Original Article
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Obesity

Branched Chain Amino Acids, Androgen Hormones, and Metabolic Risk Across Early Adolescence: A Prospective Study in Project Viva

Wei Perng [1,2], *Sheryl L. Rifas-Shiman*[3], *Marie-France Hivert*[3], *Jorge E. Chavarro*[4,5], *and Emily Oken*[3,4]

Objective: This study aimed to examine the associations of two obesity-related metabolite patterns with changes in metabolic biomarkers during early adolescence.

Methods: Multivariable linear regression was used to examine associations of branched chain amino acid (BCAA) and androgen hormone patterns with changes in glycemia (fasting glucose, insulin, homeostatic model assessment of insulin resistance), adipokines (leptin, adiponectin), inflammation (C-reactive protein, interleukin-6), lipid profile, and blood pressure during ~5 years of follow-up among 213 children aged 6 to 10 years at baseline. Covariates included baseline age, pubertal status, biomarker level, and BMI percentile, and age at follow-up. Interactions with sex and baseline BMI percentile were also considered.

Results: The median age at baseline was 7.7 years; 48.8% were boys. In adjusted models, each 1 unit of the BCAA pattern corresponded with a 4.82 (95% CI: 0.92 to 8.71) mg/dL decrease in fasting glucose in boys. In girls, the BCAA pattern was associated with an increase in triglycerides (4.17 [0.03 to 8.32] mg/dL). The androgen pattern was associated with decreased leptin (−2.35 [−4.34 to −0.35] ng/dL) and increased C-reactive protein (0.28 [0.03 to 0.54] mg/dL) in girls. These relationships did not differ by baseline BMI percentile.

Conclusions: The BCAA and androgen hormone metabolite patterns are related to changes in metabolic parameters in a sex-specific manner during early adolescence.

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Introduction

In the past two decades, the prevalence of type 2 diabetes has increased by almost a third among children and adolescents in the United States (1). These trends are alarming, as youth with chronic conditions present treatment challenges and will enter adulthood with several years of disease duration and a greater risk for early complications (2). Despite population-based efforts to reduce the risk for metabolic disease via obesity prevention and lifestyle modifications, the need to pair this strategy with targeted approaches for high-risk individuals and subgroups was recently acknowledged by the Institute of Medicine (3).

Profiling of circulating metabolites ("metabolomics") shows promise as one route to identifying specific targets for primary prevention. Studies in adults have unveiled distinct differences in plasma metabolite composition of persons who have obesity versus those who are lean (4), some of which precede the development of insulin resistance and type 2 diabetes by more than a decade, independently of weight status (5). These findings suggest that metabolite patterns have a higher discriminative capacity than weight or traditional biomarkers to identify persons at risk for type 2 diabetes earlier on the disease continuum, and that some metabolite patterns may signal risk even among individuals who do not have overweight or obesity.

Less is known of these relationships earlier in the life course. Despite a handful of recent analyses exploring cross-sectional associations of circulating metabolites with conventional biomarkers of glycemia in children and adolescents (6-10), only three studies interrogated this relationship prospectively; one study followed 17

[1] Department of Nutritional Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA. Correspondence: Wei Perng (wei.perng@gmail.com) [2] Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA [3] Division of Chronic Disease Research Across the Lifecourse, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, Massachusetts, USA [4] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA [5] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA.

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adolescents in Boston over the course of 18 months (9), another was a 2-year study of 102 Korean boys (11), and a third followed 16 nondiabetic adolescents from an obesity clinic for 2.3 years (12). The scant literature in youth is problematic given the importance of understanding the determinants and etiology of worsening metabolic health during early life for effective prevention.

Here, we used data from a cohort of children aged 6 to 10 years at baseline to attain a better understanding of the biological pathways underlying worsening metabolic health during adolescence. We investigated associations of two previously characterized metabolite patterns related to metabolic risk during midchildhood (a branched chain amino acid [BCAA] and an androgen steroid hormone metabolite pattern) (8) with change in several metabolic biomarkers during 5 years of follow-up. We also aimed to identify and characterize differences in the relationships of interest by baseline weight and pubertal status.

Methods

Study population

This study includes participants of Project Viva, a prebirth cohort recruited from a multispecialty group practice in Massachusetts (Atrius Harvard Vanguard Medical Associates). Details on study design and recruitment are reported elsewhere (13). Children in this analysis are a subset of participants in a pilot study that characterized serum metabolites associated with obesity and metabolic risk during midchildhood (age 6-10 years) (8). Of the 262 participants in the pilot study, we considered 253 who attended the early teen research visit (age 11-15 years) and provided fasting blood. The final analytic sample included 213 children with data on change in any of the metabolic biomarkers of interest. The Institutional Review Board of Harvard Pilgrim Health Care approved all study protocols. All mothers provided written informed consent and children provided verbal assent.

Blood collection

At the midchildhood (baseline) and early teen (follow-up) research visits, trained research assistants collected an 8-hour fasting blood sample from the antecubital vein. All samples were refrigerated immediately, processed within 24 hours, and stored at −80°C until time of analysis.

Exposure: plasma metabolites

We carried out untargeted metabolomic profiling in fasting plasma collected during midchildhood via a multiplatform mass-spectroscopy-based technique (14-16). Details regarding sample preparation and analysis for this population have been published (14-16) and are in the Supporting Information.

For statistical analysis, we examined metabolites in the form of a principal component analysis factor score, henceforth referred to as the BCAA and androgen pattern, both of which were cross-sectionally associated with excess adiposity and metabolic risk in this population during midchildhood (8). We also examined key metabolites within each metabolite pattern (i.e., those with a factor loading $> |0.5|$) as a z score, centered at the median, and scaled to 1 mean absolute deviation (8).

Outcome: change in metabolic biomarkers during follow-up

At both the midchildhood and early teen visits, we used fasting blood to measure plasma glucose, insulin, leptin, adiponectin, C-reactive protein (CRP), and interleukin-6 (IL-6), serum total cholesterol, triglycerides, and high-density lipoprotein (HDL). We calculated low-density lipoprotein (LDL) by using the following equation: LDL = total cholesterol − HDL − (triglycerides/5), and we estimated insulin resistance by using the homeostatic model assessment of insulin resistance (HOMA-IR) (HOMA-IR = [glucose mg/dL × insulin μIU/mL]/405). Details on assays for laboratory analyses are in the Supporting Information. We measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) by using biannually calibrated automated oscillometric monitors (Dinamap Pro100, GE Critikon, Tampa, Florida). Research assistants recorded the blood pressure on the child's upper arm up to five times at 1-minute intervals. We used the average of the five measurements for the statistical analysis.

