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Dietary Patterns among Asian Indians Living in the United States Have Distinct Metabolomic Profiles That Are Associated with Cardiometabolic Risk

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Abstract

Background: Recent studies, primarily in non-Hispanic whites, suggest that dietary patterns have distinct metabolomic signatures that may influence disease risk. However, evidence in South Asians, a group with unique dietary patterns and a high prevalence of cardiometabolic risk, is lacking.

Objective: We investigated the metabolomic profiles associated with 2 distinct dietary patterns among a sample of Asian Indians living in the United States. We also examined the cross-sectional associations between metabolomic profiles and cardiometabolic risk markers.

Methods: We used cross-sectional data from 145 Asian Indians, aged 45–79 y, in the Metabolic Syndrome and Atherosclerosis in South Asians Living in America (MASALA) pilot study. Metabolomic profiles were measured from fasting serum samples. Usual diet was assessed by using a validated food-frequency questionnaire. We used principal components analysis to derive dietary and metabolomic patterns. We used adjusted general linear regression models to examine associations between dietary patterns, individual food groups, metabolite patterns, and cardiometabolic risk markers.

Results: We observed 2 major principal components or metabolite clusters, the first comprised primarily of medium- to long-chain acylcarnitines (metabolite pattern 1) and the second characterized by branched-chain amino acids, aromatic amino acids, and short-chain acylcarnitines (metabolite pattern 2). A "Western/nonvegetarian" pattern was significantly and positively associated with metabolite pattern 2 (all participants: $\beta \pm SE = 0.180 \pm 0.090$, $P = 0.05$; participants without type 2 diabetes: $\beta \pm SE = 0.323 \pm 0.090$, $P = 0.0005$). In all participants, higher scores on metabolite pattern 2 were adversely associated with measures of glycemia (fasting insulin: $\beta \pm SE = 2.91 \pm 1.29$, $P = 0.03$; 2-h insulin: $\beta \pm SE = 22.1 \pm 10.3$, $P = 0.03$; homeostasis model assessment of insulin resistance: $\beta \pm SE = 0.94 \pm 0.42$, $P = 0.03$), total adiponectin ($\beta \pm SE = -1.46 \pm 0.47$, $P = 0.002$), lipids (total cholesterol: $\beta \pm SE = 7.51 \pm 3.45$, $P = 0.03$; triglycerides: $\beta \pm SE = 14.4 \pm 6.67$, $P = 0.03$), and a radiographic measure of hepatic fat (liver-to-spleen attenuation ratio: $\beta \pm SE = -0.83 \pm 0.42$, $P = 0.05$).

Conclusions: Our findings suggest that a "Western/nonvegetarian" dietary pattern is associated with a metabolomic profile that is related to an adverse cardiometabolic profile in Asian Indians. Public health efforts to reduce cardiometabolic disease burden in this high-risk group should focus on consuming a healthy plant-based diet. *J Nutr* 2018;148:1150–1159.

Keywords: metabolomics, dietary patterns, South Asians, Indians, diabetes

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Introduction

The human metabolome, a collection of >40,000 metabolites or small molecules, provides a comprehensive summary of an individual's chemical status and reports on the interaction between a person's genome and his or her environment. In recent years, the field of nutritional epidemiology has rapidly evolved by integrating metabolomic profiling with nutrition in complex biosystems. Metabolomics, the measurement of small molecules in biological samples, offers a unique opportunity to identify new biomarkers of diet and to examine the complex molecular mechanisms affecting disease risk (1). Diet can affect the human metabolome by either directly contributing metabolites or by indirectly influencing metabolic pathways that consume or produce specific metabolic intermediates (2). It is therefore plausible that individuals with different dietary patterns might present with distinct metabolomic signatures that could influence disease risk. For example, few studies have shown that individuals who consume a Western-style dietary pattern, defined by high intakes of red meat, potatoes, and sweets, have a metabolomic signature enriched with BCAAs (3) and short-chain acylcarnitines (ACs) (3, 4). Several studies have shown an association of a very similar metabolite cluster with obesity, insulin resistance, type 2 diabetes, and cardiovascular disease (5–11). However, associations between dietary patterns and metabolite classes related to disease risk have not been examined in a South Asian population known to have unique dietary patterns (12) and a high prevalence of cardiometabolic risk (13–16).

With the use of data from the Metabolic Syndrome and Atherosclerosis in South Asians Living in America (MASALA) pilot study, our aim was to investigate the associations between 2 dietary patterns and serum metabolomic profiles derived from a panel of 45 ACs, 15 amino acids including BCAAs and aromatic amino acids, nonesterified FAs, ketones, and lactate. To elucidate potential mechanisms by which diet may influence cardiometabolic risk in South Asians, we further examined associations between metabolomic profiles and biomarkers of cardiometabolic risk in this high-risk population.

Methods

Study population. We used cross-sectional data from the MASALA pilot population-based study. The study design and methods of the MASALA pilot study are described in detail elsewhere (17). Briefly, the study enrolled 150 participants, aged 45 to 79 y, who self-identified as Asian Indians living in the San Francisco Bay Area between August 2006 and October 2007. The pilot study was modeled after the Multi-Ethnic Study of Atherosclerosis (MESA) and used similar recruitment methods, inclusion and exclusion criteria, and study measurements. Accordingly, we excluded participants with a physician-confirmed diagnosis of cardiovascular disease or a history of coronary artery bypass graft surgery, angioplasty, valve replacement, pacemaker or defibrillator implantation, and surgery on the heart or arteries. We also excluded participants who used nitroglycerin, those undergoing active cancer treatment, those with impaired cognitive ability, those with a life expectancy <5 y, those living in a nursing home, and those planning to move. Participants who could not speak or understand Hindi or English were also excluded.

