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Metabolomic profiles of overweight/obesity phenotypes during adolescence: A cross-sectional study in Project Viva

Wei Perng[1], **Sheryl L Rifas-Shiman**[2], **Joanne Sordillo**[2], **Marie-France Hivert**[2,3], **Emily Oken**[2,3,4]

[1]Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

[2]Division of Chronic Disease Research Across the Lifecourse (CoRAL), Department of Population Medicine, Harvard Medical School/Harvard Pilgrim Health Care Institute, Boston, MA, USA

[3]Diabetes Unit, Massachusetts General Hospital, Boston, MA

[4]Department of Nutrition, T. H. Chan Harvard School of Public Health, Boston, MA, USA

Abstract

Objective: Characterize metabolomics profiles of four overweight/obese (OWOB) and metabolic risk (MetRisk) phenotypes among 524 adolescents age ~13 years.

Methods: We created a four-level phenotype variable (non-OWOB & low MetRisk, non-OWOB & high MetRisk, OWOB & low MetRisk, OWOB & high MetRisk) using BMI percentile to define OWOB, and derived high vs. low MetRisk as the 4^{th} vs. 1^{st}–3^{rd} quartiles of a z-score calculated as the average of 5 externally-standardized z-scores for waist circumference, HOMA-IR, HDL, triglycerides, and SBP. We then examined associations of nine metabolite patterns derived from principal components analysis with phenotype after accounting for age, sex, race, and pubertal status.

Results: Five metabolite patterns differed with respect to phenotype: Factor 1 comprised long-chain fatty acids and was lower among non-OWOB & high MetRisk (−0.90 [95% CI: −1.39, −0.42]) vs. non-OWOB & low MetRisk (referent). Factors 5 (branched chain amino acids; BCAAs), 8 (diacylglycerols) and 9 (steroid hormones) were highest among OWOB & high MetRisk. Factor 7 (long-chain acylcarnitines) was higher among non-OWOB & high MetRisk (0.47 [0.04, 0.91]) and lower among OWOB & low MetRisk (−0.36 [−0.68, −0.04]).

Conclusions: Long-chain fatty acids, BCAAs, acylcarnitines, diacylglycerols, and steroid hormones differed by weight status and metabolic phenotype.

Keywords

metabolomics; obesity; metabolic risk; branched chain amino acids

Contact information: Wei Perng, 12474 East 19th Ave, Room 208, Aurora, CO 80045, Wei.Perng@CUAnschutz.edu.

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INTRODUCTION

Child and adolescent obesity is one of the greatest current public health concerns as it is a major risk factor for type 2 diabetes and cardiovascular disease. There are several distinct and overlapping pathways linking obesity to these chronic conditions, including but not limited to insulin resistance, dyslipidemia, and hypertension (1).

Higher body mass index (BMI, kg/m^2) is an indicator of overall body size that correlates with excess fat mass and a worse metabolic profile (2). This makes sense given that adipose tissue is an endocrine organ that secretes biologically active molecules that could interfere with normal physiological processes (3). However, researchers have recently uncovered subgroups of adults with normal metabolic profiles despite being overweight or obese (BMI ≥25 kg/m^2), as well as persons who are not overweight/obese (BMI <25 kg/m^2) but exhibit metabolic abnormalities. Existence of these discordant phenotypes would have important implications for the use of BMI to identify high-risk persons for further metabolic assessment. From a research perspective, characterizing physiological differences between the various obesity phenotypes may provide insight into etiological pathways of metabolic disease.

Metabolomics, or the systematic and comprehensive study of low-molecular-weight compounds in biological tissues and fluids, has emerged as a powerful tool to refine our knowledge of more nuanced differences in metabolic phenotypes. To date, a handful of small studies in adults have used metabolomics to characterize differences in circulating metabolites that differ between metabolically healthy vs. metabolically unhealthy obesity in adult populations (4–8). These studies identified differences in compounds on branched chain amino acid and lipid metabolism pathways between metabolically healthy vs. unhealthy obesity. A major limitation to current literature is the lack of knowledge regarding the existence of these phenotypes earlier in life, when there is greater potential to re-route adverse health trajectories.

In this analysis, we sought to: (1) characterize prevalence of four obesity/metabolic risk phenotypes among multi-ethnic adolescents in the Project Viva cohort: non-overweight/obese with low metabolic risk, non-overweight/obese with high metabolic risk, overweight/obese with low metabolic risk, and overweight/obese with high metabolic risk; and (2) use untargeted metabolomics profiling to identify metabolite profiles that differ between these phenotypes to gain insight into underlying pathways.

