Metabolomic profiles and childhood obesity

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Abstract

Objective—To identify metabolite patterns associated with childhood obesity, to examine relations of these patterns with measures of adiposity and cardiometabolic risk, and to evaluate associations with maternal peripartum characteristics.

Design and Methods—We employed untargeted metabolomic profiling to quantify metabolites in plasma of 262 children (6–10 years). We used principal components analysis to consolidate 345 metabolites into 18 factors and identified two that differed between obese (BMI 95%ile; *n*=84) and lean children (BMI<85%ile; *n*=150). We then investigated relations of these factors with adiposity (fat mass, BMI, skinfold thicknesses) and cardiometabolic biomarkers (HOMA-IR, triglycerides, leptin, adiponectin, hsCRP, IL-6) using multivariable linear regression. Finally, we examined associations of maternal pre-pregnancy obesity, gestational weight gain, and gestational glucose tolerance with the offspring metabolite patterns.

Results—A branched-chain amino acid (BCAA)-related pattern and an androgen hormone pattern were higher in obese vs. lean children. Both patterns were associated with adiposity and worse cardiometabolic profiles. For example, each increment in the BCAA and androgen pattern scores corresponded with 6% (95% CI: 1%, 13%) higher HOMA-IR. Children of obese mothers had 0.61 (0.13, 1.08) higher BCAA score than their counterparts.

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Conclusions—BCAA and androgen metabolites were associated with adiposity and cardiometabolic risk during mid-childhood. Maternal obesity may contribute to altered offspring BCAA metabolism.

Keywords

branched-chain amino acids; androgen; steroid hormones; DHEA-S; metabolomics; obesity; insulin resistance; untargeted

INTRODUCTION

Metabolomics, the quantitative analysis of metabolites and low-molecular-weight intermediates, has emerged as a tool to understand how metabolites reflect physiological states. Studies comparing obese and lean adults found substantial differences in circulating markers of amino acid (1–4) and lipid (2, 4) metabolism. Furthermore, perturbations in some of these metabolic pathways not only correlated with established cardiometabolic risk biomarkers, such as insulin resistance, independently of weight status (5, 6), but were also predictive of future disease (7, 8).

In attempt to replicate the adult findings in children, a handful of pediatric investigations examined the relation of weight status and/or glycemia with amino acid (8–11) and fatty acid metabolite profiles (9, 11). However, animal models (12) and two studies in adolescents (9, 10) indicate that physiological responses to metabolic disturbances may be fundamentally different in early life. Therefore, approaches aimed at validating findings from adults may fail to identify relevant metabolic pathways in youth. Metabolomic studies employing unbiased, data-driven approaches are needed to identify metabolic alterations unique to early life.

In this study, our objectives were threefold. First, using untargeted metabolic profiling we sought to identify metabolite patterns associated with childhood obesity. Next, we evaluated whether these patterns were also associated with continuous measures of adiposity and cardiometabolic risk biomarkers. Finally, we examined whether the extent to which maternal peripartum characteristics related to excess offspring weight, specifically pre-pregnancy obesity, excessive gestational weight gain (GWG), and gestational diabetes mellitus (GDM) - were associated with offspring metabolite profiles in mid-childhood.

METHODS

Study population

Participants were enrolled in Project Viva, an ongoing pre-birth cohort study recruited from a multi-specialty group practice in eastern Massachusetts (Harvard Vanguard Medical Associates). Details on study design and recruitment are reported elsewhere (13). The Institutional Review Board of Harvard Pilgrim Health Care approved all study protocols. All mothers provided written informed consent and children provided verbal assent.

Of 2128 live singleton births in Project Viva, 1116 mother-child pairs attended an in-person mid-childhood visit at 6–10 years, during which anthropometry was measured in all children

and fasting blood samples were obtained from 648 assenting children. We excluded 21 participants with missing data on maternal prenatal characteristics. Among the remaining 627 mother-child pairs, funding was available for 300 metabolomic assays. We included all 113 cases of GDM and/or child obesity (n=87 child obesity, n= 22 GDM, n=4 both) to ensure sufficient numbers for each, and randomly selected 187 of the remaining plasma samples for analysis. After further exclusion of 38 children with inadequate plasma volume for the metabolomic assays, the final analytic sample included 262 mother-child pairs. As expected given the sampling strategy, children in this analysis had greater adiposity and a worse cardiometabolic profile than those not included (n=365). The sample also included a higher proportion of black (24.1% vs. 17.9%) and Hispanic (6.9% vs. 3.6%), nulliparous (48.9% vs. 40.1%), and single mothers (13.8% vs. 8.8%).

Maternal peripartum characteristics

Maternal obesity—At enrollment, mothers reported their pre-pregnancy weight and height, from which we calculated pre-pregnancy BMI. We used standard adult criteria (14) to define obesity (BMI 30 kg/m^2).

Excessive gestational weight gain—We determined GWG as the difference between the last clinically-measured weight within 4 weeks prior to delivery and self-reported pre-pregnancy weight. We categorized GWG using current Institute of Medicine (IOM) guidelines (15).

Gestational diabetes mellitus—Obstetric clinicians screened all women for gestational diabetes at 26–28 weeks of gestation with a non-fasting oral glucose challenge to categorize women as having GDM, impaired glucose tolerance, isolated hyperglycemia or normoglycemia (16).

