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A systematic review of metabolomic studies of childhood obesity: State of the evidence for metabolic determinants and consequences

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Summary

Childhood obesity has become a global epidemic and carries significant long-term consequences to physical and mental health. Metabolomics, the global profiling of small molecules or metabolites, may reveal the mechanisms of development of childhood obesity and clarify links between obesity and metabolic disease. A systematic review of metabolomic studies of childhood obesity was conducted, following Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines, searching across Scopus, Ovid, Web of Science and PubMed databases for articles published from January 1, 2005 to July 8, 2020, retrieving 1271 different records and retaining 41 articles for qualitative synthesis. Study quality was assessed using a modified Newcastle–Ottawa Scale. Thirty-three studies were conducted on blood, six on urine, three on umbilical cord blood, and one on saliva. Thirty studies were primarily cross-sectional, five studies were primarily longitudinal, and seven studies examined effects of weight-loss following a life-style intervention. A consistent metabolic profile of childhood obesity was observed including amino acids (particularly branched chain and aromatic), carnitines, lipids, and steroids. Although the use of metabolomics in childhood obesity research is still developing, the identified metabolites have provided additional insight into the pathogenesis of many obesity-related diseases. Further longitudinal research is needed into the role of metabolic profiles and child obesity risk.

KEYWORDS
child, metabolomics, obesity, STOP project

Abbreviations: AAA, aromatic amino acid; BMI, body mass index; BCAA, branched-chain amino acid; CVD, cardiovascular disease; DHEA-S, dehydroisoandrosterone sulfate; FIA-MS, flow injection analysis mass spectrometry; GGT, gamma-glutamyltransferase; GC, gas chromatography; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IS, insulin resistance; LC, liquid chromatography; LDL, low-density lipoprotein; LPCs, lysophosphatidylcholines; NMR, nuclear magnetic resonance spectroscopy; MS, mass-spectrometry; MUO, metabolically unhealthy obese; NOS, Newcastle–Ottawa Scale; NMR, nuclear magnetic resonance; PECO, Participants, Exposure, Comparator and Outcomes; PCs, phosphatidylcholines; PRISMA, Preferred Reporting Items for Systematic Reviews; PCA, principal component analysis; SMs, sphingomyelins; TG, triglycerides; TMAO, trimethylamine N-oxide; T2D, type 2 diabetes; VLDL, very-low-density lipoprotein; WoS, web of science.

1 | INTRODUCTION

Childhood obesity has become a global epidemic in developed as well as in developing countries.[1] Increased body mass index (BMI) and adiposity during childhood carries significant long-term consequences including increased risk of later development of chronic disease such as type 2 diabetes (T2D) and cardiovascular disease (CVD) and worse psychological health, social and economic outcomes.[2–4] Furthermore, gaining weight in childhood is likely to lead to lifetime overweight and obesity.[5] Behavioral dimensions such as diet and physical activity, and an "obesogenic environment" that shapes those behaviors, have contributed to the spread of childhood obesity.[6,7] Elucidating the mechanisms of development of childhood obesity at a molecular level may contribute to identifying potential targeted intervention approaches to prevent childhood obesity and clarify the links between obesity and metabolic disease.

Metabolomics, the study of the set of small molecules or metabolites (<1500 KDa) in a biological sample, can improve understanding of biological responses due to changes at the genetic, epigenetic or protein level and also due to environmental exposures such as diet, physical activity, microbiome, and toxins. Assessment of the metabolome has typically been conducted through two analytical chemistry techniques: nuclear magnetic resonance spectroscopy (NMR) or mass-spectrometry (MS) coupled to various chromatographic separations such as liquid or gas chromatography (LC or GC). Furthermore, analyses may be untargeted if they aim to assess a comprehensive range of metabolite classes or targeted if the chemical analysis is optimized to focus on particular classes of molecules, which can provide gains in precision, quantification and identification. While the field is relatively young and rapidly evolving, metabolomics may help to define molecular phenotypes and better characterize the metabolic alterations associated with obesity, such as processes related to insulin resistance (IR)[8] and inflammation.[9]

While the literature regarding application of metabolomics to obesity in adults is relatively mature, fewer studies have been conducted specifically in child populations.[10] Metabolic signatures of obesity in children may differ from a signature observed in adults for reasons including a relatively shorter duration of obesity, ongoing linear growth, and pubertal hormones. Furthermore, metabolic alterations early in life may affect child propensity to overweight and obesity. We therefore conducted a systematic review of the literature related to obesity, BMI or other measures of adiposity and metabolomics in children.

2 | METHODS

This systematic review was accomplished based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements.[11]

A systematic search of Scopus, Ovid, Web of Science (WoS) and PubMed was conducted to identify the available evidence on metabolic signatures of childhood obesity. Different keywords were combined to retrieve metabolomics papers in the outcomes of interest, "adiposity or BMI" in "child." The search was carried out using free-text search terms (Table S1) with truncations to allow for different spellings as well as using Boolean operators. We included filters related to main language, the type of document and publication year. The search strategy is provided in the supporting information (Tables S2–S5). Covidence software[12] was used for importing articles from Scopus, Ovid, WoS and PubMed removing duplicates and screening the articles.

A two-stage screening process were followed: First, literature search and study selection based on titles and abstracts were carried out independently by two researchers (EC and CHL). Then, a full text screening was contacted on first stage eligible papers. Any disagreements in selection process were resolved by the involvement of a third reviewer (OR). Articles titles were screened using the "Participants," "Exposure," "Comparator," and "Outcomes" (PECO) statement components. A study was included if: it was conducted in human children (age ≤ 18 years) (P); analyzed the association between metabolomics (including metabonomics and metabolic profile, defined as studies that apply NMR or MS, coupled to various types of chromatography, of urine, serum, saliva or plasma samples, to measure at least 10 molecules) (E) and childhood obesity/overweight (or any of modification in body sizes related to obesity/overweight including BMI, weight, waist circumference, adiposity, fat mass, waist-to-hip ratio) (O); had as control group with children without obesity/overweight (or compared continuous/categorical variation of body size measurements) (C). Additionally, eligibility criteria were[1]: full-text is available,[2] the paper is written in English,[3] the paper describes an observational study (cross-sectional studies and longitudinal studies including prospective and retrospective cohort studies) excluding controlled experiments conducted in manipulated rather than naturalistic settings (clinical trial involving administration of drugs)[4] the paper is peer-reviewed[5] and the paper is not a letter, editorial, study/review protocol, or review article. Finally, we considered only studies published from Jan 1st 2005 to July 8, 2020.

