients 10, 1387.

Khatoon, N., and Prakash, J. (2004). Nutritional quality of microwave-cooked and pressure-cooked legumes. International Journal of Food Sciences and Nutrition 55, 441-448.

Kim, M., Choi, S.Y., Lee, P., and Hur, J. (2015). Neochlorogenic Acid Inhibits Lipopolysaccharide-Induced Activation and Pro-inflammatory Responses in BV2 Microglial Cells. Neurochemical research 40, 1792-1798.

Knowler, W.C., Barrett-Connor, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Walker, E.A., Nathan, D.M., and Diabetes Prevention Program Res, G. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. New England Journal of Medicine 346, 393-403.

Krebs-Smith, S.M., Pannucci, T.E., Subar, A.F., Kirkpatrick, S.I., Lerman, J.L., Tooze, J.A., Wilson, M.M., and Reedy, J. (2018). Update of the Healthy Eating Index: HEI-2015. J Acad Nutr Diet 118, 1591-1602.

Krebs, M., Krssak, M., Bernroider, E., Anderwald, C., Brehm, A., Meyerspeer, M., Nowotny, P., Roth, E., Waldhäusl, W., and Roden, M. (2002). Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes 51, 599-605.

Kulling, S.E., and Rawel, H.M. (2008). Chokeberry (Aronia melanocarpa) - A review on the characteristic components and potential health effects. Planta Med 74, 1625-1634.

La Reau, A.J., Meier-Kolthoff, J.P., and Suen, G. (2016). Sequence-based analysis of the genus Ruminococcus resolves its phylogeny and reveals strong host association. Microb Genom 2, e000099-e000099.

La Reau, A.J., and Suen, G. (2018). The Ruminococci: key symbionts of the gut ecosystem. Journal of Microbiology 56, 199-208.

Lahti, L., Salonen, A., Kekkonen, R.A., Salojärvi, J., Jalanka-Tuovinen, J., Palva, A., Orešič, M., and de Vos, W.M. (2013). Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data. PeerJ 1, e32-e32.

Lairon, D., Lopez-Miranda, J., and Williams, C. (2007). Methodology for studying postprandial lipid metabolism. European Journal of Clinical Nutrition 61, 1145-1161.

Lam, Y.Y., Ha, C.W.Y., Campbell, C.R., Mitchell, A.J., Dinudom, A., Oscarsson, J., Cook, D.I., Hunt, N.H., Caterson, I.D., Holmes, A.J., et al. (2012). Increased Gut

Permeability and Microbiota Change Associate with Mesenteric Fat Inflammation and Metabolic Dysfunction in Diet-Induced Obese Mice. Plos One 7, e34233.

Langsted, A., Freiberg, J.J., and Nordestgaard, B.G. (2008). Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 118, 2047-2056.

Langsted, A., and Nordestgaard, B.G. (2019). Nonfasting versus fasting lipid profile for cardiovascular risk prediction. Pathology 51, 131-141.

Laparra, J.M., and Sanz, Y. (2010). Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacological Research 61, 219-225.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.M., Kennedy, S., et al. (2013). Richness of human gut microbiome correlates with metabolic markers. Nature 500, 541-546.

Le Roy, T., Llopis, M., Lepage, P., Bruneau, A., Rabot, S., Bevilacqua, C., Martin, P., Philippe, C., Walker, F., Bado, A., et al. (2013). Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut 62, 1787-1794.

Leclercq, S., Matamoros, S., Cani, P.D., Neyrinck, A.M., Jamar, F., Stärkel, P., Windey, K., Tremaroli, V., Bäckhed, F., Verbeke, K., et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A 111, E4485-4493.

Lee, Y.S., Li, P.P., Huh, J.Y., Hwang, I.J., Lu, M., Kim, J.I., Ham, M., Talukdar, S., Chen, A., Lu, W.J., et al. (2011). Inflammation Is Necessary for Long-Term but Not Short-Term High-Fat Diet Induced Insulin Resistance. Diabetes 60, 2474-2483.

Lemmer, I.L., Willemsen, N., Hilal, N., and Bartelt, A. (2021). A guide to understanding endoplasmic reticulum stress in metabolic disorders. Mol Metab 47, 101169-101169.