In addition to examining individual biomarkers, we derived a metabolic syndrome z score that calculated the mean of five age- and sex-specific internal z scores for waist circumference, inverted HDL, natural log-transformed triglycerides (because of nonnormal distribution of the original variable), natural log-transformed HOMA-IR, and SBP. This score is a modified version proposed by Viitasalo et al. (17). Specifically, we used HOMA-IR in lieu of fasting glucose and insulin, as this index has been shown to be a better assessment of glycemic homeostasis in children than glucose or insulin alone (18-20); we included HDL and triglycerides individually rather than as a ratio given evidence of limited utility of this ratio in children (21), and we used SBP instead of the average of SBP and DBP because SBP is more reliably measured in children and is a stronger predictor of future health (22).

In the analysis, we focused on change in each biomarker and metabolic syndrome z score between the two research visits.

Covariates

Assessment of covariates, including perinatal and sociodemographic characteristics as well as child anthropometry and pubertal status are in the Supporting Information.

Data analysis

Step 1. We examined bivariate associations of the BCAA and androgen metabolite pattern factor scores (see Perng et al. (8) for details on factor creation) with background and sociodemographic characteristics. This step, in conjunction with our knowledge of determinants of metabolic health, informed covariate selection.

Step 2. We investigated relations of the two metabolite patterns separately, with changes in each of the biomarkers, by using multivariable linear regression models that accounted for the child's age, pubertal status, the biomarker of interest at baseline, and age at follow-up (Model 1). We also further accounted for BMI percentile at baseline (Model 2) because weight status is a determinant of future metabolic risk (23). Because tests for interactions indicated effect modification by sex ($P < 0.05$), we ran all models separately for boys and girls. We used complete case analysis, which resulted in a decreasing sample size with the addition of covariates. To assess for potential bias because of missing data, we examined

TABLE 1 Descriptive statistics (mean ± SD) for BCAA factor score, age, and metabolic biomarkers of 104 boys and 109 girls in the Project Viva cohort during midchildhood (baseline) and early teens (follow-up)

	Boys ($n = 104$)				Girls ($n = 109$)			
	n	Baseline	Follow-up	*P*[a]	*n*	Baseline	Follow-up	*P*[a]
Factor 4 (BCAA pattern)		−0.02 ± 1.51				0.02 ± 1.51		
Factor 9 (androgen pattern)		−0.02 ± 1.56				−0.02 ± 1.56		
Age (y)	104	7.9 ± 0.8	13.0 ± 0.7	0.0007	109	7.9 ± 0.8	13.1 ± 0.7	
BMI (kg/m^2)	104	17.8 ± 3.3	21.3 ± 5.0	<0.0001	109	19.2 ± 4.2	23.5 ± 5.7	<0.0001
BMI percentile[b]	104	66.7 ± 27.3	63.2 ± 30.7	0.04	109	71.0 ± 31.0	72.3 ± 28.7	0.37
Insulin (μU/mL)	78	8.1 ± 5.8	17.4 ± 14.8	<0.0001	85	11.2 ± 7.6	18.7 ± 11.6	<0.0001
Glucose (mg/dL)	66	102.0 ± 17.8	96.2 ± 27.5	0.08	79	96.0 ± 14.4	93.7 ± 14.5	0.33
HOMA-IR	63	2.1 ± 2.0	3.9 ± 3.7	0.0007	77	2.5 ± 1.6	4.3 ± 3.1	<0.0001
Leptin (ng/mL)	81	5.9 ± 7.4	9.9 ± 14.6	0.001	86	10.2 ± 10.9	22.3 ± 18.7	<0.0001
Adiponectin (ng/mL)	81	14.7 ± 8.3	6.0 ± 2.6	<0.0001	86	15.5 ± 10.5	6.3 ± 2.8	<0.0001
CRP (mg/L)	82	0.97 ± 2.90	0.93 ± 1.97	0.88	83	1.73 ± 4.98	1.19 ± 2.08	0.28
IL-6 (pg/mL)	80	0.92 ± 1.10	1.13 ± 1.45	0.25	86	1.15 ± 1.55	1.44 ± 2.03	0.24
Total cholesterol (mg/dL)	83	163.5 ± 26.9	155.3 ± 29.4	0.005	87	164.6 ± 22.5	157.2 ± 27.7	0.003
HDL (mg/dL)	83	59.9 ± 22.7	55.4 ± 15.3	0.0006	87	55.5 ± 12.3	55.1 ± 13.4	0.74
LDL (mg/dL)	83	92.3 ± 25.0	86.2 ± 24.5	0.02	87	97.0 ± 20.7	89.2 ± 22.6	0.0001
Triglycerides (mg/dL)	83	56.9 ± 22.7	68.7 ± 31.2	0.0008	87	60.3 ± 25.5	64.2 ± 29.5	0.26
SBP (mmHg)	103	96.0 ± 9.0	110.0 ± 8.9	<0.0001	108	95.8 ± 9.0	105.8 ± 9.4	<0.0001
DBP (mmHg)	103	54.9 ± 5.3	61.3 ± 7.4	<0.0001	108	54.3 ± 5.4	62.7 ± 6.7	<0.0001
MetS *z* score[c]	62	0.17 ± 0.61	0.21 ± 0.72	0.57	76	0.17 ± 0.68	0.05 ± 0.63	0.12

[a]From paired *t* test.
[b]According to Centers for Disease Control and Prevention growth reference for children 2 to 19 years of age.
[c]Calculated as average of five internally standardized sex-specific *z* scores for inverted HDL, waist circumference, natural log-transformed HOMA-IR, natural log-transformed triglycerides, and SBP.
HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MetS *z* score, metabolic syndrome *z* score; CRP, C-reactive protein; IL-6, interleukin-6.

background characteristics across each of the subsamples (Supporting Information Table S1).

