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Abstract

Introduction

Metabolomics may identify biological pathways predisposing children to the risk of overweight and obesity. In this study, we have investigated the cord blood metabolic signatures of rapid growth in infancy and overweight in early childhood in four European birth cohorts.

Methods

Untargeted liquid chromatography-mass spectrometry metabolomic profiles were measured in cord blood from 399 newborns from four European cohorts (ENVIRONAGE, Rhea, INMA and Piccolipiu). Rapid growth in the first year of life and overweight in childhood was defined with reference to WHO growth charts. Metabolome-wide association scans for rapid growth and overweight on over 4500 metabolic features were performed using multiple adjusted logistic mixed-effect models and controlling the false discovery rate (FDR) at 5%. In addition, we performed a look-up analysis of 43 pre-annotated metabolites, previously associated with birthweight or rapid growth.

Results

In the Metabolome-Wide Association Study analysis, we identified three and eight metabolites associated with rapid growth and overweight, respectively, after FDR correction. Higher levels of cholestenone, a cholesterol derivative produced by microbial catabolism, were predictive of rapid growth ($p = 1.6 \times 10^{-3}$). Lower levels of the branched-chain amino acid (BCAA) valine ($p = 8.6 \times 10^{-6}$) were predictive of overweight in childhood. The area under the receiver operator curve for multivariate prediction models including these metabolites and traditional risk factors was 0.77 for rapid growth and 0.82 for overweight, compared with 0.69 and 0.69, respectively, for models using traditional risk factors alone. Among the 43 pre-annotated metabolites, seven and five

metabolites were nominally associated ($P<0.05$) with rapid growth and overweight, respectively.

The BCAA leucine, remained associated (1.6×10^{-3}) with overweight after FDR correction.

Conclusion

The metabolites identified here may assist in the identification of children at risk of developing obesity and improve understanding of mechanisms involved in postnatal growth. Cholestenone and BCAAs are suggestive of a role of the gut microbiome and nutrient signalling respectively in child growth trajectories.

Introduction

Childhood obesity has become a global epidemic in developed as well as in developing countries [1], with significant long-term consequences on both physical and psychological health, social and economic outcomes [2]. Behavioural dimensions such as diet and physical activity, and an 'obesogenic environment' that shapes those behaviours, have contributed to the spread of childhood obesity [3, 4]. In the last decades, there has been a growing interest in the idea that the early life environment can have lasting effects on the physiology and metabolism of the fetus and is associated with the early metabolic programming of human health [5,6,7]. Recent studies have revealed that several in utero exposures such as maternal socioeconomic status, clinical and environmental factors are associated with growth in infancy and with the subsequent development of childhood overweight or obesity [8,9,10,11,12,13]. The prenatal environment can affect fetus weight homeostasis and may result in a 'thrifty phenotype' that stores excess calories and predisposes children to weight gain [14]. Hence, a metabolic signature at birth may help elucidate the mechanisms involved in metabolic health later in life.

Metabolomics, the profiling of circulating small molecules, has been increasingly applied to investigate biological mechanisms associated with childhood obesity [15, 16]. However, few studies have investigated metabolic changes in cord blood that may predict subsequent infant growth and overweight and obesity [17]. Isganaitis, Rifas-Shiman et al. [18] analysed the metabolome in cord blood plasma from 26 cases and 26 controls differing in their postnatal weight trajectories using targeted mass spectrometry (MS) analysis of 415 metabolites, nested in an American cohort. There was a trend for lower levels of tryptophan metabolites in children that followed a rapid growth to obesity at 7 years trajectory. Sorrow, Maguire et al. [19] similarly applied a targeted MS analysis of 384 metabolites in cord blood of 25 obese and non-obese American children at 3–5 years. Children

with obesity had elevated lipid species, acetaminophen metabolites and acylcarnitines compared with non□obese children, although no multiple testing correction was applied. Hellmuth, Uhl et al. [20] applied a range of targeted LC-MS assays to assess 209 metabolites in cord blood of 700 German children in relation to birthweight, postnatal weight gain and BMI throughout adolescence. Although many metabolites were associated with weight at birth, no associations with postnatal measures survived multiple testing correction. Although initial studies have so far been based on small numbers of children or limited numbers of molecules, they reveal the potential of metabolic profiling in detecting biomarkers and pathways related to rapid growth in infancy as well as to overweight and obesity in early childhood. Identifying markers that are predictive of obesity onset may assist in the development of targeted intervention programmes for at-risk groups of children.

In this study, we have investigated the cord blood metabolic signatures of rapid growth in infancy and overweight in early childhood in four European birth cohorts, using untargeted LC-MS-based metabolic profiling. Our aims were twofold: firstly, to identify markers associated with rapid growth and overweight risk to provide mechanistic insight and elucidate causal pathways to obesity; and secondly to improve prediction of obesity risk in neonates through assessment of the predictive performance of models incorporating identified metabolites, in comparison with models based on traditional risk factors alone.

Materials and methods

Study population

The study population included participants from four population-based birth participating in the STOP project: ENVIRONAGE [21] (Belgium), INMA [22] (Spain), Piccolipiu [23] (Italy) and Rhea [24] (Greece). Ethical approval was obtained from the local Research Ethics Committees from each centre. Informed consent was obtained from the parents of the children. Further details of blood sampling, clinical, dietary and socioeconomic data of cohort individuals are given in the respective references and supporting information 1.

Untargeted metabolomics

Cord blood samples were analysed in randomised order as a single uninterrupted batch with a UHPLC-QTOF-MS system (Agilent Technologies), as previously described [25]. Further details of the acquisition and structural annotation of features are given in supporting information 1.

