Metabolomic Determinants of Metabolic Risk in Mexican Adolescents

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Objective: The goal of this study was to identify metabolites associated with metabolic risk, separately by sex, in Mexican adolescents.

Methods: Untargeted metabolomic profiling was carried out on fasting serum of 238 youth aged 8 to 14 years, and metabolites associated with a metabolic syndrome risk z-score (MetRisk z-score) were identified separately for boys and girls, using the simulation and extrapolation algorithm. Associations of each metabolite with MetRisk z-score were examined using linear regression models that accounted for maternal education, child's age, and pubertal status.

Results: Of the 938 features identified in metabolomics analysis, 7 named compounds (of 27 identified metabolites) were associated with MetRisk z-score in girls, and 3 named compounds (of 14 identified) were associated with MetRisk z-score in boys. In girls, diacylglycerol (DG) 16:0/16:0, 1,3-dielaidin, myo-inositol, and urate corresponded with higher MetRisk z-score, whereas N-acetylglycine, thymine, and dodecenedioic acid were associated with lower MetRisk z-score. For example, each z-score increment in DG 16:0/16:0 corresponded with 0.60 (95% CI: 0.47-0.74) units higher MetRisk z-score. In boys, positive associations of DG 16:0/16:0, tyrosine, and 5′-methylthioadenosine with MetRisk z-score were found.

Conclusions: Metabolites on lipid, amino acid, and carbohydrate metabolism pathways are associated with metabolic risk in girls. Compounds on lipid and DNA pathways correspond with metabolic risk in boys.

Introduction

Metabolomics, the systematic study of low-molecular-weight compounds in biological tissues, is a powerful tool to study disease onset, severity, and progression. In adults, metabolomics analyses of serum and plasma have provided novel insights into biomarkers and the etiology of metabolic disorders, the most commonly studied being type 2 diabetes (1). Given that the pathophysiological processes underlying metabolic disease development (e.g., adiposity, insulin resistance, dyslipidemia, hypertension) begin as early as 2 to 4 years of age (2-4) and may be established for life by the end of adolescence (5,6), there is a need to elucidate the relationship between circulating metabolites and conventional metabolic biomarkers earlier in life.

Few metabolomics analyses have been carried out in youth, and the majority of published studies have sought to replicate findings from adult populations by focusing on associations of specific compounds on amino acid (7-10) and fatty acid pathways (7,10) with weight status and/or glycemia. However, although adult studies (11,12) and two metabolomics analyses in school-aged children (13,14) have found that higher circulating branched chain amino acids (BCAAs) correlated with insulin resistance and/or type 2 diabetes risk, animal models (15) and data from a clinical cohort of adolescents with and without diabetes (7,8) have revealed that these metabolites actually corresponded with better glycemia and enhanced mitochondrial function. A potential explanation for the discrepancy is that physiological responses to metabolic disturbances may be fundamentally

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different during the pubertal transition, as it is a time of rapid change and hormonal fluctuation. Therefore, approaches aimed at validating findings from adults or prepubertal children may fail to identify relevant metabolic pathways in youth undergoing puberty.

Here, our primary aim was to take a data-driven approach to identify metabolites associated with a cluster of metabolic syndrome risk factors during puberty, a life stage characterized by rapid growth and development that may also be a sensitive period for development of excess adiposity and metabolic risk (16). As a secondary aim, we examined associations of BCAAs (leucine, isoleucine, valine) with the metabolic syndrome risk factors given their relevance to metabolic risk in previous metabolomics studies in youth.

Methods

Study population

This study included participants of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) Project, a cohort of pregnant women and their offspring in Mexico City, Mexico (17). In 2010, we recontacted a subset of the offspring ($n = 250$) who were then 8 to 14 years of age based on availability of archived prenatal biospecimens to participate in follow-up studies. At research visits that took place in 2010 (the "peripubertal" visit), the participants provided an 8-hour fasting blood sample and participated in anthropometric assessment. Our study sample included 238 children with data on anthropometry, metabolic biomarkers, and adequate serum volume for metabolomics analyses. The institutional review boards of the Mexico National Institute of Public Health and the University of Michigan approved the research protocols.

Untargeted metabolomics profiling

The Michigan Regional Comprehensive Metabolomics Resource Core quantified metabolites from fasting serum collected at the peripubertal visit on an untargeted platform that utilized liquid chromatography and mass spectrometry. The procedure yielded 9,303 features. After removal of redundant compounds and those with >70% of values missing, the final data set comprised 938 unique chemical features, 332 of which were "known" compounds, e.g., those whose spectral peaks, fragmentation patterns, and retention times matched with compounds within the Michigan Regional Comprehensive Metabolomics Resource Core chemical library. While we considered all metabolites in the statistical analysis, our interpretation of results focuses on known compounds. Prior to formal analyses, we removed batch effects, imputed values below the detection using the K-nearest neighbor algorithm (K = 5) with the *IMPUTE* package in R, and standardized each metabolite as a z-score using a rank-based inverse normal transformation.

