Plant-based versus animal-source food consumption, trimethylamine N-oxide, and incident metabolic syndrome: The Multi-Ethnic Study of Atherosclerosis (MESA)

by

Seyedeh Nooshan Mir Mohammad Ali

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Approved by:

Co-Major Professor
Brian Lindshield, Ph.D.

Approved by:

Co-Major Professor
Sara K. Rosenkranz, Ph.D.

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Abstract

Background: Several studies have shown that diets rich in unprocessed plant-based foods are associated with positive health outcomes, whereas diets rich in animal-source foods are associated with increased incidence of some chronic diseases including metabolic syndrome (MetS). Gut microbiota metabolites, particularly Trimethylamine N-Oxide (TMAO) have recently been identified as potential mechanisms for increased MetS risk. Therefore, the aims of the current study were to investigate the associations between plant-based dietary intake as compared with animal-source dietary intake, and MetS incidence, with a potential mediation role for TMAO.

Methods: The Multiethnic Ethnic Study of Atherosclerosis (MESA) dataset was used to conduct a longitudinal secondary analysis to meet our stated aims. The Plant-based Dietary Index (PDI) scoring system was used to score the MESA food frequency questionnaire (FFQ) according to four different indices (PDI), healthy (hPDI), unhealthy (uPDI), animal-source (ADI). We used a Cox proportional hazards mixed effects model to predict the incidence of MetS, and a linear regression model to perform mediation analysis for the potential mediation role of TMAO.

Results: The MESA cohort of 5,870 adults aged 45–84 years, of whom 2,449 were diagnosed with MetS at exam 1. Excluding those who had MetS, after 18 years, at exam 6, 1,673 of the 4,582 remaining participants had MetS. Cox proportional hazard mixed effects models examining the effects of PDI, hPDI, and uPDI on incident MetS demonstrated a significant effect for PDI (HR = 0.93, p = 0.03). Mediation analysis with PDI as an independent variable, indicated

demonstrated a significant indirect effect for TMAO (β = -0.0002, p = 0.002) on incident MetS, with no significant direct effect (β = -0.001, p = 0.36).

Conclusion: These preliminary results suggested that high plant-based dietary intake, decreased the risk of MetS at exam 6, indicating a protective effect for a plant-based dietary pattern when controlling for other factors that might have an effect on incident MetS. The TMAO mediation analysis indicated that the effect of PDI on incident MetS was reduced, suggesting that higher TMAO levels diminished the positive effects of PDI.

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I would like to express my sincerest gratitude to Dr. Dariush Mozaffarian for providing me with the academic opportunity to work on the Multiethnic Study of Atherosclerosis (MESA) Dataset. The knowledge and skills I gained during this experience will undoubtedly be invaluable in my future academic and professional pursuits. Also, I would like to express my appreciation for MESA's commitment to providing academic opportunities to students. Their dedication to supporting and empowering young scholars is an inspiration, and I am honored to have been a recipient of their generosity."

Being a student in a foreign country was not easy, but my parents were always there to provide me with all I needed to pursue my dreams. Their belief in me and my abilities gave me the strength and confidence to overcome the challenges that I faced. Their love, guidance, and support have been the bedrock of my success, and I am forever grateful for their presence in my life.

Dedication

This thesis is dedicated to my loving parents, Fattaneh and Dariush. Their unconditional love, unwavering support, and constant encouragement have been the driving force behind my academic success. I am grateful for the sacrifices my parents have made to ensure that I had the resources and opportunities necessary to pursue my academic goals. They have always been my role models, and their guidance and wisdom have been invaluable in shaping my worldview and approach to life.

Thank you, Mom and Dad, for your unwavering love and support. This thesis is a small token of my gratitude for everything you have done for me. I hope to make you proud and continue to honor your legacy in all that I do.

Chapter 1 - Literature Review

Introduction

Among the primary contributors to non-communicable chronic diseases and early mortality are poor dietary quality and high levels of sedentary behavior (López-Taboada et al., 2020). These lifestyle behaviors account for approximately 11% deaths globally (Afshin et al., 2019). Due to a growing body of research on the effects of nutrients on non-communicable diseases (NCDs) such as cardiovascular diseases (CVDs), type 2 diabetes mellitus (T2DM), obesity, some cancers, and chronic lung diseases, there is disagreement regarding the optimal diet for disease prevention (Cena & Calder, 2020). However, some dietary patterns such as Mediterranean diets, the Dietary Approaches to Stop Hypertension (DASH) diet, the 2020 Dietary Guidelines for Americans, and the Harvard Healthy Eating Plate, have been shown to be healthful eating patterns with associations with positive health outcomes and decreased risks for chronic diseases (Challa et al., 2022; *Dietary Guidelines for Americans, 2020-2025 and Online Materials | Dietary Guidelines for Americans*, n.d.; Guasch-Ferré & Willett, 2021; Willett & Stampfer, 2013). All of these dietary patterns are rich in unprocessed vegetables and fruits, plant-based fats and proteins, nuts, and whole grains (A et al., 2018). However, the Western dietary pattern, or standard American diet, is low in fruits and vegetables, and high in added sugar, saturated fat, red meat. This dietary pattern has been shown to be associated with increased risk for NCDs (Rakhra et al., 2020). Unhealthful dietary patterns are partially responsible for the current obesity epidemic; the Centers for Disease Control and Prevention (CDC) estimates that 14.7 million children and adults have obesity (*Childhood Obesity Facts | Overweight & Obesity | CDC*, 2022). Additionally, almost half of the U.S. population is suffering from chronic NCDs including cardiovascular diseases, diabetes, cancers, and chronic lung illnesses. (Nathan et al.,

2012). Non-communicable chronic diseases are illnesses with prolonged symptoms and gradual progression which occur due to several risk factors, including unhealthful dietary intake, insufficient physical activity, high levels of sedentary behavior, and other behaviors and genetic factors (Gowshall & Taylor-Robinson, 2018). Recently, in order to try to combat these high levels of obesity and chronic diseases, researchers have examined the role that plant-based diets (PBD) may play for promoting beneficial health outcomes when compared to more meat-based dietary patterns (Key et al., 2022).

Dietary patterns play an important role in the onset of many non-communicable chronic diseases such as CVDs, T2DM, and Metabolic Syndrome (MetS) (Jayedi et al., 2020). The 2020 Dietary Guidelines for Americans Advisory Committee report recommends shifting to PBD patterns (Dietary Guidelines Advisory Committee, 2020). However, not all plant source foods are healthful and some are associated with adverse health outcomes such as high intakes of refined grains, white potatoes, and sugar-sweetened beverages (SSBs) (Veronese et al., 2017; Q. Yang et al., 2014). Several studies have shown that unhealthful PBD patterns consisting of high amounts of processed foods, are positively associated with chronic diseases such as coronary heart disease, chronic obstructive pulmonary disease, and higher overall mortality risk (Li et al., 2022; Satija et al., 2016; Varraso et al., 2023). Therefore, understanding the healthfulness of plant-based foods, as compared with animal source food (ASF) intake, is important for understanding the role of PBD patterns in chronic diseases. Plant-based foods can be categorized according to their relative contribution to a healthful dietary pattern or an unhealthful dietary pattern using the Healthy Plant-based Diet Index (hPDI), the Unhealthy Plant-based Diet Index (uPDI), and the overall Plant-based Diet Index (PDI). Whole grains, fruits, vegetables, nuts, legumes, vegetable oils, and tea/coffee are scored positively on the hPDI. Whereas, fruit juices,

