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Associations of Prenatal Metabolomics Profiles with Early Childhood Growth Trajectories and Obesity Risk in African Americans: the CANDLE Study

Qi Zhao, MD, PhD[1], **Zunsong Hu, PhD**[1], **Mehmet Kocak, PhD**[1], **Jiawang Liu, PhD**[2,3], **Jay H. Fowke, PhD**[1], **Joan C. Han, MD**[4], **David Kakhniashvili, PhD**[5], **Kaja Z. Lewinn, ScD**[6], **Nicole R. Bush, PhD**[7], **W. Alex Mason, PhD**[1], **Frances A. Tylavsky, DrPH**[1]

[1]Department of Preventive Medicine, College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38163, USA

[2]Medicinal Chemistry Core, Office of Research, University of Tennessee Health Science Center, Memphis, TN 38163, USA

[3]Department of Pharmaceutical Science, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA

[4]Departments of Pediatrics and Physiology, University of Tennessee Health Science Center, and Children's Foundation Research Institute, Le Bonheur Children's Hospital, Memphis, TN 38103, USA

[5]Proteomics and Metabolomics Core, Office of Research, University of Tennessee Health Science Center, Memphis, TN 38163, USA

[6]Department of Psychiatry, University of California San Francisco, San Francisco, CA 94118, USA

[7]Departments of Pediatrics and Psychiatry, University of California San Francisco, San Francisco, CA 94118, USA

Abstract

Objective—Prenatal metabolomics profiles, providing measures of in utero nutritional and environmental exposures, may improve the prediction of childhood outcomes. We aimed to identify prenatal plasma metabolites associated with early childhood body mass index (BMI) trajectories and overweight/obesity risk in offspring.

Corresponding Author: Qi Zhao, MD, PhD, Associate Professor of Epidemiology, Department of Preventive Medicine, College of Medicine, University of Tennessee Health Science Center, 66 Pauline St, Room 639, Memphis, TN 38163, Tel: 901.448.2382, Fax: 901.448.7041, qzhao11@uthsc.edu.

Contributions

QZ and FAT–designed research; QZ, JL, DK, and FAT–conducted metabolomics data collection; QZ, ZH, and MH–conducted data analysis; KZL, NRB, WAM, and FAT–collected clinical data and biosamples and management of the CANDLE study; QZ and FAT–drafted the manuscript; JHF, JCH, and KZL– provided critical review and revisions of the manuscript; QZ– had primary responsibility for final content; and all authors: reviewed and approved the final version of the manuscript. The authors report no conflicts of interest.

Conflict of Interest

The authors declare that they have no conflict of interest.

Methods—This study included 450 African American mother-child pairs from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood Study. An untargeted metabolomics analysis was performed on the mothers' plasma samples collected during the second trimester. The children's BMI-z-score trajectories from birth to age 4 [rising-high- (9.8%), moderate- (68.2%), and low-BMI (22.0%)] and overweight/obesity status at age 4 were the main outcomes. The least absolute shrinkage and selection operator (LASSO) was used to select the prenatal metabolites associated with childhood outcomes.

Results—The mothers were 24.5 years old on average at recruitment, 76.4% having education less than 12 years and 80.0% with Medicaid or Medicare. In LASSO, seven and five prenatal metabolites were associated with the BMI-z-score trajectories and overweight/obese at age 4, respectively. These metabolites are mainly from/relevant to the pathways of steroid biosynthesis, amino acid metabolism, vitamin B complex, and xenobiotics metabolism (e.g., caffeine and nicotine). The odds ratios (95% CI) associated with a one SD increase in the prenatal metabolite risk scores (MRSs) constructed from the LASSO-selected metabolites were 2.97 (1.95–4.54) and 2.03 (1.54–2.67) for children being in the rising-high-BMI trajectory group and overweight/obesity at age 4, respectively. The MRSs significantly improved the risk prediction for childhood outcomes beyond traditional prenatal risk factors. The increase (95% CI) in the area under the receiver operating characteristic curves were 0.10 (0.03–0.18) and 0.07 (0.02–0.12) for the rising-high-BMI trajectory (P=0.005) and overweight/obesity at age 4 (P=0.007), respectively.

Conclusions—Prenatal metabolomics profiles advanced prediction of early childhood growth trajectories and obesity risk in offspring.