Two authors independently extracted information on a predefined spreadsheet about study authors and year of study publishing, study population (country, size, and age), main outcomes, biofluid matrix, analytical platform and metabolite coverage, statistical analysis, covariate adjustment, and main findings. Metabolites extracted from these manuscripts were systematically annotated and stored for enhancing a synthetic data interpretation.

The risk of bias of included studies was assessed with a modified Newcastle–Ottawa Scale,[13] with additional fields related to metabolome coverage and metabolite level of identification as proposed by Sumner et al.[14] (Tables S7 and S8). We considered high quality articles as those that scored more than six stars.

The systematic review is registered with the International Prospective Register of Systematic Reviews (PROSPERO) database with registration number CRD42020208836.

3 | RESULTS

3.1 | Overview of studies

In the primary search, 1916 records were identified from four databases. After removing duplicates, 1271 publications were screened for abstracts and title. 1169 publications were then excluded after review of the abstracts for not meeting the inclusion/exclusion criteria. Among the 101 remaining articles, 59 were excluded because: 24 were not observational, 17 did not have full text manuscripts, 18 did not analyze the defined outcome, and 1 had the wrong population. Finally, the remaining 41 papers were subjected to systematic review and are summarized in Tables S8–S11. A PRISMA flow chart of the paper selection process is shown in Figure S1.

These articles were published between 2009 and 2020, with 12 published before 2015. Twelve studies were based in the USA, seven in Germany, five in Korea, four across multiple European countries, two in each of Spain, China and Italy, and one in each of Canada, Australia, Belgium, Czech Republic, and Denmark. While most studies included a broad range of ages, eight studies included children aged 5 years or younger, five studies only included children 10 years or younger and six studies included only children aged above 14 years. Sixteen studies included less than 100 participants while eight studies included more than 500 participants. Six studies used NMR analysis and 36 studies applied MS based assays. Seven MS studies used the Biocrates (Austria) kit that allows semi-quantitative measurement of up to 200 molecules from several classes, while six of the untargeted MS studies used the metabolic profiling service provided by the Metabolon company (USA) that uses a range of GC-MS and LC-MS assays to profile many hundreds of molecules. Seventeen were conducted on plasma, 15 were conducted on serum, six were conducted on urine (two of which also analyzed serum or plasma), three were conducted on umbilical cord blood, one study was conducted on dried blood spots, and one was conducted on saliva. Regarding quality of the studies, 24 were regarded as high quality (score >6) based on the adapted scoring assessment (Table S7).

Three categories of articles were identified: Cross-sectional studies that assessed metabolic profiles and adiposity at the same timepoint and formed most studies; longitudinal studies where metabolomic assessment was conducted in infancy prior to adiposity assessment; and intervention studies where metabolic profiles were assessed in relation to a lifestyle intervention designed to reduce BMI. The three categories are presented in separate sections below.

3.2 | Cross-sectional studies: Describing the metabolic signature of obesity

The 30 included cross-sectional studies are presented in Table S8, with extracted metabolite associations given in Table S9. Metabolites reported by at least two studies to be increased or decreased in blood with the adiposity measure are presented in Figure 1A. 22 studies analyzed BMI as categories of normal weight, overweight and/or obesity, seven studies used continuous BMI as the primary outcome, while one study used visceral fat as the primary outcome.

Three studies applied NMR analyses to blood. Bertram et al.[15] found no apparent effects of BMI on NMR profiles among 75 Danish adolescents, however the vast majority of participants were of normal weight. Bervoets et al.[16] compared obesity cases to controls among 112 Belgium children and adolescents (8–18 years). Cases had higher levels of lipids in lower density lipoproteins, N-acetyl glycoproteins, and lactate and lower levels of α-ketoglutarate, glucose, citrate, cholinated phospholipids (sphingomyelins and phosphatidylcholines), cysteine, histidine, glutamine, and proline. In addition, obesity cases were stratified by metabolically unhealthy obese (MUO) versus metabolically healthy based on additional clinical parameters relating to metabolic syndrome. MUO had higher levels of lipids and lactate, and lower levels of several amino acids (histidine, isoleucine, and glutamine) and cholinated phospholipids. Saner et al.[17] examined associations between various adiposity measures and serum NMR profiles among an Australian cohort of 214 children, all with obesity (aged 6–18 years), and reported increases in phenylalanine and decreases in acetate with BMI *z* score after false discovery rate (FDR) correction. Patterns were similar with the various measures although there were some differences in associations passing FDR significance, suggesting fat distribution may contribute to metabolic profiles. They also observed stronger associations among postpubescent boys.

Four studies used metabolomic analysis provided by the Metabolon company to profile overweight and obesity. Perng et al.[18] detected 345 compounds in plasma of 84 American children with obesity, 28 overweight, and 150 normal-weight of median age 7.7 years. They applied a principal component analysis (PCA) to reduce the metabolomic data to 18 components. A branched-chain amino acid (BCAA)-related pattern and an androgen hormone pattern were higher in children with obesity comparing to lean children. Both patterns were associated with biomarkers of cardiometabolic risk relating to IR, triglycerides and inflammation. Butte et al.[19] assessed 304 metabolites in plasma in 803 Hispanic children (mean ± SD age: 11.1 ± 3.9 years) of whom 56% (450) had obesity. This relatively well-powered and comprehensive study demonstrates the wide-spread disturbance of obesity in childhood on the metabolome, with 62 (20%) metabolites increased and 46 (15%) metabolites decreased within the group with obesity, after Bonferroni correction. BCAAs (leucine, isoleucine, and valine) and their catabolites, propionylcarnitine (C3) and butyrylcarnitine (C4), were elevated in children with obesity, while lysolipids and dicarboxylated fatty acids were lower. Steroid derivatives were markedly higher in children with obesity as were markers of inflammation, such as the peptide bradykinin. Tyrosine was the highest-ranked metabolite based on its contribution to the obesity classification. In principal component analysis, the BCAAs/aromatic amino acid (AAA) component and another AA component (asparagine, glycine, and serine) made the largest contributions to BMI, and two acylcarnitine components made the largest contributions to adiposity. Pitchika et al.[20] explored metabolomic profiles of serum among 485 children participating in a German prospective cohort (aged 6–16 years). Children who were overweight had