Child adiposity and cardiometabolic biomarkers

Anthropometry and DXA scan—Trained research assistants (RAs) measured children's weight to the nearest 0.1 kg using an electronic scale (Tanita Inc., Arlington Heights, IL) and height to the nearest 0.1 cm with a calibrated stadiometer (Shorr Productions, Olney, MD). We defined obesity as body mass index (BMI; kg/m^2) 95th percentile for age and sex according to the Centers for Disease Control growth reference (17). RAs also measured subscapular (SS) and triceps (TR) skinfold thickness to the nearest 0.1 mm using calipers (Holtain, Cross-well, UK). We used these values to calculate the sum (SS+TR) and ratio (SS/TR) of skinfold thicknesses. Waist circumference was measured to the nearest 0.1 cm with a non-stretchable measuring tape (Hoechstmass Balzer GmbH, Sulzbach, Germany). RAs administered whole-body DXA scans (Hologic, Bedford, MA). A single RA checked scans for positioning, movement, and artifacts, and defined the body regions. Intra-rater reliability for body compartments was high (r=0.99). We used DXA to obtain measures of total and trunk fat mass (kg).

We measured plasma fasting glucose enzymatically, and fasting insulin using an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN). We estimated insulin resistance using the homeostasis model assessment for insulin resistance

(HOMA-IR). Triglycerides were measured enzymatically with correction for endogenous glycerol. We measured plasma leptin and adiponectin concentrations with a radioimmunoassay (Linco Research, St Charles, MO). We used an immunoturbidimetric high-sensitivity assay to determine C-reactive protein (hsCRP) concentrations (Roche Diagnostics, Indianapolis, IN). Plasma interleukin-6 (IL-6) was measured by ultrasensitive ELISA. Inter-assay CVs are reported in Supporting Information Appendix A.

Metabolic profiling

We carried out untargeted metabolomic profiling in plasma samples using a mass spectrometry-based approach. All analyses were conducted by Metabolon Inc® according to published methods (18). All samples were maintained at −80°C until processed. Details are reported in Supporting Information Appendix B.

Covariates

Using interviews and questionnaires, we collected information on maternal race/ethnicity, age, education, and parity; household income; child race/ethnicity; and paternal weight and height. We obtained birth characteristics including sex, birthweight, and delivery date from medical records. We determined birthweight-for-gestational-age z-scores ('fetal growth') using U.S. reference data (19). Small-for-gestational-age (SGA) and large-for-gestational-age (LGA) were defined as <10th percentile and 90th percentile for age and sex, respectively. Mothers reported on breastfeeding duration in postpartum questionnaires. At the mid-childhood visit, mothers provided information on their child's dietary and lifestyle characteristics, and reported on pubarchal/pubertal phenotype based on appearance of body hair, breast development for girls, and deepening of voice for boys.

Data analysis

Prior to the main analysis, we examined bivariate associations by comparing background characteristics of obese vs. lean children to identify potential confounders.

We then carried out the analysis 3 steps, conforming to the study aims. First, we divided the children in to obese and lean, and compared their metabolite profiles. Second, we examined whether the metabolite profiles associated with obesity were also related to continuous indicators of adiposity and cardiometabolic risk using multivariable linear regression. Third, we evaluated the extent to which maternal peripartum characteristics influenced the metabolite patterns.

Step 1—The metabolomic analysis identified 345 compounds. For metabolites with concentrations below the lower limit of detection, we estimated them as half the minimum detected value. We used principal components analysis (PCA) to consolidate the raw metabolite variables into 18 groups of linearly independent components known as 'factors,' which accounted for 48.4% of total variance in the original metabolites (see Supporting Information Appendix C for details on PCA). The factors can be interpreted as the strength with which an individual's metabolite profile followed the pattern captured by that particular cluster of metabolites.

To identify metabolite patterns of interest, we compared factor scores between obese (BMI 95th percentile; *n*=84) and lean (BMI<85th percentile; *n*=150) children and assessed the statistical significance of differences with the Wald test. Of the 18 factors, two were higher in obese children, even after accounting for multiple comparisons (α=0.05/18). We also compared concentrations of key constituent metabolites (those with factor loadings >0.5) within the two factors by obesity status. For these comparisons, metabolite concentrations were evaluated as z-scores centered at the median ([metabolite quant ion count − median value]/1 SD) after natural log-(ln)-transformation of non-normal distributions.

Step 2—Among all 262 children, we investigated whether the two factors associated with obesity were also related to continuous measures of overall (DXA total fat, BMI z-score, SS +TR) and central adiposity (DXA trunk fat, waist circumference, SS/TR) using linear regression models in which we assessed each adiposity indicator as an independent variable and the factor scores as outcomes. We selected covariates for multivariable analyses based on bivariate associations and known predictors of childhood obesity. We included maternal education and child age, sex, race/ethnicity, and fast-food intake in Model 1, followed by parent-reported pubarchal/pubertal phenotype in Model 2. Further adjustment for additional characteristics that differed by obesity status in Table 1 did not change results and were not included in the final models.