Outcome assessment

Rapid growth in infants in the first 12 months was categorised based on the definition of Ong et al. [25]. According to this definition, a clinically significant increment that indicates rapid growth occurs when there is a gain in weight of at least 0.67 standard deviations between different target ages. In this study, length data at birth were not available. Hence, rapid growth was defined as the weight *z* score change of >0.67 standard deviations (SD) between birth and twelve months of age based on World Health Organisation (WHO) growth charts [26]. A two-step prediction approach was used for calculating sex- and age-specific weight at exactly 12 months, using fractional polynomials of age by gender in each cohort [27] (supporting information 1).

To maintain sample size for the analysis of overweight in early childhood, we used a single measurement at an age greater than four years and as close to 6 years as available. The classification for healthy and overweight was based on WHO sex-adjusted and age-adjusted BMI *z* scores. WHO provides different classifications scheme for children under the age of 5 years (0–5< years) [28] and over the age of 5 years (5–18 years) [29]. Following the WHO proposed classification by De Onis and Lobstein [30], children younger than 5 years were classified as overweight if they had a BMI *z* scores >1 SD and children over 5 years were classified as overweight if they had a BMI z-score greater than 2 SDs [30].

Statistical analysis

A Metabolome-Wide Association Study (MWAS) was applied to investigate the association between cord blood metabolomics and infant rapid growth/childhood overweight using multiple mixed-effect logistic regression models using the lme4 R package [31]. The basic model (Model 1) was adjusted for sex and age of the child at outcome measurement, ethnicity and we used a random-effect for cohort. To account for multiple testing, a Benjamini–Hochberg false discovery rate (FDR) [32] was applied using a cutoff of 5%.

We then applied additional covariate adjustment to significant features identified in the MWAS analysis. A directed acyclical graph was used to visualise assumptions regarding covariates for further model adjustment (Figure S1). Covariates were chosen based on a bivariate analysis of their correlation with outcomes (Logistic Regression). The resulting model (Model 2) included Model 1 covariates and maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity and mode of delivery.

Pathway enrichment analysis on significant features was conducted using the *Mummichog* programme [33], supplemented with manual curation of the metabolite identities assigned by *Mummichog* (supporting information 1).

A look-up analysis, using the same statistical approach as the MWAS analysis (including 5% FDR), was conducted on 43 metabolites that had been previously annotated in the same data set as used in this study, due to their associations with birthweight [34, 35] or because they had previously been reported to predict a rapid growth leading to overweight in childhood trajectory, and could also be identified with high confidence through retention time and MS/MS matching in our data set [18].

In sensitivity analyses, we re-ran Model 2 for metabolites associated with rapid growth or overweight, stratified by cohort, sex and size for gestational age and additionally adjusted for birthweight.

We further assessed how well rapid growth in infancy or overweight in early childhood are predicted using metabolites in comparison with traditional factors using Random Forest classification models [36] (supporting information 1). We used three different sets of variables for each of the outcomes: (1) traditional risk factors (sex, birthweight, ethnicity, maternal BMI, paternal BMI, gestational age, maternal weight gain during pregnancy, paternal education, maternal passive and active smoking status during pregnancy, parity and mode of delivery), (2) significantly associated metabolites from the MWAS analysis and (3) significantly associated metabolites from MWAS analysis in combination with traditional risk factors. A bootstrap method of 1000 repetitions was advocated to quantify optimism and evaluate the generalisation of the model. A threefold cross-validation routine was performed on the training set (random 80% of the total observations) to each model to determine the optimum probability threshold. The model performance was evaluated on the relevant test set (remaining 20% of the total observations) using receiver operating characteristic (ROC curve) and area under the curve or AUROC for assessing the goodness-of-fit of the classifier. To further evaluate the predictive model, we performed a leave□one□out analysis by repeating the modelling process on a combined data set with one cohort retained as the validation set (supporting information 1).

Results

Participant Information and metabolomic data

Table 1 shows the characteristics of the population used in the analysis of rapid growth in infancy and overweight in early childhood (stratification by cohort, including available dietary information, is presented in Table S1, S2. In bivariate analyses (Table 1), birthweight, parity, maternal weight gained during pregnancy, mode of delivery and gestational age were all significantly associated ($P<0.05$) with rapid growth, while maternal passive and active smoking during pregnancy, maternal BMI, paternal education level, paternal BMI and rapid growth in infancy were significantly associated with overweight in early childhood. After data filtering procedures, 4714 metabolic features were available for statistical analysis.

Cord blood metabolomics and rapid growth in the first year of life

The analysis of rapid growth included 391 children, with 114 (28.9%) classed as rapid growers in the first year of life. In MWAS analysis, adjusting for age at the outcome measurement, sex, cohort and ethnicity (Model 1), six metabolic features were significantly associated (FDR<5%) with rapid growth in the first year of life (Fig. 1A). Table S3 contains the retention time as well as the exact mass of all significantly associated features, including unassigned metabolites. The metabolic features were grouped into four metabolites after grouping of ions originating from the same molecule (matched by retention time and pairwise feature correlation, Table S3). One metabolite could be identified as cholestenone (4-cholesten-3-one; HMDB0000921), a steroid lipid in the class of cholesterols. Upon adjustment for further covariates (Model 2), three of the four associated metabolites, including cholestenone, remained significantly associated with rapid growth (Fig. 2A). Cholestenone levels were higher in the cord blood of rapid growers, whereas levels of the rest of the metabolites were lower in the cord blood of rapid growers.