Conventional biomarkers

The primary outcome of interest was a metabolic syndrome risk z-score (MetRisk z-score) calculated as the average of five internally standardized z-scores for waist circumference, fasting glucose, fasting C-peptide (as a surrogate for fasting insulin (18)), the ratio of triglycerides to high-density lipoprotein (HDL), and the average of systolic (SBP) and diastolic blood pressure (DBP). This score is a modification (e.g., use of C-peptide rather than insulin) of a score proposed by Viitasalo et al. in a study that examined correlations among the metabolic biomarkers in children and adults, and established its association with incident type 2 diabetes and cardiovascular mortality (19).

Waist circumference. Research staff measured waist circumference at the level of the umbilicus to the nearest 0.1 cm using a non-stretchable measuring tape (QM2000; QuickMedical, Issaquah, Washington).

Fasting glucose, C-peptide, and lipids. Using 8-hour fasting blood, we measured serum glucose enzymatically. Serum C-peptide was quantified using an automated chemiluminescence immunoassay (Immulite 1000, Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania). These biomarkers provide a measure of glycemic control, as fasting glucose is an indicator of glucose metabolism and a diabetes screening tool, and C-peptide is a marker of insulin function that is secreted in quantities equal to insulin from pancreatic β-cells (18). We quantified serum triglycerides and HDL using a biochemical analyzer (Cobas Mira Plus, Roche Diagnostics, Indianapolis, Indiana).

Blood pressure. Research staff measured SBP and DBP in duplicate to the nearest mmHg in the seated position (BPTru monitors; Coquitlam, British Columbia, Canada). Because the intraclass correlation (ICC) between the measurements was high (ICC$_{SBP}$ = 0.95; ICC$_{DBP}$ = 0.89), we used the average in the analysis.

Supporting Information Table S1 shows correlations among the MetRisk z-score components.

Covariates

At enrollment, mothers reported on age, reproductive history, and lifestyle and sociodemographic characteristics. A pediatrician assessed each child to determine Tanner stage on a scale of 1 (no development) to 5 (full development) for testicles, breasts, and pubic hair.

Data analysis

Step 1: Bivariate analysis. We examined the distribution of MetRisk z-score across categories of background characteristics to identify potential confounders.

Step 2: Identification of relevant metabolites. To identify metabolites associated with metabolic risk, we examined sex-specific associations (given the differences in tempo of maturation during adolescence (20)) of individual metabolites ($n = 938$) with MetRisk z-score, while also adjusting for maternal education and pubertal status, using the simulation extrapolation (SIMEX) algorithm (*SIMEX* package in R). Our rationale for using SIMEX for the initial "first pass" to identify relevant metabolites is to correct for additive measurement error (e.g., residual batch effects, unmeasured variation in laboratory methods) in the assessment of metabolite concentrations. In the first step, the SIMEX algorithm simulates multiple datasets with varying degrees of measurement error in the metabolite of interest in order to estimate a range of coefficients that plausibly represent the error-free relationship between the metabolite and MetRisk z-score. Next, SIMEX derives an estimate for a covariate that represents the impact of the unknown source(s) of error based on the simulated data and extrapolates an error-corrected estimate for the association of interest. As a result, SIMEX estimates for regression parameters are larger in magnitude and more precise than those derived from standard regression. For confirmatory purposes, we also quantified associations of individual

metabolites with MetRisk *z*-score using standard linear regression. To reduce the likelihood of false-positive associations, we only retained metabolites for further analyses if they were associated with MetRisk *z*-score at a *P* value of < 0.05/938 (Bonferroni's correction).

Step 3: Main analysis. We used multivariable linear regression to assess the impact of potential confounders to the relationship of the known metabolites identified in step 2, as well as the BCAAs, with MetRisk *z*-score. We selected covariates based on our *a priori* knowledge and the bivariate analysis from Step 1. Because little is known regarding the metabolomics of pubertal status, a potential confounder and mediator to the relationship between metabolism and metabolic risk, we also examined crude associations of the metabolites of interest with pubertal status to ascertain its relevance as a covariate (Supporting Information Table S2). In Model 1, we accounted for confounders: maternal education and child's age. In Model 2, we further adjusted for pubertal status. Using Model 2, we also investigated associations of all metabolites of interest with components of the *z*-score.

Unless otherwise stated, all statistical analyses were performed using SAS 9.4 (Cary, North Carolina).

Results

Median age of the participants was 10.0 years (range: 8.1-14.7), and approximately half of the participants (47.5%; *n* = 113) were boys. Mean ± SD MetRisk *z*-score and the individual components of the score are displayed in Table 1.

In bivariate analysis (Table 2), older children and those at more advanced pubertal stages exhibited higher metabolic risk. As compared to participants younger than 10 years of age, those 10 to 12 years and > 12 years of age exhibited 0.20 (95% CI: 0.03-0.38) and 0.36 (95% CI: 0.16-0.55) units higher MetRisk *z*-score. In both boys and girls, we observed a positive relationship between all puberty indicators and MetRisk *z*-score.