METHODS

Study population

This study includes adolescent participants of Project Viva, an ongoing pre-birth cohort study recruited from a multi-specialty group practice in eastern Massachusetts (Atrius Harvard Vanguard Medical Associates) (9). Of the 2128 children enrolled at birth, 1038 attended the “early teen” research visit at age 11–16 years, 636 of whom provided fasting blood. Of them, we excluded 76 with inadequate serum volume for untargeted metabolomics profiling. For the present study, we further excluded those missing information on key

variables for the analysis including: race/ethnicity (n=1), pubertal status (n=2), body mass index (n=1), and the metabolic syndrome risk z-score (n=32), leaving an analytical sample of 524 participants. 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 early teen visit, trained phlebotomists 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.

Overweight/obesity and metabolic risk phenotype

We created a four-level categorical variable comprising the different combinations of overweight/obesity (yes vs. no) and metabolic risk ("MetRisk," high vs. low). We classified individuals as overweight/obese (OWOB) vs. non-OWOB based on weight (kg) measured via an electronic scale (Tanita Corporation of America, Inc., Arlington Heights, IL) and height (cm) measured using a calibrated stadiometer (Shorr Productions, Olney, MD). We used these values to calculate BMI (kg/m^2), and age and sex standardized percentiles using the Centers for Disease Control and Prevention (CDC) growth reference (10). We defined OWOB as BMI≥85^{th} percentile for age and sex, and as non-overweight/obese (non-OWOB) otherwise. We noted that there were 17 underweight participants with BMI<5^{th} percentile, and excluded them in sensitivity analysis.

Following Viitasalo et al. (11), we derived a metabolic syndrome risk z-score as the average of externally age- and sex-standardized values for the following components: waist circumference (cm), the homeostatic model of insulin resistance (HOMA-IR), serum high density lipoprotein cholesterol (HDL) levels, serum triglycerides, and systolic blood pressure (SBP). We measured waist circumference at the level of the umbilicus via a non-stretchable measuring tape, and standardized these values using data from the CDC reference data for children and adults 2011–2014 (12). We calculated HOMA-IR (glucose *mg/dL* x insulin *μIU/mL*)/405) using fasting glucose values assessed enzymatically, and fasting insulin measured via an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN). We standardized HOMA-IR values using data from 12-to-19-year old participants of the National Health and Nutrition Examination Survey (NHANES) 1999–2002 (13). Lipid profile was measured enzymatically and standardized according to age- and sex-specific data for participants aged 12–19 years in NHANES III. We measured systolic (SBP) and diastolic blood pressure (DBP) in quintuplicate using biannually-calibrated automated oscillometric monitors (Dinamap Pro100, Tampa, Florida). We used the average of the five measurements for the statistical analysis and standardized the values using the American Academy of Pediatrics' age-, sex-, and height-specific data (14). After deriving the external z-scores for each of the components, we took the average across the five variables (with HDL z-score multiplied by −1) and defined "high MetRisk" as being in the fourth quartile of the MetRisk z-score.

Untargeted metabolomics profiling

We carried out untargeted metabolomic profiling in fasting plasma collected at the early teen research visit via Metabolon's multi-platform technique comprising ultra-performance liquid chromatography/mass spectrometry (UPLC-MS/MS) with a heated electrospray ionization source and mass analyzer (15–17). Subsequently, metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standards that included retention time, molecular weight (m/z), adducts, in-source fragments, and associated mass spectrometry spectra using software developed at Metabolon. Details are in the Online Supplemental Material (OSM).

The laboratory analysis yielded 1135 metabolites. In the present study, we considered only endogenous metabolites, of which there were 1005 in this data set. Prior to formal statistical analysis, we imputed missing values for metabolites as ½ the minimum detected value, and $\log_{10}$-transformed each compound. We assessed for batch effects via principal components analysis plots but observed no notable clustering race/ethnicity or sex.

Covariates

While numerous lifestyle (e.g., diet, physical activity, sleep) and environmental (e.g., exposure to toxicants, air pollution) characteristics have potential to impact the relationship between overweight/obesity phenotype and circulating metabolites, this initial descriptive analysis seeks to identify metabolite profiles that may differentiate between the different phenotypes after accounting for a parsimonious set of biological covariates that impact metabolism in youth – namely, child's sex, age, race/ethnicity, and pubertal status. At the early-teen research visit, participants' parents reported on their pubarchal/pubertal phenotype based on appearance of body hair, breast development for girls, and body hair, facial hair, and deepening of voice for boys on a scale of 1 (no development) to 4 (full development). We combined the characteristics as an ordinal summary score of breast development and body hair for girls, and the mean of deepening of voice, facial hair, and body hair for boys for use as a covariate in multivariable models, as well as dichotomized pubertal status as pre-pubertal (puberty score=1) vs. pubertal (puberty score>1) for use as a covariate.

Data analysis

First, we created our explanatory variable of interest: a categorical variable comprising the four combinations of overweight/obesity ("OWOB," yes vs. no) and metabolic risk ("MetRisk," high vs. low) such that "not OWOB & low MetRisk" was the reference category, and the other categories included "not OWOB & high MetRisk," "OWOB & low MetRisk," and "OWOB & high MetRisk."