Eligible participants completed an in-person interview to ascertain their age, sex, medical history, smoking and alcohol use, physical activity, and dietary intake. In addition, fasting blood samples were collected by a certified phlebotomist after a 12-h fast. For the current analyses, we excluded 4 participants who reported implausible energy intakes (<500 or >5000 kcal/d) and 1 participant with missing data on metabolites. The present study included 145 participants with complete data at the time of analyses. All of the study protocols were approved by the University of California, San Francisco, Institutional Review Board, and all of the participants provided written informed consent.

Metabolomics analysis and metabolite patterns. Targeted metabolomics analyses were performed on fasting serum samples at the Stedman Center/Duke Molecular Physiology Institute Metabolomics Core at Duke University. Concentrations of lactate, total ketones, and nonesterified FAs were measured by standard clinical chemistry methods on a Beckman DxC600 autoanalyzer. With the use of a targeted MS-based approach, we determined the concentrations of 45 ACs and 15 amino acids. Proteins were first removed by precipitation with methanol. Aliquoted supernatants were dried and esterified with either hot acidic methanol (for ACs) or *n*-butanol (for amino acids) (18, 19). Analysis was done by using tandem flow injection MS with a TQD instrument (Waters Corporation). Quantitation of the "targeted" intermediary metabolites was facilitated by the addition of mixtures of known quantities of stable-isotope internal standards, as detailed previously (20). The use of internal standards enabled absolute quantitation in micromolar units, and values below the practical lower limits of quantitation were reported and analyzed as "0".

Dietary intake. Habitual food consumption and nutrient intakes were captured by using an ethnicity-specific semi-quantitative FFQ, designed and validated among South Asians in the Study of Health Assessment and Risk in Ethnic Groups (SHARE) (21). The FFQ consists of 163 items, including 61 items unique to the South Asian diet. The total quantity of consumption (servings per day) for each food item was computed from the frequency of consumption (assessed as per day, per week, per month, per year, or never) and the serving size (average, small, or large). For each food item, the average serving size was provided. A small serving size was considered to be 0.5 of the average serving size, whereas a large serving size was 1.5 of the average. Foods were categorized in 29 predefined subgroups on the basis of similarity of nutrient content, likeness, and their culinary use in an Asian-Indian diet (12) (**Supplemental Table 1**). Foods such as coffee were retained as individual categories given their high reported intake. The FFQ was previously validated against 7-d diet records. Energy-adjusted and deattenuated (corrected for measurement error) correlation coefficients for micronutrients ranged from 0.45 for total protein intake to 0.73 for intakes of vitamin E and cholesterol (21).

Measurement of covariates. Weight was measured by using a standard balance-beam scale, and height was measured by using a stadiometer. Three blood pressure measurements were obtained in the seated position with the use of an automated blood pressure monitor (Philips-Agilent V24C). Mean systolic and diastolic blood pressures were computed as averages of the second and third readings. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or the use of antihypertensive medication.

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Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: AC, acylcarnitine; CAC, coronary artery calcium; CT, computed tomography; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America; MESA, Multi-Ethnic Study of Atherosclerosis.

Biomarkers of cardiometabolic risk. Fasting plasma glucose was measured by using a glucose oxidase method (YSI 2300 STAT Plus; YSI Life Sciences). Total adiponectin and fasting insulin were measured by RIA (Linco Research, Inc.). HOMA-IR was calculated as follows: glucose (mmol/L) × insulin (mU/L)/22.5 (22). Participants were administered 75 g oral glucose, and blood samples were obtained at 120 min for the measurement of plasma glucose and insulin. Diabetes was defined as the use of a hypoglycemic medication or fasting plasma glucose ≥126 mg/dL or a 2-h postchallenge glucose concentration of ≥200 mg/dL (23). Prediabetes was defined as a fasting plasma glucose concentration of 100–125 mg/dL or a 2-h postchallenge glucose concentration of 140–199 mg/dL (23). Participants with normal glucose tolerance had both fasting plasma glucose concentrations <100 mg/dL and a 2-h glucose concentration <140 mg/dL. Insulin sensitivity index was computed as 10,000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during an oral-glucose-tolerance test) (24).

Total cholesterol, TGs, and HDL cholesterol were measured by enzymatic methods (Quest Diagnostics). LDL cholesterol was calculated by using the Friedewald formula (25).Visceral fat area was measured by using DXA (Hologic Discovery-Wi) at the L4–L5 level after participants were placed in a supine position. We obtained nonenhanced computed tomography (CT; Philips Medical Systems) images of liver and spleen density to quantify hepatic fat content. The presence of fatty liver was defined by a liver-to-spleen attenuation ratio of <1, and lower values represent higher amounts of hepatic fat (26–28). A trained vascular technician performed a carotid ultrasound examination to quantify maximal intimal medial thickness of the internal and common carotid artery (17). Cardiac CT scans were performed by using a gated-cardiac CT scanner with the use of either the 16D scanner (Philips Medical Systems) or the MSD Aquilion 64 model (Toshiba Medical Systems). All CT scans were read centrally at Harbor–University of California Los Angeles Medical Center. Coronary artery calcium (CAC) Agatston scores were reported for each of the 4 major coronary arteries, and the summed score was used (15). For the current analysis, we modeled CAC scores as a binary outcome (<10 compared with ≥10).