Next, we examined whether the metabolite patterns were related to established cardiometabolic biomarkers, including HOMA-IR, fasting insulin and glucose, triglycerides, leptin, adiponectin, hsCRP and IL-6. Using linear regression models where the biomarkers were the outcomes and the two factors were evaluated separately as independent variables, we obtained adjusted estimates using the same set of covariates described above. We ln-transformed all biomarkers because of non-normality; estimates for ln-transformed outcomes may be exponentiated and interpreted as a % change. We also examined associations of the key metabolites within the two factors with select biomarkers (HOMA-IR, leptin, hsCRP) using Model 1.

Step 3—We explored the extent to which maternal pre-pregnancy obesity, excessive GWG, and GDM were each associated offspring metabolite patterns. Using a separate linear regression model for each maternal characteristic, we assessed its relation with the factor scores as outcomes. In the multivariable models, we accounted for maternal education, father's BMI, and child age, sex, and race/ethnicity; models for GWG and gestational glucose tolerance also included continuous pre-pregnancy BMI. To evaluate the extent to which the associations were mediated by child adiposity, we further adjusted for child BMI z-score. After we observed an association of maternal pre-pregnancy obesity with one of the factors, we also examined its relation with the key constituent metabolites.

We conducted tests for a statistical interaction with sex for all associations and found evidence that the relation between the adiposity indicators and one of the factors was stronger in boys than girls, so we examined these associations separately by sex. We also noted that this factor included several sex steroid hormone precursors, so we conducted a *post hoc* analysis comparing factor scores of the 74 children whose mothers reported signs of pubarche (body hair) or puberty (breast development for girls, voice deepening for boys)

vs. the 175 with no pubarchal/pubertal signs. Excluding the pubarchal/pubertal participants yielded no substantial difference in the results, so we included all children in the analysis.

All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Our sample included 84 obese (35.9%), 28 overweight (20.7%), and 150 normal weight (57.3%) children. Median age was 7.7 years; 50% were boys. Additional details on the study population are in Table 1.

Metabolite factors associated with obesity

Of the 18 metabolite patterns characterized via PCA, Factor 4 (mean±SD: 0.00±1.52), characterized by the branched-chain amino acids (BCAA) valine, leucine, and isoleucine and related intermediate metabolites, and Factor 9 (mean±SD: 0.00±1.62), composed of several androgen hormones, including dihydroepiandrostrone sulfate (DHEA-S), and their metabolites, were significantly higher in obese than lean children (Figure 1). Concentrations of key constituent metabolites (factor loadings>0.5) within the two patterns also differed by obesity status (Table 2 for metabolite z-scores, Table S1 for metabolite quant ion counts).

Continuous adiposity measures and metabolite factors

After accounting for mother's education, child sex, age, race/ethnicity, and fast-food intake, all adiposity measures were directly related to Factor 4 (Table 3). For example, each standard deviation (SD) of DXA total fat mass corresponded with 0.47 (95% CI: 0.27, 0.67) higher Factor 4 score. Further adjustment for pubarchal/pubertal phenotype did not attenuate the association (0.48 [0.28, 0.68]). The association with Factor 9 was stronger in boys (i.e. 1 SD of DXA total fat mass corresponded with 0.80 [0.48, 1.11] vs. 0.17 [–0.13, 0.47] higher Factor 9 score for boys vs. girls, respectively). Accounting for pubarchal/pubertal status slightly strengthened the association in boys (0.88 [0.56, 1.20]), but attenuated the magnitude by about half in girls (0.08 [–0.25, 0.40]) (Table 3).

Metabolite factors and cardiometabolic biomarkers

Both metabolite factors were associated with biomarkers of cardiometabolic risk, including higher HOMA-IR, fasting insulin, leptin, triglycerides, and greater inflammation (Figure 2). For example, after accounting for confounders, each increment of Factor 4 and Factor 9 corresponded with 0.06 (0.01, 0.12) ln-transformed HOMA-IR, which translates to 6% (95% CI: 1%, 13%) higher HOMA-IR after back-transforming via exponentiation. Additionally, Factor 9 was inversely associated with adiponectin (–0.05 [–0.10, 0.00]). These associations persisted after accounting for pubarchal/pubertal status (Table S2). Many constituent metabolites of Factors 4 and 9 were also associated with the cardiometabolic biomarkers (select biomarkers presented in Table S3).

Maternal peripartum characteristics and offspring metabolite factors

Children born to obese women (41.2%) had a higher Factor 4 score than their counterparts in unadjusted analysis (0.66 [0.23, 1.10]), and after accounting for maternal education,

father's BMI, and child age, sex, and race/ethnicity (0.61 [0.13, 1.08]; Table 4). The association was somewhat attenuated after accounting for child BMI z-score (0.38 [−0.08, 0.85]). Associations between pre-pregnancy obesity and key Factor 4 metabolites are presented in Table S4.

After covariate adjustment, excessive GWG, which occurred in 8.3% of mothers, was modestly associated with Factor 4 (0.29 [−0.09, 0.67]). The association became null after adjusting for child BMI z-score (0.01 [−0.38, 0.41]). GDM was inversely related to Factor 4, though the 95% CI was wide (−0.57 [−1.22, 0.09]); accounting for child BMI z-score attenuated the estimate (−0.38 [−1.02, 0.26]). None of the peripartum characteristics was associated with Factor 9.