sugar-sweetened beverages, refined grains, potatoes, sweets and desserts, and animal foods such as dairy products, eggs, fish or shellfish, miscellaneous foods such as hamburger are scored negatively. The uPDI follows the same rationale, reversing the scoring system of the hPDI, as less healthy food groups and animal foods are scored positively, and healthy plant-based foods are scored negatively.The criteria used to diagnose MetS vary with different definitions provided by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), the International Diabetes Federation (IDF), and the World Health Organization (WHO). The NCEP-ATP III criteria are more widely used as compared to other definitions (Grundy et al., 2005). The NCEP-ATP III criteria indicate that if a patient has clustering of metabolic dysregulations, such as insulin resistance, low HDL, high triglycerides, central obesity (waist circumference), and hypertension, they would be diagnosed with MetS (Alexander et al., 2003). To be more precise, MetS is the presence of at least three of the following: 1) increased waist circumference (> 102 cm [>40 in] for men, > 88 cm [>35 in] for women); 2) increased triglycerides (≥ 150 mg/dl); 3) reduced HDL cholesterol (<40 mg/dl in men, <50 mg/dl in women); 4) hypertension (≥130/85 mmHg); and 5) impaired fasting glucose (≥ 110 mg/dl) ("Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III)," 2001). MetS is associated with incident CVDs such as myocardial infarction and stroke, and with T2DM (Fahed et al., 2022).

The World Health Organization (WHO) indicates that NCDs are the largest cause of death globally, accounting for 71% of all annual fatalities. The top four NCDs that are causal in the most global fatalities include CVDs (17.9 million annual deaths), cancers (9.0 million), respiratory illnesses (3.9 million), and diabetes (1.6 million) (*Noncommunicable Diseases*, n.d.).

In addition to the chronic diseases mentioned above, about one-third of Americans meet the diagnostic criteria for MetS, a cluster of metabolic dysregulations which is associated with increased risk for CVDs and T2DM (Fahed et al., 2022). Genetics and lifestyle factors such as unhealthful dietary patterns, inadequate physical activity and high levels of sedentary behavior, along with excess adiposity, are risk factors for MetS (Bovolini et al., 2021). Estimates of MetS prevalence suggest that approximately 2.8% of children and 4.8% of adults suffer from MetS worldwide (Noubiap et al., 2022), and it is one of the major reasons for morbidity and mortality (Swarup et al., 2022). In the U.S., more than 30% of adults aged 20y and over, meet the criteria for MetS, with significant disparities in prevalence among some racial and ethnic groups. For example, according to National Health and Nutrition Examination Survey (NHANES) data from 2011–2018, while the overall population prevalence of MetS did not increase significantly, there were increases among non-Hispanic Asians from 21.8% in 2011–12, to 31.2% in 2017–18 ((Liang et al., 2021). Among Hispanics, between 2011–2016, prevalence estimates indicated increases from 32.9% to 40.4% (Hirode & Wong, 2020). Understanding the risk factors for MetS, as well as the underlying risks across racial and ethnic groups is important for informing efforts to alleviate health disparities with regard to metabolic health. Given these prevalence estimates, research focused on mitigating risk for developing MetS is urgent (Hirode & Wong, 2020; Liang et al., 2021).

Some dietary intervention strategies have been studied with regard to their roles in future risk for incident MetS. As compared to a pattern of high ASF consumption, a PBD pattern has been shown to be associated with lower body weight (Turner-McGrievy, Mandes, and Crimarco, 2017), lower risk of CVDs (Kim et al., 2019) and T2DM (Chen et al., 2019), as well as decreased incidence of MetS (Marrone et al., 2021). Recent studies, including small populations,

have explored mechanisms behind the protective effects of PBD patterns for some chronic diseases. In addition, there has been a substantive new body of evidence around the importance of the gut microbiome for health outcomes. This area of research is emergent, and a lot more is left to learn. In order to reduce the prevalence of MetS, new risk factors have been investigated, including the role of the gut microbiota and associated metabolites such as trimethylamine (TMA). TMA is one of the gut microbiota metabolites that is primarily derived from compounds present in red meat, eggs, poultry, dairy products and fish, produced by intestinal microflora. These compounds include phosphatidylcholine/choline, carnitine, betaine, dimethylglycine, and ergothioneine (Subramaniam & Fletcher, 2018). TMA is oxidized to trimethylamine N-oxide (TMAO) by flavin-containing monooxygenase 3 (FMO3), which is a liver enzyme (Velasquez et al., 2016). The gut bacteria genera belonging to the orders *Clostridiales, Bacteroidales*, or *Desulfovibrionales* have been shown to be positively associated with circulating TMAO (Manor et al., 2018). However, the specific gut bacterial taxa that convert TMA-containing foods into TMA is unknown. Plasma levels of TMAO, produced from dietary precursors (particularly those from ASFs—due to higher conversion rates to trimethylamine (TMA))— are associated with the onset of CVDs, and less consistently with T2DM and gestational diabetes (Barrea et al., 2018; Lemaitre et al., 2021; Li et al., 2022; McArthur et al., 2022; Simó & García-Cañas, 2020; M. Wang et al., 2022; Zeisel et al., 2003). Recently, associations between total red meat consumption and the incidence of atherosclerotic cardiovascular diseases, have been shown to be partially mediated by TMAO and TMAO-related metabolites (M. Wang et al., 2022). Additionally, some studies have shown that TMAO levels may also be associated with the incidence of MetS (Chen et al., 2019; Kuo et al., 2022; Ringel et al., 2021), but these studies have been largely cross-sectional, limiting the possibility for inference regarding temporality

(with possible reverse causation). Circulating concentrations of TMAO are thought to be influenced by both environmental and host factors, including host genetics, dietary intake, and gut microbiota composition (He & Chen, 2018). Thus, increasing plant-based food intake through replacement of ASF, may be associated with positive metabolic health, and this association may be mediated by certain gut microbiota metabolites like TMAO and TMAO-related metabolites (Z. Wang et al., 2011; Seldin et al., 2016; Zhu et al., 2016). The healthfulness of PBD patterns may also play a role in both the risk of incident MetS (Kim et al., 2019; McGrath & Fernandez, 2022), and in plasma TMAO concentrations (Satija Ambika et al., 2017; McGrath & Fernandez, 2022; Kim et al., 2021); however, there are limited data available regarding the associations between the healthfulness of PBD patterns as compared with dietary patterns that include higher amounts of ASFs, and incident MetS. Further, the potential mediation role for gut microbiota metabolites, particularly TMAO and TMAO-related metabolites, requires investigation. To help to fill this gap in the available evidence, the overall objective of the proposed study was to determine whether dietary patterns that reflect PBDs (overall, healthful, and unhealthful), in comparison to patterns reflecting higher intakes of ASF (each assessed as proportions of the overall diet), were associated with incident MetS. In order to meet this primary objective as well as the aim to better understand potential racial and ethnic disparities, longitudinal data (from 2000–2018) from the Multi-Ethnic Study of Atherosclerosis (MESA) from more than 6,800 men and women between the ages of 45 and 84 years, from six American communities, were used. Further, we investigated whether any observed associations between PBD patterns and incident MetS were mediated by gut microbiota metabolites, in particular TMAO and its family of microbiome-associated metabolites.