Prior to examining associations of the four-level phenotype variable with serum metabolites, we used principal components analysis (PCA) for dimension reduction. We consolidated the 1005 endogenous metabolites into metabolite patterns using PCA with an orthogonal rotation as we have previously done (18). The procedure generates as many factors (principal components) as there are original metabolites, so we used standard criteria of the Scree plot "break" and Eigenvalues >1 to determine the number of factors to retain. We then

examined associations of the four-level variable with the retained factor scores as continuous outcomes using a multivariable linear regression model that accounted for sex, age, race/ethnicity, and pubertal status. Given the relatively small number of factors retained, we considered any factor associated with the four-level phenotype at alpha=0.05 in our discussion of results, also indicating which factors were statistically significant after Bonferroni correction based on the Type 3 *P*-value. We also conducted stratified analysis by OWOB status where we compared factor scores for participants with high vs. low MetRisk, as well as associations of each metabolic biomarker component with the factor scores of interest.

We also carried out some sensitivity analyses. First, we excluded 17 underweight participants (BMI<5th percentile) and re-ran all models. Exclusion of these persons resulted in no differences in the direction, magnitude, or precision of estimates (data not shown), so we included them in the final models. Second, in addition to defining high MetRisk as the fourth quartile of the MetRisk z-score, we also considered the following categorizations: (1) clinical metabolic syndrome based on the International Diabetes Federation (IDF) definition for adolescents (n=0) (19); (2) fourth quartile of all five components of the risk score (n=5); (3) fourth quartile of at least three components of the risk score (n=97) similar to adult definitions of metabolic syndrome; (4) upper 10th percentile of the MetRisk z-score (n=52). Given the non-existent/small sample size for definitions #1 and #2, and that use of definitions #3 (Table S1) and #4 (Tables S2) yielded similar associations to our original definition, so we focus our discussion of results using the fourth quartile of the MetRisk z-score as the threshold for high metabolic risk.

All analyses were performed using the Statistical Analyses System 9.4 software (SAS Institute Inc., Cary, NC) unless otherwise indicated.

RESULTS

Descriptive statistics

Mean±SD age of the 524 study participants was 13.0±0.7 years. Approximately half (48.3%) were female and the majority were white (63.0%). Most (63.9%) participants were non-OWOB & low MetRisk, and 19.5% were classified as OWOB & high MetRisk. Approximately 11.1% were OWOB & low MetRisk, and 5.5% were non-OWOB & high MetRisk. Table 1 shows sociodemographic characteristics for all participants, and within strata of the four-level variable. As expected, waist circumference was higher for the two OWOB categories vs. the two non-OWOB categories, and all metabolic biomarkers were higher in the categories with high MetRisk than those with low MetRisk, except for HDL, which showed the opposite trend.

Associations between phenotype and metabolite patterns

Based on the Scree plot and Eigenvalues, we retained nine factors from the PCA that accounted for 47% of variance in the original 1005 endogenous metabolites. Table 2 shows associations of the four-level phenotype (reference group=not OWOB & low MetRisk) with the factor scores. We detected differences in Factors 1, 5, 7, 8, and 9 with respect to the four-

level phenotype, with statistically significant Type 3 tests for a difference after Bonferroni correction (alpha=0.05/9=0.006) for Factors 1 (P=0.002) and 8 (P=0.001).

Table 3 shows the composition (metabolite annotations and sub-pathways) of Factors 1 (long-chain fatty acids; LCFA), 5 (branched chain amino acids; BCAAs), 7 (long-chain acylcarnitines), 8 (diacylglycerols) and 9 (steroid hormones) based on the top ten highest factor loadings into each factor. Adolescents who were non-OWOB & high MetRisk had 0.90 (95% CI: 0.42, 1.39) units lower score for Factor 1 (LCFAs) than the reference category (non-OWOB & low MetRisk). The OWOB & high MetRisk group also had a lower score for this pattern, though not as strongly compared to the non-OWOB & high MetRisk category, and with the upper CI crossing the null (−0.27 [95% CI: −0.56, 0.02]).

For Factor 5 (BCAAs), we observed the highest factor score for the OWOB & high MetRisk group (0.58 [95% CI: 0.17, 0.98] units for OWOB & high MetRisk vs. non-OWOB & low MetRisk).

For Factor 7 (long-chain acylcarnitines), participants categorized as non-OWOB & high MetRisk had a higher factor score than the referent (0.47 [95% CI: 0.04, 0.91] units), whereas those denoted as OWOB & low MetRisk had a lower score for this pattern (−0.36 [95% CI: −0.68, −0.04] units).

Factor 8 (diacylglycerols) was highest among youth with the worst metabolic health (0.88 [95% CI: 0.42, 1.35] for OWOB & high MetRisk vs. non-OWOB & low MetRisk), with a similar but non-significant effect among non-OWOB & high MetRisk participants (0.75 [95% CI: −0.04, 1.54] units).