Statistical methods. We applied a log $(x + 1)$ transformation to the metabolite data to improve normality. We used the FACTOR procedure in SAS to derive metabolomic signatures and dietary patterns. Log-transformed metabolites were entered into principal components analysis, and the varimax option was specified. Dietary patterns were derived as previously described (12). Briefly, food groups were entered into principal components analysis as servings per day and factors were rotated

TABLE 1 Descriptive characteristics of Asian Indians, aged 45–79 y, in the MASALA pilot study, by their predominant metabolomic signature[1]

Characteristic	Overall	Medium- to long-chain ACs[2]	BCAAs, AAAs, short-chain ACs	P[3]
n	145	70	75	
Age, y	57.1 ± 0.7	59.1 ± 1.1	55.2 ± 0.8	0.003
Female, %	50	60	40	0.02
BMI, kg/m^2	26.2 ± 0.4	24.8 ± 0.5	27.5 ± 0.6	0.0004
Education, %				0.80
High school or less	12.4	14.3	10.7	
Bachelor's degree or less	9.0	8.6	9.3	
More than Bachelor's degree	78.6	77.1	80.0	
Family income, %				0.17
<$40,000	14.5	18.6	10.7	
$40,000–$99,999	33.8	27.1	40.0	
≥$100,000	51.7	54.3	49.3	
Smoking, %				0.68
Never	84.1	81.4	86.7	
Past	12.4	14.3	10.7	
Current	3.5	4.3	2.7	
Metabolic equivalents of task exercise,[4] min/wk	1313 (1885)	1444 (1530)	1155 (420)	0.21
Alcoholic drinks/wk, *n*	4.1 ± 0.6	3.2 ± 0.7	4.9 ± 1.0	0.19
Diabetes, %				0.16
None	35.2	42.9	28.0	
Impaired fasting glucose and/or impaired glucose tolerance	37.2	34.3	40.0	
Type 2 diabetes	27.6	22.9	32.0	
Hypertension, %	42.1	40.0	44.0	0.63
Parental history of diabetes, %	47.6	44.3	50.1	0.72
Time in the United States, y	23.9 ± 1.0	23.2 ± 1.5	24.5 ± 1.3	0.48
Traditional Indian beliefs, %				
Weak	31.0	30.0	32.0	0.89
Intermediate	36.6	38.6	34.7	
Strong	32.4	31.4	33.3	
Score on nonvegetarian dietary pattern	−3.37 ± 0.08	−0.24 ± 0.10	0.23 ± 0.13	0.004
Score on vegetarian dietary pattern	4.21 ± 0.08	0.06 ± 0.15	−0.05 ± 0.08	0.54
Total energy intake, kcal/d	1918 ± 58	1856 ± 80	1976 ± 83	0.30

[1] Values are means ± SEMs or percentages calculated by using *t* tests for continuous variables and chi-square tests for categorical variables. AAA, aromatic amino acid; AC, acylcarnitine; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America.

[2] Participants whose factor scores for the medium- to long-chain AC pattern were higher than the BCAA, AAA, and short-chain AC pattern were assigned to the medium- to long-chain ACs group. Participants whose factor scores for the BCAA, AAA, and short-chain AC pattern were higher than those for the medium- to long-chain AC pattern were assigned to the BCAA, AAA, and short-chain AC group.

[3] *P* values comparing differences between metabolomic signature 1 and metabolomic signature 2.

[4] Due to the skewed distribution, values shown are medians (IQRs).

TABLE 2 Cross-sectional associations between dietary pattern scores and 2 major metabolomic signatures among Asian Indians, aged 45–79 y, in the MASALA pilot study[1]

	All participants ($n = 145$)[2]				Participants without type 2 diabetes ($n = 105$)[3]			
	Western/nonvegetarian dietary pattern		Vegetarian dietary pattern		Nonvegetarian dietary pattern		Vegetarian dietary pattern	
	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Metabolite pattern 1[4]								
Model 1	−0.131 ± 0.080	0.10	0.034 ± 0.078	0.67	−0.162 ± 0.090	0.08	0.027 ± 0.089	0.77
Model 2	−0.173 ± 0.100	0.09	0.070 ± 0.097	0.47	−0.188 ± 0.104	0.07	0.060 ± 0.106	0.57
Model 3	−0.173 ± 0.103	0.10	0.065 ± 0.100	0.52	−0.189 ± 0.105	0.07	0.061 ± 0.108	0.57
Metabolite pattern 2[5]								
Model 1	0.144 ± 0.075	0.06	0.008 ± 0.074	0.91	0.257 ± 0.080	0.002	−0.042 ± 0.082	0.61
Model 2	0.189 ± 0.090	0.04	0.010 ± 0.087	0.91	0.323 ± 0.089	0.0005	−0.064 ± 0.095	0.51
Model 3	0.180 ± 0.090	0.05	0.014 ± 0.088	0.87	0.323 ± 0.090	0.0005	−0.068 ± 0.097	0.48

[1] Values are βs ± SEs for each metabolite pattern score per 1-unit increase in the dietary pattern factor score calculated by using linear regression. Model 1 adjusted for age (years) and sex; model 2 adjusted as for model 1 plus for smoking (never, past smoker, or current smoker), education (high school or less, less than Bachelor's degree, Bachelor's degree, or more than Bachelor's degree), BMI (kg/m^2), physical activity (metabolic equivalent task hours per week), and total energy intake (kilocalories per day); model 3 adjusted as for model 2 plus for prevalent hypertension (yes or no). AAA, aromatic amino acid; AC, acylcarnitine; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America.

[2] Among all participants, model 3 was additionally adjusted for prevalent diabetes status (yes or no).

[3] Among participants without type 2 diabetes, a vegetarian pattern was identified in the first principal component, whereas a nonvegetarian pattern was identified in the second principal component.

[4] Medium- to long-chain ACs.