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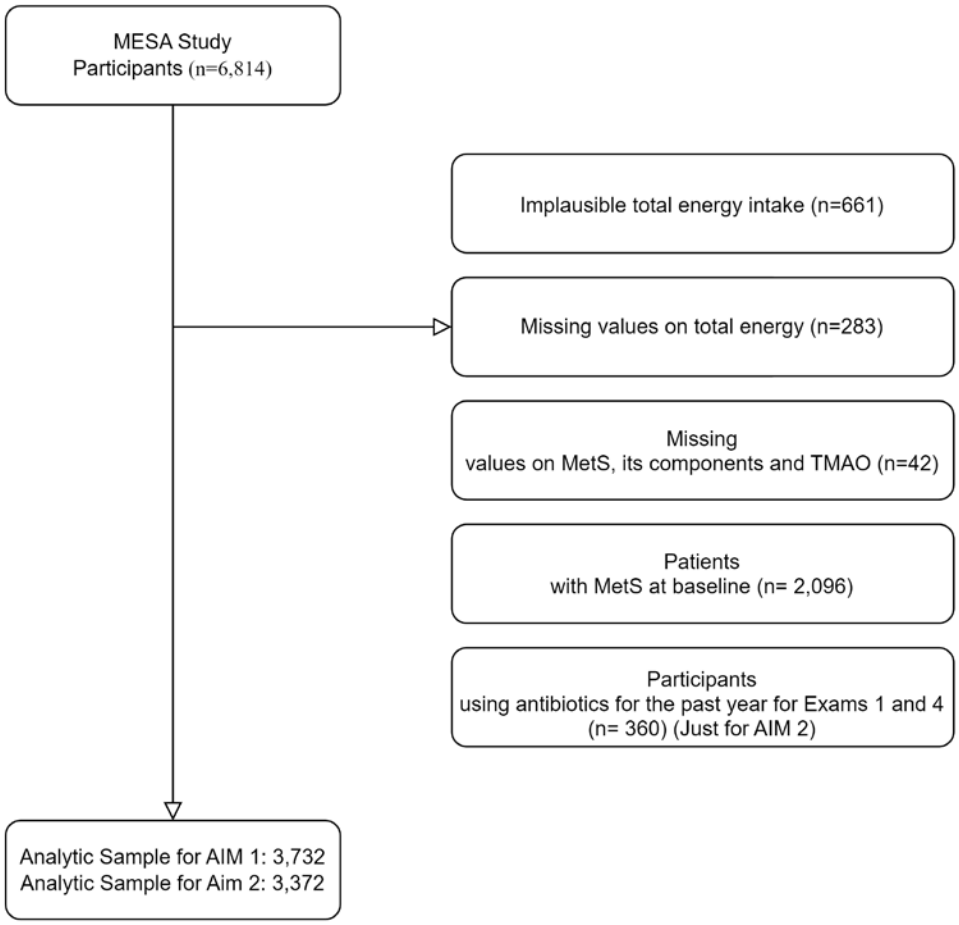
Chapter 2 - Methods

Study Population

The Multiethnic Study of Atherosclerosis (MESA), a prospective cohort study, was initiated in July 2000 to investigate the prevalence and development of subclinical CVDs. Participants were recruited from six U.S. communities and included 6,814 men and women between the ages of 45 and 84 years (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; St. Paul, MN), and concluded in 2018. The MESA cohort was 38% White (n = 2,622), 28% African American (n = 1,893), 22% Hispanic (n = 1,496) and 12% Chinese (n = 803). At the beginning of the study, participants who had undergone an invasive procedure for CVDs (coronary artery bypass graft, angioplasty, valve replacement, pacemaker placement, or other vascular surgeries), or who had a history of myocardial infarction, angina, heart failure, stroke, or transient ischemic attack were excluded (2000–2002) (Bild et al., 2002). The MESA study was approved by the Institutional Review Boards of each study site, and written informed consent was obtained from all participants.

MESA participants who had MetS at baseline, were missing primary exposure or outcome variables of interest, indicated antibiotic use at the time of TMAO measurements, and with implausible calorie intake (<600 or >3500 kcal/day for women and <800 or >4200 kcal/day for men) (Rhee et al., 2015) were excluded from the current analysis. A total of 3,732 participants (1,837 men and 1,895 women) were included in the current investigation. Among the excluded individuals: 661 had extraordinary energy intake; 283 with missing values on total energy; 42 with missing values on MetS and TMAO assessment; and 2,096 who had MetS at baseline.

Figure 2.1. Flowchart of Participants, Multi-ethnic Study of Atherosclerosis (MESA)**.**



The figure shows participant flow for included and excluded participants

MESA Data Collection

The first examination began in July 2000, conducted over a 24-month period, and was designed to be the most comprehensive of the exams. The second (July 2002 to January 2004) and third examinations (January 2004 to July 2005) were conducted over 18-month periods, and included selected baseline measurements as well as new measures that could not be included at baseline. The fourth examination (July 2005 to July 2007) and fifth examination (April 2010 to Dec 2011) were conducted over 21-month periods, and the sixth examination (Sep 2016 to June 2018) lasted 22 months. The sixth examination was the final data collection period and included

repetition of selected measures for determination of temporal trends. More details regarding the MESA Study are provided in a previously published paper (Bild et al., 2002). In order to answer the current research question, we took a longitudinal approach to analysis and included all examination time points. We used the two available MESA food frequency questionnaires (FFQs), collected during exams 1 and 5, and the available TMAO analyses which were determined at exams 1 and 4. However, most of covariates, MetS incidence and its component factors were used from all examination periods. More details are provided in Table 2.1 regarding included variables (Table 2.1).

Table 2.1 Exams for included variables

Exposures		Mediators		Outcomes		Covariates	
Variable	Exam	Variable	Exam	Variable	Exam	Variable	Exam
PDI, hPDI, uPDI, ADI	1	Gut Microbiota Metabolite: TMAO	1	MetS Incidence	1-6	Age, Sex, Study Site, Income, BMI, Smoking Status, Medical History, Kidney Disease, Physical Activity race Kidney disease, CRP	1-6

Exposure, Mediator, and Outcome Variables as well as Covariates were included in analyses.

Assessment of Dietary Intake, Its Scoring and Race/Ethnicity (Exposures)

The Insulin Resistance Atherosclerosis Study (IRAS) FFQ, which has been validated in non-Hispanic white, black, and Hispanic populations, served as a model for the development of the MESA FFQ in accordance with the validated Block format (Block et al., 1990; Mayer-Davis et al., 1999). The IRAS FFQ was altered to include distinctive Chinese foods and culinary traditions in order to cater to the needs of the MESA participant community. The resultant 120-item MESA FFQ was used to determine the typical dietary intake for the year prior to the assessment. Serving sizes (small, medium, or large) and frequencies (average times consumed per day, week, or month) of food or beverage items were provided by the participants. There were nine frequency options with the maximum frequency for foods being "rare or never" and the minimum frequency for beverages being "2 times/d". Races and ethnicities were used to

determine whether there were systematic differences in incident MetS for specific races and ethnicities. Race/ethnicity was determined as White, African American, Chinese, and Hispanic groups in all models as a PDI-by-race/ethnicity interaction term.

In order to identify the associations between healthy and unhealthy PBD versus ASF patterns, we used a similar approach to the Harvard study conducted by Satija and colleagues, which used FFQ data from the Nurse`s Health Study I and II (Satija et al., 2017). Therefore, four overarching indices for healthy and less healthy plant-based, and ASFs were determined as follows: overall plant-based diet index (PDI), healthy PDI (hPDI), unhealthy PDI (uPDI), and animal-based dietary index (ADI) To score each of the four indices, food items from the MESA FFQ were categorized into 18 food groups, according to the methods previously published from the Harvard study (Satija et al., 2017). The 18 food groups were then classified as healthful plant-based foods (i.e., whole grains, fruits, vegetables, nuts, legumes, tea and coffee); unhealthful plant-based foods (i.e., fruit juices, refined grains, potatoes, sweetened beverages, sweets and desserts and salty foods), or animal source foods (i.e., animal fat, dairy products, egg, fish or seafood, meat, and miscellaneous animal source foods). Since the MESA FFQ includes combination foods, and categorization of these foods did not align with the Harvard study food categories, we used three different approaches to determine the alignment of the MESA FFQs with the 18 food groups used in the Harvard study (Satija et al., 2017).