Keywords

childhood obesity; growth trajectory; maternal exposure; metabolomics; pregnancy

Introduction

Childhood obesity is still one of the most serious public health challenges in the US. According to the latest data of CDC, one third of children and adolescents aged 2–19 years had obesity (18.5%) or overweight (16.6%) in 2015–2016 (1). Childhood obesity is linked with a wide range of serious consequences, such as adult obesity, type 2 diabetes, and cardiovascular disease. The impact extends to increased costs throughout the health care system (2–4). Identification of early risk factors for childhood obesity would lead to interventions to prevent or delay the onset of cardiometabolic chronic diseases later in life and reduce their economic impact on the healthcare system.

According to the Developmental Origins of Health and Disease hypothesis, the intrauterine environment impacts fetal development in ways that could have irreversible and lifelong consequences (5). Indeed, prenatal risk factors reflecting maternal metabolism (6–10), maternal diet (11, 12), or exposure to environmental toxins or endocrine-disrupting chemical exposures (13–16), have been associated with an increased risk for childhood obesity. These findings strongly support that investigating in utero exposure is important for identifying early risk factors for the development of obesity.

The emerging metabolomics technology, which can systematically profile small molecules in biofluids, cells, and tissues, has been applied in the research of perinatal health (17), such as gestational diabetes (18), fetal growth restriction (19, 20), and preterm birth (21). These studies demonstrate that prenatal metabolomics changes are underlying the pathogenesis of maternal complications during pregnancy and fetal intrauterine development. Since perinatal conditions have long-term effects on children's health, changes in prenatal metabolomics profiles may also underlie the developmental origin of health conditions in offspring. However, to the best of our knowledge, no metabolomics studies have been conducted to investigate prenatal circulating metabolomics profiles associated with childhood weight gain and obesity risk. Therefore, this study aimed to conduct comprehensive metabolomics profiling among pregnant women and examine their associations with children's growth trajectories and obesity risk using a contemporary birth cohort of the US.

Methods

Study Cohort

Study subjects were drawn from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) study, a prospective birth cohort of mother-child dyads in Shelby County, Tennessee, which is a major part of the Memphis metropolitan area (6, 11, 22). The CANDLE study enrolled a total of 1 503 women aged 16–40 years during their 2nd trimester of pregnancy resulting in 1 455 live births. The sample accrued between 2006 and 2011, demographically representing Shelby County, a highly disadvantaged urban population. The study participants are primarily African Americans (AA) (65.5%) and European Americans (32.4%). In this study, we included 450 randomly selected AA mother-child pairs from the CANDLE study (Supplemental Figure 1). The characteristics of this sample were similar with all the AA mothers and children in the CANDLE study (Supplemental Table 1). The CANDLE study was conducted in accordance with the Helsinki Declaration and approved by the Institutional Review Board of The University of Tennessee Health Science Center. Informed consent was given by participants 18 years or older, while assent was given by those less than 18 years and consent provided by their legally authorized representative prior to enrollment.

Maternal Measures and Metabolomics Analysis

Self-administered questionnaires were used to collect sociodemographic information (age, race/ethnicity, education, insurance type, and marital status), lifestyle (cigarette smoking and alcohol use during pregnancy), parity, and medical history at enrollment. Self-reported height and weight prior to pregnancy were collected at enrollment and used to calculate pre-pregnancy BMI as weight (in kilograms) divided by the square of height (in meters).

The nonfasting maternal plasma samples collected in the second trimester (at recruitment) and stored at −80°C were used to conduct prenatal metabolomics profiling. The untargeted metabolomics analysis was performed using the Metabolon Discovery HD4™ Platform (Metabolon Inc., Morrisville, NC), which includes four ultra-high-performance liquid chromatography-mass spectrometry methods (Supplementary Materials). A total of 949 metabolites with known structural identity (named biochemicals) were identified in the study

samples. After excluding 69 metabolites with missing/below-the-detection-limit > 80% of the samples, 880 metabolites were included in the present study.