In a look-up analysis, we analysed associations with 43 known metabolites (retention time and *m/z* information given in Table S4) in the metabolome data set that had been previously annotated based on their associations with birthweight [34, 35], or with rapid infancy weight gain and childhood obesity [18] (including indolelactic acid, sphingosine, tryptophan and leucine)(Table S5). Fourteen metabolites were associated with rapid growth in the first year of life (Fig. 2B) after correcting for 5% FDR in basic adjustment analyses (Model 1), including higher levels of nine phosphatidylcholines (PCs) or LysoPCs, cholestenone, cholesterol, progesterone and two acylcarnitines tetradecadiencarnitine (C14:2) and decenoylcarnitine (C10:1). In additionally adjusted analyses (Model 2) cholestenone, two PCs (PC(34:2) and plasmalogen PC(36:4)/PC(O-36:5)), two acylcarnitines, docosahexaenoic acid (DHA), diacylglycerol (C36:4) and progesterone

were nominally associated (P<0.05) with rapid growth (Fig. 2B). Directions of association with rapid growth were opposite to directions observed previously with birthweight [34]. Correcting Model 2 for 5% FDR, only cholestenone remained associated with rapid growth in the first year of life.

As shown in the network graph (Fig. 2C), cholestenone was highly correlated with PC(34:2), moderately correlated with unidentified metabolite U4 and had weaker, positive correlations with the other rapid growth-associated metabolites. We noted strong correlations between DHA and plasmalogen PC(36:4)/PC(O-36:5) as well as between tetradecadiencarnitine (C14:2) and PC(34:2).

Mummichog analysis indicated enrichment among rapid growers in the 'C21-steroid hormone biosynthesis and metabolism' and 'Androgen and oestrogen biosynthesis and metabolism' pathways, with weaker support for enrichment of the 'Urea cycle/amino group metabolism' pathway (supporting information 1 and 2, Table S10).

Cord blood metabolomics and overweight in early childhood

The analysis of child overweight in early childhood included 272 children from the Piccolipiu, Rhea and INMA cohorts, of which 48 (17.6%) were classed as being overweight or obese (mean age at weight status assessment: 5.12 years (SD:1.11)). In the MWAS, adjusting for cohort and ethnicity (Model 1), 36 features were significantly associated (FDR<5%) with overweight in early childhood (Fig. 1B). After grouping ions originating from the same compound (Table S6), there were eight unique compounds associated with overweight (Fig. 3A). One compound could be annotated as valine, a branched-chain amino acid. Retention time as well as exact mass of all significantly associated features, including unassigned compounds, are available in Table S6. The inverse association of valine with overweight was strengthened upon additional covariate adjustment (Model 2) and remained significant after FDR correction.

In an analysis of the 43 pre-annotated metabolites, leucine and DHA were nominally associated ($P<0.05$) with overweight in basic analyses (Model 1) (Fig. 2B). In additionally adjusted analyses (Model 2) lower levels of leucine, progesterone, indolelactic acid, hexenoylcarnitine (C6:1), hexadecenoylcarnitine (C16:1) and DHA were nominally associated ($P<0.05$) with overweight in early childhood (Table S7). Directions of association with overweight were consistent with directions observed previously with birthweight [34]. Only leucine, a BCAA previously identified

in relation to rapid infancy weight gain and childhood obesity by Isganaitis, Rifas-Shiman et al. [18], remained significant after FDR correction.

Valine was moderately correlated with DHA and had weaker correlations with the unidentified compounds U4, U5 and U7 and stronger correlations with U3 and hexadecenoylcarnitine (C16:1). Leucine had a weak negative correlation with Valine and strong negative correlations with U1, U4, U5 and U7. Strong correlations were observed between progesterone and indolelactic acid as well as between the compounds U1, U4, U5 and U7 (Fig. 3C).

Mummichog analysis did not provide strong support for enrichment of specific pathways with childhood overweight (supporting information 1 and 3, Table S11).

Multivariate prediction models

We next utilised Random Forest classification models to evaluate the predictive performance of three different input variable sets for each of the two outcomes (Fig. 4). The rapid growth prediction model trained using only traditional risk factors exhibited a moderate predictive ability of an AUROC value of 0.69 (bootstrap 95% confidence interval (CI):0.62–0.77) (Table S8). Adding the four metabolites (cholestenone, U2, U4 and U8) identified in the MWAS analysis into the prediction model, increased the AUROC to 0.77 (bootstrap 95% CI: 0.73–0.81) (Fig. 4A). For overweight, using traditional risk factors alone, the AUROC was 0.69 (bootstrap 95%CI: 0.63–0.75), while a model using only the eight metabolites, Valine, U1, U2, U3, U4, U5, U7 and U9, identified in the MWAS analysis had an AUROC of 0.77 (bootstrap 95% CI: 0.73–0.81) (Table S8). The combined traditional risk factor and metabolite model was strongly predictive of overweight with an AUROC of 0.82 (bootstrap 95% CI: 0.79–0.85) (Fig. 4B). The leave cohort out analysis also showed improvement in predictive performance using metabolites, in the majority of cohorts (Table S9).

Sensitivity analysis

To assess the robustness and consistency of our results, we stratified our population by cohort and by sex and repeated the adjusted models (Model 2) across each subpopulation. Regarding rapid growth, results were generally consistent across cohorts for all identified metabolites, including cholesterone (Figure S2). However, opposite directions of effects were observed in the Piccolipiu cohort for PC(34:2) and plasmalogen PC(36:4)/PC(O-36:5). Regarding overweight, results were again consistent across cohorts (Figure S3), although wide confidence intervals were observed in

Piccolipiu (related to the small number of overweight cases available in this cohort). For valine, strong associations were noted in both the INMA and Rhea cohorts. For rapid growth, stronger associations were observed in boys with PC(34:2) and diacylglycerol (C36:4), while in girls stronger associations with rapid growth were observed with progesterone, tetradecadiencarnitine (C14:2), decenoylcarnitine(C10:1) and DHA (Figure S4). Very similar associations were seen with overweight upon stratification by sex (Figure S5).