Approach 1: Scoring was solely based on food items already included in the PDI, hPDI, and uPDI scoring indices provided by Sajitav et al., which does not include disaggregation of combination foods. In this approach, a total of 29 combination food items from the MESA FFQ were excluded (pea soup, miso soup, fried rice, dumplings, chowmein, stir fried beef, stir fried shrimp, stir fried vegetables, burrito, meat burrito, enchilada, meat enchilada, picadillo, arroz

pollo, meat chili ,green chili ,fried beans, salsa, tortilla, cream pasta, meat pasta, tomato pasta, meat tomato pasta, meat stew, chicken salad, pasta salad, jelly, soy milk, milk coffee/tea).

The second and third scoring approaches sought to incorporate the combination foods into the 18 defined food categories via a machine learning (ML) approach using R statistical software (version 4.2.2). A ML approach, in this case, trained a statistical algorithm using the nutrient profiles of the foods in the 18 defined categories to then assign each combination food to one of the categories based on its own nutrient profile. Specifically, once the statistical algorithm was trained, meaning that the parameters of the model are tuned via k-fold cross validation, it was then used to predict the appropriate category for the combination foods, or its relative weights across categories based upon its own individual nutrient profile. This allowed for an enfolding of the combination foods into the 18 food categories so they could then be included into the PDI, hPDI and uPDI indices.

Approach 2: A trained machine learning algorithm delivered an exact food category prediction for each combination food based on its nutrient profile.

Approach 3: A trained machine learning algorithm delivered a weighted prediction across the food categories for each combination food based on its nutrient profile. In this approach a single combination food could be probabilistically disaggregated across all the food categories. If the weight of a combination food within a food category was less than 0.06 (6%) then it was reduced to 0, as any weight of less than 6% would represent a prediction worse than chance given the number of possible food categories (N = 18) to be weighted across.

Following analyses of the PDI, hPDI, and uPDI using these three models, we checked for consistency between the models using linear regression. Table 2.2 shows the differences between

the second and third approaches pertaining to assignment of the combination foods to food categories.

Table 2.2 Categorization of MESA combination food items by machine leaning approaches.

Combination Foods	*Exact Category ML Prediction*	**Disaggregating ML Prediction**
pea soup	Legumes	Vegetables
miso soup	Legumes	Legumes
fried rice	Miscellaneous animal-based food	Refined Grains
dumplings	Refined Grains	Refined Grains
chowmein	Miscellaneous animal-based food	Miscellaneous animal-based food
stir fried beef	Vegetables	Vegetables
stir fried shrimp	Vegetables	Legumes
stir fried vegetables	Vegetables	Vegetables
burrito	Refined Grains	Refined Grains
meat burrito	Miscellaneous animal-based food	Refined Grains
enchilada	Legumes	Vegetables
meat enchilada	Miscellaneous animal-based food	Miscellaneous animal-based food
picadillo	Miscellaneous animal-based food	Miscellaneous animal-based food
arroz pollo	Miscellaneous Animal	Legumes
meat chili	Vegetables	Vegetables
red chili	Miscellaneous animal-based food	Vegetables
green chili	Miscellaneous animal-based food	Vegetables
fried beans	Legumes	Vegetables
salsa	Vegetables	Vegetables
tortilla	Refined Grains	Refined Grains
cream pasta	Refined Grains	Miscellaneous animal-based food
meat pasta	Refined Grains	Miscellaneous animal-based food
tomato pasta	Legumes	Refined Grains
meat tomato pasta	Miscellaneous animal-based food	Miscellaneous animal-based food
meat stew	Miscellaneous animal-based food	Miscellaneous animal-based food
chicken salad	Fish and Seafood	Miscellaneous animal-based food
pasta salad	Miscellaneous animal-based food	Miscellaneous animal-based food
jelly	Fruits	Fruits
soymilk	Whole Grains	Tea Coffee
milk coffee tea	Dairy	Dairy

Two different machine learning approaches to categorize combination food items into 18 food groups for the PDI scoring system

Quintile rankings according to reported consumption of foods in the 18 food groups were created and then used to assign positive or reverse scores depending on the index. For positive scores, participants who reported consumption of foods that ranked in the highest quintile for a

particular food group received a score of 5, with the lowest quintile for consumption of foods within that food group receiving a 1. When scores were reversed, the opposite pattern of scoring was used, where now the highest quintile of consumption received a 1 and the lowest quintile received a 5. The PDI index was scored by assigning positive scores to healthy and unhealthy plant-based foods, and reverse scores to ASFs. The hPDI was scored by assigning positive scores to healthy plant-based food groups and reverse scores to less healthy plant food groups and animal food groups. The uPDI was scored using positive scores for unhealthy plant-based foods and reverse scores for healthy plant-based and ASFs. Finally, the animal-based dietary index (ADI) was determined by assigning positive scores to ASFs and reverse scores to plant-based foods. Table 2.3 shows the scoring systems and food items within each category.

Table 2.3 Categorization of all MESA food items including all approaches and scoring

Plant-based Food Groups	**Initial food groups by Sajita et al.**	**Exact Category ML Prediction**	**Disaggregating ML Prediction**	**PDI**	***hPDI***	***uPDI***	***ADI***
Healthy							
Whole Grains	Whole grain breakfast cereal, other cooked breakfast cereal, cooked oatmeal, dark bread, brown rice, other grains, bran, wheat germ, popcorn	soymilk		Positive Score	Positive Score	Reverse Score	Reverse Score
Fruits	Raisins or grapes, prunes, bananas, cantaloupe, watermelon, fresh apples or pears, oranges, grapefruit, strawberries, blueberries, peaches, apricots, plums	jelly	jelly	Positive Score	Positive Score	Reverse Score	Reverse Score
Vegetables	Tomatoes, tomato juice, tomato sauce, broccoli, cabbage, cauliflower, brussels sprouts, carrots, mixed vegetables, yellow or winter squash, eggplant or zucchini, yams or sweet potatoes, spinach cooked, spinach raw, kale or mustard orchard greens, iceberg or head lettuce, romaine or leaf lettuce, celery, mushrooms, beets, alfalfa sprouts, garlic, corn	Stir fried beef, stir fried shrimp, stir fried vegetables, meat chili, salsa	Fried rice chowmein meat burrito, meat enchilada, picadillo, Arroz pollo, red chili, green chili, meat tomato pasta, meat	Positive Score	Positive Score	Reverse Score	Reverse Score