Child Measures

Birth weight and length of the children were extracted from medical charts by research assistants (23). The body weight and length/height were also measured at each annual visit until 4 years old using the methods guided by the NHANES protocol (24). The sex- and age-specific BMI-z-score for each child were calculated based on the World Health Organization growth standards (< 2 years) and the Center for Disease Control and Prevention (CDC) growth charts (≥ 2 years) as recommended by CDC (25). In addition, a 24-hour dietary recall of the child including food and beverage consumed was administered at 2, 2.5, and 3 years, respectively. All the 24-hour dietary recall data have been processed using Nutrition Data System for Research software (http://www.ncc.umn.edu/products/) to yield energy, macro, and micronutrient intakes. At the 4-year visit, the frequency of engaging in vigorous physical activity per week was also asked and collected.

Childhood Outcomes

Childhood growth trajectories have been demonstrated to be more predictive for obesity risk later in life than a single growth measurement. An early childhood high-BMI or rapid BMI gain trajectories are associated with a higher risk of obesity later in life (26). In this study, both BMI trajectories and overweight and obesity status at age 4 were the early childhood outcomes of interest. 1) Three BMI-z-score trajectories (rising-high-, moderate-, and low-BMI) among the 450 children were identified using the latent class growth modeling approach (Figure 1) (27). The rising-high-BMI trajectory was considered as the risk trajectory for obesity later in life and selected as the main trajectory outcome. 2) Childhood overweight and obesity at age 4 were defined according to CDC criteria (28). Overweight was defined as a BMI at or above the 85th percentile and below the 95th percentile for children of the same age and sex. Obesity was defined as a BMI at or above the 95th percentile for children of the same age and sex.

Statistical Analysis

We imputed the missing values for the metabolites with missing rates < 50% using the K-nearest neighbor imputation method implemented in the R package “impute”. After imputation, the values were natural log-transformed, followed by median normalization and auto-scaling. For metabolites with missing rates between 50% and 80%, the abundance of metabolites was recoded as the following: missing values were coded as 0; values below the median of the non-missing values were coded as 1; and values above the median of the non-missing values were coded as 2.

The partial least-squares discriminant analysis (PLS-DA) method was used to examine whether prenatal metabolomics profiles could distinguish children’s BMI-z-score trajectory groups and overweight/obesity status at age 4. PLS-DA is a classification method based on PLS regression (29). A permutation test was used to examine whether differences found between groups were significant (30). In the permutation test, the outcome groups were permuted and randomly assigned 1000 times. For each permutated dataset, a sum of squares

between/sum of squares within (B/W) ratio from the PLS-DA model was calculated for the class assignment predictions. A p value is calculated as the proportion of the times that class separation based on randomly assigned sample is at least as good as the one based on the original data. The least absolute shrinkage and selection operator (LASSO) regression implemented in the R package “glmnet” was used to select the most predictive metabolites in association with childhood outcomes of interest (31). In LASSO, a penalization parameter λ is added to the least-squares criterion, resulting in that the most predictive variables are selected into the model and the coefficients of the remaining variables are shrunk to zero. In our analysis, a 10-fold cross-validation approach was used to determine the parameter λ that gave the minimum mean error. We fit LASSO with the 880 metabolites and traditional prenatal risk factors for childhood obesity, including maternal age at pregnancy, pre-pregnancy BMI, parity, education levels, insurance types, alcohol drinking, and smoking during pregnancy. Gestational diabetes, gestational weight gain, birth weight, or gestational age at birth were not included in the LASSO model because these variables might be on the same pathways as the prenatal metabolites and mediate their effects on childhood outcomes. The associations between the final LASSO-selected maternal metabolites and these four potential mediators were examined.

A metabolite risk score (MRS) was constructed using the sum of selected metabolite levels weighted by the effect sizes from the LASSO model. Logistic regression models were used to examine the association between the prenatal MRS and childhood outcomes of interest with the adjustment for the traditional prenatal risk factors. Considering childhood nutrition and physical activities might also be confounding factors but had less complete data (69.3% having both variables), we did a sensitivity analysis to further adjust for the mean total energy intake of 2–3 years and weekly frequency of vigorous physical activity at age 4 in the logistic regression models. The area under the receiver operating characteristic (ROC) curve (AUC) was used to examine whether the MRS improved the risk prediction of the studied childhood outcomes beyond traditional prenatal risk factors.