Finally, Factor 9 (steroid hormones) was highest among youth classified as OWOB & high MetRisk (0.44 [95% CI: 0.16, 0.72] for OWOB & high MetRisk vs. non-OWOB & low MetRisk).

Association of phenotype with metabolite factors after stratification by weight status

Table S3 displays similar associations to Table 2, but stratified by non-OWOB (n=362) vs. OWOB (n=160) status. Associations for the non-OWOB group are similar to those in Table 2 given that in both analyses, non-OWOB & low MetRisk was the referent. Among OWOB participants, those with high MetRisk had a lower score for Factor 1 (LCFAs; −0.60 [95% CI: −0.99, −0.20]), and higher scores for Factor 5 (BCAAs; 0.69 [95% CI: 0.12, 1.25]) and 8 (diacylglycerols; 0.84 [95% CI: 0.17, 1.51]) than the low MetRisk group.

Associations between individual components of the MetRisk z-score and metabolite patterns

When we examined associations of the individual biomarkers (as externally-standardize age- and sex-specific z-scores) with factors of interest within strata of OWOB status (Table 4), we noted concordance in the direction of associations across the metabolic biomarkers.

Among participants classified as non-OWOB, HOMA-IR was inversely related to Factor 1 (−0.55 [95% CI: −0.74, −0.36]). Waist circumference was inversely associated with Factor 7

(long-chain acylcarnitines; −0.52 [95% CI: −0.95, −0.10]), whereas triglycerides (0.35 [95% CI: 0.17, 0.54]) and SBP (0.17 [95% CI: 0.02, 0.32] were positively associated with this metabolite pattern. Serum triglycerides were positively related to Factor 8 (diacylglycerols; 0.99 [95% CI: 0.63, 1.30]), and waist circumference was positively associated with Factor 9 (steroid hormones; 0.58 [95% CI: 0.11, 1.05]).

Among participants classified as OWOB, higher waist circumference, HOMA-IR, and inverted HDL each corresponded with a lower score for Factor 1 (LCFAs). Conversely, higher waist circumference and HOMA-IR corresponded with higher Factor 5 (BCAAs). Serum triglycerides were positively related to both Factor 8 (diacylglycerols) and Factor 9 (steroid hormones).

DISCUSSION

In this cross-sectional analysis of 524 multi-ethnic adolescents in the U.S., we assessed prevalence of four phenotypes based on weight status and an externally-standardized metabolic syndrome risk z-score. The majority of participants in the sample were non-overweight/obese with low metabolic risk, and the second most common group was overweight/obese with high metabolic risk. We also identified subsets of youth who were overweight/obese with low metabolic risk, and those who were not overweight/obese with high metabolic risk. We identified five metabolite patterns that differed across these groups, discussed below.

Factor 1: Long-chain fatty acids

In comparison to the healthiest participants (i.e., those who are not overweight/obese with low metabolic risk), those who were not overweight/obese but had high metabolic risk had a lower score for Factor 1. Youth classified as overweight/obese with high metabolic risk also had a lower score for this pattern (albeit at approximately 1/3 the magnitude), suggesting that this metabolite pattern is more strongly correlated with metabolic risk than weight status. Indeed, within strata of weight status, we detected a lower Factor 1 score with high vs. low metabolic risk in youth who were overweight/obese as well as non-overweight/obese.

This metabolite pattern was characterized by several long-chain fatty acids, including anti-inflammatory and anti-obesogenic polyunsaturated fatty acids (PUFAs). Given the benefits of long-chain fatty acids and PUFAs to metabolic health, our finding of an inverse relationship between this pattern and metabolic risk is not surprising. For example, linoleic acid and its derivative dihomo-linoleic acid are N-6 PUFAs involved in the biosynthesis of prostaglandins and cell membranes. While the literature on linoleic acid, obesity, and metabolic health in humans is mixed (20–22), rodent studies found beneficial effects of dietary linoleic acid administration on insulin resistance and glucose tolerance (23, 24), which aligns with our findings of an inverse relationship between HOMA-IR and Factor 1 irrespective of weight status. Other metabolites in this pattern that have been correlated with metabolic health is palmitoleate, which has recently received attention as a protective factor against heart disease risk (25).

The relatively large decrement in the factor score for this metabolite pattern among youth who are non-overweight/obese with high metabolic risk vs. the referent (non-overweight/obese and low metabolic risk) is worth noting. One explanation is that these participants consume fewer foods that contain the fatty acids captured by this pattern (e.g., plant oils and nuts as a source of eicosenoate (26); blackcurrant seed, borage, and hemp seed oils as sources of gamma-linolenic acid, from which dihomo-linoleic acid (27) is derived) which would lead to lower circulating levels of these compounds, as well as acylcarnitine intermediates of their metabolism. Another explanation is that participants with high metabolic risk exhibit disturbances to the desaturation and elongation pathways involved in metabolism of dietary precursors of fatty acids of interest, thereby leading to lower levels of compounds in this metabolite pattern and contributing to greater metabolic risk. Unfortunately, the cross-sectional and observational nature of our data does not enable us to untangle these nuances.