To assess the role of birthweight in observed associations, we additionally adjusted our models for birthweight. There was some attenuation in effect size in associations for rapid growth (Figure S6), however, the attenuation with cholestenone was modest and significance was retained. Adjustment for birthweight had little effect on associations with overweight (Figure S7). Upon stratification by size for gestational age (< and ≥33rd percentile of birthweight for gestational age, Figure S8) we observed stronger associations with cholestenone and rapid growth as well as DHA and rapid growth among larger for gestational age (≥33rd percentile) infants. We noted stronger associations with hexadecenoylacarnitine (C16:1), hexenoylcarnitine(C6:1), leucine and valine and overweight among smaller for gestational age (<33rd percentile) infants (Figure S9).

Discussion

This is the first study to date that investigates the association between untargeted metabolic profiles of cord blood and rapid growth at the first year of life and overweight/obesity in early childhood. We identified cholestenone and BCAA levels in cord blood as predictive of rapid growth and overweight/obesity, respectively, among healthy deliveries from four European populations. In multivariate analysis, we found that the addition of metabolites substantially improved prediction of both rapid growth and overweight compared with models using traditional risk factors alone.

Higher levels of cholestenone were identified as predictive of rapid growth in the MWAS analysis, with consistent effects noted across the four included cohorts. Little is known about the effects of cholestenone on health. It has previously been reported to be associated with CpG sites that are differentially methylated in relation to birthweight [35], however, birthweight did not appear to be an important contributor to the relationship between cholestenone and rapid growth in our study. Supplementation of diet with cholestenone leads to growth retardation in rodents and high levels cause hypertrophy of the adrenal glands, which may suggest potential endocrine effects [37, 38]. Cholestenone is produced by bacterial catabolism of cholesterol in the intestinal tract [39]. It therefore may be serving as a proxy indicator of the relative abundance of various microbiota present at birth, although the infant gut microbiome is generally uniform and under-developed at

this stage [40]. Indeed, gestational age, which is known to influence the composition of the neonatal gut microbiome [41], was strongly associated with cholestenone levels in our data. However, the strong association between cholestenone and rapid growth remained after adjustment for gestational age. The role of the gut microflora in obesity is increasingly recognised [42] and differences in faecal microbiota composition measured during the first year of life have been found to be associated with weight status in later childhood [43].

Lower levels of the BCAAs valine and leucine were associated with overweight/obesity in early childhood, with consistent effects across both the Rhea and INMA cohorts. Associations were somewhat stronger with valine than leucine. Lower levels of cord blood leucine were also identified as nominally associated with children on a rapid growth trajectory by the study of Isganaitis, Rifas-Shiman et al. [18]. This is in contrast with the study of Hellmuth et al., where no associations were reported between BCAAs in cord blood and weight status at 2 and 10 years, although the authors speculated that the long storage period in their study may have degraded certain metabolites such as amino acids. BCAAs levels in cord blood represent the balance of supply, from the mother and from protein degradation, and of clearance through protein synthesis, excretion and BCAA catabolism and/or oxidation. BCAAs have a complex relationship with overweight and obesity. On one hand, higher levels in blood are consistently associated with obesity, insulin resistance and type 2 diabetes. Adjustment for maternal BMI, which would be expected to increase maternal levels and the fetal supply of BCAAs, strengthened the association between cord blood BCAA levels and childhood overweight, suggesting some negative confounding. On the other hand, numerous intervention studies and animal studies have shown that increasing dietary intake of BCAAs has beneficial signalling effects, with positive effects on parameters including body composition, glycemia and satiety [44]. Multiple mechanisms for these positive effects have been proposed including direct effects on hypothalamic and brainstem processes involved in satiety [44]. Cord blood BCAAs levels could therefore influence later propensity for overweight through causal processes such as control of food intake or alternatively serve as a marker of other metabolic processes that influence both propensity for weight gain and levels of BCAAs.

Apart from the association between leucine and overweight, no other associations were observed for metabolites identified by Isganaitis, Rifas-Shiman et al. [18]. Among metabolites previously identified as associated with birthweight, we identified higher levels of progesterone, PC(34:2), plasmalogen PC(36:4)/PC(O-36:5), DHA, decenoylcarnitine (C10:1), tetradecadiencarnitine (C14:2) and diacylglycerol (C36:4) as nominally associated with rapid growth, although these did not pass multiple testing correction. Progesterone is the major progestational hormone involved

throughout all stages of pregnancy, and the pathway enrichment analysis also highlighted the role of hormonal signalling in rapid growth. DHA supplementation in milk has been shown to increase growth among preterm infants [45]. For overweight in early childhood, we noted nominal associations with lower levels of progesterone, indolelactic acid, hexenoylcarnitine (C6:1), hexadecenoylcarnitine (C16:1) and DHA. Indolelactic acid is a tryptophan catabolite that has an important role in the pathophysiology of obesity [46, 47] and is produced entirely by gut microbes [48]. Hexadecenoylcarnitine (C16:1) levels in the blood have been associated with obesity in children [49], while positive effects of DHA on obesity risk and metabolic health have been noted by multiple studies [50, 51], with proposed mechanisms including suppression of fatty-acid synthesis, enhancement of fatty-acid β-oxidation and increase of the serum adiponectin level [52]. The relatively small overlap in cord blood metabolites associated with birthweight and with rapid growth and with obesity, suggests that different mechanisms underlie these outcomes. Furthermore, despite the established association with rapid growth in infancy and later development of overweight, the different directions of effect in birthweight-related metabolites, observed with these two outcomes, suggest different contributory processes. Indeed, lower birthweight was a strong predictor of rapid growth while there was a trend for larger birthweight being associated with overweight in childhood.