Post-hoc analysis

In light of the androgen hormone composition of Factor 9, we conducted an exploratory analysis comparing factor scores for children with parent-reported pubarchal (body hair; *n*=48) or pubertal characteristics (breast development for girls, *n*=27; voice deepening for boys; *n*=8) vs. those with none (*n*=175 overall, *n*=101 boys, *n*=74 girls). Children with body hair and girls with breast development had a higher Factor 9 score than their counterparts (body hair: 0.70 [0.19, 1.21]; breast development: 0.64 [0.06, 1.21]); the associations were somewhat attenuated after adjustment for BMI z-score (body hair: 0.55 [0.04, 1.05]; breast development: 0.52 [−0.13, 1.17]). A similar trend was observed for voice deepening in boys, although there were only 8 participants in this group.

DISCUSSION

In this study of school-age children, we identified two metabolite patterns that differed between obese and lean youth: Factor 4, which was driven by branched-chain amino acids (BCAA) and related metabolites; and Factor 9, characterized by androgen hormones. Higher factor scores for these patterns corresponded with continuous measures of overall and central adiposity, and were also associated with established cardiometabolic biomakers. Additionally, maternal obesity was related to a higher score for the BCAA-related pattern in offspring.

Factor 4: BCAA-related pattern

Obese children had a higher factor score for the BCAA-related pattern than their lean counterparts. Accordingly, obese particiapnts also had higher concentrations of key constituent metabolites, including the BCAA valine, leucine, and isoleucine, and downstream intermediates of BCAA metabolism including C3- and C5-acylcarnitines, isovalerylcarnitine, isobutyrylcarnitine, 3-methyl-2-oxovalerate (keto-3-methylvalerate [KMV]), and 4-methyl-2-oxopentanoate (2-ketoisocaproate [KIC]). Additionally, obese children had higher levels of the large neutral amino acids (LNAA), phenylalanine, tyrosine, and tryptophan, which compete with BCAA for cellular transport via the LAT1 protein (20). It is possible that LAT1 expression is decreased in obese persons, thereby contributing to higher circulating levels of both LNAA and BCAA. This pattern also included kynurenine, a

tryptophan metabolite; and gamma-glutamylleucine, which is generated during protein catabolism.

All adiposity indicators were directly related to the BCAA-related pattern. We noted that the relations were slightly stronger for measures of central adiposity (DXA trunk fat and waist circumference). In children, central adiposity measured by DXA is a proxy for metabolically active visceral adipose tissue (21), which may play a role in dysregulated BCAA metabolism through its association with insulin resistance (1)..

Several mechanisms may explain the positive association between adiposity and the BCAA-related pattern. First, impairment in distal metabolic pathways could lead to incomplete BCAA oxidation (22). Second, the gut microbiome, which contains bacteria capable of *de novo* BCAA synthesis, may differ in obese vs. lean individuals and thereby contribute to circulating BCAA (23). Finally, obese persons may experience greater skeletal muscle degradation due to insulin resistance (24), resulting in greater release of BCAA into circulation.

In agreement with evidence in adults (1, 5–7), we found that higher circulating BCAA and metabolites related to BCAA metabolism were associated with insulin resistance. The relation between BCAA and insulin resistance is complex, and it is not clear whether the observed elevations in circulating BCAA among obese persons are adequate to elicit insulin resistance. While a growing literature demonstrates that plasma BCAA are higher in insulin resistant humans and animals (5, 7, 25–27), possibly due to increased mitochondrial free fatty acid oxidation with insulin resistance (28), it is possible that elevated BCAA is an effect rather than a cause of insulin resistance. Longitudinal and experimental studies are warranted to disentangle temporality and causality of the relations between excess adiposity, insulin resistance, and circulating BCAA levels.

Current literature regarding BCAA and insulin resistance in youth is sparse, and findings have not been consistent. Two cross-sectional investigations in adolescents with and without dysglycemia found that higher plasma BCAA were related to higher insulin sensitivity (9) and better β-cell function (10). The investigators postulated that the opposing trend for adolescents vs. adults could be explained by adaptive metabolic plasticity in early life that becomes dysfunctional with age and continued obesity (9, 10). However, a recent study of 69 healthy children and adolescents observed no association between BCAA and HOMA-IR at the time of recruitment, but higher BCAA at baseline predicted worsening of insulin resistance during eighteen months of follow-up among the 17 participants with complete data (8). The discrepancies in study findings are difficult to reconcile because of inter- and intra-study variation in age, pubertal status, and glycemia.

In our study, the BCAA-related pattern was also directly associated with triglycerides, leptin, hsCRP, and IL-6. Because adipocytes secrete leptin and pro-inflammatory cytokines (29), and are involved in regulation of serum lipids (29, 30), these relations could reflect endocrine properties of adipose tissue. Alternatively, some of these associations could occur independently of fat mass. For example, since leucine influences leptin secretion via the

mammalian target of rapamycin (mTOR) signaling pathway (31), higher BCAA could elicit increases in leptin independently of fat mass.