Step 3. To gain insight into biochemical pathways underlying the associations of the BCAA and androgen patterns with the conventional biomarkers, we further investigated relations of individual metabolites with a factor loading $> |0.50|$ in each of the patterns and with the changes in biomarkers that were predicted by this pattern in Step 2. In other words, if we detected a significant association ($P < 0.05$) between a metabolite pattern and change in a biomarker, we further explored the associations of individual metabolites within the metabolite pattern with this biomarker.

In all models, we tested for an interaction between the metabolite pattern and baseline BMI percentile (continuous) and pubertal status (ordinal summary score). We observed evidence of interactions between the BCAA pattern and pubertal status for one of the biomarkers in boys, so we evaluated puberty-stratified associations for this biomarker (prepubertal vs. pubertal). We also carried out sensitivity analyses to assess the impact of further adjustment for covariates that were associated with the metabolite patterns in bivariate analysis.

Because the relationships of interest involve correlated exposures (metabolites on related pathways) and outcomes that cluster and track over time (metabolic biomarkers), we did not account for multiple comparisons. Instead, we focus on the magnitude and direction of associations rather than statistical significance when interpreting results. Additionally, although the tempo of maturation during follow-up could impact findings, we controlled only for pubertal status at baseline and interpreted the estimates as the total effect of the metabolite patterns on metabolic risk during follow-up given that change in pubertal status could be on the causal pathway, and, thus, adjusting for it could introduce bias (24).

All models met standard assumptions for multivariable linear regression (linearity of exposure/outcome relationship, homoscedasticity of error, normality of residuals). All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

The median age of the participants at the baseline midchildhood visit was 7.7 years (range: 6.7-10.6); 48.8% ($n = 104$) were boys. Descriptive statistics on the factor scores, age, BMI, and the metabolic biomarkers at the midchildhood and early teen visits are presented in Table 1.

Table 2 shows bivariate associations of the BCAA and androgen factor scores with perinatal and sociodemographic characteristics.

TABLE 2 Mean ± SD of the BCAA and androgen metabolite pattern factor scores according to background characteristics of 213 Project Viva mother-child pairs

	n	BCAA pattern factor score	*P*[a]	Androgen pattern factor score	*P*[a]
Maternal and perinatal characteristics					
Annual household income			0.05		0.02
≤$70,000	81	0.25 ± 1.49		0.27 ± 1.69	
>$70,000	115	−0.17 ± 1.57		−0.25 ± 1.42	
Smoking habits			0.21		0.23
Never	141	−0.15 ± 1.56		0.09 ± 1.55	
Former	47	0.20 ± 1.43		−0.36 ± 1.62	
Smoked in early pregnancy	25	0.29 ± 1.27		0.04 ± 1.52	
Prepregnancy BMI[b]			0.008		0.25
Normal weight	114	−0.20 ± 1.54		−0.05 ± 1.56	
Overweight	53	−0.11 ± 1.31		−0.28 ± 1.41	
Obesity	46	0.53 ± 1.53		0.36 ± 1.71	
Gestational weight gain			0.18		0.27
Inadequate	23	−0.15 ± 1.95		−0.31 ± 1.30	
Adequate	65	−0.24 ± 1.59		−0.08 ± 1.47	
Excessive	125	0.12 ± 1.36		0.07 ± 1.66	
Gestational glucose tolerance			0.68		0.53
Normoglycemic	163	0.00 ± 1.52		−0.06 ± 1.61	
Isolated hyperglycemia	28	−0.18 ± 1.44		0.18 ± 1.20	
Impaired glucose tolerance	6	1.25 ± 1.28		−0.85 ± 2.21	
Gestational diabetes	17	−0.39 ± 1.42		0.32 ± 1.41	
Duration of any breastfeeding			0.52		0.20
<1 months	33	−0.05 ± 1.58		0.30 ± 1.34	
1 to < 7 months	71	0.27 ± 1.32		−0.20 ± 1.80	
7 to < 12 months	40	−0.74 ± 1.53		0.25 ± 1.60	
≥12 months	47	0.11 ± 1.48		−0.38 ± 1.31	
Child's characteristics at baseline (midchildhood visit)					
Age			0.73		0.03
<7 y	12	−0.14 ± 1.18		−0.39 ± 1.49	
7 to < 8 y	129	−0.03 ± 1.56		−0.16 ± 1.41	
≥8 y	72	0.02 ± 1.47		0.30 ± 1.79	
Race/ethnicity			0.76		0.003
White	125	−0.03 ± 1.51		−0.25 ± 1.49	
Black	46	0.15 ± 1.36		0.71 ± 1.77	
Hispanic	15	−0.27 ± 1.55		0.15 ± 1.29	
Other	28	−0.13 ± 1.72		−0.27 ± 1.36	
Sex			0.36		0.80
Male	104	−0.12 ± 1.49		−0.05 ± 1.60	
Female	109	0.07 ± 1.52		0.01 ± 1.53	
Weight status[c]			<0.0001		0.0004
Normal weight	122	−0.48 ± 1.46		−0.29 ± 1.44	
Overweight	23	0.49 ± 1.26		−0.38 ± 1.50	
Obesity	68	0.68 ± 1.38		0.59 ± 1.65	
Pubertal status			0.93		0.01
Prepubertal	144	−0.02 ± 1.51		−0.22 ± 1.53	
Pubertal	60	0.00 ± 1.48		0.38 ± 1.56	

[a]Represents test for linear trend for ordinal variables and type-3 test for difference for dichotomous and categorical (race/ethnicity and smoking habits).
[b]According to World Health Organization and Centers for Disease Control and Prevention adult weight status classification. "Normal weight" includes four women classified as "underweight."
[c]According to Centers for Disease Control and Prevention 2000 age- and sex-specific reference data. "Normal weight" includes four children classified as underweight (BMI < 5th percentile).