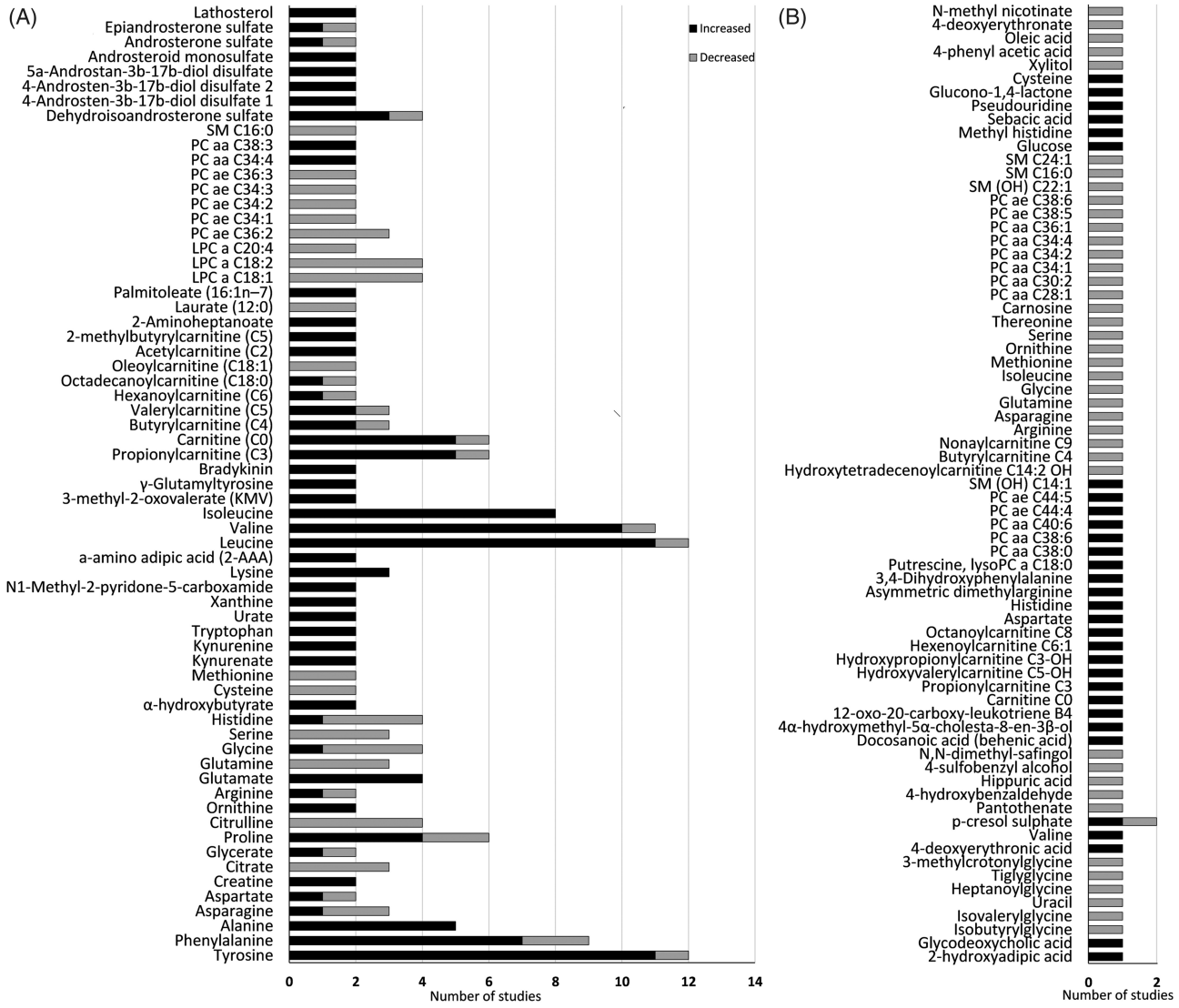

FIGURE 1 (A) Metabolites reported as associated with measures of child adiposity in at least two cross-sectional studies of blood. From 27 studies. (B) Metabolites reported as associated with measures of child adiposity in at least one cross-sectional study of urine. From four studies

significantly higher levels of various amino acids, lipids, and steroids; the co-factor n1-methyl-4-pyridone-3-carboxamide; the nucleotide urate, the peptide γ-glutamyltyrosine, and the plant derived piperine. Perng et al.[21] assessed metabolic profiles of 524 adolescents participating in the U.S. Project Viva cohort (mean age 13 years) who were classed into four groups as normal weight with and without metabolic syndrome, and overweight/obesity with and without metabolic syndrome. The 1005 metabolites were reduced to nine factors using PCA. Factors 8 (diacylglycerols) and 9 (steroid hormones) increased in overweight groups, while factor 7 (long-chain acylcarnitines) was decreased. Factors 1 (long-chain fatty acids) decreased and factor 5 (BCAAs) increased with metabolic syndrome, in both normal weight and overweight groups.

Farook et al.[22] compared untargeted LC-MS profiles of plasma among 42 Mexican American children (6–17 years). After correction for multiple testing only higher levels of bradykinin and lower levels of thyronine and the plant flavanone naringenin were observed among children with overweight and obesity. Zeng et al.[23] identified 30 endogenous metabolites following untargeted GC-MS profiling of plasma in 61 Chinese children aged 6-12 years. Metabolic profiles were not strikingly different, with only glycerate significantly higher in the obesity group in univariate analysis. Higher isoleucine, glycerate, 2,3,4-trihydroxybutyric acid and lower serine and phenylalanine were the most important predictors in two different multivariate analysis approaches that could discriminate those with obesity and normal weight groups.

Three studies employed the MS based Biocrates analysis kit. Lee et al.[24] compared weight status among 110 Korean children (aged 9–11) through analysis of plasma finding increased BCAAs, AAAs, alpha-amino adipic acid (2-AAA), free carnitine, and short-chain odd-number acylcarnitines among children with obesity. They reported that most phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), and

sphingomyelins (SMs) were lower among children with obesity. BCAAs were predictive of IR and metabolic syndrome score at follow-up 2 years later. Wahl et al.[25] applied Biocrates analysis in serum of 80 German children with obesity and 40 with normal weight between 6 and 15 years of age. Concentrations of two acylcarnitines (C12:1 and C16:1) were significantly increased in obesity compared to normal-weight group, while concentrations of glutamine, methionine, proline, and nine phospholipids were significantly decreased in children with obesity. They also found differences in 69 metabolite ratios including ratios between saturated and unsaturated LPCs, between saturated LPCs and PCs and between SMs and PCs, which were all increased in children with obesity. Lau et al.[26] assessed metabolome associations between BMI *z* score in 1192 children (aged 6–11 years) recruited from birth cohorts in six European countries using an untargeted NMR analysis in urine and Biocrates analysis in serum. Among the serum metabolites, positive associations with BMI *z* score included free carnitine, short-chain acylcarnitines (C3, C5), seven amino acids including glutamate and BCAAs, sphingolipids, multiple phosphatidylcholine species and four lysophosphatidylcholines.