Leonard, P.J., Brand, M.H., Connolly, B.A., and Obae, S.G. (2013). Investigation of the Origin of Aronia mitschurinii using Amplified Fragment Length Polymorphism Analysis. HortScience horts 48, 520-524.

Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. (2005). Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America 102, 11070-11075.

Ley, S.H., Hamdy, O., Mohan, V., and Hu, F.B. (2014). Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet 383, 1999-2007.

Ley, S.H., Schulze, M.B., Hivert, M.F., Meigs, J.B., and Hu, F.B. (2018). Risk Factors for Type 2 Diabetes. In Diabetes in America. C.C. Cowie, S.S. Casagrande, A. Menke, M.A. Cissell, M.S. Eberhardt, J.B. Meigs, E.W. Gregg, W.C. Knowler, E. Barrett-Connor, D.J. Becker, et al., eds. (Bethesda (MD) of interest.: National Institute of Diabetes and Digestive and Kidney Diseases (US)).

Li, H.-Y., Zhang, H.-L., Zhao, F.-J., Wang, S.-Q., Wang, Z.-X., and Wei, Z.-Y. (2020). Modulation of Gut Microbiota, Short-Chain Fatty Acid Production, and Inflammatory Cytokine Expression in the Cecum of Porcine Deltacoronavirus-Infected Chicks. Front. Microbiol. 11, 897-897.

Li, J., Zhao, F.Q., Wang, Y.D., Chen, J.R., Tao, J.E., Tian, G., Wu, S.L., Liu, W.B., Cui, Q.H., Geng, B., et al. (2017). Gut microbiota dysbiosis contributes to the development of hypertension. Microbiome 5, 19.

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G.A., Beguinot, F., and Miele, C. (2019). Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci 20, 2358.

Lopez-Miranda, J., Williams, C., and Lairon, D. (2007). Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br. J. Nutr. 98, 458-473.

Louca, P., Nogal, A., Wells, P.M., Asnicar, F., Wolf, J., Steves, C.J., Spector, T.D., Segata, N., Berry, S.E., Valdes, A.M., et al. (2021). Gut microbiome diversity and composition is associated with hypertension in women. Journal of Hypertension 39.

Ludwig, D.S., Majzoub, J.A., Al-Zahrani, A., Dallal, G.E., Blanco, I., and Roberts, S.B. (1999). High Glycemic Index Foods, Overeating, and Obesity. Pediatrics 103, e26-e26.

Luiken, J.J., Arumugam, Y., Dyck, D.J., Bell, R.C., Pelsers, M.M., Turcotte, L.P., Tandon, N.N., Glatz, J.F., and Bonen, A. (2001). Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. The Journal of biological chemistry 276, 40567-40573.

Machado, P.P., Steele, E.M., Levy, R.B., da Costa Louzada, M.L., Rangan, A., Woods, J., Gill, T., Scrinis, G., and Monteiro, C.A. (2020). Ultra-processed food consumption and obesity in the Australian adult population. Nutrition & Diabetes 10, 39.

MacLaughlin, H.L., Hall, W.L., Sanders, T.A., and Macdougall, I.C. (2015). Risk for chronic kidney disease increases with obesity: Health Survey for England 2010. Public health nutrition 18, 3349-3354.

Magnusdottir, S., Ravcheev, D., de Crecy-Lagard, V., and Thiele, I. (2015). Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. Frontiers in Genetics 6.

Makki, K., Deehan, E.C., Walter, J., and Bäckhed, F. (2018). The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. Cell Host & Microbe 23, 705-715.

Mamo, J.C.L., Watts, G.F., Barrett, P.H.R., Smith, D., James, A.P., and Pal, S. (2001). Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? American Journal of Physiology-Endocrinology and Metabolism 281, E626-E632.

Manco, M., Putignani, L., and Bottazzo, G.F. (2010). Gut Microbiota, Lipopolysaccharides, and Innate Immunity in the Pathogenesis of Obesity and Cardiovascular Risk. Endocrine Reviews 31, 817-844.

Maraki, M., Aggelopoulou, N., Christodoulou, N., Katsarou, C., Anapliotis, P., Kavouras, S.A., Panagiotakos, D., and Sidossis, L.S. (2011). Validity of abbreviated oral fat tolerance tests for assessing postprandial lipemia. Clinical Nutrition 30, 852-857.