TABLE 3 Associations of BCAA metabolite pattern factor score with change in metabolic biomarkers between midchildhood (~7 years) and early teens (~12 years) among boys and girls in Project Viva

	β (95% CI) per 1-unit BCAA pattern factor score[a]					
	Boys ($n = 104$)			Girls ($n = 109$)		
	Unadjusted	Model 1	Model 2	Unadjusted	Model 1	Model 2
Insulin (μU/mL)	−0.26 (−2.40 to 1.88)	1.64 (−0.35 to 3.64)	1.51 (−0.49 to 3.51)	0.57 (−1.26 to 2.41)	1.04 (−0.67 to 2.75)	0.59 (−1.14 to 2.32)
Glucose (mg/dL)	−3.42 (−7.63 to 0.79)	**−4.70 (−8.47 to −0.93)**	**−4.97 (−8.83 to −1.11)**	2.58 (−0.69 to 5.86)	2.12 (−0.22 to 4.45)	1.60 (−0.78 to 3.98)
HOMA-IR	−0.48 (−1.12 to 0.15)	0.31 (−0.17 to 0.79)	0.26 (−0.22 to 0.73)	0.35 (−0.16 to 0.85)	0.39 (−0.11 to 0.89)	0.24 (−0.26 to 0.75)
Leptin (ng/mL)	0.53 (−0.99 to 2.05)	0.23 (−1.34 to 1.80)	0.06 (−1.54 to 1.65)	0.23 (−2.21 to 2.68)	0.35 (−2.18 to 2.89)	−1.04 (−3.39 to 1.31)
Adiponectin (ng/mL)	−0.29 (−1.43 to 0.84)	−0.13 (−0.49 to 0.23)	−0.04 (−0.40 to 0.33)	−0.21 (−1.54 to 1.11)	−0.01 (−0.35 to 0.33)	0.03 (−0.33 to 0.38)
CRP (mg/L)	0.07 (−0.23 to 0.37)	0.03 (−0.17 to 0.23)	0.03 (−0.17 to 0.24)	−0.16 (−0.82 to 0.51)	0.07 (−0.21 to 0.36)	0.09 (−0.21 to 0.39)
IL-6 (pg/mL)	0.15 (−0.08 to 0.38)	0.13 (−0.08 to 0.33)	0.11 (−0.11 to 0.32)	−0.08 (−0.40 to 0.24)	−0.04 (−0.34 to 0.25)	0.05 (−0.25 to 0.35)
Total cholesterol (mg/dL)	−2.29 (−5.80 to 1.22)	−2.78 (−5.96 to 0.39)	−2.87 (−6.13 to 0.38)	−0.94 (−4.13 to 2.25)	−0.99 (−4.16 to 2.19)	−0.97 (−4.30 to 2.35)
HDL (mg/dL)	−1.06 (−2.62 to 0.51)	−1.21 (−2.71 to 0.30)	−1.02 (−2.56 to 0.51)	0.62 (−0.91 to 2.15)	0.06 (−1.44 to 1.55)	0.49 (−1.02 to 1.99)
LDL (mg/dL)	−1.54 (−4.61 to 1.53)	−1.85 (−4.56 to 0.86)	−2.15 (−4.92 to 0.61)	−2.00 (−4.54 to 0.54)	−1.61 (−4.14 to 0.92)	−1.99 (−4.62 to 0.65)
Triglycerides (mg/dL)	1.55 (−2.70 to 5.79)	1.40 (−2.70 to 5.49)	1.13 (−3.07 to 5.33)	2.19 (−2.31 to 6.69)	**4.38 (0.40 to 8.37)**	3.99 (−0.13 to 8.11)
SBP (mmHg)	−0.96 (−2.27 to 0.35)	−0.49 (−1.58 to 0.60)	−0.51 (−1.63 to 0.62)	0.33 (−0.95 to 1.62)	0.87 (−0.24 to 1.98)	1.03 (−0.14 to 2.20)
DBP (mmHg)	0.28 (−0.68 to 1.24)	0.21 (−0.72 to 1.14)	−0.03 (−0.98 to 0.92)	0.47 (−0.37 to 1.32)	0.46 (−0.36 to 1.29)	0.56 (−0.30 to 1.42)
MetS *z* score[b]	−0.03 (−0.12 to 0.07)	0.01 (−0.08 to 0.11)	0.00 (−0.10 to 0.09)	0.02 (−0.08 to 0.13)	0.06 (−0.02 to 0.15)	0.04 (−0.04 to 0.12)

Bolded values indicate statistical significance at alpha < 0.05.
[a]Model 1: Estimates adjusted for pubertal status and biomarker of interest at midchildhood visit and for age at midchildhood and early teen visits. Model 2: Model 1 + BMI percentile at baseline.
[b]Calculated as average of five internally standardized sex-specific *z* scores for inverted HDL, waist circumference, natural log-transformed HOMA-IR, natural log-transformed triglycerides, and SBP.

TABLE 4 Associations of androgen metabolite pattern factor score with change in metabolic biomarkers between midchildhood (~7 years) and early teens (~12 years) among boys and girls in Project Viva