[5] BCAA, AAA, and short-chain AC pattern.

orthogonally. To determine the number of factors to retain for both metabolomics signatures and dietary patterns, we examined principal components with Eigenvalues >1, scree plots, variance explained, factor loadings, and overall interpretability. Metabolites or food groups with factor loadings ≥0.5 were considered to contribute significantly to the principal component. We used the SCORE procedure in SAS to derive a score for each factor for each participant by summing intakes of the food groups (or metabolomic signature groups) multiplied by their respective factor loadings. Higher scores indicate a greater degree of conformance to the respective principal component. Dietary pattern scores were modeled as both continuous and categorical variables (<0 and ≥0).

Participants who received higher factor scores for metabolomic pattern 1 than for metabolomic pattern 2 were categorized into metabolomic signature 1. Likewise, those who had higher factors scores for metabolite pattern 2 than for pattern 1 were categorized into metabolomic signature 2. Differences in descriptive characteristics of study participants by metabolomic signature categories were determined with the use of *t* tests for continuous variables and chi-square analyses for categorical variables. We used Pearson correlations to examine associations between dietary pattern scores and metabolite patterns, adjusting for total energy intake. We used a general linear regression model to examine associations between dietary pattern scores (continuous and categorical: <0 and ≥0) and metabolite pattern scores (continuous). In our first model, we adjusted for age and sex. In model 2, we additionally adjusted for total energy intake, educational status, and traditional cardiometabolic risk factors, such as smoking, BMI, and physical activity. Because alcohol was listed as a food group in our principal components analysis, we did not adjust for this variable. In our final model, to account for potential reverse causation due to the diagnosis of a cardiometabolic outcome, we further adjusted our models for the presence of prevalent type 2 diabetes and hypertension. In a secondary analysis, in order to understand if associations between dietary pattern scores and metabolite patterns were driven by certain food groups, we examined cross-sectional associations between food groups with factor loadings ≥0.5 (continuous) and metabolite pattern scores adjusting for the covariates listed above.

Given our previous findings that greater adherence to a Western pattern was associated with adverse measures of glycemia and insulin resistance (29), we examined the cross-sectional associations between metabolite pattern scores and several biomarkers of cardiometabolic risk with the use of linear regression. For models with CAC as a binary outcome, we used logistic regression. In these models, in addition to the covariates listed above, we further adjusted for medication use, including cholesterol-lowering medications. Because the presence of type 2 diabetes can significantly alter the metabolomic profile (30, 31), we repeated all analyses restricting to those without type 2 diabetes. For all linear models, we checked for evidence of violations of the assumptions of normality, linearity, and homogeneity by examining plots of residuals compared with predicted values and the normal probability plots of residuals. All of the statistical analyses were conducted with the use of SAS version 9.4 (SAS Institute). A significance level of $P \leq 0.05$ was used.

Results

Dietary patterns and metabolite patterns. We derived 2 major dietary patterns: a Western/nonvegetarian pattern, characterized by high intakes of poultry, fish, red meat, coffee, pizza, and alcohol, and a vegetarian pattern, characterized by high intakes of rice, legumes, sugar-sweetened beverages, and snacks. When we restricted the analyses to those without diabetes, we observed a nonvegetarian and a vegetarian pattern. Similar to the overall population, the nonvegetarian pattern was characterized by higher intakes of red meat, poultry, and fish as well as eggs and vegetables. Foods that loaded heavily on the vegetarian pattern included rice, snacks, legumes, and sugar-containing beverages (**Supplemental Table 2**). In all of the participants, we derived 2 major metabolomic signatures. The first pattern was enriched primarily with medium-chain (C8–C14) and long-chain (C16 –C20) ACs and accounted for 15.9% of the variance. The second metabolite pattern was characterized primarily by BCAAs and aromatic amino acids plus short-chain (C2 and C5) ACs (**Supplemental Table 3**). Among participants without type 2 diabetes, we observed similar metabolomic signatures (Supplemental Table 3).

Descriptive characteristics. We included data on 145 participants with complete information on diet and metabolites. In the overall sample, half of the participants were women, and the average age was 57 y. A large majority of the participants had at least a bachelor's degree, a family income ≥$100,000, and were

never smokers. Participants with higher factor loadings on the BCAA, aromatic amino acid, and short-chain AC pattern than on the medium- to long-chain AC pattern were younger, more likely to be male, and had a significantly higher BMI. These participants were also more likely to have higher factor scores on the nonvegetarian dietary pattern (**Table 1**).

Cross-sectional associations between dietary patterns and metabolomic signatures. In the overall sample ($n = 145$), after adjusting for age, sex, smoking status, education, BMI, physical activity, total energy intake, and comorbidities including type 2 diabetes and hypertension, we found a positive association between a Western/nonvegetarian dietary pattern and a metabolite pattern enriched with BCAAs, aromatic amino acids, and short-chain ACs (**Table 2**). At the same time, compared with those with low scores (<0) on the Western/nonvegetarian dietary pattern, those with high scores (≥0) had significantly higher adjusted mean BCAAs, aromatic amino acids, and short-chain ACs in their metabolite pattern scores (**Table 3**). When we restricted the analysis to those without type 2 diabetes ($n = 105$), a nonvegetarian dietary pattern was strongly and positively associated with BCAAs, aromatic amino acids, and short-chain ACs. Likewise, compared with those with low factor scores (<0) on the nonvegetarian dietary pattern, those with high factor scores (≥0) had significantly higher BCAA, aromatic amino acid, and short-chain AC metabolite pattern scores (Table 3). In both the overall sample and among those without type 2 diabetes, we found no significant associations between a vegetarian dietary pattern and metabolomic signatures ($P > 0.05$).