Martin, D.A., Taheri, R., Brand, M.H., Draghi, A., Sylvester, F.A., and Bolling, B.W. (2014). Anti-inflammatory activity of aronia berry extracts in murine splenocytes. J. Funct. Food. 8, 68-75.

Martínez Steele, E., Baraldi, L.G., Louzada, M.L.d.C., Moubarac, J.-C., Mozaffarian, D., and Monteiro, C.A. (2016). Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. BMJ Open 6, e009892.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412-419.

McKeown, N.M., Meigs, J.B., Liu, S., Saltzman, E., Wilson, P.W., and Jacques, P.F. (2004). Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. Diabetes Care 27, 538-546.

McRorie, J.W., Jr., and McKeown, N.M. (2017). Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. J Acad Nutr Diet 117, 251-264.

Mego, M., Manichanh, C., Accarino, A., Campos, D., Pozuelo, M., Varela, E., Vulevic, J., Tzortzis, G., Gibson, G., Guarner, F., et al. (2017). Metabolic adaptation of colonic

microbiota to galactooligosaccharides: a proof-of-concept-study. Aliment Pharmacol Ther 45, 670-680.

Miller, M., Stone Neil, J., Ballantyne, C., Bittner, V., Criqui Michael, H., Ginsberg Henry, N., Goldberg Anne, C., Howard William, J., Jacobson Marc, S., Kris-Etherton Penny, M., et al. (2011). Triglycerides and Cardiovascular Disease. Circulation 123, 2292-2333.

Mokdad, A.H., Ford, E.S., Bowman, B.A., Dietz, W.H., Vinicor, F., Bales, V.S., and Marks, J.S. (2003). Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA-J. Am. Med. Assoc. 289, 76-79.

Monfort-Pires, M., Delgado-Lista, J., Gomez-Delgado, F., Lopez-Miranda, J., Perez-Martinez, P., and Ferreira, S.R.G. (2016). Impact of the Content of Fatty Acids of Oral Fat Tolerance Tests on Postprandial Triglyceridemia: Systematic Review and Meta-Analysis. Nutrients 8, 580.

Monteiro, C.A., Cannon, G., Levy, R.B., Moubarac, J.-C., Louzada, M.L.C., Rauber, F., Khandpur, N., Cediel, G., Neri, D., Martinez-Steele, E., et al. (2019). Ultra-processed foods: what they are and how to identify them. Public health nutrition 22, 936-941.

Mora, S., Rifai, N., Buring, J.E., and Ridker, P.M. (2008). Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation 118, 993-1001.

Murty, C.M., Pittaway, J.K., and Ball, M.J. (2010). Chickpea supplementation in an Australian diet affects food choice, satiety and bowel health. Appetite 54, 282-288.

Muscella, A., Stefàno, E., and Marsigliante, S. (2020). The effects of exercise training on lipid metabolism and coronary heart disease. American Journal of Physiology-Heart and Circulatory Physiology 319, H76-H88.

Musso, G., Gambino, R., and Cassader, M. (2011). Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med 62, 361-380.

Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G., and Dietz, W.H. (1999). The disease burden associated with overweight and obesity. JAMA-J. Am. Med. Assoc. 282, 1523-1529.

Nagahashi, M., Yamada, A., Katsuta, E., Aoyagi, T., Huang, W.C., Terracina, K.P., Hait, N.C., Allegood, J.C., Tsuchida, J., Yuza, K., et al. (2018). Targeting the SphK1/S1P/S1PR1 Axis That Links Obesity, Chronic Inflammation, and Breast Cancer Metastasis. Cancer Res 78, 1713-1725.

Nakajima, K., Tokita, Y., and Tanaka, A. (2020). Atherogenic postprandial remnant lipoproteins—a causal lipoproteins for the initiation of obesity and atherosclerosis. Journal of Laboratory and Precision Medicine 5.

Nixon, G.F. (2009). Sphingolipids in inflammation: pathological implications and potential therapeutic targets. Br J Pharmacol 158, 982-993.

Nordestgaard, B.G., Benn, M., Schnohr, P., and Tybjaerg-Hansen, A. (2007). Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama 298, 299-308.

Nutrient Data Laboratory. (2010). USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods. USDA.

Ogden, C.L., Carroll, M.D., Kit, B.K., and Flegal, K.M. (2014). Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. JAMA-J. Am. Med. Assoc. 311, 806-814.