	β (95% CI) per 1-unit androgen factor score[a]					
	Boys ($n = 104$)			Girls ($n = 109$)		
	Unadjusted	Model 1	Model 2	Unadjusted	Model 1	Model 2
Insulin (μU/mL)	1.04 (−1.09 to 3.18)	−0.19 (−2.15 to 1.78)	−0.48 (−2.47 to 1.52)	−1.35 (−2.96 to 0.25)	−1.12 (−2.66 to 0.43)	−1.14 (−2.64 to 0.36)
Glucose (mg/dL)	2.53 (−1.56 to 6.62)	1.90 (−1.88 to 5.68)	1.97 (−1.90 to 5.85)	−0.63 (−3.38 to 2.13)	1.27 (−0.78 to 3.32)	1.26 (−0.74 to 3.25)
HOMA-IR	0.43 (−0.17 to 1.03)	−0.09 (−0.52 to 0.35)	−0.21 (−0.65 to 0.23)	−0.32 (−0.73 to 0.10)	−0.22 (−0.66 to 0.22)	−0.21 (−0.64 to 0.22)
Leptin (ng/mL)	0.37 (−1.17 to 1.91)	−0.12 (−1.75 to 1.52)	−0.25 (−1.89 to 1.39)	−1.85 (−4.09 to 0.40)	**−2.39 (−4.76 to −0.02)**	**−2.50 (−4.61 to −0.39)**
Adiponectin (ng/mL)	0.46 (−0.68 to 1.61)	−0.01 (−0.38 to 0.38)	0.07 (−0.31 to 0.45)	1.04 (−0.18 to 2.26)	0.00 (−0.32 to 0.33)	0.01 (−0.32 to 0.33)
CRP (mg/L)	−0.23 (−0.52 to 0.05)	0.12 (−0.09 to 0.32)	0.12 (−0.09 to 0.33)	0.06 (−0.54 to 0.66)	**0.28 (0.03 to 0.54)**	**0.28 (0.03 to 0.54)**
IL-6 (pg/mL)	0.08 (−0.15 to 0.31)	0.20 (−0.02 to 0.41)	0.18 (−0.03 to 0.40)	0.06 (−0.23 to 0.36)	0.26 (−0.02 to 0.55)	0.26 (−0.02 to 0.54)
Total cholesterol (mg/dL)	−1.97 (−5.46 to 1.52)	−1.07 (−4.38 to 2.24)	−1.06 (−4.42 to 2.30)	−0.43 (−3.38 to 2.52)	−0.61 (−3.69 to 2.46)	−0.60 (−3.68 to 2.48)
HDL (mg/dL)	−0.71 (−2.28 to 0.85)	−1.02 (−2.61 to 0.58)	−0.87 (−2.47 to 0.72)	0.02 (−1.39 to 1.44)	−0.05 (−1.45 to 1.36)	0.01 (−1.36 to 1.39)
LDL (mg/dL)	−1.25 (−4.30 to 1.81)	0.30 (−2.51 to 3.11)	0.17 (−2.67 to 3.01)	−0.28 (−2.66 to 2.09)	−0.56 (−2.99 to 1.87)	−0.58 (−3.01 to 1.84)
Triglycerides (mg/dL)	−0.07 (−4.30 to 4.16)	0.12 (−4.09 to 4.32)	−0.12 (−4.37 to 4.13)	−0.85 (−5.02 to 3.32)	1.73 (−2.11 to 5.57)	1.71 (−2.10 to 5.52)
SBP (mmHg)	−0.81 (−2.02 to 0.40)	0.16 (−0.91 to 1.22)	0.16 (−0.90 to 1.22)	**−1.32 (−2.57 to −0.07)**	−0.52 (−1.63 to 0.59)	−0.52 (−1.63 to 0.59)
DBP (mmHg)	−0.17 (−1.05 to 0.72)	0.19 (−0.71 to 1.08)	0.06 (−0.83 to 0.95)	−0.17 (−1.01 to 0.68)	0.14 (−0.68 to 0.96)	0.15 (−0.67 to 0.96)
MetS *z* score[b]	−0.03 (−0.12 to 0.06)	−0.01 (−0.11 to 0.08)	−0.02 (−0.11 to 0.07)	**−0.10 (−0.18 to −0.02)**	−0.04 (−0.12 to 0.03)	−0.04 (−0.11 to 0.03)

Bolded values indicate statistical significance at alpha < 0.05.
[a]Model 1: Estimates adjusted for pubertal status and biomarker of interest at midchildhood visit and for age at midchildhood and early teen visits. Model 2: Model 1 + BMI percentile at baseline.
[b]Calculated as average of five internally standardized sex-specific *z* scores for inverted HDL, waist circumference, natural log-transformed HOMA-IR, natural log-transformed triglycerides, and SBP.

TABLE 5 Associations of metabolites within BCAA pattern with change in fasting glucose in boys and change in serum triglycerides in girls

		Boys ($n = 63$)	Girls ($n = 83$)
	Factor loading	β (95% CI) for change in fasting glucose (mg/dL)[a]	β (95% CI) for change in serum triglycerides (mg/dL)[a]
Valine	0.83	−2.91 (−6.83 to 1.02)	1.72 (−1.88 to 5.31)
Leucine	0.76	−2.12 (−5.48 to 1.24)	1.72 (−1.56 to 4.99)
Phenylalanine	0.72	−3.35 (−6.70 to 0.01)	0.78 (−2.87 to 4.44)
Isoleucine	0.71	−3.10 (−7.07 to 0.87)	2.26 (−1.16 to 5.67)
Propionylcarnitine (C3)	0.66	**−4.38 (−7.79 to −0.97)**	2.01 (−2.31 to 6.33)
2-Methylbutyrylcarnitine (C5)	0.63	−2.01 (−6.13 to 2.10)	2.74 (−0.69 to 6.16)
Isovalerylcarnitine	0.30	−2.18 (−5.13 to 0.76)	−0.46 (−3.78 to 2.86)
Isobutyrylcarnitine	0.56	**−3.54 (−6.18 to −0.90)**	−0.22 (−3.40 to 2.95)
Tryptophan	0.54	−2.65 (−6.29 to 0.98)	−1.81 (−5.79 to 2.16)
3-Methyl-2-oxovalerate (KMV)	0.52	−0.81 (−4.46 to 2.85)	1.13 (−2.99 to 5.25)
Kynurenine	0.52	−1.39 (−5.06 to 2.28)	**3.65 (0.10 to 7.21)**
Tyrosine	0.51	−3.70 (−8.35 to 0.94)	−2.38 (−6.14 to 1.38)
Gamma-glutamylleucine	0.51	−2.92 (−6.72 to 0.88)	**4.44 (1.10 to 7.79)**
4-Methyl-2-oxopentanoate (KIC)	0.51	−0.63 (−4.06 to 2.80)	−0.19 (−4.23 to 3.85)

Bolded text indicates statistical significance at alpha < 0.05.
[a]Estimates adjusted for age, pubertal status, BMI percentile, biomarker level at midchildhood visit, and age at early teen visit.