Of the 938 features quantified in laboratory analyses, the SIMEX algorithm identified 27 compounds, 7 of which were known, in girls and 14 compounds, 3 of which were known, in boys that were associated with MetRisk *z*-score after Bonferroni's correction (Table 3). A comparison of the magnitude, direction, and significance of the estimates derived from standard linear regression versus SIMEX showed that the latter yielded larger effect sizes (~1.5-2 times the magnitude) and smaller *P* values, which is expected given that the SIMEX procedure accounts for error in assessment of the metabolite concentrations.

After identification of relevant metabolites, we investigated associations (β [95% CI]) of each known compound with MetRisk *z*-score using multivariable linear regression (Table 4). In girls, we found positive relations of diacylglycerol (DG) 16:0/16:0, 1,3-dielaidin, myo-inositol, and urate with MetRisk *z*-score and inverse associations with N-acetylglycine, thymine, and dodecenedioic acid. Adjustment for maternal education and child's age (Model 1) followed by pubertal status (Model 2) did not change the results. For example, each single *z*-score increment in DG 16:0/16:0 corresponded with 0.63 (0.49-0.76) units higher MetRisk *z*-score in the unadjusted

TABLE 1 Mean ± SD of individual metabolic biomarkers and MetRisk *z*-score in 238 ELEMENT participants

	All, *n* = 238	Girls, *n* = 125	Boys, *n* = 113
Waist circumference (cm)	70.8 ± 10.8	71.6 ± 11.5	70.0 ± 10.0
Fasting glucose (mg/dL)	87.1 ± 9.4	86.2 ± 10.6	88.1 ± 7.9
C-peptide (ng/dL)	1.7 ± 1.23	1.9 ± 1.3	1.6 ± 1.2
HDL (mg/dL)	58.7 ± 11.8	57.7 ± 11.7	59.8 ± 12.0
Triglycerides (mg/dL)	87.8 ± 44.2	96.8 ± 47.2	77.9 ± 38.4
SBP (mmHg)	103 ± 10	101 ± 10	105 ± 10
DBP (mmHg)	66 ± 7	66 ± 7	66 ± 7
MetRisk *z*-score[a]	0.00 ± 0.62	0.03 ± 0.65	−0.04 ± 0.58

[a]Calculated as the average of five internally standardized *z*-scores for waist circumference, fasting glucose, C-peptide, triglyceride:HDL ratio, and the average of SBP and DBP.

model. Accounting for mother's education and child's age did not materially alter the estimate (0.61 [0.47-0.75]), nor did further inclusion of pubertal status (0.60 [0.47-0.74]). Likewise, each single *z*-score increment in thymine was associated with 0.38 (0.22-0.54) units lower MetRisk *z*-score in Model 1 and 0.39 (0.23-0.55) units lower MetRisk *z*-score in Model 2. In boys, we detected positive associations of DG 16:0/16:0 (0.42 [0.27-0.57]), tyrosine (0.37 [0.21-0.53]), and 5′-methylthioadenosine (MTA) (0.30 [0.15-0.46]) with MetRisk *z*-score in unadjusted models. These associations persisted after multivariable adjustment (Table 4).

We also examined the relationships of each metabolite with individual components of the MetRisk *z*-score: waist circumference, fasting glucose, fasting C-peptide, triglyceride:HDL ratio, and the average of SBP and DBP (Table 5). In girls, the four compounds positively associated with MetRisk *z*-score were also positively related to each *z*-score component. We observed the most consistent associations for C-peptide. For example, DG 16:0/16:0, 1,3-dielaidin, myo-inositol, and urate corresponded with 0.4 (0.2-0.6), 0.3 (0.1-0.5), 0.4 (0.2-0.6), and 0.3 (0.0-0.5) ng/mL C-peptide, respectively. We noted similar trends of these compounds with waist circumference. Similarly, the three compounds inversely related to MetRisk *z*-score (thymine, dodecenedioic acid, and N-acetylglycine) were inversely associated with the individual biomarkers—again, with the most robust associations observed for waist circumference and C-peptide.

In boys, each single *z*-score increment in DG 16:0/16:0, tyrosine, and 5′-MTA was associated with 3.1 (1.4-4.8), 2.7 (0.9-4.4), and 3.8 (2.2-5.4) cm higher waist circumference, respectively. These metabolites were also consistently positively associated with C-peptide and, to a lesser extent, the triglyceride:HDL ratio and blood pressure.