Hellmuth et al.[27] similarly examined BMI *z* score in meta-analysis of 1020 children aged 5 to 10 years in five European countries. They employed multiple MS based targeted analyses with 108 metabolites available in all studies. SM 32:2 was the metabolite that showed the strongest association with BMI *z* score followed by tyrosine, valine, PC aa 34:4, and PC aa 38:3. Only three metabolites, PC ae 36:2, PC ae 36:1, and laurate (12:0), were negatively associated with BMI *z* score at the Bonferroni corrected significance threshold. The strength of the association with SM 32:2 was striking, particularly as this is a metabolite not assayed by the other platforms included in this review. The study also reported that free carnitine, SM 32:2, SM 34:2 and acylcarnitine 3:0 measured at age 5.5 years was predictive of BMI *z* score at 8 years, in 355 children. However, these associations disappeared upon adjustment for BMI at 5 years. McCormack et al.[28] examined associations with BMI *z* score of 60 metabolites measured in serum using targeted LC-MS among 69 American children (aged 8–18) finding positive associations with glutamate, BCAAs and 3-hydroxyanthranilic acid and a negative association with citrulline. Follow-up of 17 of these children identified glutamate and BCAAs measured at baseline as predictive of IR 1.5 years later.

Most of the other targeted analyses have specifically targeted amino acids and carnitines. Hirschel et al.[29] analyzed dried blood sorts collected from 2,191 German children aged between 3 months and 18 years. They report positive association with BMI *z* score with leucine, isoleucine, tyrosine, valine, free carnitine, alanine, proline, and hydroxyproline and negative associations with citrulline. Sex differences were also observed: In boys only increases with BMI were observed for sarcosine, propionylcarnitine (C3), and acetylcarnitine (C2) and in girls only citrate and glycine were decreased with BMI.

Mihalik et al.[30] applied a targeted LC-MS analysis of short-chain acylcarnitine and amino acids in a study comparing children of normal weight, with obesity, and with obesity and T2D among 120 American children (aged 12–18 years). Although few Bonferroni corrected significant differences (for histidine and arginine) were observed between the normal and children with obesity only, significantly lower levels of acylcarnitines and amino acids were observed among children with obesity and T2D compared to normal weight controls, with generally intermediate levels among the obesity without T2D group. There was also a negative correlation for these metabolites with BMI analyses as a continuous score. The associations reported with the short chain acylcarnitines, BCAAs and tyrosine contrast with all other studies in this review. Although associations appear to be driven mainly by the T2D group, which was slightly older than the other groups, there are not obvious sources of bias in this study and T2D is widely reported to be associated with higher levels of these metabolites.[31]

Short et al.[32] investigated serum profiles of amino acids and related metabolites among 94 Indian Americans (aged 11–18) measured at baseline before participating in an exercise-based intervention trial. Higher levels of glutamate, phosphoethanolamine, aspartate, cystathionine, tyrosine, alloisoleucine, phenylalanine, leucine, alanine, valine, β-alanine, ornithine,2-aminoadipic acid, proline, histidine, and lysine and lower levels of glutamine, β-aminoisobutyric acid, cysteine, asparagine, homocysteine, γ-amino-n-butyric acid were observed among children with obesity compared to normal weight children.

Moran-Ramos et al.[33] applied a targeted analysis of 42 amino acids and fatty acids among Mexican children (aged 6–12). The study incorporated a case-control component of 1120 children, a cross-sectional analysis, and a longitudinal component. In the case-control study, following PCA, three components were associated with obesity. Amino acid and mixed chain length acylcarnitine component scores (C2, C16, and C18) were higher among children with obesity, while a medium chain acylcarnitine component score component score was lower. The amino acid component score was also associated with IR and traditional lipid markers, while the mixed chain length acylcarnitine component score was additionally associated with parameters related to kidney and liver function. The amino acids component was predictive of BMI percentile, fat mass and triglyceride levels measured two years later. Although only associations with triglycerides were significant upon adjustment for BMI percentile at baseline.

Lopez-Contreras et al.[34] measured both flow injection analysis mass spectrometry (FIA-MS) metabolic profiles in serum and microbial taxa relative abundance by 16S rRNA sequencing in 138 Mexican children (aged 6–12 years). Levels of amino acids leucine, valine, tyrosine, phenylalanine and alanine were higher among children with obesity. Interestingly, both *Bacteroides plebeius* and unclassified *Christensenellaceae* abundances, which were both reduced among children with obesity, were negatively correlated with phenylalanine.

Yoo et al.[35] measured levels of carnitines and six fatty acids, along with various clinical measures among 60 Korean girls, aged 14–16. Girls of normal weight and with obesity could be distinguished based on multivariate models built upon these analytes. Of lipids measured only total acylcarnitines were significantly different between the groups (mean 11.7 ± 2.9 μmol/L serum in the group with obesity compared to 8.0 ± 3.7 μmol/L in normal weight group). Hlavaty et al.[36] applied a targeted fatty acid assay among 380 Czech adolescents

(aged 15–18) and found palmitoleic acid (16:1n-7) to be correlated with percentage of body fat and saturated fatty acids in phospholipids positively correlated with BMI and percentage of body fat.

Syme et al.[37] applied serum lipidomics to profile 69 glycerophosphocholines among 990 Canadian adolescents, aged 12 – 18 years. They reported significant positive associations with LPC (14:1/0:0) and PC (20:0/2:0), and negative associations with LPC (20:6/0:0), PC (16:1/2:0), PC (16:0/2:0) and LPC (O-20:1/0:0), and visceral fat. Wang et al[38] analyzed targeted profiles of over 328 lipid species in 100 Chinese adolescents, aged 14-16. Multivariate discriminatory analysis of lipidomic profiles could distinguish between serum collected from normal weight and children with obesity. Five lipids that contributed most to the multivariate models (LPC18:2, LPC18:1, LPC20:2, LPC20:1, and LPC20:0) were lower in the group with obesity and these differences remained even after normalization for total glycerophospholipid level and after accounting for traditional lipid markers such as lipoprotein measurements.

Son et al.[39] performed serum profiles of 20 sterols among 253 Korean children aged 6-14 years. Among children with overweight or obesity the cholesterol precursors lanosterol and lathosterol were significantly higher, while plant sterols campesterol and sitosterol were lower, suggesting that obesity increases cholesterol synthesis while maintaining overall cholesterol homeostasis.