Children from households with an annual income < $70,000 versus > $70,000 had a higher BCAA score ($\beta = 0.43$; 95% CI: 0.01-0.85). As we have previously shown (8), children of women with obesity had a higher BCAA score than those whose mothers had overweight ($\beta = 0.64$; 95% CI: 0.06-1.22) or normal weight ($\beta = 0.74$; 95% CI: 0.23-1.24) prior to pregnancy. Children who had obesity at baseline exhibited a higher BCAA score than their counterparts with overweight ($\beta = 0.93$; 95% CI: 0.29-1.56) and normal

TABLE 6 Associations of metabolites within BCAA pattern with change (Δ) in SBP in 98 boys, stratified by pubertal status at baseline (midchildhood)

		β (95% CI) for Δ in SBP (mmHg)[a]	
	Factor loading	Prepubertal ($n = 79$)	Pubertal ($n = 19$)
BCAA factor score		−0.88 (−2.09 to 0.33)	2.48 (−0.45 to 5.40)
Individual metabolites			
Valine	0.83	−0.44 (−1.40 to 0.52)	0.91 (−1.77 to 3.59)
Leucine	0.76	−0.05 (−0.91 to 0.81)	1.48 (−0.67 to 3.63)
Phenylalanine	0.72	0.19 (−0.70 to 1.07)	1.79 (−0.23 to 3.81)
Isoleucine	0.71	−0.32 (−1.26 to 0.62)	1.32 (−1.08 to 3.73)
Propionylcarnitine (C3)	0.66	−0.77 (−1.80 to 0.26)	−0.54 (−2.71 to 1.41)
2-Methylbutyrylcarnitine (C5)	0.63	−0.17 (−1.32 to 0.98)	0.65 (−2.19 to 3.50)
Isovalerylcarnitine	0.30	−0.17 (−1.14 to 0.79)	−0.25 (−1.71 to 1.21)
Isobutyrylcarnitine	0.56	−0.50 (−1.32 to 0.33)	−0.95 (−2.72 to 0.82)
Tryptophan	0.54	−0.80 (−1.64 to 0.04)	**2.59 (0.03 to 5.15)**
3-Methyl-2-oxovalerate (KMV)	0.52	−0.33 (−1.35 to 0.68)	2.32 (−0.06 to 4.71)
Kynurenine	0.52	−0.38 (−1.39 to 0.62)	**2.53 (0.51 to 4.55)**
Tyrosine	0.51	−0.21 (−1.44 to 1.02)	−0.17 (−3.12 to 2.77)
Gamma-glutamylleucine	0.51	−0.08 (−1.27 to 1.10)	1.03 (−0.78 to 2.84)
4-Methyl-2-oxopentanoate (KIC)	0.51	−0.04 (−1.02 to 0.94)	2.31 (−0.08 to 4.70)

Bolded text indicates statistical significance at alpha < 0.05.
[a]Estimates adjusted for age, pubertal status as an ordinal variable, BMI percentile, biomarker level at midchildhood visit, and age at early teen visit.

TABLE 7 Associations of metabolites within androgen pattern with change in leptin and CRP in girls

		β (95% CI)[a]	
	Factor loading	Change in leptin (ng/dL), $n = 82$	Change in CRP (mg/dL), $n = 79$
4-Androsten-3beta,17beta-diol disulfate 1	0.86	−0.92 (−2.95 to 1.11)	0.18 (−0.07 to 0.42)
Dehydroisoandrosterone sulfate (DHEA-S)	0.84	−1.68 (−4.47 to 1.11)	0.05 (−0.29 to 0.39)
Epiandrosterone sulfate	0.79	−0.40 (−2.33 to 1.52)	0.13 (−0.11 to 0.37)
Androsterone sulfate	0.79	−1.56 (−3.83 to 0.71)	0.10 (−0.17 to 0.37)
4-Androsten-3beta,17beta-diol disulfate 2[b]	0.78	**−1.78 (−3.35 to −0.20)**	0.04 (−0.16 to 0.23)
Pregn steroid monosulfate[b]	0.76	**−2.50 (−4.87 to −0.13)**	0.02 (−0.27 to 0.31)
Pregnen-diol disulfate[b]	0.70	−1.94 (−4.11 to 0.23)	−0.02 (−0.29 to 0.25)
Pregnenolone sulfate	0.65	−0.64 (−2.66 to 1.39)	0.05 (−0.20 to 0.30)
Andro steroid monosulfate 2[b]	0.61	−1.09 (−3.14 to 0.97)	−0.02 (−0.28 to 0.24)

Bolded text indicates statistical significance at alpha < 0.05.
[a]Estimates adjusted for age, pubertal status, BMI percentile, biomarker level at midchildhood visit, and age at early teen visit.
[b]Indicates tier-2 identification in which no commercially available authentic standards could be found; however, annotated based on accurate mass, spectral, and chromatographic similarity to tier-1 identified compounds.

weight ($\beta = 1.14$; 95% CI: 0.72-1.55). We observed similar associations for the androgen factor score (Table 2). Additionally, children classified as pubertal had a higher score for this metabolite pattern than prepubertal participants ($\beta = 0.60$; 95% CI: 0.14-1.06).

Table 3 shows associations of the BCAA pattern at baseline (midchildhood) with changes in the metabolic biomarkers during follow-up (difference between the early teen and midchildhood values). A higher score for the BCAA pattern was associated with a decrease in fasting glucose in boys, even after adjusting for age, pubertal status, and fasting glucose at baseline and age at follow-up. Specifically, each 1-unit increment in the BCAA score corresponded with a 4.70 mg/dL decrease (95% CI: 0.93 to 8.47) in fasting glucose (Model 1). This association did not materially change after accounting for baseline BMI percentile (Model 2: $\beta = -4.97$; 95% CI: −8.83 to −1.11 mg/dL per 1-unit BCAA score), suggesting that the BCAA pattern predicts change in fasting glucose even after accounting for glucose levels and adiposity at baseline. In girls, each 1-unit increment in the BCAA score was associated with a 4.38 mg/dL increase (95% CI: 0.40 to 8.37) increase in serum triglyceride levels in Model 1. Adjustment for baseline BMI percentile in Model 2 attenuated the estimate by < 10% ($\beta = 3.99$; 95% CI: −0.13 to 8.11 mg/dL per 1-unit BCAA score).

We did not observe any associations of the androgen pattern with change in the metabolic biomarkers among boys (Table 4). In girls, a higher score for the androgen pattern corresponded with a decrease in leptin ($\beta = -2.39$; 95% CI: 4.76 to −0.02 ng/dL per 1-unit factor score in Model 1) and an increase in CRP ($\beta = 0.28$; 95% CI: 0.03 to 0.54 mg/dL per 1-unit factor score in Model 1). These estimates were essentially unchanged after adjustment for baseline BMI percentile in Model 2.