Table 6 shows associations of BCAAs with the metabolic biomarkers. In girls, valine corresponded with higher triglyceride:HDL ratio (0.3 [0.1-0.5]). In boys, all three BCAAs were associated with higher waist circumference, fasting C-peptide, and MetRisk *z*-score (Table 6). For example, each single *z*-score increment in leucine corresponded with 3.0 (1.4-4.5) cm higher waist circumference, 0.3 (0.2-0.5) ng/mL higher C-peptide, and 0.13 (0.03-0.24) units higher

TABLE 2 Association of background characteristics with MetRisk z-score among 238 ELEMENT mother-child pairs

		MetRisk z-score	
	N	Mean ± SD	*P*[a]
Maternal characteristics at the time of enrollment			
Age at enrollment			0.62
15-24 y	87	0.59±0.02	
25-34 y	118	0.59±0.07	
35-44 y	32	0.16±0.75	
Marital status			0.23
Married or cohabiting	212	−0.02±0.62	
Single	26	0.13±0.59	
Maternal education			0.90
<10 y	83	−0.01±0.60	
10-12 y	119	0.00±0.62	
≥13 y	34	0.00±0.65	
Parity			0.40
0	85	0.02±0.58	
1-2	137	0.00±0.62	
≥3	16	−0.15±0.77	
Smoking during pregnancy			0.30
Yes	233	−0.27±0.51	
No	5	0.01±0.62	
Delivery method			0.29
C-section	96	0.05±0.63	
Vaginal	141	−0.04±0.61	
Child characteristics at the peripubertal visit			
Sex			0.39
Male	113	−0.04±0.58	
Female	125	0.03±0.65	
Child's age			0.0002
<10 y	119	−0.14±0.63	
10 to 12 y	68	0.07±0.56	
>12 y	51	0.22±0.59	
Male puberty indicators[b]			
Pubic hair			0.01
Tanner stage 1	90	−0.05±0.60	
Tanner stage 2	15	0.18±0.62	
Tanner stage 3-5	5	0.58±0.74	
Testicle development			0.04
Tanner stage 1	54	−0.15±0.60	
Tanner stage 2	40	0.18±0.64	
Tanner stage 3	10	−0.10±0.47	
Tanner stage 4-5	5	0.36±0.91	
Female puberty indicators[c]			
Pubic hair			0.004
Tanner stage 1	94	−0.09±0.63	
Tanner stage 2	21	0.11±0.45	
Tanner stage 3	7	0.42±0.25	
Tanner stage 4-5	3	0.48±0.48	

TABLE 2. (continued).

		MetRisk z-score	
	N	Mean ± SD	*P*[a]
Breast development			0.002
Tanner stage 1	83	−0.12±0.63	
Tanner stage 2	20	0.13±0.54	
Tanner stage 3	15	0.23±0.43	
Tanner stage 4-5	7	0.39±0.22	

[a]Represents a test for linear trend in which an ordinal indicator is entered into the model as continuous variable, with the exception of binary variables (Wald test).
[b]Tanner stages combined due to small sample sizes for pubic hair stages 3-5 (stage 3: *n* = 4; stage 4: *n* = 1; stage 5: *n* = 0) and testicle development stages 4-5 (stage 4: *n* = 5; stage 5: *n* = 0).
[c]Tanner stages combined due to small sample sizes for pubic hair (stage 4: *n* = 2; stage 5: *n* = 1) and breast development (stage 4: *n* = 7; stage 5: *n* = 0).

MetRisk z-score. We observed associations of similar direction and magnitude for isoleucine and valine.

Discussion

In this study of 238 Mexican youth 8 to 14 years of age, we employed a data-driven approach to identify metabolites associated with metabolic syndrome risk factors. Use of untargeted metabolomic profiling followed by measurement-error corrected regression models revealed associations of metabolites on lipid, amino acid, and carbohydrate metabolism pathways with metabolic risk in girls, whereas compounds on lipid and DNA pathways were related to metabolic risk in boys. As a secondary end point, we examined associations of BCAAs (leucine, isoleucine, valine), which have been previously implicated in metabolic risk in youth (7-9,13,14), with the metabolic outcomes and found positive associations with metabolic risk in boys.

Data-driven approach: girls
In girls, we identified seven named metabolites that were associated with MetRisk z-score. Of these compounds, four were positively related to metabolic risk: DG 16:0/16:0, 1,3-dielaidin, myo-inositol, and urate; and three were inversely associated with metabolic risk: thymine, dodecenedioic acid, and N-acetylglycine.

Metabolites associated with higher MetRisk z-score
We found that higher serum levels of DG 16:0/16:0, a glyceride composed of two palmitic acid side chains, corresponded with higher metabolic risk, which makes sense given that DG 16:0/16:0 is a major component of palm oil, a saturated vegetable oil that is commonly used in developing nations like Mexico and that adversely impacts plasma lipid profile (21). Indeed, when we examined associations with components of MetRisk z-score, DG 16:0/16:0 was strongly associated with a larger triglyceride:HDL ratio, which has been identified as a discriminatory biomarker of metabolic syndrome in children (22). Another pathway through which

TABLE 3 Association of metabolite concentrations with MetRisk z-score among 125 girls and 113 boys using standard linear regression and SIMEX