			stew, pasta salad				
Legumes	String beans, tofu or soybeans, beans or lentils, peas or lima beans	Pea soup, miso soup, enchilada, fried beans, tomato pasta	Miso soup, Stir fried shrimp, Arroz pollo	Positive Score	Positive Score	Reverse Score	Reverse Score
Nuts	Nuts, peanut butter			Positive Score	Positive Score	Reverse Score	Reverse Score
Tea and Coffee	Tea, coffee, decaffeinated coffee		Soy milk	Positive Score	Positive Score	Reverse Score	Reverse Score
Less Healthy							Reverse Score
Fruit Juices	*Fruit Juices*			Positive Score	Reverse Score	Positive Score	Reverse Score
Refined Grains	Refined grain breakfast cereal, white bread, English muffins or bagels or rolls, muffins or biscuits, white rice, pancakes or waffles, crackers, pasta	Burrito, tortilla, cream pasta, meat pasta, dumplings	Fried rice, dumplings, burrito, meat burrito, tortilla, Tomato pasta	Positive Score	Reverse Score	Positive Score	Reverse Score
Potatoes	French fries, baked or mashed potatoes, potato or corn chips			Positive Score	Reverse Score	Positive Score	Reverse Score
Sugar Sweetened Beverages	Colas with caffeine and sugar, colas without caffeine but with sugar, other carbonated beverages with sugar, noncarbonated fruit drinks with sugar			Positive Score	Reverse Score	Positive Score	Reverse Score
Sweets and Desserts	Chocolates, candy bars, candy without chocolate, cookies (home-baked and ready-made), brownies, doughnuts, cake (home-baked and ready-made), sweet roll (home-baked and ready-made), pie (home-baked and readymade), jams or jellies or preserves or syrup or honey			Positive Score	Reverse Score	Positive Score	Reverse Score
Animal Food Groups							
Animal Fat	Butter added to food, butter or lard used for cooking			Reverse Score	Reverse Score	Reverse Score	Positive Score
Dairy	Skim low fat milk, whole milk, cream, sour cream, sherbet, ice cream, yogurt, cottage or ricotta cheese, cream cheese, other cheese	Milk in coffee/tea	Milk in coffee/tea	Reverse Score	Reverse Score	Reverse Score	Positive Score
Egg	Eggs, omelets, huevos rancheros			Reverse Score	Reverse Score	Reverse Score	Positive Score

Fish and seafood	Canned tuna, dark meat fish, other fish, shrimp or lobster or scallops	Chicken salad		Reverse Score	Reverse Score	Reverse Score	Positive Score
Meat	Chicken or turkey with skin, chicken or turkey without skin, bacon, hot dogs, meats, liver, hamburger, beef or pork or lamb mixed dish, beef or pork or lamb main dish			Reverse Score	Reverse Score	Reverse Score	Positive Score
Miscellaneous animal-based foods	Pizza, chowder or cream soup, mayonnaise or other creamy salad dressing	Fried rice chowmein meat burrito meat enchilada, picadillo, arroz pollo, red chili, green chili, meat tomato pasta, meat stew, pasta salad	Chowmein, Meat enchilada, picadillo, cream pasta, meat pasta, meat tomato pasta, meat stew, chicken salad, pasta salad	Reverse Score	Reverse Score	Reverse Score	Positive Score

PDI: Plant-based Dietary Index; hPDI: healthy Plant-based Dietary Index uPDI: unhealthy Plant-based Dietary Index; ADI; Animal-based Dietary Index.

Assessment of Metabolic Syndrome (Outcomes)

As previously reported, an automated oscillometer sphygmomanometer (Dinamap PRO 100; Critikon) was used to measure resting seated blood pressure three times, including the average of the last two readings for analysis. Use of anti-hypertensive medication or having a systolic or diastolic blood pressure over 130 mmHg and 85 mmHg were used to characterize hypertension. Using a steel measuring tape with a typical 4-oz tension, waist circumference was measured at the umbilicus to the nearest 0.1 cm and the average value was used for analyses. A central lab examined fasting blood glucose using a Vitros analyzer to measure serum glucose (Johnson & Johnson Clinical Diagnostics) and fasting blood lipids using a standardized kit, plasma lipids (triglycerides, HDL cholesterol, and total cholesterol) were determined (Roche Diagnostics) (Grundy et al., 2005a). The Friedewald formula was used to compute LDL cholesterol (Friedewald et al., 1972). Using the Linco Human Insulin Specific RIA kit, the

radioimmunoassay method was used to measure serum insulin (Linco Research). MetS was defined in accordance with the updated Adult Treatment Panel III standards of the National Cholesterol Education Program (Grundy et al., 2005b). The specific thresholds for each MetS component were as follows: triglycerides >150 mg/dl, HDL < 40 mg/dl (men) or < 50 mg/dl (women), waist circumference ≥ 88 cm for women or ≥ 102 cm for men, glucose ≥ 100 mg/dl, use of anti-hypertensive medication or blood pressure ≥ 130/85 mm/Hg. Individuals meeting the thresholds for at least three of MetS components were considered to meet the criteria for having MetS. Presence or absence of MetS as well as presence or absence of each of the MetS components was determined for each of the six examination periods.

Assessment of Plasma TMAO (Mediator)

As indicated in previously published studies, samples of blood were obtained following an 8–12 hour fast, and then frozen and transported on dry ice after being stored for a brief time at –70˚C (Z. Wang, Levison, et al., 2014). Using a stable-isotope dilution assay in conjunction with high-performance liquid chromatography, online electrospray ionization tandem mass spectrometry, and a Shimadzu 8050 mass spectrometer, each microbiota metabolite was measured using its deuterium-isotopologue as an internal reference (Z. Wang, Levison, et al., 2014). The Cleveland Clinic Lerner Research Institute conducted all laboratory tests, with a reported coefficient of variance of less than 10% for each metabolite, as previously reported (Z et al., 2019). A total of 6,796 samples were obtained at the baseline visit (Exam 1: 2000–2002), with 3,587 (52.8%) of those samples from female participants. At the follow-up visit (Exam 4: 2005–2007), 2,967 (52.7%) of the 5,633 samples were from females.

Assessment of Covariates

The MESA study dataset provides information on sociodemographic, lifestyle factors, anthropometrics, medical history, and medications at each exam timepoint. In order to assess the associations between pattern of dietary intake and incident MetS, we adjusted all models for other variables that were likely to be related to incident MetS: age, biological sex, study site, annual household income, baseline BMI, smoking status, medication; medical history including kidney disease, rheumatoid heart disease, and history of cancers; hormone therapy and menopausal status; and physical activity (MET minutes/week). Alcohol use was not included in the PDI, hPDI, or uPDI indices, but was adjusted for in the analyses for all 3 scoring approaches. In addition, C-reactive protein (CRP) was measured using a high-sensitivity ELISA kit, and Cystatin-C and creatinine were measured and used to calculate estimated glomerular filtration rate (eGFR) with the Chronic Kidney Disease Epidemiology Collaboration equation (Steubl et al., 2019; Weiner et al., 2004).

Statistical Analysis

All data were analyzed using R Statistical Software version (4.2.2) and p-values < 0.05 were considered as statistically significant. We examined sociodemographic characteristics, health status, dietary intake, using percentages for categorical variables, and means ± SD for continuous variables.

AIM 1: To investigate prospective associations of overall (PDI) and quality of plant-based food consumption (using uPDI and hPDI), as well as ASF consumption (using ADI), with incident MetS.

Participants who had MetS (≥3 MetS risk factors) at exam 1 were not included in the analysis. We used a Cox proportional hazards fixed effects model and a Cox proportional

hazards fixed effects model with study site and participant as random nested effects to predict incidence of MetS (MetS = 1, No-MetS = 0). The primary predictor variables were PDI, hPDI and uPDI. The models included age, biological sex, race/ethnicity, education, and annual household income . smoking status, total alcohol intake (per week), anthropometric measurements, physical activity (METs), and history of heart failure and kidney disease as covariates. We also performed these analyses with PDI, hPDI, and uPDI metrics that included the ML predicted categories of the combination foods. Continuous variables were mean centered and divided by their standard deviations to control for collinearity among independent variables.

AIM 2: To investigate whether gut microbiota-generated trimethylamine N-oxide (TMAO) mediated the relationship between plant-based food intake and incidence of MetS.

To determine whether TMAO mediated the relationship between plant-based food intake and incidence of MetS we used linear regression models to perform mediation analysis for direct and indirect model construction. Since TMAO was skewed, it waslog-transformed and standardized by mean and standard deviation.