Our analysis using a Random Forest classification model revealed that the coupling of the strongly associated molecules and demographic and clinical factors has a high ability to predict overweight/obesity in early childhood. Isganaitis, Rifas-Shiman et al. [18] suggested that cord blood metabolic signatures could be associated with early childhood obesity trajectories demonstrating, in a similar way with our analysis, that prediction models based on prenatal obesity factors (maternal age, pre-pregnancy BMI and breastfeeding duration) can be improved by adding cord blood associated metabolites. Although models would need to be validated in cohorts that are independent of the selection of metabolites, our results highlight a potential practical application of metabolomics to identify children at risk of obesity and support the potential merit of routine screening of cord blood [53].

A strength of our study includes the use of cord blood from multiple birth cohorts, enabling assessment of the metabolome prior to infant growth, limiting reverse causality. We included a number of prenatal sociodemographic and clinical factors in our analysis. However, we did not have complete data related to maternal nutrition and physical activity that could be linked to both the metabolome and the family environment later in life. Nevertheless, we used paternal socioeconomic factors and maternal clinical factors such as BMI that can reflect general patterns of family nutrition

[54] and physical activity [55,56,57]. Future studies, with high-quality dietary data available, should explore the role of maternal nutrition on the cord blood metabolome.

Although the samples were analysed within a single analytical run in random order, we observed heterogeneity across the cohort metabolomic signatures, mainly explained by the processing of cord blood into plasma or serum. This heterogeneity can influence the observed associations, and for this reason, we added in the model a random effect variable for the cohort. Another limitation was that the sample was selected from the general population and we, therefore, had a relatively low number of overweight children. Furthermore, the use of BMI *z* scores to classify children as overweight is a blunter assessment of adiposity than direct measures such as dual-energy X-ray absorptiometry [58]. We used WHO obesity classification criteria, which have higher sensitivity and lower specificity in identifying obese subjects than the International Obesity Task Force cutoffs. The untargeted approach is both a strength and limitation: while it provides wide metabolome coverage [59], identification of the features can be challenging. Indeed, we were also unable to characterise all the significant features in the MWAS analysis.

Conclusion

We have demonstrated metabolic profiles associated with rapid growth in infancy and overweight/obesity in early childhood, highlighting the role of multiple metabolites in various pathways. We presented evidence that cholestenone and BCAAs are associated with rapid growth in infancy and overweight/obesity in early childhood, respectively, and provide new insights on the potential mechanism underlying overweight risk, particularly early in development. Our findings present a potential route to the identification of at-risk children for the provision of targeted interventions to improve outcomes for children living in obesogenic environments.

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Contributions

O.R., L.C. and P.V. conceived the study. E.H. performed most statistical analyses. E.H. and O.R. draughted the manuscript. P.K.R., N.R. and A.S. acquired the MSy data and conducted structural annotation. T.R. contributed additional analyses for age prediction models. M.P. and T.N. coordinated data/sample collection in ENVIRONAGE, S.B. and L.R. coordinated data/sample collection in Piccolipiu, L.M. and M.V. coordinated data/sample collection in INMA, T.R. and L.C. coordinated data/sample collection in Rhea, R.A., E.H. and O.R. prepared and supervised data collection and curation. O.R. coordinated and supervised. All authors critically reviewed the manuscript.

Cord blood metabolic signatures predictive of childhood overweight and rapid growth

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Supporting information

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96 *Table S1: Demographic, anthropometric and clinical outcome variables. Values are given in mean (standard*
97 *deviation, SD) or percent (%) for rapid growth at twelve months of age by cohort.*