Olson, K.A., Schell, J.C., and Rutter, J. (2016). Pyruvate and Metabolic Flexibility: Illuminating a Path Toward Selective Cancer Therapies. Trends Biochem Sci 41, 219-230.

Oszmianski, J.A.N., and Sapis Jean, C. (1988). Anthocyanins in Fruits of Aronia Melanocarpa (Chokeberry). Journal of Food Science 53, 1241-1242.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306, 457-461.

Palócz, O., Pászti-Gere, E., Gálfi, P., and Farkas, O. (2016). Chlorogenic Acid Combined with Lactobacillus plantarum 2142 Reduced LPS-Induced Intestinal Inflammation and Oxidative Stress in IPEC-J2 Cells. Plos One 11, e0166642.

Peach, J.T., Wilson, S.M., Gunderson, L.D., Frothingham, L., Tran, T., Walk, S.T., Yeoman, C.J., Bothner, B., and Miles, M.P. (2021). Temporal metabolic response yields a dynamic biosignature of inflammation. iScience 24, 102817.

Pedersen, S.B., Jønler, M., and Richelsen, B. (1994). Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. The Journal of Clinical Endocrinology & Metabolism 78, 1354-1359.

Perera, T., Russo, C., Takata, Y., and Bobe, G. (2020). Legume Consumption Patterns in US Adults: National Health and Nutrition Examination Survey (NHANES) 2011-2014 and Beans, Lentils, Peas (BLP) 2017 Survey. Nutrients 12.

Pérez-Martin, A., Dumortier, M., Raynaud, E., Brun, J.F., Fédou, C., Bringer, J., and Mercier, J. (2001). Balance of substrate oxidation during submaximal exercise in lean and obese people. Diabetes Metab 27, 466-474.

Perreault, L., Lavely, J.M., Kittelson, J.M., and Horton, T.J. (2004). Gender differences in lipoprotein lipase activity after acute exercise. Obes Res 12, 241-249.

Perry, R.J., Peng, L., Barry, N.A., Cline, G.W., Zhang, D.Y., Cardone, R.L., Petersen, K.F., Kibbey, R.G., Goodman, A.L., and Shulman, G.I. (2016). Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. Nature 534, 213-+.

Pluskal, T., Castillo, S., Villar-Briones, A., and Orešič, M. (2010). MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 11, 395.

Pyun, C.-W., Seo, T.-S., Kim, D.-J., Kim, T.-W., and Bae, J.-S. (2020). Protective Effects of Ligularia fischeri and Aronia melanocarpa Extracts on Alcoholic Liver Disease (In Vitro and In Vivo Study). BioMed research international 2020, 9720387-9720387.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., and Zhang, F. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 490.

Rabe, K., Lehrke, M., Parhofer, K.G., and Broedl, U.C. (2008). Adipokines and insulin resistance. Mol Med 14, 741-751.

Rauber, F., da Costa Louzada, M.L., Steele, E.M., Millett, C., Monteiro, C.A., and Levy, R.B. (2018). Ultra-Processed Food Consumption and Chronic Non-Communicable Diseases-Related Dietary Nutrient Profile in the UK (2008⁻2014). Nutrients 10.

Reedy, J., Lerman, J.L., Krebs-Smith, S.M., Kirkpatrick, S.I., Pannucci, T.E., Wilson, M.M., Subar, A.F., Kahle, L.L., and Tooze, J.A. (2018). Evaluation of the Healthy Eating Index-2015. J Acad Nutr Diet 118, 1622-1633.

Research, C.I.f.A. (1999). FITNESSGRAM Test Administration Manual. (Champaign, IL: Human Kinetics).

Robertson, M.D., Henderson, R.A., Vist, G.E., and Rumsey, R.D.E. (2002). Extended effects of evening meal carbohydrate-to-fat ratio on fasting and postprandial substrate metabolism. The American Journal of Clinical Nutrition 75, 505-510.

Rohr, M.W., Narasimhulu, C.A., Rudeski-Rohr, T.A., and Parthasarathy, S. (2020). Negative Effects of a High-Fat Diet on Intestinal Permeability: A Review. Advances in Nutrition 11, 77-91.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P.I., Godneva, A., Kalka, I.N., Bar, N., et al. (2018). Environment dominates over host genetics in shaping human gut microbiota. Nature 555, 210-+.