To investigate the mediation of TMAO, direct association, indirect association, and mediation proportion (i.e., the percent excess risk explained by the mediators) were estimated by comparing the multivariable-adjusted model (Model 1 from aim 1) without TMAO, to the model that included TMAO. We also included additional covariates to assess additional biological mediators that were associated with MetS incidence but were independent from the proposed PDI/TMAO indirect pathway. Specifically, we included blood analytes (C-reactive protein (CRP), eGFR at each TMAO time measurement) as covariates. Direct associations were estimated by the odds ratios in the model with TMAO, and indirect associations were estimated

by the differences between the twoodds ratios in the model, with and without TMAO, respectively. Mediation proportion was estimated using a ratio where the numerator included the difference between the TMAO unadjusted and TMAO adjusted odds ratio, and the denominator included the TMAO unadjusted excess odds [i.e., (ORunadj – Oradj)/ (Orunadj – 1)]. Confidence intervals for indirect associations and mediation proportions were estimated using bootstrap percentile intervals based on 1000 bootstrap samples.

In order to determine the role of race/ethnicity in these associations, we used a pairwise comparison by marginal hazard ratio for aim 1 and performed a separate mediation analysis with race/ethnicity as an independent variable and MetS incidence as the outcome variable and TMAO as a mediation variable for aim 2.

Chapter 3 - Results

Participant Characteristics

Among all MESA participants, 3,372 adults, free of MetS at baseline, were included. Among participants at baseline, the mean (SD) age was 61.6 (10.4) years, 50.4% were female, and 42.4% were White. Educational attainment ranged from no schooling (0.8%) to graduate school (21.9%). Among participants, 4.4% had high triglycerides, 32% hypertension, 11.3% low HDL, a mean of 93.5 (13.0) cm waist circumference, and 89.4 (19.6) mg/dl fasting blood glucose (Table 3.1). PDI scores ranged from 26 to 69 for both women and men; however, hPDI scores ranged from 28 to 70 for women, and 27 to 74 for men. The uPDI scores ranged from 27 to 73 for women, and 32 to 70 for men.

Table 3.1 Participants Characteristics at Baseline.

	Included Participants
N	3372
Biological Sex	
Female	1700 (50.4%)
Male	1672 (49.6%)
Age	61.6 ±10.4
energy	1661.1 ± 675.5
PDI	47.9 ± 6.7
hPDI	48.2 ± 6.8
uPDI	48.1 ± 6.1
Smoking Status	
0: Never	1694 (50.3%)
1: Former	1253 (37.2%)
2: Current	422 (12.5%)
Income	
1: < $5,000	66 (2%)
2: $5,000-7,999	90 (2.8%)
3: $8,000-11,999	148 (4.5%)
4: $12,000-15,999	196 (6%)
5: $16,000-19,999	145 (4.4%)
6: $20,000-24,999	226 (6.9%)
7: $25,000-29,999	190 (5.8%)

8: $30,000-34,999	223 (6.8%)
9: $35,000-39,999	186 (5.7%)
10: $40,000-49,999	331 (10.1%)
11: $50,000-74,999	587 (18%)
12: $75,000-99,999	337 (10.3%)
13: $100,000 +	538 (16.5%)
Education	
0: Education	28 (0.8%)
1: Grades 1-8	262 (7.8%)
2: Grades: 9-11	201 (6%)
3: Completed High School/GED	557 (16.5%)
4: Some college but no degree	524 (25.6%)
5: Technical school certificate	235 (7%)
6: Associate degree	169 (5%)
7: Bachelor`s degree	655 (19.4%)
8: Graduate or professional school	737 (21.9)
Race	
1: White	1431 (42.4%)
2: Chinese	429 (12.7%)
3: Black:	878 (26%)
4: Hispanic/Latino	634 (18.8%)
BMI	26.72 ± 4.78
MetS components according to NCEP ATP III definition	
Triglyceride (mg/dl)	
1: Normal <150	3000 (89%)
2: Borderline high. 150-199	224 (6.6%)
3: High, 200-499	147 (4.4%)
4: Very High, 500+	0 (0%)
Hypertension by JNC VI (1997) criteria	
0: Yes	2294 (68%)
1: No	1078 (32%)
HDL Cholesterol (mg/dl)	
1: High, 60+	1106 (32.8%)
2: 40-59	1885 (55.9%)
3: Low, <40	381 (11.3%)
Waist circumference (cm)	93.4 ± 13.0
Fasting Blood Glucose ((mg/dl)	89.4 ± 19.6
Cancer	
0: Yes	3107 (92%)
1: No	256 (7.6%)

9: Do not know	4 (0.1%)
Kidney Disease	
0: Yes	3303 (98%)
1: No	60 (1.8%)
9: Do not know	9 (0.3%)
Medications	
Insulin or oral Hypoglycemics for Diabetes	
0: Yes	3253 (96.5%)
1: No	99 (2.9%)
9: Do not know	20 (0.6%)
Hypertension Medication	
0: Yes	2523 (74.8%)
1: No	849 (26.2%)
Any lipid-lowering medication	
0: Yes	2907 (86.2%)
1: No	465 (13.8%)
Nitrates	
0: Yes	3368 (99.9%)
1: No	4 (0.1%)
Fibrates	
0: Yes	3351 (99.4%)
1: No	21 (0.6%)

The table shows the baseline characteristics of included MESA participants reported as mean ± SD for continuous variables and number (%) for categorical variables.

Effects of PDI, hPDI, and uPDI on the hazard rate of MetS

The results of the Cox proportional hazard mixed effects model examining the effect of PDI, hPDI, and uPDI on incidence of MetS demonstrated a significant effect of PDI (HR = 0.93, p = 0.03). This represents a protective effect, i.e., the greater the PDI the lower the risk of MetS, when all other variables are held equal. Specifically, one standard deviation increase above the mean PDI of the sample would indicate a 7% decrease in the risk of MetS. Therefore, low PDI / high ADI was as significant risk factor for MetS, meaning that the higher the ADI score, the greater the incidence of MetS. Since ADI was the inverse score for PDI, we did not include it in

the analysis to avoid collinearity. To confirm the protective effect of uPDI, we plotted uPDI against the log Hazard rate across all time points (Fig 3.2). A total of 6,757 observations were deleted due to missingness. There were 10,009 events out of 27,487 potential events. Table 3.2 shows the hazard ratios and significance levels for independent variables and covariates for the Cox proportional hazards model.

Table 3.2 Hazard ratios for all independent and control variables.