	RHEA (n=100)	Missing	ENVIRONAGE (n=109)	Missing	Piccolipiu (n=95)	Missing	INMA (n=87)	Missing
cohort								
RHEA	100 (100%)		-		-		-	
ENVIRONAGE	-		109 (100%)		-		-	
Piccolipiu	-		-		95 (100%)		-	
INMA	-		-		-		87 (100%)	
gender								
male	53 (53.0%)		55 (50.5%)		54 (56.8%)		42 (48.3%)	
female	47 (47.0%)		54 (49.5%)		41 (43.2%)		45 (51.7%)	
maternal parity before this pregnancy		2 (2.0%)		0 (0%)		0 (0%)		1 (1.1%)
nulliparous	28 (28.0%)		65 (59.6%)		45 (47.4%)		44 (50.6%)	
uniparous	47 (47.0%)		44 (40.4%)		42 (44.2%)		36 (41.4%)	
multiparous	23 (23.0%)		0 (0%)		8 (8.4%)		6 (6.9%)	
maternal age		0 (0%)		0 (0%)		1 (1.1%)		0 (0%)
Mean (SD)	30.0 (4.99)		29.1 (3.63)		33.3 (4.46)		31.7 (4.03)	
mother's education		1 (1.0%)		4 (3.7%)		0 (0%)		0 (0%)
primary school	8 (8.0%)		9 (8.3%)		6 (6.3%)		18 (20.7%)	
secondary school	57 (57.0%)		29 (26.6%)		40 (42.1%)		40 (46.0%)	
university or higher	34 (34.0%)		67 (61.5%)		49 (51.6%)		29 (33.3%)	
father's education		2 (2.0%)		10 (9.2%)		0 (0%)		1 (1.1%)
primary school	21 (21.0%)		10 (9.2%)		16 (16.8%)		23 (26.4%)	
secondary school	57 (57.0%)		45 (41.3%)		43 (45.3%)		44 (50.6%)	
university or higher	20 (20.0%)		44 (40.4%)		36 (37.9%)		19 (21.8%)	
maternal smoking		1 (1.0%)		0 (0%)		0 (0%)		0 (0%)
no	79 (79.0%)		102 (93.6%)		76 (80.0%)		67 (77.0%)	
yes	20 (20.0%)		7 (6.4%)		19 (20.0%)		20 (23.0%)	
passive smoke exposure		5 (5.0%)		2 (1.8%)		0 (0%)		1 (1.1%)
no	13 (13.0%)		100 (91.7%)		74 (77.9%)		46 (52.9%)	
yes	82 (82.0%)		7 (6.4%)		21 (22.1%)		40 (46.0%)	
maternal height (cm)		1 (1.0%)		5 (4.6%)		0 (0%)		1 (1.1%)
Mean (SD)	163 (5.65)		167 (7.07)		164 (5.67)		163 (6.66)	
maternal weight (kg)		1 (1.0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	66.8 (15.6)		67.4 (14.0)		61.1 (11.2)		63.1 (11.5)	
maternal BMI		1 (1.0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	25.1 (5.37)		24.1 (4.52)		22.7 (3.91)		23.7 (3.99)	
maternal weight gain (kg)		11 (11.0%)		0 (0%)		1 (1.1%)		0 (0%)
Mean (SD)	13.1 (5.89)		14.4 (5.20)		12.4 (4.44)		14.2 (4.82)	
paternal height (cm)		1 (1.0%)		5 (4.6%)		0 (0%)		1 (1.1%)
Mean (SD)	176 (7.21)		179 (7.51)		177 (6.31)		177 (6.48)	
paternal weight (kg)		0 (0%)		5 (4.6%)		0 (0%)		1 (1.1%)
Mean (SD)	85.0 (14.5)		81.2 (10.8)		78.3 (9.65)		81.3 (13.7)	
paternal age (years)		0 (0%)		4 (3.7%)		1 (1.1%)		0 (0%)
Mean (SD)	34.2 (5.04)		31.7 (4.77)		36.8 (5.48)		33.6 (4.06)	
delivery		0 (0%)		0 (0%)		0 (0%)		1 (1.1%)
vaginal	38 (38.0%)		103 (94.5%)		64 (67.4%)		79 (90.8%)	
caesarean	62 (62.0%)		6 (5.5%)		31 (32.6%)		7 (8.0%)	
pregnancy diabetes		0 (0%)		0 (0%)		0 (0%)		33 (37.9%)
no	89 (89.0%)		107 (98.2%)		88 (92.6%)		49 (56.3%)	
yes	11 (11.0%)		7 (7.4%)		11 (11.0%)		2 (1.8%)	
birth weight (g)		0 (0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	3270 (428)		3420 (551)		3230 (406)		3290 (402)	
gestational age (weeks)		0 (0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	38.4 (1.32)		39.0 (1.61)		39.6 (1.60)		39.9 (1.54)	
ethnicity		0 (0%)		0 (0%)		0 (0%)		0 (0%)
non native	5 (5.0%)		18 (16.5%)		8 (8.4%)		4 (4.6%)	
native	95 (95.0%)		89 (81.7%)		87 (91.6%)		83 (95.4%)	
paternal BMI		1 (1.0%)		5 (4.6%)		0 (0%)		1 (1.1%)
Mean (SD)	27.2 (3.90)		25.3 (3.10)		24.9 (2.70)		25.8 (3.63)	
breast feeding		4 (4.0%)		109 (100%)		3 (3.2%)		0 (0%)
no	12 (12.0%)		0 (0%)		12 (12.6%)		7 (8.0%)	
yes	84 (84.0%)		0 (0%)		80 (84.2%)		80 (92.0%)	
breast feeding duration (weeks)		4 (4.0%)		109 (100%)		19 (20.0%)		0 (0%)
Mean (SD)	19.3 (20.7)		NA (NA)		42.5 (28.9)		23.3 (17.4)	
rapid growth		0 (0%)		13 (4.7%)		0 (0%)		0 (0%)
no	60 (60.0%)		76 (69.7%)		82 (83.7%)		62 (71.3%)	
yes	40 (40.0%)		33 (30.3%)		16 (16.3%)		25 (28.7%)	
Vegetables (serves/day)		0 (0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	4.04 (2.87)		1.79 (0.829)		1.13 (0.630)		2.36 (1.17)	
Fruits (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	2.12 (2.35)		NA (NA)		0.956 (0.441)		2.88 (1.58)	

Milk products (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	2.42 (1.45)		NA (NA)		1.08 (0.593)		3.08 (1.34)	
Fish (serves/day)		0 (0%)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	0.196 (0.198)		2.21 (1.05)		0.175 (0.101)		0.774 (0.559)	
Pulses (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	0.422 (0.513)		NA (NA)		0.198 (0.195)		0.250 (0.286)	
Sugar (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	1.29 (1.33)		NA (NA)		0.526 (0.378)		4.04 (2.56)	
Eggs (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	0.178 (0.297)		NA (NA)		0.179 (0.132)		0.379 (0.179)	
Grains (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	3.15 (3.45)		NA (NA)		1.17 (0.354)		2.28 (0.974)	
Meat (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	0.477 (0.436)		NA (NA)		1.30 (0.774)		0.882 (0.344)	
Processed meat (serves/day)		10 (10.0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	0.340 (0.433)		NA (NA)		0.222 (0.237)		0.359 (0.303)	
Potatoes (serves/day)		0 (0%)		109 (100%)		0 (0%)		0 (0%)
Mean (SD)	0.596 (0.612)		NA (NA)		0.228 (0.154)		0.526 (0.317)	