Metabolite patterns and food groups. In a secondary analysis, we examined cross-sectional associations between servings of individual food groups with factor loadings ≥0.5 and metabolite patterns. In the overall sample, we found a positive and significant association between red meat intake and BCAAs, aromatic amino acids, and short-chain ACs. Among those without type 2 diabetes, higher intakes of poultry, red meat, and vegetables were associated with a BCAA, aromatic amino acid, and short-chain AC metabolomic signature (**Table 4**). However, after performing Bonferroni correction ($P < 0.006$), only vegetables were associated with a BCAA, aromatic amino acid, and short-chain AC metabolomic signature. We found no association between any of the individual food groups and the medium- to long-chain AC metabolite pattern.

Metabolomic signatures and biomarkers of cardiometabolic risk. Among all participants and in those without type 2 diabetes, we found that higher scores on the medium- to long-chain AC pattern were associated with higher concentrations of total adiponectin (**Table 5**). In the entire sample, higher scores on the BCAA, aromatic amino acid, and short-chain AC metabolomic pattern were significantly associated with higher fasting insulin and 2-h insulin concentrations, higher insulin resistance, and lower concentrations of total adiponectin and a lower insulin sensitivity index. At the same time, the BCAA, aromatic amino acid, and short-chain AC metabolomic pattern was also positively associated with total cholesterol and TG concentrations and lower liver-to-spleen attenuation. In analyses restricted to those without type 2 diabetes, this metabolomic pattern was associated with higher concentrations of fasting glucose, lower concentrations of total adiponectin, and a lower insulin sensitivity index.

TABLE 3 Adjusted mean metabolite pattern scores according to dietary pattern score among Asian Indians, aged 45–79 y, in the MASALA pilot study[1]

	All participants ($n = 145$)[2]						Participants without type 2 diabetes ($n = 105$)[3]					
	Western/Nonvegetarian dietary pattern			Vegetarian dietary pattern			Nonvegetarian dietary pattern			Vegetarian dietary pattern		
	Low scores (<0)	High scores (>0)	*P*	Low scores (<0)	High scores (>0)	*P*	Low scores (<0)	High scores (>0)	*P*	Low scores (<0)	High scores (>0)	*P*
Metabolite pattern 1[4]												
Model 1	0.118 ± 0.100	−0.180 ± 0.123	0.06	0.049 ± 0.101	−0.074 ± 0.122	0.44	0.138 ± 0.117	−0.091 ± 0.134	0.20	0.136 ± 0.114	−0.108 ± 0.140	0.18
Model 2	0.232 ± 0.192	−0.100 ± 0.208	0.07	0.148 ± 0.197	0.028 ± 0.217	0.56	0.267 ± 0.207	0.081 ± 0.218	0.35	0.276 ± 0.207	0.040 ± 0.235	0.31
Model 3	0.236 ± 0.195	−0.098 ± 0.211	0.07	0.149 ± 0.199	0.023 ± 0.222	0.55	0.266 ± 0.209	0.079 ± 0.222	0.35	0.276 ± 0.210	0.039 ± 0.234	0.31
Metabolite pattern 2[5]												
Model 1	−0.152 ± 0.093	0.220 ± 0.115	0.01	0.010 ± 0.095	−0.022 ± 0.115	0.83	−0.066 ± 0.106	0.280 ± 0.122	0.03	0.126 ± 0.105	0.020 ± 0.130	0.53
Model 2	−0.374 ± 0.170	0.030 ± 0.185	0.01	−0.174 ± 0.177	−0.270 ± 0.194	0.60	−0.157 ± 0.182	0.205 ± 0.193	0.04	0.089 ± 0.186	−0.141 ± 0.211	0.27
Model 3	−0.350 ± 0.169	0.042 ± 0.182	0.02	−0.168 ± 0.175	−0.224 ± 0.194	0.76	−0.160 ± 0.185	0.202 ± 0.196	0.04	0.084 ± 0.189	−0.148 ± 0.214	0.27

[1] Values are adjusted mean ± SE metabolite pattern scores calculated by using linear regression. Model 1 adjusted for age (years) and sex; model 2 adjusted as for model 1 plus for smoking (never, past smoker, or current smoker), education (high school or less, less than Bachelor's degree, Bachelor's degree, or more than Bachelor's degree), BMI (kg/m^2), physical activity (metabolic equivalent task hours per week), and total energy intake (kilocalories per day); model 3 adjusted as for model 2 plus for prevalent hypertension (yes or no). AAA, aromatic amino acid; AC, acylcarnitine; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America.

[2] Among all participants, model 3 was additionally adjusted for prevalent diabetes status (yes or no).

[3] Among participants without type 2 diabetes, a vegetarian pattern was identified in the first principal component, whereas a nonvegetarian pattern was identified in the second principal component.

[4] Medium- to long-chain ACs.

[5] BCAA, AAA, and short-chain AC pattern.

TABLE 4 Cross-sectional associations between food groups (servings per day) and metabolite pattern scores among Asian Indians, aged 45–79 y, in the MASALA study[1]