	Linear regression		SIMEX	
	β[a]	*P* value	β[a]	*P* value
Girls (*n* = 125)				
Diacylglycerol (DG) 16:0/16:0	**0.34**	**1.09E-10**	**0.61**	**1.26E-14**
C35 H63 Cl N4 O2	0.33	2.24E-10	0.59	4.39E-14
C33 H67 N4 O4 P	0.31	4.73E-09	0.55	2.46E-12
C35 H76 N2 P2 S	0.30	1.58E-09	0.55	2.65E-12
C35 H73 N4 O4 P	0.29	1.10E-08	0.53	6.34E-12
C33 H69 N4 O4 P	0.30	1.69E-08	0.53	1.63E-11
C44 H68 S	0.29	7.75E-08	0.53	7.03E-11
1,3-Dielaidin	**0.27**	**3.55E-07**	**0.49**	**8.34E-10**
C42 H79 P S3	0.26	1.66E-06	0.47	6.67E-09
C36 H65 N8 P	0.27	2.71E-06	0.50	9.41E-09
N-Acetylglycine	**−0.28**	**4.88E-06**	**−0.51**	**1.99E-08**
Myo-inositol	**0.24**	**1.96E-05**	**0.42**	**4.16E-07**
C46 H74 S2	0.24	5.10E-05	0.44	6.25E-07
Urate	**0.24**	**6.07E-05**	**0.42**	**2.11E-06**
C37 H69 N4 O4 P	0.23	9.07E-05	0.41	2.69E-06
PA 38:7	0.23	5.86E-05	0.40	3.39E-06
C40 H69 Cl N4 O	0.22	1.12E-04	0.37	6.62E-06
C44 H66 N4 O	0.22	2.32E-04	0.39	7.24E-06
885.586@25.532227	−0.22	2.21E-04	−0.40	7.63E-06
C37 H62 N6 O3	0.21	2.66E-04	0.39	8.78E-06
Thymine	**−0.21**	**3.08E-04**	**−0.38**	**1.04E-05**
765.394@26.4027	0.23	1.43E-04	0.39	1.07E-05
925.6017@25.308508	0.23	1.28E-04	0.36	1.36E-05
781.3842@26.705156	0.22	1.73E-04	0.37	1.44E-05
783.5786@24.554457	0.21	3.75E-04	0.37	1.96E-05
C37 H71 N2 O4 P	0.22	1.44E-04	0.35	2.17E-05
Dodecenedioic acid	**−0.18**	**1.18E-04**	**−0.34**	**2.90E-05**
Boys (*n* = 113)				
Diacylglycerol (DG) 16:0/16:0	**0.23**	**8.72E-06**	**0.43**	**5.18E-08**
993.5887@25.33285	0.22	3.83E-06	0.38	1.06E-07
C35 H63 Cl N4 O2	0.22	2.52E-05	0.41	3.37E-07
C33 H69 N4 O4 P	0.20	1.65E-04	0.39	2.30E-06
869.5421@24.07403	0.18	1.09E-04	0.33	3.45E-06
923.6981@28.415735	−0.22	7.20E-06	−0.34	6.15E-06
765.394@26.4027	0.19	1.98E-04	0.34	9.11E-06
5′-Methylthioadenosine (MTA)	**0.18**	**2.59E-04**	**0.35**	**1.12E-05**
885.586@25.532227	−0.20	7.66E-05	−0.36	1.47E-05
Tyrosine	**0.18**	**5.29E-04**	**0.35**	**2.54E-05**
895.6661@26.81271	−0.21	2.50E-05	−0.32	2.75E-05
991.6704@28.157875	−0.20	2.02E-04	−0.36	2.92E-05
C35 H73 N4 O4 P	0.19	9.81E-04	0.36	2.94E-05
C35 H76 N2 P2 S	0.19	8.41E-04	0.36	3.30E-05

Bolded estimates indicate statistical significance after Bonferroni's correction ($P < 5.33E-05$).
[a]Estimates are adjusted for mother's education level and child's age.

DG 16:0/16:0 may influence metabolic risk is by inducing insulin resistance. In a study that compared muscle tissue DG composition of $n = 6$ adults without diabetes or obesity, $n = 6$ adults with type 2 diabetes, and $n = 6$ endurance-trained athletes, Bergman et al. reported higher levels of three DG species, one of which was DG 16:0/16:0, in the myocyte membranes of patients with diabetes than for the other two groups (23). Further, the authors observed an increasing monotonic relationship between the DGs and insulin resistance in all three groups (23). a phenomenon that could be explained by the capacity of DGs to activate protein kinase-C, an enzyme implicated in the pathogenesis of skeletal muscle insulin resistance (24). Accordingly, this compound was associated with higher fasting C-peptide, a surrogate marker of insulin (18), in girls.