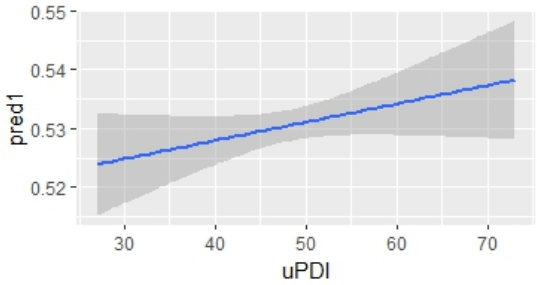
	Hazard Ratio (C0)	P value
Biological Sex		
Female	1	
Male	0.93 (0.86-1.00)	0.05
Age	1.00 (1.00-1.001)	0.06
PDI	0.97 (0.93-1.00)	0.27
hPDI	0.90 (0.85-0.95)	0.006
uPDI	0.93 (0.89 – 0.98)	0.0004
Smoking Status		
0: Never	1	
1: Former	1.02 (0.95-1.09)	0.53
2: Current	1.08 (0.96- 1.21)	0.19
Income		
1: < $5,000	1	
2: $5,000-7,999	1.02 (84-1.24)	0.81
3: $8,000-11,999	0.95 (0.79-1.15)	0.65
4: $12,000-15,999	0.90 (0.75-1.09)	0.30
5: $16,000-19,999	0.94 (0.78-1.14)	0.58
6: $20,000-24,999	0.81 (0.67-0.98)	0.03
7: $25,000-29,999	0.82 (0.68-1.00)	0.05
8: $30,000-34,999	0.77 (0.63-0.93)	0.007
9: $35,000-39,999	0.89 (0.74-1.08)	0.258
10: $40,000-49,999	0.83 (0.69-1.00)	0.05
11: $50,000-74,999	0.76 (0.63-0.90)	0.002
12: $75,000-99,999	0.66 (0.54-0.81)	<0.001
13: $100,000 +	0.66 (0.54-0.80)	<0.001
Education		
0: Education	1	
1: Grades 1-8	0.93 (0.64-1.09)	0.19
2: Grades: 9-11	0.77 (0.57-1.02)	0.07
3: Completed High School/GED	0.69 (0.52-0.90)	0.007
4: Some college but no degree	0.71 (0.54-0.94)	0.01
5: Technical school certificate	0.63(0.47-0.84)	0.001
6: Associate degree	0.66(0.49-0.89)	0.007
7: Bachelor`s degree	0.57 (0.43-0.76)	<0.001

8: Graduate or professional school	0.51 (0.38-0.69)	<0.001
Race		
1: White	1	
2: Chinese	0.74 (0.63-0.86)	<0.001
3: Black:	1.11 (1.01-1.22)	0.02
4: Hispanic/Latino	1.23 (1.10-1.37)	<0.001
Heart Disease		
0: Yes	1	
1: No	1.53 (1.23-1.91)	<0.001
9: Do not know	1.36 (0.91-2.03)	0.12
Kidney Disease		
0: Yes	1	
1: No	1.73 (1.40-2.13)	<0.001
9: Do not know	2.80 (1.65-4.74)	<0.001

Hazard ratios (Cl) and p values for all independent variables and covariates in the Cox proportional hazards model.

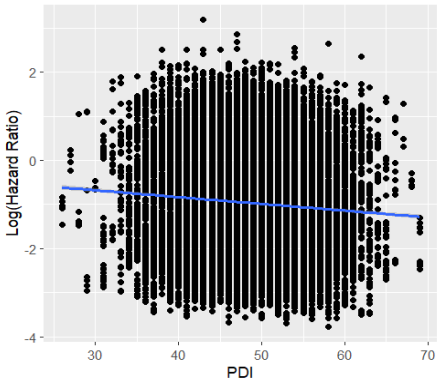
In the hazard mixed effects model, there were no significant effects of hPDI and uPDI on incident MetS. However, in the Cox proportional hazards fixed effects model, we demonstrated significant protective effects for both hPDI and uPDI on incident MetS. Since PDI, hPDI, uPDI are linked to one another and potentially confounding, we performed the Cox proportional hazard models with individual effects of PDI, uPDI, hPDI separately in each model. Unlike the Cox proportional hazards fixed effects model, which demonstrated a significant protective effect of PDI, hPDI, and uPDI, the results for Cox proportional hazards mixed effects model did not change. Both models showed a significant protective effect of hPDI and PDI on MetS incidence. However, the Cox proportional hazards fixed effect model showed a significant protective effect of uPDI, which is in contrast to the association between uPDI and loglikelihood risk of MetS shown in Figure 3.1.

Figure 3.1 The association between uPDI and loglikelihood risk of MetS.



The association between uPDI and risk of incident MetS, indicating that uPDI is a risk factor for incident MetS

Figure 3.2 PDI plotted against Log Hazard Ratio across all time points. The blue line represents the least squares line.

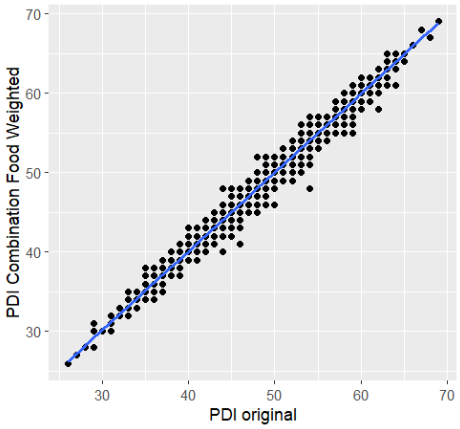


PDI has a protective effect for incident MetS across all time points, higher PBD decreases the risk of incident MetS.

Effects of PDI, hPDI, and uPDI weighted by combination food on the hazard rate of MetS

We used the third ML driven approach to include the combination foods, which were disaggregated across all food groups through weighting the combination food items based on their nutrient profiles. The results of this Cox proportional hazard models demonstrated no differences in results as compared with the model that did not include combination foods (PDI HR = 0.93, p = 0.03). Combination food weighting resulted in only a small change in the PDI scoring overall, as evidenced by Fig 3.3. A paired t-test between PDI combination food weighting scores and PDI original scores was not significant (p = 0.93).

Figure 3.3 The observed differences in individual PDI scores when combination foods were weighted, compared to the original PDI scores.



PDI scoring without combination foods compared with PDI scoring after inclusion of combination foods, indicating the negligible impact of including the combination foods on PDI scoring.

Mediation role of TMAO in relationship between plant-based food intake and incidence of MetS (Aim2)

The results of the mediation analysis that used PDI as an independent variable, TMAO as the mediator variable, and incident MetS as the outcome, demonstrated a significant indirect effect ($\beta = -0.0002$, $p = 0.002$), with no significant direct effect ($\beta = -0.001$, $p = 0.36$). Given that the indirect effect is negative, this result indicates that the effect of PDI on incident MetS is reduced when TMAO is taken into account, i.e., TMAO suppresses the effect of PDI on incident MetS. We performed an additional mediation analysis with race/ethnicity as the independent variable, TMAO as the mediator variable, and MetS as the outcome variable. Results of the model demonstrated a significant direct effect of race/ethnicity MetS incidence ($\beta = 0.07$, $p = 0.01$), with no significant indirect effect ($\beta = -0.0007$, $p = 0.6$). This result indicates that the effect of race/ethnicity on incident MetS, acts independently of TMAO, where the level of TMAO does not differentially suppress or enhance incidence of MetS by race or ethnicity.

Chapter 4 - Discussion

In this large cohort of U.S. adults, high PBD intake as compared with high ASF intake, is associated with a reduction in incident MetS, TMAO mediated this association significantly, by suppressing the effect of PDI and MetS. Further, according to hPDI, uPDI, and loglikelihood risk of MetS, hPDI is a protective factor, and uPDI is a risk factor for incident MetS. Finally, there was no significant effect for race/ethnicity when entered into the mediation analysis, indicating that the relationship between TMAO and incident MetS was the same across races and ethnicities.

To the best of our knowledge, this is the first longitudinal study assessing the associations between PBDs versus ASFs using PDI, hPDI, uPDI, and ADI scoring indices with three different analytical approaches. Further, this is the first prospective cohort study to investigate the potential mediation role of TMAO in this association. Prior studies have primarily determined the associations between TMAO and other chronic diseases or have been cross sectional studies, not allowing for determination of temporality.