Factor 5: Branched chain amino acids

Factor 5 included compounds that are part of a branched chain amino acid (BCAA) metabolite pattern previously identified as a correlate of obesity and insulin resistance in this (18) and other populations (28). Metabolites within this factor include: valine, one of three BCAAs that have been implicated in incident insulin resistance and development of type 2 diabetes in adults (29–32); C3 and C5 acylcarnitines (2-methylbutyrylcarnitine, isovalerylcarnitine, propionylcarnitine) that are intermediates of BCAA catabolism; and kynurenate, a metabolite of the large neutral amino acid tryptophan, which is often elevated in concomitance with BCAAs since it competes for a shared protein transporter (33).

The score for this metabolite pattern was highest among youth classified as overweight/obese with high metabolic risk, and we noted no differences in the score with respect to high vs. low metabolic risk among non-overweight/obese participants. The metabolic biomarkers most strongly associated with Factor 5 were waist circumference and HOMA-IR, which align with findings from a joint human/rodent study that identified elevated BCAAs as a correlate of excess adiposity that promotes insulin resistance (28). The fact that this metabolite pattern was associated with metabolic risk only in the context of overweight/obesity suggests a unique contribution of excess adiposity above and beyond that of the metabolic biomarkers.

Factor 7: Acylcarnitines

In comparison to the reference group, Factor 7 was higher among youth who were not overweight/obese with high metabolic risk, but lower among participants classified as overweight/obese with high metabolic risk. Uon stratifying by weight status, the only association that persisted was a higher factor score among youth who were non-overweight/obese with high metabolic risk. When we examined associations of this pattern with individual biomarkers, Factor 7 exhibited associations only among non-overweight/obese participants. Specifically, we noted inverse associations with waist circumference and direct relations with serum triglycerides and SBP.

Top annotated metabolites in Factor 7 were long-chain acylcarnitines (e.g., oleoylcarnitine, linoleoylcarnitine (C18:2), dihomo-linolenoylcarnitine (C20:3n3 or 6), arachidonoylcarnitine (C20:4), and dihomo-linoleoylcarnitine (C20:2)) that accumulate during specific metabolic conditions, including fasting (34). In a small study of 7 normal weight and 7 women with obesity aged 20–53 years, Hoppel et al. (35) reported elevated acylcarnitines while fasting – including the long-chain species – among both normal weight and participants with obesity. In this study, we noted elevations in these metabolites only among non-overweight/obese participants. This could be indicative of lipodystrophy, a condition that does not necessarily co-occur with excess adiposity and is characterized by defective carnitine biosynthesis and carnitine accumulation in cells and organs (36). The discrepancy in findings in our study vs. those in adults could also be due to fundamental differences in metabolism in adolescents vs. adults, and the fact that Hoppel et al. study was a controlled feeding study whereas participants of Project Viva provided blood after an 8-hour fast. Factor 7 also included compounds on energy production pathways – e.g., 5-oxoproline, which is a marker of oxidative stress associated with hepatic glutathione production (37) that modulates DNA synthesis (38); and pyruvate, which is a product of glycolysis in the citric acid cycle.

Factor 8: Diacylglycerols

Factor 8 was composed of diacylglycerols (DAGs). The score for this pattern was highest among youth who were overweight/obese with high metabolic risk. With respect to the individual biomarkers, triglycerides were most strongly associated with this metabolite pattern, which makes sense given that DAGs are precursors to triglycerides.

Several metabolites within this pattern, including palmitoyl-linoleoyl-glycerol (16:0/18:2) and palmitoyl-linoleoyl-glycerol (16:1/18:2), are common emulsifiers used in bakery products, shortening, whipped toppings and other confections. Endogenously, alterations in DAG composition could be indicative of either lipolytic or lipogenic activity – a distinction that we are not able to make due to the cross-sectional nature of our data. In a prospective study of rhesus macaques, Polewski et al. (39) identified differences in plasma DAG composition (i.e., decreased products of palmitate desaturation; increased essential N-6 fatty acids) that served as markers of worsening insulin resistance and metabolic syndrome onset. Additional research in humans is warranted to further interrogate the extent to which differences in plasma DAG composition is involved in metabolic disease etiology.

Factor 9: Androgen steroid hormones

Factor 9 was composed of several androgen steroid hormones, including dehydroisoanderosterone sulfate (DHEA-S), androstenediol disulfate, and androstenediol disulfate. As with Factor 5, we previously identified this metabolite pattern in Project Viva during mid-childhood as a correlate of obesity and several metabolic biomarkers (18). Given the steroid hormone composition of this pattern, it likely represents increased androgen synthesis, which is expected given the age range of the study sample. Additionally, elevated DHEA-S is a marker of polycystic ovarian syndrome, a common endocrine disorder among women of reproductive-age that is associated with adverse metabolic health – most notably, insulin resistance (40).