To obtain a more granular understanding of specific compounds driving the associations detected with respect to the factor scores, we further examined relations of key metabolites within the BCAA pattern with change in fasting glucose among boys and with serum triglycerides among girls based on results shown in Table 3 (Table 5). Among boys, all metabolites were associated with a decrease in fasting glucose, with the strongest association for propionylcarnitine ($\beta = -4.38$; 95% CI: −7.79 to −0.97 mg/dL per 1 z score) and isobutyrylcarnitine ($\beta = -3.54$; 95% CI: −6.18 to −0.90 mg/dL per 1 z score). Among girls, the majority of compounds exhibited a positive relationship with change in triglycerides, with the strongest association detected for kynurenine ($\beta = 3.65$; 95% CI: 0.10 to 7.21 mg/dL per 1 z score) and gamma-glutamylleucine ($\beta = 4.44$; 95% CI: 1.10 to 7.79 mg/dL per 1 z score).

When testing for effect modification by pubertal status, we found evidence of an interaction with respect to SBP in boys, so we ran these models within strata of baseline pubertal status (Table 6). The BCAA pattern was inversely related to change in SBP in prepubertal boys ($n = 79$; Model 1: $\beta = -0.86$; 95% CI: −2.06 to 0.33 mmHg per 1-unit BCAA score) but was positively associated with change in SBP in pubertal boys ($n = 19$; Model 1: $\beta = 1.67$; 95% CI: −0.82 to 4.16 mmHg per 1-unit BCAA score). Further adjustment for baseline BMI percentile did not change these findings (prepubertal boys: $\beta = -0.88$; 95% CI: −2.09 to 0.33; pubertal boys: $\beta = 2.48$; 95% CI: −0.45 to 5.40 mmHg per 1-unit BCAA score). We examined these relationships with individual metabolites and identified associations with respect to tryptophan ($\beta = 2.59$; 95% CI: 0.03 to 5.15 mmHg per 1 z score of the metabolite) and kynurenine ($\beta = 2.53$; 95% CI: 0.51 to 4.55 mmHg per 1 z score of the metabolite) (Table 6).

Table 7 shows associations of metabolites in the androgen pattern with the change in leptin and CRP in girls (outcomes selected based on findings from Table 4). All individual metabolites were consistently inversely associated with a change in leptin, with the strongest associations for 4-androsten-3beta,17beta-diol disulfate 2 ($\beta = -1.78$; 95% CI: −3.35 to −0.20 ng/dL per 1 z score) and pregn steroid monosulfate ($\beta = -2.50$; 95% CI: −4.87 to −0.13 ng/dL per 1 z score). The majority of metabolites were positively associated with a change in CRP, but none of the estimates was statistically significant.

None of the relationships assessed differed by baseline BMI percentile. In sensitivity analyses, we evaluated the impact of adjustment for background characteristics associated with the metabolite patterns in bivariate analysis, namely prepregnancy BMI, annual household income, and child's race and/or ethnicity. The inclusion of these variables did not change the results, so we did not include them for the sake of parsimony. An assessment of background characteristics of each of the subsamples analyzed in multivariable analyses yielded no notable differences, thus indicating that the risk of bias because of missing data is likely low (Supporting Information Table S1).

Discussion

In this prospective study of 253 children 6 to 10 years of age at baseline, we examined associations of two previously derived metabolite patterns, a BCAA metabolite pattern and an androgen hormone pattern (8), with changes in conventional metabolic biomarkers during ~5 years of follow-up. Counter to findings from adults (5,25), the BCAA pattern was not associated with worsening metabolic health in this cohort of adolescents, as the majority of the relationships we examined were null. However, we did find an inverse association of the BCAA pattern with a change in fasting glucose in boys and a direct relation of this pattern with a change in serum triglycerides in girls. The androgen hormone pattern, which, to our knowledge, has not been evaluated in relation to metabolic risk in other populations, was related to decreased leptin and increased CRP during follow-up in girls.

BCAA metabolite pattern

During midchildhood, the BCAA pattern was associated with obesity as well as higher fasting glucose, insulin, HOMA-IR, leptin, CRP, and IL-6. When we examined associations with change in the biomarkers during follow-up, the majority of estimates were null, with the exceptions of an inverse relationship between the BCAA pattern and fasting glucose in boys and a direct association with serum triglycerides in girls.

BCAA pattern and change in fasting glucose in boys. This finding was counter to what we expected based on findings in adults (5,25) as well as two prospective studies in adolescents that did not account for glycemia at baseline (9,11). However, our findings aligned with results of two cross-sectional analyses in similarly aged youth. In a study of 139 adolescents ~13 years of age, Michaliszyn et al. found that several compounds in the BCAA pattern, including leucine, isoleucine, valine, phenylalanine, and propionylcarnitine, were associated with higher insulin sensitivity (7). In the same population, Mihalik et al. unveiled evidence that these metabolites were also associated with enhanced fatty acid oxidation (6). The investigators hypothesized that the discrepancy in direction of association in adults versus adolescents may be due to an adaptive increase in mitochondrial function (and, accordingly, an improvement in glycemia) during early life that eventually wanes with age and continued metabolic dysregulation (26). Another explanation for our finding relates to the nutrient-signaling properties of BCAAs. For example, leucine activates the mammalian target of rapamycin, a nutrient sensor involved in the neurocircuitry of energy balance, food intake, and glycemic regulation (27), that has been shown to improve glucose tolerance in mice (28). The fact that we observed positive, albeit nonsignificant, associations with fasting insulin and HOMA-IR supports this mechanism.

Of note, a recent study of 16 children 8 to 13 years of age who had obesity but were nondiabetic at baseline found that, although circulating BCAAs were associated with worse glycemia throughout the course of an oral glucose tolerance test at baseline, these metabolites were not associated with glucose control or indices of insulin secretion and/or sensitivity over 2.3 years of follow-up (12). These findings are generally in line with those of the present analysis given our predominantly null results with respect to other glycemia biomarkers (fasting insulin, HOMA-IR).