Results

Table 1 shows the characteristics of the CANDLE mothers and children included in this study. The average (SD) age of the mothers at recruitment was 24.5 (5.1) years, with relatively low education (76.4% of ≤ 12 years of education) and a high rate of being covered by Medicaid or Medicare (80.0%). Nearly 60% of the mothers were overweight (24.2%) or obese (33.6%) before pregnancy. Almost 30% of the children were overweight (13.1%) or obese (15.5%) at 4 years old.

Prenatal Metabolic Profiles and Childhood Outcomes

Using the PLS-DA method, we found that the overall prenatal metabolomics profiles could significantly separate the children in the different BMI-z-score trajectories (*P for imputation* = 0.017). Although the PLS-DA model was not statistically significant for the BMI groups (normal/overweight/obese groups) at age 4 (*P for imputation = 0.267*), we still saw a trend for separation, suggesting the difference in metabolomics profiles among the three groups (Figure 2).

LASSO-selected Prenatal Metabolites Associated with Childhood Outcomes

The LASSO method selected seven and five metabolites associated with the BMI-z-score trajectories and overweight/obesity at age 4, respectively (Table 2). The metabolite β-sitosterol was negatively associated with both the rising-high-BMI trajectory and overweight/obesity risk at age 4. Most of the metabolites are involved in/relevant to the pathways of lipid and amino acid metabolism. Interestingly, three of the identified metabolites are related to xanthines (5-acetylamino-6-amino-3-methyluracil, 1,3-dimethyluric acid, and 1-methyluric acid), and one derives from nicotine metabolism (hydroxycotinine). Most of the outcome-related metabolites showed very low or no correlations with each other (Supplemental Figure 2) and were not associated with gestational diabetes, gestational weight gain, gestational birth weight, or gestational age at birth (Supplemental Table 2).

Associations of the MRSs and Childhood Outcomes

After adjusting for prenatal covariates (maternal age at pregnancy, pre-pregnancy BMI, parity, education level, insurance type, alcohol drinking, and smoking during pregnancy), the odds ratios (ORs) (95% CI) associated with an SD increase in the MRSs were 2.97 (1.95–4.54) and 2.03 (1.54–2.67) for the rising-high-BMI trajectory ($P = 4.6\times10^{-7}$) and overweight/obesity ($P = 4.3\times10^{-7}$) in the children, respectively. With additional adjustment for childhood energy intake and physical activity, the associations were attenuated but still significant. The ORs (95% CI) of the children decreased to 2.30 (1.40–3.77) and 1.84 (1.36–2.50) for being in the rising-high-BMI trajectory group ($P = 9.5\times10^{-4}$) and overweight/obese ($P = 7.6\times10^{-5}$), respectively.

Prediction Improvement with the MRSs

Adding the MRSs to the models with the traditional prenatal risk factors significantly improved the prediction of childhood outcomes (Figure 3). For the childhood outcome of being in the rising-high-BMI trajectory, the AUC of ROC (95% CI) significantly increased from 0.72 (0.64–0.80) to 0.82 (0.76–0.88) with the ΔAUC (95% CI) of 0.10 (0.03–0.18) ($P = 0.005$). For the childhood outcome of being overweight/obese at age 4, the AUC of ROC (95% CI) significantly increased from 0.67 (0.61–0.79) to 0.74 (0.68–0.79) with the ΔAUC (95% CI) of 0.07 (0.02–0.12) (P=0.007).

Discussion

In this birth cohort of African American women and their children, we identified 11 maternal prenatal metabolites associated with early childhood growth trajectories and/or overweight/obesity risk in offspring, highlighting the importance of the maternal metabolism of lipids, amino acids, vitamins, caffeine, and nicotine in child development. Furthermore, the prenatal MRS constructed by the metabolites significantly improved the prediction of the studied childhood growth outcomes beyond traditional prenatal risk factors.

The maternal diet provides energy and key nutrients for fetal development and provides programming for post-natal growth. Our study highlighted the importance of the metabolism of lipids (β-sitosterol) and proteins (hydroxyproline and hydroxyasparagine) in the

developmental origins of childhood obesity. Mechanistically, β-sitosterol, a phytosterol, decreases the intestinal absorption of cholesterol thereby limiting the transfer of cholesterol to the fetal circulation via placental transfer (32). Protective effects of lower maternal cholesterol are probably mediated through in utero programming of adipocyte development (33), epigenetics changes (34), and offspring's eating behavior (35). Evidence is emerging that phytosterol supplementation during pregnancy can normalize cholesterol in both mothers and children in observational studies of human and animal studies (36, 37). More human studies including clinical trials are still warranted to investigate the protective effect of prenatal β-sitosterol on postnatal growth in the future.