Factor 9: androgen hormone pattern

Androgen levels were higher in obese than lean children, including DHEA-S and its derivatives epiandrosterone and androsterone sulfate. Obese children also had higher levels of several intermediates androgen steroidogenesis from cholesterol, including pregnenolone sulfates and the testosterone precursors 4-androsten-3beta, 17beta-diol disulfate 1 and 17beta-diol disulfate 2.

The metabolites in Factor 9 likely represent increased androgen synthesis. Accordingly, children with parent-reported pubarchal/pubertal characteristics had a higher score for this pattern than their counterparts, even after accounting for BMI z-score, which itself is related to pubarche/puberty onset. Children with greater adiposity had a higher score for the androgen hormone pattern. This pattern was also related to higher HOMA-IR, fasting insulin, triglycerides, leptin, hsCRP, and IL-6, and lower adiponectin. These findings are consistent with the physiological shifts related to early sexual maturation (32) driven by sex steroid stimulation of the insulin-like growth factor/growth hormone (IGF/GH) axis (33). In addition to exacerbating insulin resistance, the changes in IGF/GH axis may also increase leptin synthesis (34), inflammation (35), and lipid profiles (33, 36). Considering that early adrenarche may increase risk of cardiometabolic disorders in later life, such as metabolic syndrome and polycystic ovary syndrome (37), future studies exploring the relation of specific metabolites within this pattern with prospective changes in metabolic risk may help to identify preventive strategies.

Associations with maternal peripartum characteristics

Maternal pre-pregnancy obesity predicted higher BCAA and related metabolites in offspring. Although this relation was somewhat mediated by child adiposity, our finding suggests that maternal obesity may have an independent influence on offspring metabolism. While we are not aware of other human studies investigating the influence of peripartum characteristics on offspring metabolite patterns, a study of maternal-fetal macaque pairs identified differences in global metabolomic profiles of offspring born to obese vs. lean dams independently of maternal diet during breeding and pregnancy (38).

Our study has some limitations. The sample size for obese children was relatively small, so the study may be underpowered to detect smaller effects. Also, the cross-sectional assessment of metabolic profiles with mid-childhood adiposity and cardiometabolic risk precludes temporal inference. Strengths of this study include the systematic metabolomic profiling analysis, an unbiased data-driven approach to indentifying metabolite patterns associated with childhood obesity, research-quality measures of body composition and cardiometabolic biomarkers, and rich data on important covariates.

In conclusion, higher circulating levels of BCAA and androgen hormones were associated with adiposity and established cardiometabolic biomarkers in school-age children. Longitudinal studies are necessary to determine causality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

BCAA	branched-chain amino acid
BMI	body mass index
CI	confidence interval
DHEA-S	dehydroepiandrosterone - sulfate
DXA	dual x-ray absorptiometry
HDL-C	high density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment insulin resistance
hsCRP	high sensitivity C-reactive protein
IL-6	interleukin-6
IRS-1	insulin receptor substrate 1
LNAA	large neutral amino acids
mTOR	mammalian target of rapamycin
PCA	principal components analysis

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What is already known

- Disturbances in certain metabolic pathways, such as fatty acid and amino acid metabolism, are associated with obesity, subclinical cardiovascular markers, and future disease risk in adults.

- Because aberrations in metabolite pathways precede clinical disease, identifying metabolic profiles associated with obesity and metabolic derangements early in life could provide insight on pathophysiology and enhance preventive efforts.

What this study adds

- Obesity during mid-childhood (6–10 years) is associated with higher serum levels of branched chain amino acids (BCAA) and related metabolites of BCAA metabolism, and androgen sex steroid hormones.

- Both metabolite patterns were also directly related to continuous measures of adiposity and cardiometabolic biomarkers, including insulin resistance, adipocytokines, and inflammation.

- Maternal obesity prior to pregnancy may play a role in dysregulation of BCAA metabolism in offspring.
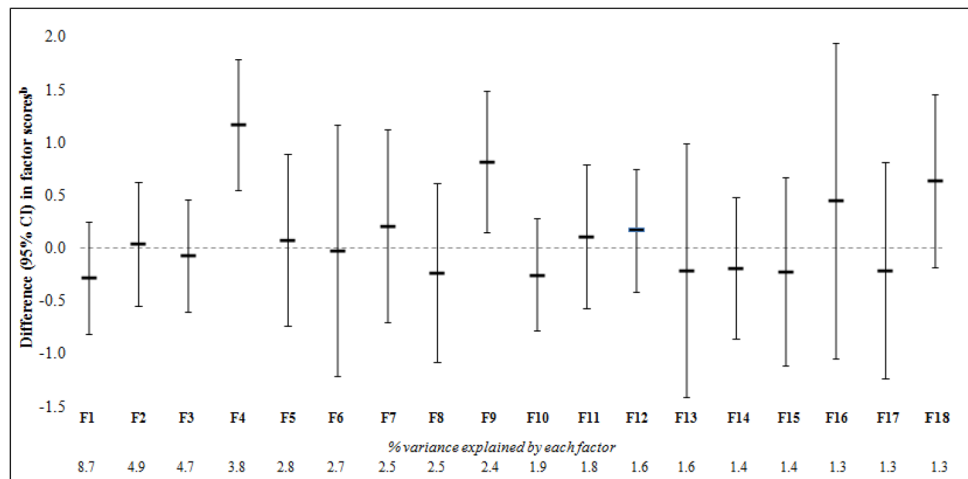
Figure 1.
Comparison of 18 metabolite factor scores between obese (BMI ≥ 95th %ile) and lean
(BMI<85th %ile) children[a]

a Estimates are adjusted for child sex, age, and race/ethnicity.