	All participants ($n = 145$)[3]				Participants without type 2 diabetes ($n = 105$)			
	Metabolite pattern 1 (Medium- to long-chain AC pattern)		Metabolite pattern 2 (BCAA, AAA, and short-chain AC pattern)		Metabolite pattern 1 (Medium- to long-chain AC pattern)		Metabolite pattern 2 (BCAA, AAA, and short-chain AC pattern)	
Food groups[2]	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Rice	0.069 ± 0.090	0.45	0.060 ± 0.079	0.45	0.070 ± 0.087	0.42	0.032 ± 0.078	0.68
Legumes	0.085 ± 0.095	0.37	−0.070 ± 0.082	0.40	0.033 ± 0.103	0.75	−0.080 ± 0.092	0.38
Sugary drinks	0.156 ± 0.180	0.39	0.071 ± 0.157	0.66	0.127 ± 0.174	0.47	−0.011 ± 0.157	0.94
Snacks	0.031 ± 0.101	0.76	−0.040 ± 0.088	0.65	0.060 ± 0.110	0.59	−0.067 ± 0.099	0.50
Poultry	−0.168 ± 0.198	0.40	0.118 ± 0.173	0.50	−0.193 ± 0.345	0.58	0.760 ± 0.300	0.01
Fish	−0.318 ± 0.378	0.40	0.624 ± 0.328	0.06	−1.001 ± 0.577	0.09	0.935 ± 0.518	0.07
Red meat	0.031 ± 0.249	0.90	0.512 ± 0.213	0.02	−0.197 ± 0.262	0.45	0.534 ± 0.229	0.02
Coffee	−0.084 ± 0.077	0.28	0.006 ± 0.068	0.93	—	—	—	—
Pizza	−0.666 ± 0.527	0.21	−0.732 ± 0.460	0.11	—	—	—	—
Alcohol	0.062 ± 0.116	0.60	0.040 ± 0.101	0.69	—	—	—	—
Eggs	—	—	—	—	−0.392 ± 0.209	0.06	0.198 ± 0.191	0.30
Vegetables	—	—	—	—	−0.045 ± 0.035	0.21	0.087 ± 0.031	0.005

[1] Values are βs ± SEs for each metabolite pattern score per 1-serving increase in the corresponding food group calculated by using linear regression. Models were adjusted for age (years), sex, smoking (never, past smoker, or current smoker), education (high school or less, less than Bachelor's degree, Bachelor's degree, or more than Bachelor's degree), BMI (kg/m^2), physical activity (metabolic equivalent task hours per week), total energy intake (kilocalories per day), and prevalent hypertension (yes or no). AAA, aromatic amino acid; AC, acylcarnitine; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America.

[2] Food groups are those with factor loadings ≥0.5 in all dietary patterns.

[3] Models in all participants additionally adjusted for prevalent diabetes status (yes or no).

In both the overall sample and the subgroup restricted to those without type 2 diabetes, we found no associations between metabolomic patterns, visceral fat area, and subclinical measures of atherosclerosis.

Discussion

In this cross-sectional pilot study in middle-aged immigrant Asian Indians living in the United States, we confirmed 2 major dietary patterns—a Western/nonvegetarian and a vegetarian dietary pattern—and documented 2 major metabolomic profiles, including one enriched with medium- to long-chain ACs and another characterized by higher concentrations of BCAAs, aromatic amino acids, and short-chain (C2, C5) ACs. In both the overall sample and in the subset of participants without type 2 diabetes, a Western or a nonvegetarian dietary pattern was positively associated with a BCAA, aromatic amino acid, and short-chain AC metabolomic pattern. In particular, higher intakes of red meat were positively associated with this metabolite pattern. Among those without type 2 diabetes, in addition to red meat intake, intakes of fish and vegetables were additionally associated with the BCAA, aromatic amino acid, and short-chain AC pattern. We also found this metabolomic pattern to be significantly and positively associated with measures of glycemia, insulin resistance, TGs, and liver-to-spleen attenuation ratio.

A direct comparison of our findings with the existing literature is not straightforward because, to our knowledge, no previous study has examined associations between a South Asian dietary pattern and metabolomic profiles. However, our results are broadly consistent with studies that examined dietary sources of metabolites and with studies that identified a "Western" or "meat" pattern. For example, in a cross-sectional analysis of 210 participants, aged 18 to 50 y, recruited from the greater Quebec City area, a Western dietary pattern defined by higher intakes of refined-grain products, desserts, sweets, and processed meats was positively associated with a metabolomic signature comprised of leucine, methionine, arginine, phenylalanine, proline, ornithine, histidine, and ACs C0, C3, C4, and C5 ($r = 0.38$, $P = 0.03$) (3). In the Oxford arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, consistent with our finding that a "nonvegetarian" pattern was associated with short-chain ACs, Schmidt et al. (4) found that meat and fish eaters had the highest concentrations of tryptophan and ACs C0, C4, and C5. At the same time, fish eaters had the highest concentrations of the BCAAs leucine and valine and the amino acids methionine and tyrosine (4, 32), all 4 of which are essential amino acids. When examining individual food groups, similar to our findings, several studies documented elevated concentrations of acetylcarnitine (C2) with meat consumption. In a short-term feeding study, acetylcarnitine concentrations were significantly elevated in 12 healthy men after a "high-meat" diet (33). In a double-blind, randomized, placebo-controlled dietary intervention trial in 160 participants, a positive association between red meat intake and urinary concentrations of *O*-acetylcarnitine was documented (34). Likewise, in a recent randomized, controlled, crossover trial, urinary concentrations of *O*-acetylcarnitine were significantly higher with increases in red meat intake (35). In a case-control study, red and processed meat intake was found to be positively associated with urinary, but not serum, concentrations of acetylcarnitines (36). In a more recent analysis, 3 short-chain ACs, including acetylcarnitine (C2), propionylcarnitine (C3), and 2-methylbutyrylcarnitine (C5), were found to be generic markers of meat and fish intake (37). Our finding of higher concentrations of the ACs C2 and C5 with the "nonvegetarian" pattern is consistent with these findings. Interestingly, C3 and C5 ACs are generated from BCAA catabolism and have been previously reported to be associated with obesity and insulin resistance. It is worth noting that red meat is a major source of L-carnitine, a trimethylamine, which could contribute to the association of particular AC species with increased red meat and fish consumption (38). In fact, acetylcarnitine has been reported to