When we examined associations of individual metabolites within the BCAA pattern with glucose during follow-up in boys, we found that propionylcarnitine and isobutyrylcarnitine were the strongest determinants of decreasing glucose during follow-up. Both compounds are downstream intermediates of BCAA catabolism, and the latter of which (isobutyrylcarnitine) is also product of acyl coenzyme A beta oxidation, a process that feeds into the citric acid cycle for energy production (29). Although we were not able to locate any published studies on isobutyrylcarnitine in relation to glycemia, a pilot trial of 24 patients who had obesity and type 2 diabetes found that intravenous administration of propionylcarnitine improved glycemic control (30). Proposed mechanisms include propionylcarnitine's involvement in muscle metabolism, endothelial function, and carbohydrate oxidation (31,32).

The BCAA pattern was also associated with increasing SBP among pubertal but not prepubertal boys. Specific metabolites driving this relationship were tryptophan and its downstream metabolite, kynurenine. These results support findings of Wolf et al., who demonstrated that intraperitoneal tryptophan administration reduced blood pressure in hypertensive rodents but increased blood pressure in normotensive animals (33). The investigators speculated that the duality of tryptophan's effect on blood pressure is complex and regulated by numerous physiological processes involved in the control of blood pressure, such as brain monoamines and catecholamines (33). In the present study population, the majority of children were normotensive at baseline (98.8%); therefore, the positive relationship between tryptophan and SBP corroborates results from rodent models. The fact that this relationship was only observed among pubertal boys may reflect the sex-specific differences in blood pressure change during puberty (34).

BCAA pattern and change in serum triglycerides in girls. In girls, a higher score for the BCAA pattern was associated with increased serum triglycerides. When we examined component metabolites of the BCAA pattern, we detected the strongest relations with respect to kynurenine and gamma-glutamylleucine.

Although there have not been any publications specifically relating kynurenine to triglycerides, growing evidence has suggested that deregulation of the tryptophan-to-kynurenine pathway is involved in atherosclerotic cardiovascular disease, possibly through perturbations in immune pathways (35). Despite an equally scanty literature on gamma-glutamylleucine in relation to lipid profile, the positive association between this compound and triglycerides may reflect defective hepatic lipid metabolism as gamma-glutamylleucine has been

associated with nonalcoholic fatty liver disease, a condition characterized by the accumulation of triglycerides in the liver, and elevated circulating triglycerides (36,37). Because blood lipid levels exhibit high within-person variability until after puberty (38), whether or not these compounds serve as early indicators of dyslipidemia deserves further investigation beyond adolescence.

Androgen hormone metabolite pattern

During midchildhood, the androgen hormone metabolite pattern, which we speculated was an indicator of pubarchal and/or pubertal advancement, was associated with higher HOMA-IR and marginally higher serum triglycerides, leptin, and IL-6, and lower adiponectin (8). When we examined these relationships prospectively, we found that this metabolite pattern corresponded with a decrease in leptin and an increase in CRP in girls. The androgen hormone pattern was not associated with change in any of the biomarkers in boys.

Androgen pattern and change in leptin in girls. A higher score for this pattern at baseline corresponded with a decrease in leptin in girls, which was unexpected given that this metabolite pattern is associated with more advanced pubarchal and/or pubertal status at baseline (8) and the fact that leptin levels increase throughout adolescence in females (39,40). Although this finding could be due to variability in the tempo of maturation during follow-up (a variable that we did not control for because it could be on the causal pathway), additional studies are warranted to confirm our results. When we examined associations with specific metabolites, we detected the strongest relations with the testosterone precursor 4-androsten-3beta,17beta-diol disulfate 1, and pregn steroid monosulfate which is an intermediate in the steroidogenesis of androgen hormones from cholesterol.

Androgen pattern and change in CRP in girls. The androgen hormone pattern, but not the individual metabolites, was also related to increasing CRP in girls. This finding aligns with published data on the precipitous increase in CRP in females, but not males, during adolescence, particularly during the later stages of puberty (41). Future investigations are warranted to ascertain whether this relationship persists beyond puberty.

Strengths and weaknesses

This study had several weaknesses. First, assessment of plasma metabolites at a single point in time precluded our ability to infer on upregulation versus downregulation of specific pathways. Second, we did not implement challenge testing (e.g., oral glucose tolerance test) or measure glycated hemoglobin and, thus, were not able to evaluate the entire range of glycemia. Third, the use of complete case analysis is subject to missing data bias; however, a comparison of background characteristics among each of the subsamples provided no indication of differences in background characteristics of participants within the subsamples. Fourth, there may be residual confounding from variation in the tempo of sexual maturation during follow-up. Finally, as is the case with most analyses of high-dimensional omics data, we cannot rule out the possibility of false-positive associations, although we aimed to examine and compare associations (many of which are on correlated biochemical pathways) in order to gain insight on etiology rather than to predict outcomes.

Strengths of this investigation included our ability to examine the relationship of two metabolite patterns with prospective change in multiple metabolic biomarkers in a cohort of multiethnic youth. Each of these elements are key improvements upon published studies, which have mostly been cross-sectional (6-9), of smaller sample size and shorter duration of follow-up ($n = 17$ for 18 months (9); $n = 102$ for 2 years (11)), and conducted in rather specific populations (e.g., Korean boys) (11).

Conclusion

We detected sex-specific associations of the BCAA and androgen hormone metabolite patterns with a change in conventional metabolic biomarkers during ~5 years of follow-up in this population of youth aged 6 to 10 years at baseline. Specifically, the BCAA pattern corresponded with decreasing fasting glucose in boys and increasing serum triglycerides in girls. However, given that the majority of associations were null (which may be an artifact of small sample sizes), the BCAA metabolite pattern does not appear to be an indicator of worsening metabolic health during early adolescence. The androgen hormone pattern, which, to our knowledge, has not been reported in other populations, was associated with a decrease in leptin and an increase in CRP in girls; these findings are likely related to the timing of sexual maturation.

Given that puberty is a time of rapid physiological change, future studies are required to evaluate these relationships beyond adolescence and to interrogate the capacity of these metabolite patterns to predict metabolic disease progression beyond that of conventional biomarkers. Nevertheless, given that many metabolic risk factors, including blood pressure (42,43), lipid profile (43,44), and glycemia (43), track from late childhood or early adolescence into adulthood, a better understanding of early biomarkers of metabolic risk is a crucial step toward the identification of targets for primary prevention.O

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