The score for this metabolite pattern was highest among children categorized as overweight/obese with high metabolic risk, although stratification by weight status yielded no difference in the factor score for participants with high vs. low metabolic risk. When we examined associations of individual metabolic biomarkers with this factor, we noted a positive association with waist circumference among non-overweight/obese participants, and a positive association with serum triglycerides among participants with overweight/obesity. The former could be related to excess central adiposity among participants who are farther along in the pubertal transition (41), whereas the latter may reflect the increase in serum triglycerides that occurs throughout adolescence (42) – a phenomenon that may be more pronounced among youth with overweight/obesity.

Strengths & limitations

Strengths of this study include the comprehensive metabolomics profiling analysis; large sample size; multi-ethnic study population, which may enhance generalizability of findings; and ability adjust for key covariates like pubertal status that contribute to variability in metabolism.

Limitations include the fact that we assessed metabolomics from fasting serum samples collected at a single time, which precludes our ability to infer on upregulation vs. downregulation of pathways. We also had a relatively small sample size (n=29) for non-overweight/obese with high metabolic risk group, which prevented us from conducting further sub-group analyses (e.g., assessing for race/ethnic or sex-specific associations) and may have reduced our ability to detect significant associations.

Conclusions

By age 11–16 years, we were able to identify not only the expected phenotypes of overweight/obese with high metabolic risk and normal weight with low metabolic risk, but also the rarer subtypes of overweight/obese with low metabolic risk and normal weight with high metabolic risk. Leveraging untargeted metabolomics data, we identified five metabolite patterns (long-chain fatty acids, BCAAs, acylcarnitines, diacylglycerols, and androgen steroid hormones) that differed across the phenotypes and provided insight into underlying biological pathways and mechanisms. Future research is required to validate our findings, evaluate the extent to which these metabolite patterns are associated with future health risks, and to identify dietary and lifestyle determinants of metabolites of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known on this topic?

- In adults, researchers have observed evidence of metabolically-healthy obesity and metabolically-unhealthy normal weight.
- Little is known regarding the existence of these phenotypes earlier in the life course, and the underlying biological pathways remain unclear.

What does this study add?

- In 524 adolescents, we found evidence of the existence of metabolically-healthy overweight/obesity (OWOB) and metabolically-unhealthy normal weight (non-OWOB), in addition to the usual phenotypes of metabolically-unhealthy OWOB and metabolically-healthy non-OWOB.
- Using untargeted metabolomics profiling of fasting plasma, we identified five metabolite patterns that differed with respect to OWOB status and high vs. low metabolic risk: long-chain fatty acids, branched chain amino acids, diacylglycerols, steroid hormones, and long-chain acylcarnitines.

Table 1

Background characteristics of 524 Project Viva participants.

	Overall *n*=524	Non-OWOB & Low MetRisk *n*=335	Non-OWOB & High MetRisk *n*=29	OWOB & Low MetRisk *n*=58	OWOB & High MetRisk *n*=102
Background characteristics					
Age (years)	13.0 ± 0.7	12.9 ± 0.7	13.0 ± 0.7	13.0 ± 0.8	13.0 ± 0.7
% Female	48.3% (253)	49.6% (166)	37.9% (11)	55.2% (32)	43.1% (44)
Race/ethnicity					
Black	15.5% (81)	11.7% (39)	6.9% (2)	22.4% (13)	26.5% (27)
Hispanic	4.6% (24)	4.5% (15)	6.9% (2)	3.5% (2)	4.9% (5)
White	63.0% (330)	66.8% (223)	75.9% (22)	51.7% (30)	53.9% (55)
Other	16.8% (88)	17.1% (57)	10.3% (3)	22.4% (13)	14.7% (15)
% started puberty	88.2% (462)	87.8% (294)	86.2% (25)	87.9% (51)	90.2% (92)
MetRisk z-score & its components					
Waist circumference (cm)	73.5 ± 11.7	67.3 ± 5.5	69.3 ± 5.4	82.1 ± 7.1	90.3 ± 10.7
SBP (mmHg)	107 ± 9	105 ± 8	117 ± 9	107 ± 8	112 ± 9
HDL (mg/dL)	55.9 ± 13.2	59.0 ± 13.1	49.8 ± 10.4	58.2 ± 11.8	45.9 ± 9.3
HOMA-IR	3.22 ± 2.34	2.40 ± 1.18	4.97 ± 3.02	3.11 ± 1.15	5.49 ± 3.47
Triglycerides (mg/dL)	68.7 ± 30.0	61.5 ± 23.4	100.9 ± 40.1	63.4 ± 27.3	86.1 ± 34.2
MetRisk z-score	−0.14 ± 0.46	−0.38 ± 0.28	0.37 ± 0.27	−0.14 ± 0.21	0.48 ± 0.39

Overweight/obese (OWOB) defined as ≥85%ile of age and sex according to the CDC 2000 growth reference for children 2–19 years. Metabolic risk (MetRisk) defined as being in the 4th quartile of an externally standardized metabolic syndrome risk z-score comprised of waist circumference, systolic blood pressure, reversed HDL, triglycerides, and HOMA-IR.