We also found positive associations of 1,3-dielaidin, myo-inositol, and urate with MetRisk z-score. Although there are no published studies relating 1,3-dielaidin, a glycerol with two monounsaturated fatty acid chains, to metabolic health, a few mechanisms could underlie our findings for the other two compounds. Myo-inositol plays an important role as a precursor for secondary cellular messengers such as inositol phosphates phosphatidylinositol and phosphatidylinositol phosphate-containing lipids (25). While myo-inositol occurs naturally in fruits, grains, beans, and nuts, it is also generated endogenously from D-glucose to augment cellular glucose uptake (26), a process that may be upregulated under conditions of hyperglycemia and/or insulin resistance (27) to encourage glucose utilization. This notion is supported by our finding that myo-inositol corresponded with higher fasting glucose and C-peptide.

We observed a positive association between urate and MetRisk z-score, which aligns with literature pointing toward elevated serum uric acid as a risk factor for cardiometabolic disease. Proposed mechanisms include uric acid's ability to induce oxidative stress (28) and stimulate proinflammatory pathways (29), both of which interfere with metabolic homeostasis.

Metabolites associated with lower MetRisk z-score

In girls, we identified three metabolites that were inversely associated with metabolic risk: N-acetylglycine, dodecenedioic acid, and thymine. A few studies have identified N-acetylglycine as a correlate of weight status. In an untargeted metabolomics study of adults in three cohorts, Moore et al. found an inverse relationship between N-acetylglycine and BMI under both fasting and nonfasting conditions (30). Similarly, in an untargeted analysis of fasting plasma from 803 Hispanic children, Butte et al. reported higher plasma N-acetylglycine in children without obesity compared to children with obesity (14). While the exact mechanism linking this compound to excess adiposity remains to be elucidated, a study in 73 elderly adults found higher levels of N-acetylglycine in the gut microbiome following ingestion of high-fiber foods (31). Given that dietary fiber intake is consistently associated with lower adiposity and metabolic risk in epidemiologic studies (32), mechanistic investigations are warranted to determine whether N-acetylglycine is simply a marker of fiber metabolism or whether it imparts a direct protective effect against metabolic risk.

The inverse relationship between dodecenedioic acid and metabolic risk could transpire from its antioxidant properties. A recent *in vitro* study of human skin fibroblasts showed that administration of

TABLE 4 Sex-specific associations of individual metabolites with MetRisk z-score among 238 ELEMENT adolescents

	β (95% CI) MetRisk z-score per 1 unit increment in metabolite z-score		
	Unadjusted	**Model 1**	**Model 2**
Girls (n = 125)			
Diacylglycerol (DG) 16:0/16:0	**0.63 (0.49 to 0.76)**	**0.61 (0.47 to 0.75)**	**0.60 (0.47 to 0.74)**
1,3-Dielaidin	**0.51 (0.36 to 0.66)**	**0.49 (0.35 to 0.64)**	**0.49 (0.35 to 0.63)**
Myo-inositol	**0.44 (0.28 to 0.59)**	**0.43 (0.27 to 0.58)**	**0.43 (0.27 to 0.58)**
Urate	**0.44 (0.27 to 0.61)**	**0.42 (0.25 to 0.58)**	**0.40 (0.23 to 0.56)**
Thymine	**−0.33 (−0.49 to −0.17)**	**−0.38 (−0.54 to −0.22)**	**−0.39 (−0.55 to −0.23)**
Dodecenedioic acid	**−0.33 (−0.47 to −0.17)**	**−0.34 (−0.49 to −0.19)**	**−0.35 (−0.50 to −0.20)**
N-Acetylglycine	**−0.42 (−0.58 to −0.25)**	**−0.51 (−0.68 to −0.35)**	**−0.53 (−0.69 to −0.36)**
Boys (n = 113)			
Diacylglycerol (DG) 16:0/16:0	**0.42 (0.27 to 0.57)**	**0.43 (0.29 to 0.58)**	**0.42 (0.28 to 0.56)**
Tyrosine	**0.37 (0.21 to 0.53)**	**0.35 (0.19 to 0.50)**	**0.33 (0.17 to 0.48)**
5′-Methylthioadenosine (MTA)	**0.30 (0.15 to 0.46)**	**0.35 (0.20 to 0.49)**	**0.32 (0.18 to 0.46)**

Model 1: Adjusted for maternal education level and child's age.
Model 2: Model 1 + pubertal status.
Bolded estimates indicate statistical significance at $P < 0.05$.

dodecenedioic acid decreased phospholipid peroxidation (33), a process that is upregulated under conditions of hyperglycemia.

Finally, our finding that higher serum concentrations of thymine corresponded with lower metabolic risk could stem from the physiological effects of its catabolite β-aminoisobutyrate, a compound released by skeletal muscle that enhances glucose tolerance (34).

Data-driven approach: boys

As in girls, DG 16:0/16:0 was positively associated with MetRisk z-score in boys, emphasizing the relevance of this compound to metabolic health regardless of sex. In addition, we found that higher serum levels of tyrosine and 5′-MTA corresponded with greater metabolic risk among males.