Hydroxyproline and hydroxyasparagine are posttranslationally modified amino acids proline and asparagine, respectively. Hydroxyproline is a major component of the protein collagen and plays key roles in collagen stability. Increased hydroxyproline levels in amniotic fluid have been associated with neural tube defects (38). Hydroxyproline is also a biomarker for the consumption of processed meat which has relatively high collagen content and is undesirable because of deficiency in essential amino acids (39, 40). Prenatal intake of processed meat has been associated with childhood obesity (41, 42). Hydroxyasparagine is found in fibrillin which is essential for the formation of elastic fibers in connective tissue. Mutations of the coding gene of fibrillin lead to abnormalities of cardiovascular development, such as Marfan syndrome (43). However, like hydroxyproline, the mechanisms underlying the effect of maternal hydroxyasparagine on child development and obesity risk are still unclear.

The metabolites, methylmalonic acid and FAD, implicated the potential importance of the metabolism of B complex vitamins during pregnancy. Specifically, the B complex vitamins are required for decarboxylation, transamination, acylation, oxidation, and reduction of substrates that ultimately are used for energy utilization (44). These processes are used in the metabolism of amino acid, fatty acid, cholesterol, steroid, glucose synthesis, methylgroup transfer and the supply of single-carbon units for DNA synthesis. Elevation of methylmalonic acid can be caused by vitamin B12 deficiency. Maternal vitamin B12 deficiency has been associated with an increased risk of preterm delivery and intrauterine growth retardation (45, 46). FAD is derived from vitamin B2 and an indispensable cofactor for the cellular antioxidant defense of oxidative stress which has been linked with adiposity development and elevated risks of metabolic disorders in offspring (47, 48). Our findings suggest further investigation targeting these B complex vitamins may be a plausible pathway for their preventive effects on childhood obesity.

We identified some other metabolites which might be indicators of food/drug intake. Metabolic indicators of caffeine (5-acetylamino-6-amino-3-methyluracil) and theophylline (1,3-dimethyluric acid, and 1-methyluric acid), were found to be positively associated with accelerated infant growth and childhood obesity. Both caffeine and theophylline are xanthines, a group of purine alkaloids, and can be from the intake of coffee, tea, and chocolate. Theophylline also can be one of the products of caffeine metabolic processing in the liver (49). Our findings are in line with previous epidemiology studies which reported maternal caffeine intake during pregnancy is a risk factor for childhood obesity (50–52). Caffeine is a neural stimulant and can penetrate the placental barrier and enter

fetal circulation (53). Caffeine can affect fetal neurological development which regulates appetite and metabolic processes (54, 55). In addition, prenatal caffeine exposure induces a lower level of fetal blood leptin, resulting in greater appetite and energy storage (56). We also identified another protective metabolite for accelerated early childhood growth, 2,6-dihydroxybenzoic acid, which is a potential marker of consumption of beers and olives (e.g., olive oil). However, the biological mechanisms underlying these associations with the childhood outcome are still largely unknown.

The metabolomics study also identified a couple of metabolic indicators of environmental exposures. Hydroxycotinine along with cotinine is the main metabolite of nicotine and a biomarker of tobacco exposure from either active or passive smoking. Maternal smoking during pregnancy has been associated with an increased risk of many adverse health outcomes in offspring, including overweight and obesity (13, 57). Nicotine can be transported across the placenta. Animal studies have shown that the administration of nicotine to pregnant mothers results in smaller birthweight but increased body fat and faster postnatal weight gain (58). Fetal exposure to nicotine causes abnormal cell proliferation, differentiation, and synaptic activity in the brain and the peripheral autonomic pathways, which may lead to permanent changes in the hypothalamic regulation of food intake and energy expenditure (59). Another interesting metabolite is isoeugenol sulfate which derives from isoeugenol. Isoeugenol is a commonly used fragrance added to many commercially available hair and skin products and occurs naturally in the essential oils of plants. An animal study has shown the developmental toxicity of isoeugenol in rats, including intrauterine growth retardation (60).