Table 2

Associations of overweight/obesity (OW/OB) with high or low meteabolic risk (MetRisk) with metabolite factor scores.

	β (95% CI)				
	Non-OWOB & Low MetRisk ***n*=335**	**Non-OWOB & High MetRisk** ***n*=29**	**OWOB & Low MetRisk** ***n*=58**	**OWOB & High MetRisk** ***n*=102**	***P*-value**
Factor 1	0.00 (Reference)	**−0.90 (−1.39, −0.42)**	0.27 (−0.09, 0.63)	−0.27 (−0.56, 0.02)	**0.0002** *
Factor 2	0.00 (Reference)	0.13 (−0.85, 1.11)	−0.37 (−1.10, 0.35)	0.01 (−0.57, 0.59)	0.75
Factor 3	0.00 (Reference)	0.04 (−0.64, 0.71)	−0.12 (−0.61, 0.38)	−0.32 (−0.72, 0.08)	0.47
Factor 4	0.00 (Reference)	−0.01 (−0.88, 0.85)	−0.38 (−1.02, 0.26)	−0.32 (−0.84, 0.19)	0.48
Factor 5	0.00 (Reference)	0.25 (−0.42, 0.91)	−0.06 (−0.55, 0.43)	**0.58 (0.17, 0.98)**	**0.03**
Factor 6	0.00 (Reference)	0.07 (−0.74, 0.88)	−0.32 (−0.92, 0.28)	−0.17 (−0.65, 0.31)	0.70
Factor 7	0.00 (Reference)	**0.47 (0.04, 0.91)**	**−0.36 (−0.68, −0.04)**	−0.13 (−0.39, 0.12)	**0.01**
Factor 8	0.00 (Reference)	0.75 (−0.04, 1.54)	0.11 (−0.47, 0.69)	**0.88 (0.42, 1.35)**	**0.001** *
Factor 9	0.00 (Reference)	−0.03 (−0.50, 0.45)	0.25 (−0.10, 0.60)	**0.44 (0.16, 0.72)**	**0.01**

[a] Estimates are adjusted for age, sex, race/ethnicity, and pubertal status. OWOB is defined as ≥85th percentile of age- and sex-specific BMI according to the CDC 2000 growth reference; high MetRisk defined as being in the 4th quartile of an externally standardized metabolic syndrome risk z-score.

Bold font indicates statistical significance at alpha = 0.05.

* Denotes statistical significance after Bonferroni correction ($P < \alpha < 0.05/9=0.006$)

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Table 3

Metabolite composition of factors of interest.

	Factor 1	
Metabolite	**Subpathway**	**Factor loading**
Oleate/vaccenate (18:1)	Long-chain fatty acid	0.91
Eicosenoate (20:1)	Long-chain fatty acid	0.88
Myristoleoylcarnitine (C14:1) *	Fatty acid metabolism (acylcarnitine)	0.87
10-Heptadecenoate (17:1n7)	Long-chain fatty acid	0.87
Dihomo-linoleic acid (20:2n6)	Polyunsaturated Fatty Acid (n3 and n6)	0.87
3-Hydroxylaurate	Fatty Acid, Monohydroxy	0.87
Palmitate (16:0)	Long-chain fatty acid	0.87
Linoleic acid (18:2n6)	Polyunsaturated Fatty Acid (n3 and n6)	0.86
Unknown	--	0.86
Palmitoleate (16:1n7)	Long-chain fatty acid	0.85
	Factor 5	
Metabolite	**Subpathway**	**Factor loading**
Valine	Leucine, Isoleucine and Valine Metabolism	0.68
Urea	Urea cycle; Arginine and Proline Metabolism	0.66
2-Methylbutyrylcarnitine (C5)	Leucine, Isoleucine and Valine Metabolism	0.65
Isovalerylcarnitine (C5)	Leucine, Isoleucine and Valine Metabolism	0.64
Unknown	Urea adduct	0.62
Propionylcarnitine (C3)	Fatty Acid Metabolism (also BCAA Metabolism)	0.62
2-Oxoarginine *	Urea cycle; Arginine and Proline Metabolism	0.61
Argininate *	Urea cycle; Arginine and Proline Metabolism	0.59
Dihydroorotate	Pyrimidine Metabolism, Orotate containing	0.59
Kynurenate	Tryptophan metabolism	0.57
	Factor 7	
Metabolite	**Subpathway**	**Factor loading**
Unknown	--	0.83
Oleoylcarnitine (C18:1)	Fatty Acid Metabolism(Acyl Carnitine)	0.66
Linoleoylcarnitine (C18:2) *	Fatty Acid Metabolism(Acyl Carnitine)	0.64