TABLE 5 Cross-sectional associations between metabolite pattern scores and biomarkers of cardiometabolic risk among Asian Indians, aged 45–79 y, in the MASALA study[1]

	All participants ($n = 145$)				Participants without type 2 diabetes ($n = 105$)			
	Metabolite pattern 1 (medium- to long-chain AC pattern)		Metabolite pattern 2 (BCAA, AAA, and short-chain AC pattern)		Metabolite pattern 1 (medium- to long-chain AC pattern)		Metabolite pattern 2 (BCAA, AAA, and short-chain AC pattern)	
	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Laboratory measures[2]								
Fasting glucose, mg/dL	−1.44 ± 1.98	0.47	4.36 ± 2.30	0.06	−1.66 ± 0.99	0.10	3.19 ± 1.17	0.008
2-h Glucose, mg/dL	−3.61 ± 6.42	0.57	10.8 ± 7.42	0.15	0.68 ± 3.39	0.84	−0.19 ± 4.19	0.96
Fasting insulin, μU/mL	−0.69 ± 1.12	0.54	2.91 ± 1.29	0.03	−0.32 ± 0.66	0.63	0.61 ± 0.81	0.45
2-h Insulin, μU/mL	2.61 ± 9.02	0.77	22.1 ± 10.3	0.03	−2.23 ± 10.4	0.83	18.5 ± 12.5	0.14
HOMA-IR, glucose (mmol/L) × insulin (mU/L)/22.5	−0.27 ± 0.36	0.46	0.94 ± 0.42	0.03	−0.14 ± 0.18	0.44	0.18 ± 0.22	0.42
Total adiponectin, μg/mL	0.91 ± 0.40	0.03	−1.46 ± 0.47	0.002	0.87 ± 0.44	0.05	−1.16 ± 0.53	0.03
Insulin sensitivity index	0.24 ± 0.21	0.25	−1.07 ± 0.22	<0.0001	0.33 ± 0.28	0.24	−1.12 ± 0.32	0.0006
Lipids[2,3]								
Total cholesterol, mg/dL	1.05 ± 2.93	0.72	7.51 ± 3.45	0.03	0.34 ± 3.36	0.92	1.83 ± 4.08	0.66
TGs, mg/dL	−7.09 ± 5.63	0.21	14.4 ± 6.67	0.03	−8.79 ± 6.35	0.17	3.34 ± 7.79	0.67
LDL cholesterol, mg/dL	0.27 ± 2.59	0.92	4.39 ± 3.08	0.16	−0.14 ± 2.94	0.96	−0.40 ± 3.57	0.91
HDL cholesterol, mg/dL	2.00 ± 1.08	0.07	0.42 ± 1.31	0.75	1.94 ± 1.47	0.19	1.72 ± 1.79	0.34
Body composition[2,3]								
Visceral fat area, cm^2	−0.79 ± 3.59	0.83	3.02 ± 4.29	0.48	−1.61 ± 3.93	0.68	−1.81 ± 4.78	0.71
Liver-to-spleen attenuation, Hounsfield units	−0.12 ± 0.35	0.74	−0.83 ± 0.42	0.05	−0.02 ± 0.44	0.96	−0.62 ± 0.53	0.25
Subclinical atherosclerosis[2,3]								
Common carotid intima medial thickness, mm	−0.003 ± 0.014	0.84	0.026 ± 0.017	0.12	0.017 ± 0.015	0.27	0.004 ± 0.018	0.82
Internal carotid intima medial thickness, mm	0.007 ± 0.032	0.82	0.054 ± 0.037	0.15	0.035 ± 0.032	0.27	0.047 ± 0.038	0.23
Coronary artery calcium, Agatston scores <10 vs. >10	−0.152 ± 0.236	0.52	−0.421 ± 0.287	0.14	0.118 ± 0.328	0.72	−0.711 ± 0.424	0.09

[1] Values are βs ± SEs for each corresponding cardiometabolic outcome per 1-unit increase in the metabolite pattern score calculated by using linear regression. AAA, aromatic amino acid; AC, acylcarnitine; MASALA, Metabolic Syndrome and Atherosclerosis in South Asians Living in America.
[2] Models were adjusted for age (years), sex, smoking (never, past smoker, or current smoker), education (high school or less, less than Bachelor's degree, Bachelor's degree, or more than Bachelor's degree), BMI (kg/m^2), physical activity (metabolic equivalent task hours per week), total energy intake (kilocalories per day), prevalent hypertension (yes or no), medication for high cholesterol (yes or no), and statin use (yes or no).
[3] In all participants, models with lipids, body-composition measures, and measures of subclinical atherosclerosis were additionally adjusted for prevalent diabetes status (yes or no).

be a marker for prediabetes and diabetes in previous studies (39, 40).

Our finding of a positive association between vegetable consumption and a metabolite pattern characterized by BCAAs, aromatic amino acids, and short-chain ACs was unexpected. Given that previous studies have found these metabolites to be associated with higher meat consumption and because vegetables are traditionally included in some South Asian meat preparations, it is likely that the observed associations could be confounded by meat intake. However, when we additionally adjusted for red meat and poultry, the association, although attenuated, remained significant. It may also be that the major unhealthy components of the vegetarian pattern, such as rice, sugar-containing beverages, and snacks, could confound this association. It is also likely that oils and clarified butter, typically rich in saturated fats, which are added to vegetable curries during the cooking process, could be driving this association.