b 95% CIs reflect Bonferroni's correction for multiple comparisons (α = 0.05/18).
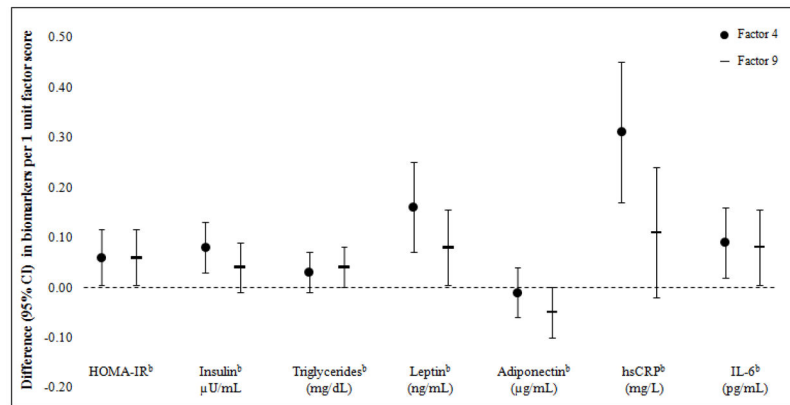
Figure 2.
Associations between Factor 4 and 9 scores and cardiometabolic risk biomarkers[a]
a Estimates are adjusted for maternal education and child age, sex, race/ethnicity, and fast-food intake.
b Natural log transformed due to non-normal distributions.

Table 1

Characteristics of the study sample, overall and according to obese (BMI ≥95th percentile) and lean (BMI<85th percentile) status among 262 Project Viva mother-child pairs[a]

|	All n = 262	Obese n = 84	Lean n = 150	P[b]
Family & background characteristics				
Sociodemographic characteristics				
Child's age (years)	8.0 ± 0.9	8.2 ± 1.0	7.9 ± 0.8	0.006
Female, % (N)	50 (131)	61.9 (52)	44.0 (66)	0.009
Child's race/ethnicity, % (N)				0.0009
White	56.3 (147)	38.1 (32)	63.1 (94)	
African American	24.1 (63)	40.5 (34)	17.5 (26)	
Hispanic	6.9 (18)	8.3 (7)	7.4 (11)	
Asian	0.8 (2)	0.0 (0)	1.3 (2)	
Other	11.9 (31)	13.1 (11)	10.7 (16)	
Mother's age at enrollment (years)	32.0 ± 5.8	30.4 ± 5.7	32.4 ± 5.8	0.009
Mother completed university education, % (N)	22.2 (58)	16.7 (14)	28.9 (43)	0.04
Annual household income < 20 K, % (N)	4.2 (10)	7.0 (5)	3.6 (5)	0.27
Father's BMI (kg/m^2)	26.8 ± 4.0	27.9 ± 4.4	25.9 ± 3.5	0.001
Perinatal & early life characteristics				
Mother's pre-pregnancy BMI (kg/m^2)	26.0 ± 6.0	28.7 ± 7.1	24.4 ± 4.8	<0.0001
Excessive gestational weight gain, %(N)	58.8 (154)	64.3 (54)	54.0 (81)	0.13
Gestational diabetes, % (N)	8.4 (22)	4.8 (4)	10.7 (16)	0.12
C-section delivery, % (N)	19.5 (51)	19.1 (16)	15.4 (23)	0.48
Mother smoked during pregnancy, % (N)	13.7 (36)	21.4 (18)	11.3 (17)	0.04
Birthweight for gestational age z-score	0.25 ± 0.97	0.19 ± 1.00	0.21 ± 0.93	0.83
Duration of breastfeeding (months)	5.9 ± 4.5	4.8 ± 4.4	6.5 ± 4.6	0.01
Characteristics in mid-childhood				
Overall adiposity				
DXA total fat mass (kg)	9.4 ± 5.5	15.5 ± 5.1	5.7 ± 1.6	<0.0001
BMI z-score	0.8 ± 1.1	2.07 ± 0.29	−0.04 ± 0.72	<0.0001
SS + TR (mm)	24.5 ± 13.3	39.6 ± 11.2	15.7 ± 4.4	<0.0001
Central adiposity				
DXA trunk fat mass (kg)	3.4 ± 2.5	6.1 ± 2.4	1.8 ± 0.6	<0.0001
Waist circumference (cm)	63.8 ± 11.3	76.6 ± 9.5	56.5 ± 4.3	<0.0001
SS/TR	0.75 ± 0.23	0.91 ± 0.27	0.67 ± 0.15	<0.0001
Height z-score	0.41 ± 1.04	0.92 ± 0.83	0.08 ± 1.01	<0.0001
Conventional cardiometabolic biomarkers				
Fasting insulin (μIU/mL)	9.7 ± 7.5	14.5 ± 9.7	6.9 ± 3.6	<0.0001
Fasting glucose (mg/dL)	96.9 ± 16.5	97.5 ± 16.9	97.1 ± 16.9	0.87
Triglycerides (mg/dL)	62.4 ± 50.8	70.6 ± 35.6	54.9 ± 20.4	<0.0001
hsCRP (mg/dL)	1.2 ± 3.7	2.7 ± 6.0	0.4 ± 1.1	<0.0001