Two studies specifically targeted steroid hormones. Lee et al.[40] reported lower levels of DHEA-S, pregnenolone sulfate, cholesterol sulfate among Korean girls with obesity, suggesting alteration to steroid phase II metabolism. Mauras et al.[41] applied a targeted LC-MS assay of 12 Estrogens and their metabolites in 35 prepubertal American girls. Estradiol and its genotoxic metabolite 16α-Hydroxy (16α-OH)-estrone were higher among girls with obesity while levels of the chemoprotective metabolite 2-Methoxy-estrodiol were lower. These results may have relevance for long- term breast cancer risk.

Two studies analyzed both blood and urine in the same participants. Concepcion et al.[42] applied targeted LC-MS analysis of 273 analytes in plasma and urine samples from 90 American adolescents (age 13–19 years) comparing obesity cases with T2D, obesity cases without T2D and normal weight controls. Compared to normal weight controls, children with obesity had elevated levels of glutamate, 2-hydroxybutyrate, BCAAs, saica-riboside, 3-hydroxyisobutyric acid, xanthine, 3-methyl-2-oxovaleric acid and pyridoxal in plasma, and 2-hydroxyadipic acid and glycodeoxycholic acid in urine. Lower levels of isobuyrylglycine and 2-oxoglutarate/glutamate ratios in plasma, and isobutyrylglycine, isovalerylglycine, uracil, heptanoylglycine, tiglyglycine, 3-methylcrotonylglycine in urine were observed in children with obesity compared to normal weight controls. The authors found that urinary BCAAs and their intermediates behaved as a more specific biomarker for T2D, while plasma BCAAs were associated with the obesity, insulin resistant state independent of diabetes status. Lau et al.,[26] as previously described, assessed metabolic profiles in 1192 European children using an untargeted NMR analysis in urine and targeted LC-MS (Biocrates) in serum. In urine they reported positive associations between the sugar acid 4-deoxyerythronic acid and the BCAA valine and negative associations with urinary p-cresol sulfate (a microbial metabolite) and pantothenate (vitamin B5). Associations with other BCAAs were only observed in serum, which would support the findings of Concepcion et al. in this healthy population.

Two studies included only analysis of urine. Cho et al.[43] analyzed urine to compare Korean adolescents without obesity ($n = 91$) and with obesity ($n = 93$), using both untargeted MS analysis and targeted Biocrates based analysis. Untargeted LC-MS metabolomics identified lowered levels among children with obesity of four metabolites produced by the gut microbiome (4-hydroxybenzaldehyde, hippuric acid, 4-sulfobenzyl alcohol and N,N-dimethyl-safingol). Other metabolites associated with inflammation such as docosanoic acid were elevated in the group with obesity. They found that 45 metabolites were differentially expressed ($P < 0.05$) in urine in the targeted analysis, many overlapping with other Biocrates based studies in serum. Triosi et al.[44] applied an untargeted GC-MS analysis of urine from 36 Italian children (aged 5–16 years) stratified by normal weight, obesity, and obesity with steatosis. Metabolites that contributed to multivariate discriminatory models including higher levels of p-cresol-sulfate, glucose, methyl histidine, sebacic acid, pseudouridine, glucono-1,4-lactone and cysteine and lower levels of xylitol, 4-phenyl acetic acid, oleic acid, 4-deoxyerythronate and N-methyl nicotinate among children with obesity. Glucose and methyl histidine were higher among children with steatosis, while xylitol and 4-phenyl acetic acid appeared to relate to diet quality. Figure 1B summarizes metabolites associated with adiposity in studies of urine.

Triosi et al.[45] performed a pilot study using saliva samples from 41 Italian children, aged 7–15 years. Multivariate discriminatory analysis of GC-MS metabolic profiles could separate children with obesity from normal weight children and also performed well separating children according to metabolic syndrome, although less well in separating children with obesity by steatosis. Valine and isoleucine were among the AAs more prevalently involved in the obesity-deranged pathways, but they did not appear to accurately reflect specific hepatic or metabolic involvement. Two saturated fatty acids, palmitic, acid and myristic acid were also higher in the group with obesity.

3.3 | Prospective studies: Predicting obesity risk

Three studies analyzed cord blood and two studies analyzed plasma collected during infancy to predict child weight status in later life (Table S10). Isganaitis et al[46] compared the cord blood metabolomic profiles, measured by LC-MS (Metabolon, 415 metabolites), of cases ($n = 26$) based on top quartile of change in weight-for-age 0–6 months and overweight in mid-childhood to matched controls ($n = 26$) in an American cohort. Tryptophan metabolites serotonin, tryptophan betaine, and tryptophyl leucine were lower in cases respectively, as were 2 methyl donors, dimethylglycine and N-acetylmethionine. While nominally significant, these changes did not pass FDR correction. Pathway analysis identified enrichment in "Tryptophan Metabolism" and "Excitatory neural signalling through 5HTR4/6/7 and serotonin" pathways. Sorrow et al.[47] similarly applied

a Metabolon analysis of 384 metabolites in cord blood of 25 American children with and without obesity at 3–5 years. Children with obesity had elevated lipid species, acetaminophen metabolites and acylcarnitines, although no multiple testing correction was applied. Hellmuth et al.[48] applied a range of targeted LC-MS assays (209 metabolites) to profile cord blood of 700 German children to predict rapid weight gain, and BMI at 2 and 7 years. While many metabolites were associated with weight at birth, no associations with postnatal measures survived multiple testing correction. Cord blood metabolites that were associated with increased birth weight showed a tendency to be associated with lower postnatal weight gain *z* scores and BMI *z* scores at ages 2 and 15 years, while the converse was observed for metabolites with birth weight-lowering effects.

Rzehak et al.[49] applied LC-MS to profile 168 metabolites using the Biocrates kit to analyze plasma samples collected at age 6 months from 726 infants participating a European multi-center randomized trial (Childhood Obesity Programme, CHOP), randomized to a high- or low-protein content formula and breast-fed infants. Tyrosine and citrulline, PC aa C34:4 and LPC a C14:0 were associated with rapid growth during the first 6 months of life. However, metabolic signatures were significantly different by feeding group and after adjusting for feeding group, only LPC a C14:0 remained significantly associated with rapid weight gain. Intriguingly, LPC a C14:0 at age 6 months was also predictive of subsequent overweight at age 6 years, suggesting a metabolically programmed effect of infant weight gain on the later obesity risk. Similarly, Fleddermann et al.[50] analyzed 129 metabolites (fatty acids, carnitines, and AAs) in plasma collected at age 4 months from 250 infants participating in a randomized control trial examining reduced protein content formula milk on infant growth trajectories. After adjustment for feeding group, six metabolites (asparagine, lysine, methionine, phenylalanine, tryptophan, and tyrosine) were positively associated with change in weight-for-age *z* score and one metabolite (tyrosine) was positively associated with change in BMI-for-age *z* score between 1 and 4 months of age. No metabolites predicted anthropometry at 4 years.