Consistent with our findings, several studies have suggested that BCAAs and aromatic amino acids are predictors of insulin resistance and cardiometabolic risk (41–43). In large-scale cohorts such as the Framingham Heart Study, the Framingham Heart Study Offspring Study, and the Insulin Resistance Atherosclerosis Study, higher BCAA concentrations were inversely associated with insulin sensitivity (43) and positively with insulin resistance (42, 44) and fasting glucose (44) and TG (42, 44) concentrations. However, unlike in the Framingham cohorts (42, 44), we were unable to find an association with HDL cholesterol. Only 3 other studies in South Asians have examined associations between metabolomic profiles and measures of glycemia and insulin resistance (9, 45, 46). In a cohort of nondiabetic South Asian men from the Southall and Brent Revisited (SABRE) study, BCAAs or aromatic amino acids were not correlated with fasting or 2-h glucose, fasting or 2-h insulin, and HOMA-IR. However, both BCAAs and aromatic amino acids were strongly and adversely associated with incident diabetes, even after adjusting for metabolic risk factors (46). In a small cross-sectional study in 83 Asian-Indian men in Singapore, strong associations, independent of BMI and dietary protein, were reported between BCAAs, aromatic amino acids, methionine, and insulin resistance (9). In a third study in Indians from South India, BCAAs and aromatic amino acids were elevated in participants with a high BMI and type 2 diabetes and in obese, nondiabetic participants (45).

To our knowledge, this is the first study to evaluate associations between dietary patterns, metabolomic signatures, and cardiometabolic risk in an Asian-Indian population that is a

distinctly high-risk phenotypic group. The strengths of the current study include the availability of validated dietary data, absolute quantitation of plasma metabolites, and a well-phenotyped study population. Still, our results need to be interpreted in the context of a few limitations. First, given the overall small sample size of our pilot study, the possibility of a type II error cannot be excluded. Second, given the cross-sectional nature of the study, we cannot ascertain if changes in diet before the start of the study or the presence of disease at baseline changed a participant's metabolomic profile. However, when we restricted our analyses to those without type 2 diabetes at baseline, our results remained largely consistent. Third, the use of an FFQ to measure diet may introduce some degree of measurement error. However, given that measurement errors in diet are unlikely to be related to objective measures of metabolite data, observed associations are likely to be attenuated toward the null. Fourth, our study population was limited to Asian Indians living in the United States, and it remains unclear if our findings can be generalized to other ethnic groups or South Asians living in South Asia. Still, the consistency of our findings with those in European populations and in a smaller study in south Indians in India enhances the external validity of our findings. Fifth, although we carefully adjusted for several known factors that could confound the association between diet and metabolites, reverse causation due to prevalent disease and residual confounding remain a strong possibility. Nevertheless, to address the issue of reverse causation, when we restricted our analyses to those without type 2 diabetes, the results remained largely consistent with the overall study sample. Finally, although there is some evidence from European populations that polymorphisms associated with insulin resistance may have an effect on circulating concentrations of BCAAs (47), to our knowledge, no such study exists in Asian Indians. Furthermore, it is unlikely that any such polymorphisms, which would differentially affect BCAA concentrations, would be more prevalent among those who consume a Western dietary pattern.

Taken together, our findings shed some insights into potential mechanistic pathways through which diet can influence cardiometabolic risk. Because a Western/nonvegetarian pattern was associated with a metabolomic signature known to be related to disease risk, our findings support public health recommendations to adopt a healthy eating pattern that focuses on a high variety of vegetables from all subgroups, fruit, whole grains, fat-free or low-fat dairy, a variety of plant proteins (e.g., nuts, legumes, seeds, and soy products), seafood, and lean meats and poultry, and limiting saturated fats, *trans* fats, added sugars, and sodium (48). Although Asian Indians are at a higher risk of cardiometabolic diseases than their white counterparts (17), it is important to note that nearly 90% of the study participants were immigrants and many may have acculturated and adopted a "Western"-style diet, thereby potentially compounding their risk for cardiometabolic diseases. Although the vegetarian diet pattern was not associated with an adverse metabolic profile in this study, it is worth mentioning that this pattern was not "healthy" because it loaded heavily on unhealthy foods such as rice (primarily white rice), sugar-containing beverages, and snacks, which have been shown previously to increase cardiometabolic risk (49, 50). In fact, the only healthy food group that defined this vegetarian pattern was legumes. Although we do not have data from the Indian subcontinent, the composition of the vegetarian pattern may be a reflection of the global nutrition transition in which traditional diets rich in whole grains, legumes, fruit, and vegetables are being replaced with more refined grains, sugar-sweetened beverages, and increasing intakes of meat products and salt (51). In fact, we found that among those without type 2 diabetes, vegetable consumption was positively associated with a BCAA, aromatic amino acid, and short-chain AC metabolite pattern which is likely due to unmeasured confounding due to these other unhealthy components. It is therefore imperative to examine how "vegetarianism," cooking methods of vegetables, particularly with regard to exposure to such refined fats as ghee, and the overall quality of a vegetarian diet among Asian Indians relate to cardiometabolic risk.

In conclusion, we found that a Western/nonvegetarian dietary pattern in Asian Indians was positively associated with a metabolomic signature characterized by BCAAs, aromatic amino acids, and short-chain ACs, which, in turn, were adversely associated with measures of glycemia, lipid measures, and the liver-to-spleen attenuation ratio. Public health efforts to lower cardiometabolic risk should focus on adapting a healthy plant-based diet as outlined in the 2015 Dietary Guidelines for Americans (48). Given the cross-sectional nature of our study, future prospective studies should confirm our findings and explore the association between the quality of plant-based diets, metabolomic signatures, and cardiometabolic risk. At the same time, to elucidate the diet-metabolome-disease relation, it is important to examine whether changes in diet can influence the metabolic profile and the development of cardiometabolic disease.

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