Our findings are consistent with previous studies showing associations between less healthy PBD / high ASF and adverse health outcomes. For instance, the Nurse`s Health Study (NHS) I and II studies indicated that higher uPDI scores have been shown to be associated with greater weight gain (Satija et al., 2019) and increased T2DM incidence (Satija et al., 2016). In addition, less healthful PBD patterns are associated with incident hypertension in middle-aged U.S. adults (Kim et al., 2020), incident MetS in Korean adults (Kim et al., 2021), and cardiometabolic risk factors among individuals with South Asian ancestry (Bhupathiraju et al., 2022). The high content of sugar and refined carbohydrate in less healthy PBD may lead to increased abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT)

(McKeown et al., 2010). In contrast, higher intake of dietary fiber and whole grains in healthier PBD patterns are associated with lower inflammation, as determined by circulating C-reactive Protein (CRP) (Jiao et al., 2015), as well as lower LDL and total cholesterol (Hollænder et al., 2015). However, the results from a cross-sectional study of Iranian older adults, showed no significant associations between PBD intake and risk of MetS (Amini et al., 2021), which may be due to the nature of the study design. Similar to our results, patterns of higher amounts of ASF consumption, as compared with PBDs (low PDI/high ADI), contribute to increased risk for incident chronic diseases. For example a longitudinal study of U.S. older adults (> 65 years) showed positive associations between ASF and incident atherosclerotic vascular disease (ASCVD) (M. Wang et al., 2022). Similarly another multinational cohort study found that processed meat was associated with CVDs and its associated mortality in 21 countries (Iqbal et al., 2021). Several studies have shown a positive associations between ASF intake and MetS risk (Azemati et al., 2021; Chung et al., 2020; Pasdar et al., 2020). High intake of ASF has also been shown to be associated with increased risk of MetS components such as increased waist circumference in men (Z. Wang, Zhang, et al., 2014), high cholesterol levels (Igl et al., 2013), abdominal and general obesity (Alkerwi et al., 2015; Shang et al., 2017), and insulin resistance (Z. Chen et al., 2020). Several mechanisms have been proposed to explain the positive associations between ASF and MetS. The nutrient composition of such foods, including the abundance of cholesterol, saturated fatty acids, iron, and nitrite/nitrate in red meat are potential ASF components which have been shown to be associated with poor chronic disease outcomes (Männistö et al., 2010; Micha et al., 2012). However, a cross-sectional study in Iranian adults showed a negative association between protein intake both from animal and plant-based food and

MetS incidence, which again, may be due to the cross-sectional design of the study, which does not allow for determination of causality (Jamshidi et al., 2022).

Recently, gut microbiota-generated metabolites have emerged as potential mechanisms for the associations between dietary patterns and chronic disease incidence. The current results indicated a positive association between less PBD patterns and ASF intake, with TMAO levels. These results agree with the results obtained by previous studies showing inverse associations between PBDs and plasma TMAO levels (S et al., 2021). In contrast to our results, a 13-month study of 620 healthy men in the U.S., found a weak association between red meat consumption and plasma TMAO, and an inverse association between uPDI and TMAO levels (Hamaya et al., 2020). These contrasting results may be due, in part, to smaller sample size, shorter study durations, and use of red meat only in comparison with ASF in the current study. An international pooled analysis of 16 studies on the association between TMAO and dietary patterns showed that circulating TMAO was associated with red meat, eggs, and plant protein consumption in the U.S., and with fish and shellfish consumption in Asian countries (J. J. Yang et al., 2021). Authors of this pooled analysis concluded that populations in different countries vary with regard to associations between consumption of different dietary patterns and TMAO levels due, potentially due to the differences in dietary patterns by population, resulting in gut microbiome composition differences. Therefore, TMAO levels are dependent on a variety of factors, and cannot be considered as a marker of an unhealthful dietary pattern in absolute terms.

Wang and colleagues showed that ASF intake was positively associated with ACSVD, and that relationship was partially mediated by carnitine-related gut microbiota metabolites of TMAO (M. Wang et al., 2022). A cross-sectional study also showed an association between higher levels of plasma TMAO and increased risk of MetS (Kuo et al., 2022). The associations

between TMAO and other carnitine-related gut microbiota result from the creation of the protein kinase RNA-like endoplasmic reticulum kinase which in turn produces the protein FoxO1. High levels of this protein may result in metabolic illnesses (S. Chen et al., 2019), which is consistent with the current results, indicating a mediation role for TMAO in the relationship between PBD consumption and incident MetS. However, an observational study showed that low plasma betaine concentrations were associated with increased risk of CVDs in non-diabetic patients, suggesting that metabolites related toTMAO may not always be associated with negative health outcomes (Lever et al., 2014).

Incident MetS varies between races/ethnicities. For example, according to NHANES data from 2011–2018, while the overall population prevalence of MetS did not increase significantly, there were increases among non-Hispanic Asians from 21.8% in 2011–12 to 31.2% in 2017–18 (Liang et al., 2021). In Hispanics, between 2011–2016, prevalence estimates indicated increases from 32.9% to 40.4% (Hirode & Wong, 2020). However, the current analyses indicated that the mediation role of TMAO in the relationship between PDI and incident MetS was independent of race/ethnicity.

The current study had several strengths. To our knowledge, this was the first study comparing PBD patterns with ASF patterns regarding long-term risk for incident MetS, using established dietary pattern indices in a large prospective cohort study. A significant new piece of evidence in the puzzle of nutrition and health, the microbiome, was included in our analyses to determine whether there was a mediation role for a novel group of metabolites produced from the gut microbiome. Specifically, we investigated the role of TMAO using large dataset from a community-based cohort with good representation of the U.S. population, we were able to determine whether the associations between TMAO and incident MetS differed according to race

and ethnicity. We also used a novel approach for translating the MESA FFQ into the established indices, in order to account for the MESA combination foods, employing machine learning to best match food items to the appropriate food groups, a method that may be useful in future studies. Further, the three models we used were adjusted for several potential confounders

While the current study has several strengths, there are some limitations that should be noted. While the study was longitudinal, which adds to the case that can be made for causality, the observational nature of the study simultaneously limits the opportunity for that determination. The FFQs used in the MESA study, and some confounding variables were self-reported, which could result in measurement inaccuracies. Since the MESA FFQ had not been used to score the PDI, hPDI, and uPDI indices, the MESA FFQ combination foods did not fit well into the 18 predetermined food groups. We took a rigorous approach to avoid exclusion of multiple types of foods from the MESA FFQ, which could lead to poor validity regarding actual dietary intake. Additionally, we took steps to appropriately categorize combination foods according to the foods groups that were the most similar in terms of nutrient content. Although our ML approaches hold promise for translation of other dietary intake data, further studies need to validate these methods. Our data were collected from a U.S. population of adults between the ages of 44–84 years, and therefore the current findings may not be generalizable to other populations and ages. As has been previously reported in other studies using the MESA FFQ data, due to a coding error that was apparently systematically applied to a subset of Exam 1 FFQ variables in a subset of the patients, FFQ information for 1,904 participants were imputed using multinomial regression or ordinal regression (when appropriate). These analyses were performed by the Nutrition Coordinating System at University of Minnesota (Oh et al., 2022). In the MESA FFQ, animal fat and oil were reported as a binary variable, while the servings/day was calculated

for fats and oils combined, therefore it was not possible to score fats and oils for use in the indices. Because of this limitation, we opted to adjust for servings/day of fats and oils in the overall analyses. The lack of inclusion of this important dietary factor in the indices, may have reduced the validity of the current results

Chapter 5 - Conclusion

In this large cohort of U.S. adults, higher PBD patterns (indicating low ASF intake), were associated with lower incident MetS after 18 years of follow up. These associations were mediated by TMAO. This secondary data analysis affirmed the importance of PBD patterns, particularly healthier PBD patterns, for beneficial metabolic health outcomes. Further research, especially clinical trials, is warranted to confirm these findings and to determine causal relationships in different populations with different patterns of dietary intake.

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