Our study has several strengths. First, this study is the initial study to investigate prenatal metabolomics profiles in association with childhood growth and obesity risk. Second, the contemporary birth cohort included repeated measurements during early childhood, which enabled longitudinal growth trajectory analysis among offspring. Third, we included African Americans mother-child pairs, as we targeted an understudied population with a high obesity rate in the US (61). Fourth, an untargeted or global metabolomics platform which is a hypothesis-free approach was used to maximize the study's ability to identify novel prenatal biomarkers for childhood growth outcomes of interest. Furthermore, a comprehensive set of confounding factors was controlled for in the analyses examining the associations of prenatal plasma metabolites with childhood outcomes. No fasting was required for the blood sample collection considering the potential difficulty for the research clinic visit of some pregnant women. This might have introduced variance to the metabolites because of diet. However, the nonfasting metabolomics profiles might be more likely to reflect the metabolic status of the pregnant women during most time of the day and more relevant to the fetus development. This may be partially supported by the previous findings that similar with fasting measures, nonfasting glucose and triglycerides were still associated with ischemic heart disease (62, 63). A potential limitation is the lack of replication samples for this metabolomics study. However, we identified the associations of the plasma metabolites of caffeine and nicotine with childhood growth outcomes, which are in line with previous studies based on self-reported measures of these two substances (13, 50–52, 57). This may support the robustness of our study findings to some extent.

In conclusion, our study has provided novel metabolites and metabolic pathways for advancing the understanding of the in utero environment and the obesity development in offspring. The outcome-related metabolites provide early prenatal markers for better predicting the development of childhood obesity, and most of them are likely to be modifiable through maternal dietary and behavioral interventions during pregnancy. Future studies are warranted to validate our findings and to clarify the biological mechanisms underlying the effects of these prenatal metabolites on childhood outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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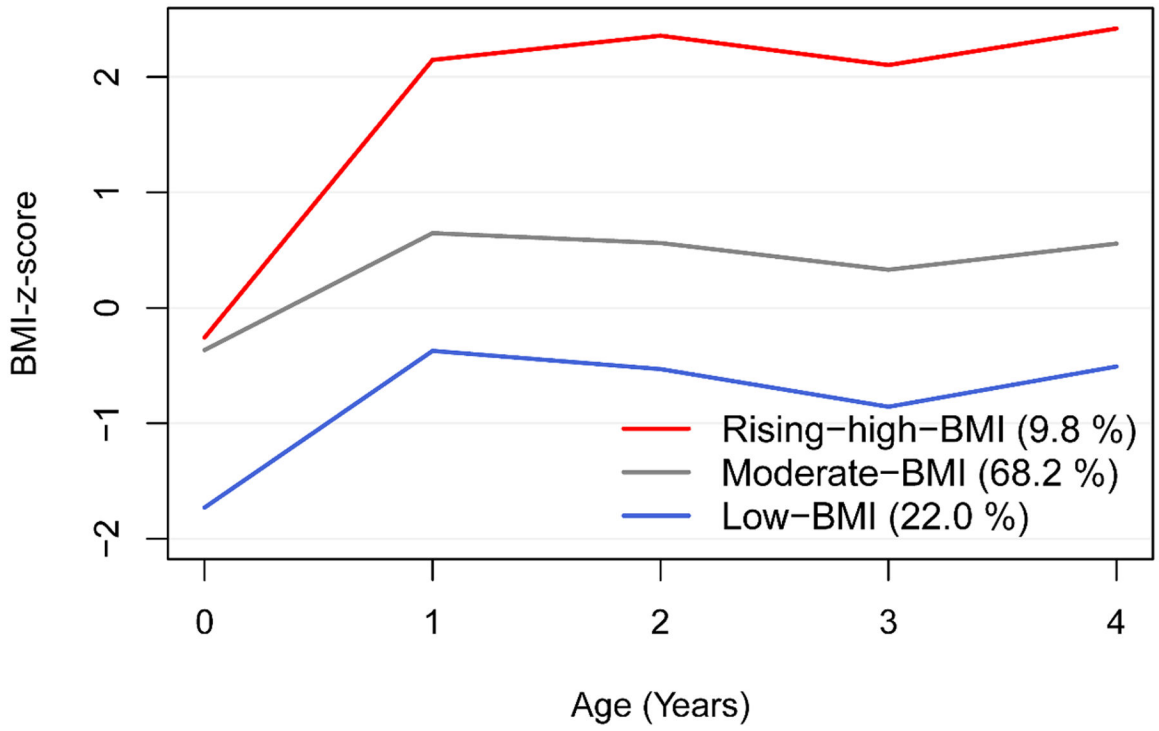

Figure 1.
BMI-z-score trajectories of the studied children.