3.4 | Intervention studies: Is the metabolic signature of obesity reversible?

Seven studies examined changes in metabolites measured before and after a weight loss intervention program (Table S10). Untargeted LC-MS profiling of plasma[51] and NMR profiling of urine[52] was conducted at baseline and after a 6-month-long lifestyle intervention program in up to 35 children (7–10 years) with obesity in Spain. The intervention significantly reduced BMI, HbA1c (%) and total cholesterol levels, and increased adherence to a healthy diet. In the LC-MS analysis, PCA identified one component (PC1) significantly altered by the intervention. A sphingolipid metabolism-related signature was identified as the major contributor to PC1. Sphingolipid metabolites were decreased by the intervention, and included multiple SMs, ceramide, glycosylsphingosine, and sulfatide species. However, changes of individual metabolites such as sphingomyelin 23:0 were associated only with changes in HbA1c (%) and not with changes in BMI. Among the urinary metabolites measured by NMR, levels of trimethylamine N-oxide (TMAO), 3-hydroxyisovalerate, and dimethylglycine were decreased and xanthosine increased after the intervention. However, no metabolite changes were correlated with change in BMI and changes in TMAO appeared to be driven by increase in dietary fiber.

In NMR urinary analysis measured before and after an American 3-week immersion healthy lifestyle camp in up to 12 adolescents with obesity,[53] lower levels of 2-Oxoisocaproate, which arises from the incomplete breakdown of BCAAs, predicted weight loss, while lower levels of tyrosine, taurine and glycine were observed among participants after the intervention with weight loss, at nominal statistical significance. Short al.[32] did not detect any differences in serum amino acid profiles among 58 Indian American children with obesity (aged 11–18), measured before and after a 48-week incentivized exercise program, despite a small but significant reduction in BMI.

Three studies reported results from a German 1-year lifestyle intervention in children and adolescents with obesity. In Biocrates-based LC-MS analysis of serum from 80 children at baseline,[54] 17 metabolites were predictive of a decrease in BMI including arginine, LPC a C18:0, and 15 long-chain and unsaturated PCs (13 diacyl and two acyl-alky). Analysis of 14 metabolites,[55] which had been identified previously as associated with obesity,[25] in serum before and after the intervention from 160 children identified significant increases among children with substantial weight loss in glutamine, methionine, LPC a C18:1, LPC a C18:2, and LPCa20:4, as well as the acyl–alkyl PC PCaeC36:2. The same group also reported reductions in steroid hormones DHEA-S, cortisol and corticosterone in 40 adolescent girls with obesity who achieved substantial weight loss following the intervention program.[56]

4 | DISCUSSION

Among the 27 cross-sectional studies based on blood we identified, 227 different metabolites were reported to be associated with adiposity measures (Table S9). Despite wide differences in sample processing, metabolome coverage and analytical technique, 64 metabolites were reported by more than one study (Figure 1A). The most widely reported and consistent associations were for BCAAs and for the aromatic AAs tyrosine and phenylalanine, followed by many other AAs. However, other groups of molecules were consistently reported by at least one study including acylcarnitines (particularly those of shorter chain length), steroid hormones, glycerophospholipids, sphingolipids, polyamines, peptides, purines and single metabolites from other classes. Figure 2 summarizes our main conclusions.

Of the BCAAs, eight studies report an increase with BMI for isoleucine[19,18,24,23,29,28,33,42] 10 studies reported an increase for valine,[19,18,20,24,27,29,28,32,42,34] and 11 studies reported an increase for leucine.[19,18,24,26,29,28,32,33,42,34] An increase in tyrosine was reported by 11 studies[19,17,18,20,24,27,29,32–34] and phenylalanine by seven studies.[19,17,18,24,26,32,33,.34] Only one study reported decreases in leucine, valine and tyrosine[30] and two studies reported a decrease in

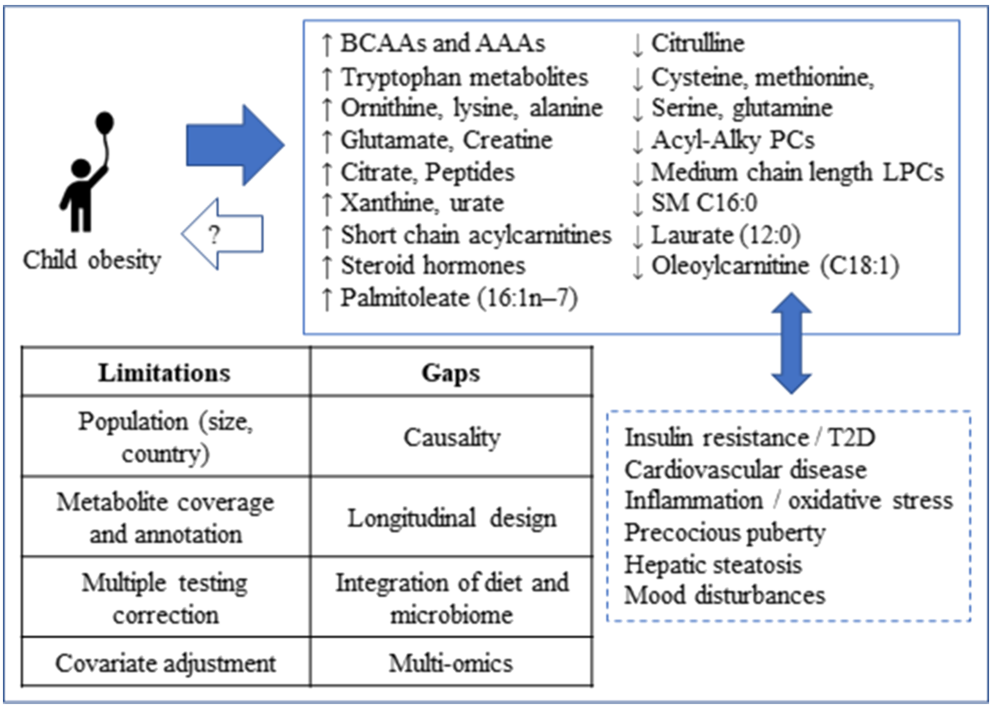

FIGURE 2 Schematic of main conclusions. Blue box shows metabolites consistently reported to be increased or decreased in cross-sectional studies. Metabolites most likely to be consequence of childhood obesity (blue arrow) rather than determinants (white arrow) for which evidence is lacking. Dashed blue box show health conditions that have been associated with these metabolites. Table summarizes limitations and gaps in the literature. BCAAs: Branched-chain amino acids. AAAs: Aromatic amino acids. PCs: Phosphatidylcholines. LPCs: Lysophosphatidylcholines. T2D: Type 2 diabetes. SM: Sphingomyelin