	All n = 262	Obese n = 84	Lean n = 150	P^b
IL-6 (pg/mL)	1.1 ± 1.3	1.3 ± 1.3	0.9 ± 1.3	0.04
Leptin (ng/mL)	8.2 ± 9.7	15.1 ± 12.2	4.7 ± 5.7	<0.0001
Highest quartile of physical activity, % (N)	26.5 (66)	16.7 (13)	31.5 (46)	0.02
Sugar-sweetened beverage intake (servings/day)	0.4 ± 0.7	0.5 ± 0.8	0.3 ± 0.5	0.06
Dietary intake >4 times/week, % (N)				
Fast-food	0.8 (2)	2.6 (2)	0.0 (0)	0.05
Eggs	8.1 (20)	6.5 (5)	9.0 (13)	0.52
Whole dairy	43.7 (108)	42.9 (33)	43.5 (63)	0.93
Margarine	3.2 (8)	2.6 (2)	3.5 (5)	0.73
Meat	8.1 (20)	5.2 (4)	9.0 (13)	0.31
Pubertal status				
Body hair, % (N)	19.3 (48)	30.8 (24)	14.4 (21)	0.004
Breast development, girls only % (N)	32.5 (41)	55.1 (27)	13.6 (9)	<0.0001
Deepening of voice, boys only % (N)	6.5 (8)	6.9 (2)	6.3 (5)	0.90

[a] Values represent mean ± SD unless otherwise indicated.

[b] Pearson's chi-squared test for categorical variables; Wald test for continuous variables.

Table 2

Plasma metabolite concentrations of obese versus lean children

		Metabolite concentration z-score		
		Obese	**Lean**	
	Factor loading	**BMI 95th %ile** $n = 84$	**BMI <85th %ile** $n = 150$	P^b
Factor 4: mean ± SD = 0.00 ± 1.52				
Valine	0.83	1.17 ± 2.75	−0.54 ± 2.47	<0.0001
Leucine	0.76	1.38 ± 2.93	−0.37 ± 2.66	<0.0001
Phenylalanine	0.72	1.23 ± 3.02	−0.27 ± 2.66	<0.0001
Isoleucine	0.71	1.18 ± 2.84	−0.37 ± 2.47	<0.0001
Propionylcarnitine (C3)	0.66	0.95 ± 2.66	−0.44 ± 2.29	<0.0001
2-methylbutyrylcarnitine (C5)	0.63	0.80 ± 2.66	−0.03 ± 2.38	0.0002
Isovalerylcarnitine	0.60	1.34 ± 2.75	−0.33 ± 2.38	<0.0001
Isobutyrylcarnitine	0.56	1.19 ± 3.21	0.33 ± 2.84	0.001
Tryptophan	0.54	1.07 ± 3.02	0.21 ± 2.66	0.0007
3-methyl-2-oxovalerate (KMV)	0.52	0.92 ± 2.75	−0.11 ± 2.38	<0.0001
Kynurenine	0.52	0.56 ± 2.93	−0.19 ± 2.57	0.002
Tyrosine	0.51	1.54 ± 2.57	0.04 ± 2.29	<0.0001
Gamma-glutamylleucine	0.51	0.53 ± 2.93	−0.23 ± 2.57	0.002
4-methyl-2-oxopentanoate (KIC)	0.51	0.73 ± 2.75	−0.23 ± 2.38	<0.0001
Factor 9: mean ± SD: 0.00 ± 1.62				
4-androsten-3beta,17beta-diol disulfate 1[*]	0.86	0.61 ± 2.84	−0.49 ± 2.47	<0.0001
Dehydroepiandrosterone sulfate (DHEA-S)	0.84	0.45 ± 2.47	−0.41 ± 2.11	<0.0001
Epiandrosterone sulfate	0.79	0.14 ± 2.93	−0.70 ± 2.66	0.0007
Androsterone sulfate	0.79	0.30 ± 2.66	−0.38 ± 2.38	0.002
4-androsten-3beta,17beta-diol disulfate 2[*]	0.78	0.48 ± 3.21	−0.74 ± 2.84	<0.0001
Pregn steroid monosulfate[*]	0.76	0.70 ± 2.75	−0.22 ± 2.38	<0.0001
Pregnen-diol disulfate[*]	0.70	0.30 ± 2.75	−0.14 ± 2.38	0.05
Pregnenolone sulfate	0.65	0.71 ± 3.02	−0.09 ± 2.66	0.002
Andro steroid monosulfate 2[*]	0.61	0.41 ± 2.66	−0.46 ± 2.38	<0.0001

[a] Estimates represent mean ± SD and are adjusted for child's sex, age, and race/ethnicity.

[b] From the Wald test.

[*] 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.