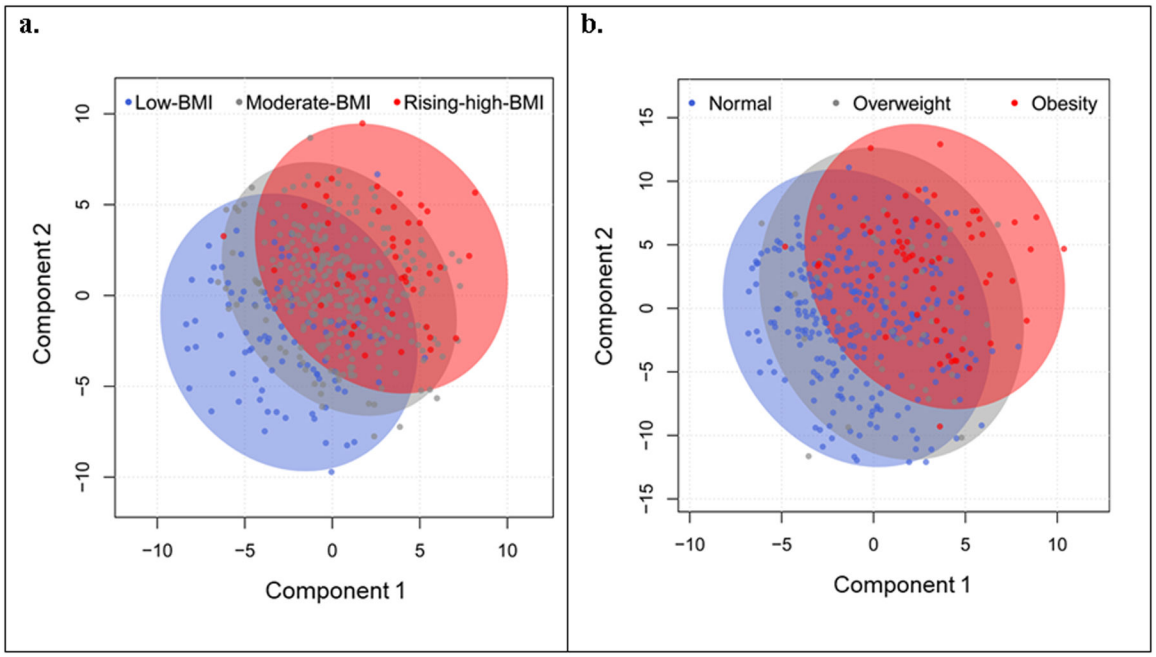

Figure 2.

The classification of the childhood growth trajectory groups and weight groups at age 4 using PLS-DA. PLS-DA, partial least-squares discriminant analysis. a. PLS-DA analysis for BMI-z-score trajectories groups; b. PLS-DA analysis for weight groups.