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99 *Table S2: Individual number, observation number, demographic, anthropometric and clinical outcome variables*
100 *average values (standard deviation) or percent (%) for overweight throughout early childhood by cohort.*

	RHEA (n=97)	Missing	Piccolipiu (n=79)	Missing	INMA (n=96)	Missing
cohort						
RHEA	97 (100%)		-		-	
ENVIRONAGE	-		-		-	
Piccolipiu	-		79 (100%)		-	
INMA	-		-		96 (100%)	
age at weight status assessment		0 (0%)		0 (0%)		0 (0%)
	5.53 (1.03)		4.43 (0.105)		6.16 (0.635)	
	6.02 [4.01, 7.07]		4.42 [4.17, 4.75]		6.14 [4.04, 7.49]	
gender						
male	53 (54.6%)		44 (55.7%)		48 (50.0%)	
female	44 (45.4%)		35 (44.3%)		48 (50.0%)	
maternal parity before this pregnancy		2 (2.1%)		0 (0%)		1 (1.0%)
nulliparous	26 (26.8%)		37 (46.8%)		51 (53.1%)	
uniparous	46 (47.4%)		37 (46.8%)		37 (38.5%)	
multiparous	23 (23.7%)		5 (6.3%)		7 (7.3%)	
maternal age (years)		0 (0%)		1 (1.3%)		0 (0%)
Mean (SD)	30.2 (4.94)		33.7 (4.61)		31.6 (4.09)	
Median [Min, Max]	29.8 [20.3, 41.7]		33.9 [19.9, 42.8]		31.8 [23.6, 41.3]	
mother's education		1 (1.0%)		0 (0%)		0 (0%)
primary school	7 (7.2%)		4 (5.1%)		18 (18.8%)	
secondary school	55 (56.7%)		33 (41.8%)		44 (45.8%)	
university or higher	34 (35.1%)		42 (53.2%)		34 (35.4%)	
father's education		2 (2.1%)		0 (0%)		1 (1.0%)
primary school	20 (20.6%)		11 (13.9%)		27 (28.1%)	
secondary school	55 (56.7%)		37 (46.8%)		46 (47.9%)	
university or higher	20 (20.6%)		31 (39.2%)		22 (22.9%)	
maternal smoking		1 (1.0%)		0 (0%)		1 (1.0%)
no	77 (79.4%)		65 (82.3%)		74 (77.1%)	
yes	19 (19.6%)		14 (17.7%)		21 (21.9%)	
passive smoke exposure		5 (5.2%)		0 (0%)		2 (2.1%)
no	13 (13.4%)		62 (78.5%)		48 (50.0%)	
yes	79 (81.4%)		17 (21.5%)		46 (47.9%)	
maternal height (cm)		1 (1.0%)		0 (0%)		2 (2.1%)
Mean (SD)	163 (5.56)		164 (6.04)		163 (6.59)	
maternal weight (kg)		1 (1.0%)		0 (0%)		0 (0%)
Mean (SD)	67.1 (15.7)		59.8 (10.6)		62.3 (10.5)	
maternal BMI		1 (1.0%)		0 (0%)		0 (0%)
Mean (SD)	25.2 (5.42)		22.2 (3.67)		23.4 (3.64)	
maternal weight gain (kg)		11 (11.3%)		0 (0%)		0 (0%)
Mean (SD)	13.0 (5.78)		12.3 (4.19)		14.3 (4.97)	
paternal height (cm)		1 (1.0%)		0 (0%)		2 (2.1%)
Mean (SD)	177 (7.14)		178 (6.24)		177 (6.80)	