Sadur, C.N., and Eckel, R.H. (1982). Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. The Journal of clinical investigation 69, 1119-1125.

Saltiel, A.R., and Kahn, C.R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414, 799-806.

Satija, A., Bhupathiraju, S.N., Rimm, E.B., Spiegelman, D., Chiuve, S.E., Borgi, L., Willett, W.C., Manson, J.E., Sun, Q., and Hu, F.B. (2016). Plant-Based Dietary Patterns and Incidence of Type 2 Diabetes in US Men and Women: Results from Three Prospective Cohort Studies. PLOS Medicine 13, e1002039.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75, 7537-7541.

Schmidt, T.S.B., Raes, J., and Bork, P. (2018). The Human Gut Microbiome: From Association to Modulation. Cell 172, 1198-1215.

Sender, R., Fuchs, S., and Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. Plos Biology 14.

Serino, M., Luche, E., Gres, S., Baylac, A., Berge, M., Cenac, C., Waget, A., Klopp, P., Iacovoni, J., Klopp, C., et al. (2012). Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 61, 543-553.

Sharma, A., Gupta, N.K., and Dixit, V.K. (2010). Complexation with phosphatidyl choline as a strategy for absorption enhancement of boswellic acid. Drug Delivery 17, 587-595.

Sharrett, A.R., Heiss, G., Chambless, L.E., Boerwinkle, E., Coady, S.A., Folsom, A.R., and Patsch, W. (2001). Metabolic and Lifestyle Determinants of Postprandial Lipemia

Differ From Those of Fasting Triglycerides. Arteriosclerosis, Thrombosis, and Vascular Biology 21, 275-281.

Shkoporov, A.N., Chaplin, A.V., Shcherbakova, V.A., Suzina, N.E., Kafarskaia, L.I., Bozhenko, V.K., and Efimov, B.A. (2016). Ruthenibacterium lactatiformans gen. nov., sp. nov., an anaerobic, lactate-producing member of the family Ruminococcaceae isolated from human faeces. International Journal of Systematic and Evolutionary Microbiology 66, 3041-3049.

Sievenpiper, J.L., Kendall, C.W.C., Esfahani, A., Wong, J.M.W., Carleton, A.J., Jiang, H.Y., Bazinet, R.P., Vidgen, E., and Jenkins, D.J.A. (2009). Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. Diabetologia 52, 1479.

Singh, R.P., Halaka, D.A., Hayouka, Z., and Tirosh, O. (2020). High-Fat Diet Induced Alteration of Mice Microbiota and the Functional Ability to Utilize Fructooligosaccharide for Ethanol Production. Frontiers in Cellular and Infection Microbiology 10.

Smith, K., McCoy, K.D., and Macpherson, A.J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Seminars in Immunology 19, 59-69.

Snelson, M., Clarke, R.E., Nguyen, T.V., Penfold, S.A., Forbes, J.M., Tan, S.M., and Coughlan, M.T. (2021). Long Term High Protein Diet Feeding Alters the Microbiome and Increases Intestinal Permeability, Systemic Inflammation and Kidney Injury in Mice. Mol Nutr Food Res 65, e2000851.

Sommer, F., and Backhed, F. (2013). The gut microbiota - masters of host development and physiology. Nature Reviews Microbiology 11, 227-238.

Sonnenburg, E.D., Smits, S.A., Tikhonov, M., Higginbottom, S.K., Wingreen, N.S., and Sonnenburg, J.L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. Nature 529, 212-215.

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I., and Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 579, 200-213.

Sparks, L.M. (2017). Exercise training response heterogeneity: physiological and molecular insights. Diabetologia 60, 2329-2336.

Spiegel, S., and Milstien, S. (2011). The outs and the ins of sphingosine-1-phosphate in immunity. Nature Reviews Immunology 11, 403-415.

Stremmel, W., Ehehalt, R., Staffer, S., Stoffels, S., Mohr, A., Karner, M., and Braun, A. (2012). Mucosal protection by phosphatidylcholine. Dig Dis 30 Suppl 3, 85-91.

Stremmel, W., Vural, H., Evliyaoglu, O., and Weiskirchen, R. (2021). Delayed-Release Phosphatidylcholine Is Effective for Treatment of Ulcerative Colitis: A Meta-Analysis. Dig Dis 39, 508-515.