Table 3

Associations between adiposity indicators and factor scores

| | | | | | | | Difference (95% CI) in factor scores | | | | |
	N	Unadjusted	*N*	Model 1	*N*	Model 2
Factor 4						
per 1 SD DXA total fat mass (kg)	237	0.40 (0.22, 0.58)	222	0.47 (0.27, 0.67)	222	0.48 (0.28, 0.68)
per 1 SD BMI z	262	0.49 (0.31, 0.66)	246	0.49 (0.31, 0.68)	246	0.52 (0.33, 0.71)
per 1 SD SS + TR (mm)	262	0.50 (0.32, 0.67)	245	0.56 (0.36, 0.76)	245	0.58 (0.38, 0.78)
per 1 SD DXA trunk fat mass (kg)	237	0.42 (0.24, 0.60)	222	0.50 (0.30, 0.69)	222	0.50 (0.30, 0.71)
per 1 SD waist circumference (cm)	261	0.51 (0.33, 0.68)	245	0.59 (0.39, 0.78)	245	0.60 (0.41, 0.80)
per 1 SD SS/TR	261	0.25 (0.07, 0.43)	245	0.29 (0.09, 0.48)	245	0.29 (0.10, 0.48)
Factor 9						
Boys						
per 1 SD DXA total fat mass (kg)	112	0.77 (0.49, 1.05)	103	0.80 (0.48, 1.11)	103	0.88 (0.56, 1.20)
per 1 SD BMI z	131	0.50 (0.22, 0.78)	121	0.48 (0.17, 0.79)	121	0.51 (0.20, 0.82)
per 1 SD SS + TR (mm)	130	0.78 (0.49, 1.06)	120	0.75 (0.44, 1.07)	120	0.82 (0.50, 1.13)
per 1 SD DXA trunk fat mass (kg)	112	0.78 (0.50, 1.06)	103	0.79 (0.48, 1.10)	103	0.86 (0.55, 1.18)
per 1 SD waist circumference (cm)	131	0.68 (0.41, 0.95)	121	0.67 (0.37, 0.97)	121	0.72 (0.41, 1.03)
per 1 SD SS/TR	130	0.58 (0.30, 0.85)	120	0.55 (0.27, 0.84)	120	0.59 (0.30, 0.88)
Girls						
per 1 SD DXA total fat mass (kg)	125	0.23 (−0.04, 0.51)	119	0.17 (−0.13, 0.47)	119	0.08 (−0.25, 0.40)
per 1 SD BMI z	131	0.23 (−0.03, 0.49)	125	0.26 (−0.02, 0.53)	125	0.20 (−0.10, 0.51)
per 1 SD SS + TR (mm)	131	0.36 (0.10, 0.62)	125	0.32 (0.03, 0.60)	125	0.31 (−0.01, 0.63)
per 1 SD DXA trunk fat mass (kg)	125	0.27 (−0.01, 0.54)	119	0.18 (−0.12, 0.49)	119	0.10 (−0.23, 0.43)
per 1 SD waist circumference (cm)	130	0.32 (0.05, 0.58)	124	0.23 (−0.07, 0.53)	124	0.17 (−0.16, 0.51)
per 1 SD SS/TR	131	0.31 (0.06, 0.56)	125	0.20 (−0.08, 0.49)	125	0.15 (−0.14, 0.44)

Model 1: Adjusted for maternal education and child age, sex, race/ethnicity, and fast-food intake.

Model 2: Model 1 + parent-reported pubarchal/pubertal phenotype.

Table 4

Associations of maternal peripartum factors with offspring metabolite factor scores in mid-childhood among 262 mother-child pairs in Project Viva

	Difference (95% CI) in factor score		
	Unadjusted *n = 262*	**Model 1** *n = 247*	**Model 2** *n = 247*
Factor 4			
Maternal obesity[a]	0.66 (0.23, 1.10)	0.61 (0.13, 1.08)	0.38 (−0.08, 0.85)
Excessive GWG[b]	0.25 (−0.12, 0.62)	0.29 (−0.09, 0.67)	0.01 (−0.38, 0.41)
Gestational diabetes[c]	−0.35 (−1.01, 0.31)	−0.57 (−1.22, 0.09)	−0.38 (−1.02, 0.26)
Factor 9			
Maternal obesity[a]	0.22 (−0.25, 0.69)	0.09 (−0.41, 0.59)	−0.08 (−0.58, 0.43)
Excessive GWG[b]	0.07 (−0.33, 0.47)	0.13 (−0.27, 0.53)	0.13 (−0.27, 0.53)
Gestational diabetes[c]	0.29 (−0.42, 1.00)	0.27 (−0.42, 0.96)	0.44 (−0.24, 1.13)

Model 1 Adjusted for mother's education; father's BMI; and child's age, sex, and race/ethnicity. Models for GWG and GDM were additionally adjusted for pre-pregnancy BMI.

Model 2 Adjusted for Model 1 + child's BMI z-score.

[a] Defined as BMI 30 kg/m^2 (*n* = 59) vs. BMI <30 kg/m^2 (*n* = 203)

[b] GWG: gestational weight gain; Excessive GWG (*n* = 54) vs. normal/inadequate GWG (*n* = 108).

[c] GDM: *n* = 22 vs. no GDM: *n* = 240.