phenylalanine[23,30] Although the number of studies reporting associations with these metabolites partly results from the ability of most analytical techniques in the included studies to assess these molecules, it should be noted that these were among the highest ranked metabolites to be associated with BMI in studies that assessed a broad range of metabolite classes.[19,18,27] Associations between serum BCAAs levels and obesity and IR were first reported half a century ago;[57] however, the advantage of the metabolomic approach is putting these changes in the context of concomitant changes in other metabolites. Many studies included here also linked BCAAs to an insulin resistance or T2D.[16,18,27,33,42] Tyrosine also was among the strongest associates with IR among generally healthy European children.[27] Potential mechanisms for increased levels of AAs include increasing protein degradation, impairment of efficient oxidative metabolism in some tissues,[58] or even reduced *de novo* synthesis by the gut microbiome as indicated by one study in this review.[34] 3-methyl-2-oxovalerate, produced from the incomplete breakdown of branched-chain amino acids was also reported decreased by two studies.[18,42] Whether BCAAs may cause IR (for instance through activation of the mammalian target of rapamycin complex 1 (mTORC1)) or more likely reflect metabolic changes related to the IR state is still unclear.[58]

Acylcarnitines play a crucial role in transport of fatty acids to the mitochondria for β-oxidation and plasma levels have been linked to IR[59] and CVD.[60] Seventeen different acylcarnitines were reported as associated with BMI. In particular, increases in short chain acylcarnitines were commonly reported including free carnitine,[19,20,24,26,29] acetylcarnitine (C2),[29,33] propionylcarnitine (C3),[19,18,24,29,26] butyrylcarnitine (C4),[19,20] valerylcarnitine (C5),[24,26] and 2-methylbutyrylcarnitine (C5).[19,18] Most studies reported rises in acylcarnitines alongside BCAAs, and the increases likely represents increased availability of acyl-CoAs from BCAA catabolism. Increases were generally not reported for the longer-chained acylcarnitines and a decrease in oleoylcarnitine (C18:1) was reported by two studies[19,26] likely reflecting reduced fatty acid catabolism.[19]

Tryptophan was reported to be increased by two studies[19,18] as were related polyamines, kynurenate[19,20] and kynurenine.[19,26] These compounds may reflect immune activation or low-grade systemic inflammation, and increases can result from upregulation of indoleamine 2,3-dioxygenase activity. The enzyme has been closely related to obesity, potentially resulting from reduced serotonin production and mood disturbances, depression, and impaired satiety, finally leading to increased caloric uptake.[61] Interestingly, enrichment of tryptophan and serotonin pathways were observed in cord blood of neonates who went on the become overweight in childhood.[46]

Lysine was reported increased by three studies[19,26,32] while related metabolite a-amino adipic acid was decreased in two studies.[24,32] Mixed associations were reported for amino acids involved in the urea cycle: Proline was reported to be increased in four studies[26,29,32,33] and decreased in two,[16,25] ornithine was

increased in two studies[19,32] arginine was increased in one study[33] and decreased in another,[30] while citrulline was reported to be decreased in four studies.[19,29,28,30] The reasons for decreased citrulline are unclear but it may reflect hepatic steatosis that is often present in children with obesity.[10] The sterol lathosterol was increased in two studies[19,39] and may also reflect hepatic and gastroenteric involvement.[62]

Glutamate was reported to be increased by three studies[19,16,29] while glutamine was reported decreased by three studies.[19,23,30] Cysteine[16,32] and methionine[25,30] were reported decreased by two studies each. Glutamine and methionine were also reported to be decreased following weight loss.[55] These changes would be consistent with increased glutathione demand due to increased oxidative stress. Serine was decreased in three studies,[19,23,30] while glycine was decreased in three studies[19,29,30] and increased in one other.[26] Reduced levels of glycine and serine may indicate increased gluconeogenesis which is observed with insulin resistance. The organic acid α-hydroxybutyrate was increased in two studies[19,42] and is produced as a result of the conversion of cystathionine to cysteine and is produced downstream from glycine and serine.[63] α–hydroxybutyrate has been associated with increased glutathione demand and disrupted mitochondrial metabolism and shown to derive from hepatic glutathione stress.[64] Citrate, reported as decreased in three studies[19,16,29] also indicates altered energy metabolism in the mitochondria.

Purine metabolites, urate,[19,20] and xanthine[19,42] were increased in two studies each. It is possible that hyperuricemia may be causative of obesity through increase of hepatic and peripheral lipogenesis[65] although as urate is a scavenger of oxidative species, increases may reflect oxidative stress. Xantine is also involved in the inflammatory response.[19] Peptides γ-glutamyltyrosine[19,20] and bradykin[19,22] were increased in two studies each. Bradykin too, is indicative of inflammation and the activation of an immune response.[19]

Increases in steroid hormones, in particular androgens, were reported by multiple studies, including 4-Androsten-3b-17b-diol disulfate,[19,18] 5a-Androstan-3b-17b-diol disulfate,[19,20] androsteroid monosulfate,[19,18] and DHEA-S,[19,18,20] although one study also reported a decrease in DHEA-S.[40] DHEA-S was also reported to be decreased in a study of girls following weight-loss.[56] Obesity has been associated with puberty timing, particularly for girls,[66] and Perng et al.[18] reported an association between the androgen hormone pattern and parent-reported puberty characteristics. Since pubertal timing may increase risk of cardiometabolic disorders in adult life, steroid hormones may represent a mechanism linking childhood obesity to later onset of CVD.[67]