Stuckler, D., McKee, M., Ebrahim, S., and Basu, S. (2012). Manufacturing Epidemics: The Role of Global Producers in Increased Consumption of Unhealthy Commodities Including Processed Foods, Alcohol, and Tobacco. PLOS Medicine 9, e1001235.

Swinburn, B., Sacks, G., and Ravussin, E. (2009). Increased food energy supply is more than sufficient to explain the US epidemic of obesity. American Journal of Clinical Nutrition 90, 1453-1456.

Taira, T., Yamaguchi, S., Takahashi, A., Okazaki, Y., Yamaguchi, A., Sakaguchi, H., and Chiji, H. (2015). Dietary polyphenols increase fecal mucin and immunoglobulin A and ameliorate the disturbance in gut microbiota caused by a high fat diet. Journal of clinical biochemistry and nutrition 57, 212-216.

Tall, A.R., and Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. Nature Reviews Immunology 15, 104.

Tan, W., Zhang, Q., Dong, Z., Yan, Y., Fu, Y., Liu, X., Zhao, B., and Duan, X. (2020). Phosphatidylcholine Ameliorates LPS-Induced Systemic Inflammation and Cognitive Impairments via Mediating the Gut–Brain Axis Balance. Journal of agricultural and food chemistry 68, 14884-14895.

Tanaka, H., Monahan, K.D., and Seals, D.R. (2001). Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology 37, 153-156.

Tang, W.H.W., Bäckhed, F., Landmesser, U., and Hazen, S.L. (2019). Intestinal Microbiota in Cardiovascular Health and Disease: JACC State-of-the-Art Review. J Am Coll Cardiol 73, 2089-2105.

Team, R.C. (2019). R: A Language and Environment for Statistical Computing. (Vienna, Austria: R Foundation for Statistical Computing).

Teeman, C.S., Kurti, S.P., Cull, B.J., Emerson, S.R., Haub, M.D., and Rosenkranz, S.K. (2016). Postprandial lipemic and inflammatory responses to high-fat meals: a review of the roles of acute and chronic exercise. Nutr Metab 13, 80.

Tominaga, M., Eguchi, H., Manaka, H., Igarashi, K., Kato, T., and Sekikawa, A. (1999). Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care 22, 920-924.

Treede, I., Braun, A., Jeliaskova, P., Giese, T., Füllekrug, J., Griffiths, G., Stremmel, W., and Ehehalt, R. (2009). TNF-alpha-induced up-regulation of pro-inflammatory cytokines is reduced by phosphatidylcholine in intestinal epithelial cells. BMC Gastroenterol 9, 53.

Treede, I., Braun, A., Sparla, R., Kühnel, M., Giese, T., Turner, J.R., Anes, E., Kulaksiz, H., Füllekrug, J., Stremmel, W., et al. (2007). Anti-inflammatory effects of phosphatidylcholine. The Journal of biological chemistry 282, 27155-27164.

Tremaroli, V., and Backhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. Nature 489, 242-249.

Tripathy, D., Mohanty, P., Dhindsa, S., Syed, T., Ghanim, H., Aljada, A., and Dandona, P. (2003). Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 52, 2882-2887.

Trosvik, P., de Muinck, E.J., and Stenseth, N.C. (2015). Biotic interactions and temporal dynamics of the human gastrointestinal microbiota. Isme Journal 9, 533-541.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444.

Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., and Gordon, J.I. (2009). The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. Sci. Transl. Med. 1, 6ra14.

USDHHS (2020). Dietary Guidelines for Americans, 2020-2025.

Uusitupa, M., Khan, T.A., Viguiliouk, E., Kahleova, H., Rivellese, A.A., Hermansen, K., Pfeiffer, A., Thanopoulou, A., Salas-Salvadó, J., Schwab, U., et al. (2019). Prevention of Type 2 Diabetes by Lifestyle Changes: A Systematic Review and Meta-Analysis. Nutrients 11.

Uysal, K.T., Wiesbrock, S.M., Marino, M.W., and Hotamisligil, G.S. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389, 610-614.

Verdam, F.J., Fuentes, S., de Jonge, C., Zoetendal, E.G., Erbil, R., Greve, J.W., Buurman, W.A., de Vos, W.M., and Rensen, S.S. (2013). Human intestinal microbiota

composition is associated with local and systemic inflammation in obesity. Obesity 21, E607-E615.