Our finding of a positive association between tyrosine, a large neutral amino acid, and MetRisk z-score, as well as with adiposity (larger waist circumference) and worse glycemia (higher C-peptide), corroborates literature in both adults and children. Because tyrosine relies on the same protein (LAT1) as BCAAs for membrane transport, circulating tyrosine concentrations directly correlate with BCAAs. Tyrosine is often captured as part of the BCAA "metabolic signature" that is associated with obesity and insulin resistance in cross-sectional studies of adults (11) and children (13,14) and with incident type 2 diabetes among young adults in the Framingham cohort (12). Of particular relevance to our findings are two recent investigations that have identified tyrosine as a discriminatory marker of metabolic risk. In an analysis of 96 healthy Slovenian adults 25 to 49 years of age, Mohorko et al. (35) quantified 10 amino acids in fasting serum and categorized participants as having zero, one, or two components of metabolic syndrome in order to determine whether certain amino acids had greater capacity than others to discriminate between the three levels of subclinical disease severity. The investigators found that tyrosine was higher among persons who met criteria for one versus none of the components of

metabolic syndrome, suggesting that it may serve as an early indicator of metabolic syndrome risk. Similarly, in a recent analysis of 80 European children with obesity, Hellmuth et al. (36) examined changes in glycemia and circulating levels of 163 metabolites related to amino acid metabolism before versus after a 1-year weight loss intervention and identified tyrosine as the only compound that correlated with insulin resistance at baseline, after the intervention, and with change in insulin resistance during the course of the study (36).

We also found a direct association between 5′-MTA with metabolic risk. While there have not been any studies specifically reporting on 5′-MTA with respect to metabolic health, 5′-MTA is a by-product of polyamine synthesis in DNA turnover cycles that increases with inflammation to modulate cellular stress (37). Considering that metabolic diseases are characterized by persistent chronic inflammation, it is plausible that the relationship between 5′-MTA and the MetRisk z-score is driven by proinflammatory pathways.

BCAAs and metabolic risk

In addition to our data-driven approach, we also examined associations of BCAAs with the metabolic syndrome risk factors based on their relevance to metabolic health in previous studies of children and adolescents (7-9,13,14). While the results were generally null for girls (with the exception of a positive relationship between valine and triglyceride:HDL), all three BCAAs corresponded with higher waist circumference, fasting C-peptide, and the MetRisk z-score in boys. These results corroborate the existing literature in youth regarding the relationship of BCAAs with excess adiposity and worse glycemia (9,13,14), but they warrant additional investigations in other populations to confirm the sex-specific nature of our findings.

Strengths and weaknesses

Strengths of this study include our data-driven approach, research-quality measures of body composition and metabolic biomarkers,

TABLE 5 Sex-specific associations of metabolites with MetRisk z-score components among 238 ELEMENT adolescents

	Mean ± SD metabolite z-score[b]	β (95% CI) for each biomarker per 1 unit increment in metabolite z-score[a]				
		Waist circumference, cm	Fasting glucose, mg/dL	Fasting C-peptide, ng/mL	Triglyceride: HDL ratio	(SBP + DBP)/2, mmHg
Girls (n = 125)						
Diacylglycerol (DG) 16:0/16:0	0.07 ± 1.03	**3.5 (1.8 to 5.1)**	1.2 (−0.6 to 2.9)	**0.4 (0.2 to 0.6)**	**0.7 (0.6 to 0.9)**	**1.3 (0.0 to 2.5)**
1,3 to Dielaidin	0.14 ± 1.03	**2.6 (1.0 to 4.4)**	1.7 (−0.1 to 3.5)	**0.3 (0.1 to 0.5)**	**0.6 (0.5 to 0.9)**	0.6 (−0.7 to 1.9)
Myo to inositol	−0.12 ± 1.01	0.6 (−1.1 to 2.4)	**6.3 (4.8 to 7.7)**	**0.4 (0.2 to 0.6)**	0.1 (−0.1 to 0.3)	0.7 (−0.6 to 2.0)
Urate	−0.05 ± 0.96	**3.8 (2.1 to 5.6)**	0.3 (−1.6 to 2.2)	0.3 (0.0 to 0.5)	**0.2 (0.0 to 0.4)**	**2.5 (1.2 to 3.8)**
Thymine	0.09 ± 0.98	**−2.3 (−4.1 to −0.5)**	**−2.7 (−4.4 to 0.8)**	−0.2 (−0.5 to 0.0)	−0.1 (−0.3 to 0.1)	**−2.6 (−3.9 to −1.3)**
Dodecenedioic acid	−0.09 ± 1.06	**−3.1 (−4.7 to −1.5)**	−1.5 (−3.3 to 0.2)	−0.2 (−0.4 to 0.0)	**−0.3 (−0.4 to −0.1)**	−0.3 (−1.6 to 1.0)
N-Acetylglycine	0.08 ± 0.94	**−3.6 (−5.5 to −1.8)**	**−2.5 (−4.5 to −0.6)**	**−0.5 (−0.7 to −0.3)**	−0.2 (−0.4 to 0.0)	**−1.9 (−3.4 to −0.5)**
Boys (n = 113)						
Diacylglycerol (DG) 16:0/16:0	−0.07 ± 0.96	**3.1 (1.4 to 4.8)**	−0.7 (−2.2 to 0.8)	**0.3 (0.1 to 0.5)**	**0.5 (0.4 to 0.6)**	**1.5 (0.1 to 2.9)**
Tyrosine	0.09 ± 1.03	**2.7 (0.9 to 4.4)**	0.8 (−0.8 to 2.3)	**0.4 (0.2 to 0.6)**	0.1 (−0.1 to 0.2)	0.7 (−0.8 to 2.2)
5'-Methylthioadenosine (MTA)	0.00 ± 1.00	**3.8 (2.2 to 5.4)**	−0.4 (−1.8 to 1.1)	**0.3 (0.2 to 0.5)**	**0.2 (0.0 to 0.3)**	**2.4 (1.0 to 3.7)**