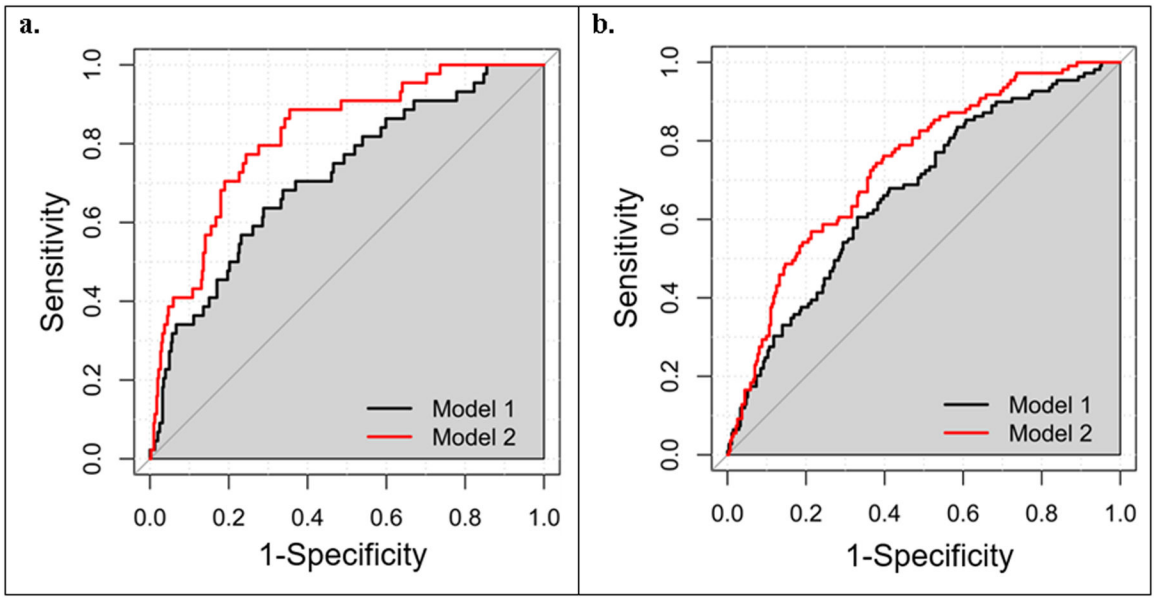

Figure 3.

ROC curves of predictive models. a. ROC curves for the rising-high-BMI trajectory; b. ROC curves for overweight/obesity at age 4. Model 1: traditional risk factors including maternal age, education, health insurance, smoking and drinking status, parity, and pre-pregnancy BMI; Model 2: Model + metabolite risk score. ROC, receiver operating characteristic.

Table 1.

Characteristics of the CANDLE mothers and children

Variables	Mean (SD) or percentage (N=450)
Mothers	
Age, years	24.5 (5.1)
Education (≤ 12 years), %	76.4
Insurance (Medicaid or Medicare), %	80.0
Smoking during pregnancy, %	9.1
Alcohol drinking during pregnancy, %	5.8
Parity (primiparous), %	27.1
Pre-pregnancy BMI, kg/m2	28.3 (8.3)
Pre-pregnancy overweight, %	24.2
Pre-pregnancy obesity, %	33.6
Gestational weight gain, kg	14.4 (8.3)
Gestational diabetes mellitus, %	4.5
Children	
Male, %	51.8
Gestational age at birth, weeks	38.5 (2.3)
Birth weight, kg	3.1 (0.6)
BMI-z-score at birth	−0.7 (1.4)
BMI-z-score at age 1	0.7 (1.2)
BMI-z-score at age 2	0.2 (1.2)
BMI-z-score at age 3	0.3 (1.3)
BMI-z-score at age 4	0.5 (1.2)
Overweight at age 4, %	13.1
Obesity at age 4, %	15.5

BMI, body mass index; SD, standard deviation.

Table 2.

Childhood outcome-associated metabolites selected by LASSO

Metabolite	Class	Pathways[a]	β/OR[b]
Rising-high-BMI trajectory			
β-Sitosterol	Lipid	Steroid biosynthesis	−0.194/0.824
Methylmalonic acid	Organic acid	Branch-chain amino acid, pyrimidine, and propanoate metabolism	−0.005/0.996
Hydroxyasparagine	Amino acid	Alanine and aspartate metabolism	0.143/1.153
Hydroxyproline	Amino acid	Arginine and proline metabolism	0.052/1.054
5-Acetylamino-6-amino-3-methyluracil	Xenobiotics	Xanthine metabolism	0.069/1.072
Hydroxycotinine	Xenobiotics	Tobacco metabolite	0.068/1.071
2,6-Dihydroxybenzoic acid	Xenobiotics	Food component/plant	−0.043/0.958
Overweight/obesity at age 4			
FAD	Cofactors and Vitamins	Riboflavin metabolism	−0.142/0.867
β-Sitosterol	Lipid	Steroid biosynthesis	−0.133/0.875
Isoeugenol sulfate	Xenobiotics	Food component/plant	0.087/1.091
1,3-Dimethyluric acid	Xenobiotics	Xanthine metabolism	0.015/1.015
1-Methyluric acid	Xenobiotics	Xanthine metabolism	0.005/1.005

LASSO, least absolute shrinkage and selection operator.

a. Involved in or relevant to the pathways;

b. Associated with a standard deviation increase in the metabolite level