Virgin, Herbert W., and Todd, John A. (2011). Metagenomics and Personalized Medicine. Cell 147, 44-56.

Volpato, S., Guralnik, J.M., Ferrucci, L., Balfour, J., Chaves, P., Fried, L.P., and Harris, T.B. (2001). Cardiovascular disease, interlenkin-6, and risk of mortality in older women - The women's health and aging study. Circulation 103, 947-953.

Wang, Y.F., Beydoun, M.A., Liang, L., Caballero, B., and Kumanyika, S.K. (2008). Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. Obesity 16, 2323-2330.

Warburton, D.E.R., Nicol, C.W., and Bredin, S.S.D. (2006). Health benefits of physical activity: the evidence. Canadian Medical Association Journal 174, 801-809.

Weickert, M.O., Roden, M., Isken, F., Hoffmann, D., Nowotny, P., Osterhoff, M., Blaut, M., Alpert, C., Gögebakan, O., Bumke-Vogt, C., et al. (2011). Effects of supplemented isoenergetic diets differing in cereal fiber and protein content on insulin sensitivity in overweight humans. Am J Clin Nutr 94, 459-471.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation 112, 1796-1808.

Weiss, E.P., Fields, D.A., Mittendorfer, B., Haverkort, M.A., and Klein, S. (2008). Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test. Metabolism 57, 1479-1485.

Welch, R.W., and Mitchell, P.C. (2000). Food processing: a century of change. British Medical Bulletin 56, 1-17.

White, K.T., Moorthy, M.V., Akinkuolie, A.O., Demler, O., Ridker, P.M., Cook, N.R., and Mora, S. (2015). Identifying an Optimal Cutpoint for the Diagnosis of Hypertriglyceridemia in the Nonfasting State. Clinical chemistry 61, 1156-1163.

Wickham, H. (2009). Elegant graphics for data analysis. (New York: Springer-Verlag).

Wiklund, P., Zhang, X., Pekkala, S., Autio, R., Kong, L., Yang, Y., Keinänen-Kiukaanniemi, S., Alen, M., and Cheng, S. (2016). Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Scientific Reports 6, 24540.

Wilson, S.M., Maes, A.P., Yeoman, C.J., Walk, S.T., and Miles, M.P. (2021). Determinants of the postprandial triglyceride response to a high-fat meal in healthy overweight and obese adults. Lipids in Health and Disease 20, 107.

Windey, K., De Preter, V., and Verbeke, K. (2012). Relevance of protein fermentation to gut health. Mol Nutr Food Res 56, 184-196.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., and Keilbaugh, S.A. (2011a). Linking long-term dietary patterns with gut microbial enterotypes. Science 334.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., et al. (2011b). Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science 334, 105-108.

Wu, Y., Ding, Y., Tanaka, Y., and Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci 11, 1185-1200.

Wulan, S.N., Schrauwen-Hinderling, V.B., Westerterp, K.R., and Plasqui, G. (2020). Substrate utilization and metabolic profile in response to overfeeding with a high-fat diet in South Asian and white men: a sedentary lifestyle study. International Journal of Obesity 44, 136-146.

Xiao, L., Sonne, S.B., Feng, Q., Chen, N., Xia, Z., Li, X., Fang, Z., Zhang, D., Fjære, E., Midtbø, L.K., et al. (2017). High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome 5, 43.

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., et al. (2015). Personalized Nutrition by Prediction of Glycemic Responses. Cell 163, 1079-1094.

Zhai, S., Qin, S., Li, L., Zhu, L., Zou, Z., and Wang, L. (2019). Dietary butyrate suppresses inflammation through modulating gut microbiota in high-fat diet-fed mice. FEMS Microbiol Lett 366.

Zhao, Y., Liu, L., Yang, S., Liu, G., Pan, L., Gu, C., Wang, Y., Li, D., Zhao, R., and Wu, M. (2021). Mechanisms of Atherosclerosis Induced by Postprandial Lipemia. Frontiers in Cardiovascular Medicine 8.

Zhong, Y., Nyman, M., and Fåk, F. (2015). Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. Molecular Nutrition & Food Research 59, 2066-2076.