Bolded estimates indicate statistical significance at P < 0.05.
[a]Adjusted for maternal education and child's sex and pubertal status.

TABLE 6 Sex-specific associations of branched chain amino acids (BCAA) with MetRisk z-score and its components among 238 ELEMENT adolescents

	Mean ± SD metabolite z-score[b]	β (95% CI) for each biomarker per 1 unit increment in metabolite z-score[a]					
		Waist circumference, cm	Fasting glucose, mg/dL	Fasting C-peptide, ng/mL	Triglyceride: HDL ratio	(SBP + DBP)/2, mmHg	MetRisk z-score
Girls (n = 125)							
Leucine	0.04 ± 0.91	0.0 (−2.1 to 2.2)	−1.1 (−3.2 to 1.0)	0.0 (−0.2 to 0.2)	0.1 (−0.1 to 0.3)	−0.1 (−1.7 to 1.4)	−0.01 (−0.13 to 0.11)
Isoleucine	−0.02 ± 0.93	0.9 (−1.2 to 3.0)	0.0 (−2.0 to 2.1)	0.1 (−0.2 to 0.3)	0.2 (0.0 to 0.4)	−0.3 (−1.8 to 1.2)	0.05 (−0.06 to 0.16)
Valine	0.06 ± 0.93	1.3 (−0.7 to 3.4)	−0.5 (−2.5 to 1.6)	0.1 (−0.1 to 0.4)	**0.3 (0.1 to 0.5)**	−0.1 (−1.6 to 1.4)	0.09 (−0.03 to 0.20)
Boys (n = 113)							
Leucine	−0.01 ± 1.09	**3.0 (1.4 to 4.5)**	−0.8 (−2.2 to 0.6)	**0.3 (0.2 to 0.5)**	0.0 (−0.1 to 0.2)	1.2 (−0.1 to 2.6)	**0.13 (0.03 to 0.24)**
Isoleucine	0.04 ± 1.08	**3.1 (1.5 to 4.8)**	−1.3 (−2.7 to 0.2)	**0.2 (0.0 to 0.4)**	0.0 (−0.1 to 0.2)	0.8 (−0.6 to 2.2)	0.10 (−0.01 to 0.21)
Valine	−0.07 ± 1.07	**2.4 (0.7 to 4.0)**	−0.8 (−2.2 to 0.7)	**0.3 (0.1 to 0.5)**	0.1 (−0.1 to 0.2)	1.4 (−0.1 to 1.9)	**0.14 (0.03 to 0.24)**

Bolded estimates indicate statistical significance at P < 0.05.
[a]Adjusted for maternal education and child's sex and pubertal status.

data on important covariates including physician-assessed pubertal status, and use of the SIMEX algorithm to correct for unmeasured error in assessment of metabolite concentrations.

Our study has some limitations. First, the sample size was relatively small, although it is comparable to other untargeted metabolomics studies carried out in children and adolescents (13,14). Second, the cross-sectional design impedes our ability to ascertain temporality. Third, assessment of the metabolites took place at a single time point; thus, we are not able to make inferences on metabolic flux. Finally, we cannot rule out the possibility of unmeasured confounding.

Conclusion

In this study of 238 Mexican youth 8 to 14 years of age, metabolites on lipid, amino acid, and carbohydrate metabolism pathways corresponded with metabolic risk in girls, and compounds on lipid, DNA, and BCAA pathways corresponded with greater metabolic risk in boys. The sex-specific nature of the associations between metabolites and metabolic risk have been observed in prepubertal children with obesity (38) and adolescents with obesity (39) in relation to insulin resistance and with respect to metabolic syndrome in young adults (40), emphasizing the importance of considering sex differences in studies of metabolism. While our findings shed light on potential mechanisms underlying metabolic risk during adolescence, future studies are necessary to investigate associations of these compounds with prospective change in conventional metabolic biomarkers.O

Acknowledgments

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