Regarding lipids, multiple associations were reported for fatty acids, long-chain fatty-acids, lysolipids, LPCs, PCs, and SMs. Lipids have varied roles as metabolic substrates, cellular membrane components and signaling molecules, with the complexity of their biological roles related to the chain-length and degree of unsaturation of the fatty acid components. Among the PCs, only negative associations were observed with the acyl-alky PCs, with two studies[24,25] reporting decreases with PC ae C34:1, PC ae C34:2 and PC ae C34:2, and decreases also reported for PC ae C36:2[24,25,27] and PC ae C36:3.[24,25] PC ae C36:2 was also the only acyl-alky PC to be reported to increase following weight loss[55] Associations were mixed within the class of diacyl PCs with no overlap across studies for individual diacyl PCs, except for two studies[26,27] that both reported increases in PC aa C34:4 and PC aa C38:3. Among the LPCs, negative associations with BMI measures were reported for the shorter chain length species LPC a C14:0 and LPC a C16:1,[26] while only positive associations were reported for the longer chain length species.[24–26,38] LPC a C18:1 and LPC a C18:2 were reported increased by four studies.[24,25] LPC a C18:1, LPC a C18:2, and LPCa20:4 were also reported to increase following weight loss.[55] One study that applied lipidomics, which provides greater detail on the fatty acid chain structures of lipids than general metabolomics, found decreased PC 16:0/2:0 and increased LPC 14:1/0:0 mediated the effect of visceral fat on CVD risk factors.[37] Mixed associations were observed for the sphingomyelins, with only SM C16:0 reported by more than one study with both studies[24,26] reporting the lipid to be decreased. A Sphingolipid principal component was also significantly decreased following a weight loss program.[51] The fatty acids laurate (12:0)[19,27] and palmitoleate (16:1n–7)[19,36] were reported to be decreased and increased respectively by two studies each. Palmitoleate has been suggested to act as an adipose tissue-derived lipid hormone[68]

One of the aims of this review was to identify candidate metabolites or profiles that may act as determinants of childhood obesity, by indicating a metabolic shift that increases obesity risk or even through enhanced assessment of the obesogenic environment, such as particular aspects of diet. However, the overwhelming proportion of studies identified were cross-sectional, with no formal testing of causal direction such as through the use of Medelian Randomization or repeated testing of both BMI and metabolome. Two of these studies also tested prediction of BMI at later follow-up; however, no associations were apparent after adjustment for baseline BMI[27.33] These findings together with intervention studies that indicated changes in some obesity-associated metabolites following weight loss[55,56] and causal studies in adults,[69] suggest profiles identified in these cross-sectional studies are a consequence of obesity. Only five studies addressed metabolites as determinants specifically, through analysis of the metabolome among neonates or infants and later assessment of BMI. Although there were indications that some metabolites (e.g., leucine and tryptophan metabolites,[46] fatty acids and acylcarnitines,[47] LPC a C14:0[49]) reported in the cross-sectional studies may also predict later obesity risk of neonates and infants, studies are still small and few in this area to draw firm conclusions. After the search period of this review, we recently published an analysis of the cord blood metabolome among 399 new-borns from four European cohorts.[70] We found that lower levels of BCAAs valine and leucine to be predictive of overweight in childhood, replicating the association with leucine reported by Isganaitis et al.[46] This supports the notion that metabolic profiles can identify determinative factors and improve identification of children at risk of developing obesity, supporting further longitudinal studies in this area.

While higher blood levels of BCAAs result from physiological changes associated with obesity, BCAAs are also reflective of

nutritional quality, particularly protein intake,[71] and paradoxically higher intake can have positive effects on satiety and regulation of body weight.[58] Metabolomic analysis can simultaneously profile diet, including breast milk constituents,[72] products of microbial metabolism and physiological changes. Future metabolomic studies of child obesity, particularly prospective studies, should carefully consider these factors, considering their close relationship to child obesity.[73] We found only four studies analyzing metabolic profiles in urine, with 71 metabolite associations reported (Figure 1B). Only p-cresol sulfate was reported by more than one study with contrasting direction of associations.[44,26] This may be expected as p-cresol sulfate is a microbially produced metabolite of tyrosine, so reflects both increased tyrosine levels and an altered gut microbiome. Generally, many associations reported in urine were also consistently reported in blood, although as indicted in the two studies that measured both matrices,[42,26] the biological interpretation of metabolites present in blood and urine may differ. Many more diet-specific and microbial metabolites were also detected in urine than blood. Increased use of urinary metabolomics, in conjunction with analysis in blood, may help clarify the role of these factors in obesity-related profiles. Diet is difficult to accurately assess, particularly in overweight populations where reporting bias may be greater and was not accounted for in many of the included studies. Urinary metabolomics is increasingly being used to provide more objective dietary assessments[74] and can also provide a more practical solution for microbiome analysis than incorporation of metagenomic analysis of faecal samples.[34] Another research gap is the integration of metabolomics with "omic" assessment at other biological layers. Epigenetics has attracted great interest in child obesity research due to in role in foetal programming, sensitivity to environmental factors including potential transgenerational effects, and may influence metabolism.[75] Metabolomics can clarify the role of observed epigenetic factors and their integration can provide a more complete picture of mechanistic pathways.[76]

Reviewing metabolomics studies presents challenges: Structural annotation in metabolomics remains an issue and many included studies did not report identification levels according to current community standards,[14] so misclassification of reported metabolites is possible. The breadth of metabolome coverage and also measurement precision and quantification differed widely between studies which makes assessing consistency of associations and quantitative synthesis challenging. Also, compared to the genome, the metabolome is much more highly correlated, and its size is not known and can vary across samples, which makes accounting for multiple testing difficult, particularly in untargeted studies where many features may represent analytical noise. Permutation based procedures[77] may be considered the gold-standard in addressing the multiplicity problem. We did not formally test publication bias as this is not readily applicable to omics studies where many features are tested. Only one study did not report any associations, but it should be noted that a large proportion of studies, particularly earlier studies, did not apply any multiple testing correction, increasing the likelihood of having associations to report. There are currently over 100,000 metabolites in the Human Metabolome Database,[78] while the highest number of molecules assayed by studies in this review was just over 1,000. Future untargeted studies will need to both increase metabolite coverage, through technological development and combining analytical platforms, alongside improvements in structural annotation[78] and appropriate statistical methodology, to provide comprehensive assessment and generate new hypotheses. In parallel, further targeted studies are required to further explore classes of molecules and test hypotheses. Both steroids and lipids appear from this review to be promising avenues.

In conclusion, a consistent metabolic profile of childhood obesity was observed including amino acids (particularly BCAAs and AAAs), carnitines, lipids and steroids. These signatures appear largely concordant with those in adult studies.[10] Although the use of metabolomics in childhood obesity research is still developing, the identified metabolites have provided additional insight into the pathogenesis of many obesity-related diseases. Further longitudinal research is needed into the role of metabolic profiles and child obesity risk.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

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