5-Oxoproline	Glutathione metabolism	0.64
Dihomo-linolenoylcarnitine (C20:3n3 or 6)*	Fatty Acid Metabolism(Acyl Carnitine)	0.64
Heme	Hemoglobin and Porphyrin Metabolism	0.64
Arachidonoylcarnitine (C20:4)	Fatty Acid Metabolism(Acyl Carnitine)	0.61
Pyruvate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.57
Dihomo-linoleoylcarnitine (C20:2)*	Fatty Acid Metabolism(Acyl Carnitine)	0.57
Adrenoylcamitine (C22:4)*	Fatty Acid Metabolism(Acyl Carnitine)	0.56
	Factor 8	
Metabolite	**Subpathway**	**Factor loading**
Diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	Diacylglycerol	0.64
Palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]*	Diacylglycerol	0.64
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	Diacylglycerol	0.63
Palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]*	Diacylglycerol	0.63
Oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	Diacylglycerol	0.60
Oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	Diacylglycerol	0.59
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]*	Diacylglycerol	0.58
Palmitoleoyl-arachidonoyl-glycerol (16:1/20:4) [2]*	Diacylglycerol	0.57
Oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	Diacylglycerol	0.56
Oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]*	Diacylglycerol	0.54
	Factor 9	
Metabolite	**Subpathway**	**Factor loading**
Androstenediol (3beta,17beta) disulfate (2)	Androgenic Steroids	0.82
21-Hydroxypregnenolone disulfate	Pregnenolone Steroids	0.74
Unknown	--	0.74
Androstenediol (3beta,17beta) disulfate (1)	Androgenic Steroids	0.70
Unknown	--	0.69
Unknown	--	0.69
Androstenediol (3beta,17beta) monosulfate (2)	Androgenic Steroids	0.69
Unknown	--	0.68
Dehydroisoandrosterone sulfate (DHEA-S)	Androgenic Steroids	0.67

Pregnenediol disulfate (C21H34O8S2) *	pregnenediol disulfate (C21H34O8S2) *	0.67

* 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 4

Associations of metabolic syndrome components with factor scores among adolescents classified as non-overweight/obese (non-OWOB) and overweight/obese (OWOB).

	β (95% CI) per 1 unit of each age- and sex-specific z-score[a]				
	Waist circ.	**HOMA-IR**	**Inverted HDL**	**Triglycerides**	**SBP**
Non-OWOB (n=364)[b]					
Factor 1	0.15 (−0.34, 0.63)	**−0.55 (−0.74, −0.36)**	−0.14 (−0.34, 0.05)	−0.20 (−0.41, 0.01)	−0.13 (−0.30, 0.04)
Factor 5	−0.29 (−0.93, 0.35)	0.08 (−0.18, 0.34)	0.01 (−0.24, 0.27)	−0.01 (−0.29, 0.27)	0.08 (−0.14, 0.30)
Factor 7	**−0.52 (−0.95, −0.10)**	0.09 (−0.08, 0.26)	0.14 (−0.03, 0.31)	**0.35 (0.17, 0.54)**	**0.17 (0.02, 0.32)**
Factor 8	0.25 (−0.51, 1.00)	0.22 (−0.09, 0.53)	0.22 (−0.07, 0.52)	**0.99 (0.68, 1.30)**	0.06 (−0.20, 0.33)
Factor 9	**0.58 (0.11, 1.05)**	0.04 (−0.15, 0.23)	0.07 (−0.11, 0.26)	0.06 (−0.14, 0.27)	−0.04 (−0.21, 0.13)
OWOB (n=160)[b]					
Factor 1	**−0.39 (−0.73, −0.05)**	**−0.26 (−0.40, −0.12)**	**−0.32 (−0.61, −0.03)**	0.07 (−0.19, 0.33)	−0.07 (−0.30, 0.16)
Factor 5	**0.75 (0.27, 1.23)**	**0.25 (0.04, 0.45)**	0.17 (−0.26, 0.59)	−0.30 (−0.67, 0.07)	0.06 (−0.27, 0.39)
Factor 7	0.26 (−0.05, 0.55)	0.00 (−0.12, 0.13)	0.22 (−0.04, 0.05)	0.02 (−0.20, 0.24)	0.05 (−0.15, 0.24)
Factor 8	0.28 (−0.29, 0.87)	0.07 (−0.19, 0.33)	0.40 (−0.11, 0.90)	**0.95 (0.54, 1.37)**	0.05 (−0.35, 0.44)
Factor 9	0.00 (−0.32, 0.32)	0.06 (−0.08, 0.20)	0.09 (−0.18, 0.37)	**0.23 (−0.00, 0.47)**	−0.01 (−0.22, 0.20)

[a] Estimates are adjusted for age, sex, race/ethnicity, and pubertal status

[b] OWOB and non-OWOB defined as ≥85th and <85th percentile, respectively, of age- and sex-specific BMI according to the CDC growth reference.

Bold font indicates statistical significance at alpha = 0.05.