paternal weight (kg)		0 (0%)		0 (0%)		2 (2.1%)
Mean (SD)	85.1 (14.4)		79.1 (11.1)		81.1 (13.3)	
paternal age (years)		0 (0%)		1 (1.3%)		0 (0%)
Mean (SD)	34.2 (5.02)		36.7 (5.50)		33.5 (4.32)	
delivery		0 (0%)		0 (0%)		1 (1.0%)
vaginal	36 (37.1%)		50 (63.3%)		84 (87.5%)	
caesarean	61 (62.9%)		29 (36.7%)		11 (11.5%)	
pregnancy diabetes		0 (0%)		0 (0%)		41 (42.7%)
no	87 (89.7%)		73 (92.4%)		50 (52.1%)	
yes	10 (10.3%)		6 (7.6%)		5 (5.2%)	
birth weight (g)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	3270 (428)		3230 (406)		3290 (402)	
gestational age (weeks)		0 (0%)		0 (0%)		0 (0%)
Mean (SD)	38.4 (1.30)		39.7 (1.49)		39.8 (1.51)	
Median [Min, Max]	38.4 [34.2, 41.1]		39.6 [36.6, 44.6]		39.9 [34.3, 44.7]	
ethnicity		0 (0%)		0 (0%)		0 (0%)
native	4 (4.1%)		5 (6.3%)		4 (4.2%)	
non native	93 (95.9%)		74 (93.7%)		92 (95.8%)	
paternal BMI		1 (1.0%)		0 (0%)		2 (2.1%)
Mean (SD)	27.2 (3.82)		24.9 (3.18)		25.8 (3.46)	
breast feeding		4 (4.1%)		1 (1.3%)		0 (0%)
no	12 (12.4%)		10 (12.7%)		8 (8.3%)	
yes	81 (83.5%)		68 (86.1%)		88 (91.7%)	
breast feeding duration (weeks)		4 (4.1%)		16 (20.3%)		0 (0%)
Mean (SD)	18.8 (20.4)		42.9 (28.8)		23.1 (17.4)	
overweight/obesity[a] population		0 (0%)		0 (0%)		0 (0%)
no	66 (68.0%)		75 (94.9%)		67 (69.8%)	
yes	31 (32.0%)		4 (5.1%)		29 (30.2%)	
rapid growth		0 (0%)		0 (0%)		12 (12.5%)
no	59 (60.8%)		82 (83.7%)		60 (62.5%)	
yes	38 (39.2%)		16 (16.3%)		24 (25.0%)	
Vegetables (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	4.09 (2.88)		1.13 (0.665)		2.31 (1.18)	
Fruits (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	2.15 (2.38)		0.951 (0.458)		2.81 (1.54)	
Milk products (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	2.42 (1.47)	1.10 (0.574)	1.10 (0.574)		3.12 (1.30)	
Fish (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	0.195 (0.200)	0.175 (0.106)	0.175 (0.106)		0.764 (0.551)	
Pulses (serves/day)		0 (0%)		0 (0%)		
Mean (SD)	0.427 (0.519)		0.186 (0.157)		0.249 (0.277)	1 (1.0%)
Sugar (serves/day)		0 (0%)		0 (0%)		
Mean (SD)	1.30 (1.34)		0.548 (0.389)		3.97 (2.54)	
Eggs (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	0.177 (0.298)		0.175 (0.115)		0.381 (0.173)	
Grains (serves/day)		0 (0%)		0 (0%)		
Mean (SD)	3.12 (3.47)		1.18 (0.343)		2.23 (0.958)	1 (1.0%)
Meat (serves/day)		0 (0%)		0 (0%)		
Mean (SD)	0.480 (0.442)		1.28 (0.774)		0.869 (0.336)	
Processed meat (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	0.337 (0.438)		0.222 (0.229)		0.344 (0.212)	
Potatoes (serves/day)		0 (0%)		0 (0%)		1 (1.0%)
Mean (SD)	0.597 (0.617)		0.216 (0.143)		0.524 (0.313)	

[a]Classification based on WHO sex-adjusted and age-adjusted BMI z-scores

Table S3: All metabolomic features significantly associated (FDR 5%) with rapid growth at in first year of life. In case of more than one feature per compound were detected, the feature with highest intensity is written in bold.

Compound	*m/z*	Rt(min)	Annotation	Estimate	Std Error	t-score	p-value*
1	**385.3487**	**9.076708**	**Cholestenone**	**0.725**	**0.132**	**5.492**	**1.88E-04**
1	407.3299	9.073516	Cholestenone	0.642	0.127	5.076	8.66E-04
2	269.1894	5.3084226	Unidentified(U8)	-0.571	0.125	-4.558	6.09E-03
3	289.2157	4.8316393	Unidentified (U6)	-0.563	0.125	-4.502	6.34E-03
4	482.2392	3.6582649	Unidentified (U4)	-0.538	0.127	-4.238	1.77E-02

*Model was adjusted for child's sex and age at outcome measurement and ethnicity. We used a random *effects* model by cohort

Table S4: Pre-annotated metabolites in cord blood that have been previously identified in the same dataset associated with birthweight (Robinson et al.; Alfano et al.), or because they have previously been reported to predict rapid growth leading to overweight in childhood trajectory (Isganaitis et al.).

ID	Metabolite name	*m/z*	retention time (minutes)	Reference
1	Leucine	132.1021	1.4519173	Isganaitis et al., 2015
2	Tryptophan	205.0965	2.4842238	Isganaitis et al., 2015
3	Indolelactic acid	206.0822	3.8289883	Robinson et al., 2018
4	Methoxykynurenic acid	220.5393	3.6709497	Robinson et al., 2018
5	Butyrylcarnitine/Isobutyrylcarnitine (C4:0)	232.1537	1.9274178	Robinson et al., 2018
6	Hexenoylcarnitine (C6:1)	258.1699	2.8306587	Robinson et al., 2018
7	Retinol	269.2278	7.2190323	Robinson et al., 2018
8	Octanoylcarnitine (C8:0)	288.2171	4.4222255	Robinson et al., 2018
9	Sphingosine	300.2905	6.4203	Isganaitis et al., 2015
10	Decenoylcarnitine (C10:1)	314.2321	4.8776007	Robinson et al., 2018
11	Progesterone	315.232	6.3944817	Robinson et al., 2018
12	Decanoylcarnitine (C10:0)	316.2489	5.1387444	Robinson et al., 2018
13	Docosahexaenoic acid	329.2482	7.2322	Robinson et al., 2018
14	Dodecenoylcarnitine (C12:1)	342.2641	5.422301	Robinson et al., 2018
15	Dodecanoylcarnitine (C12:0)	344.2797	5.647444	Robinson et al., 2018
16	Tetradecadiencarnitine (C14:2)	368.2793	5.631012	Robinson et al., 2018
17	Cholesterol	369.3521	9.60744	Alfano et al., 2019
18	Tetradecenoylcarnitine (C14:1)	370.2955	5.840157	Robinson et al., 2018
19	Tetradecanoylcarnitine (C14:0)	372.3112	6.56033	Robinson et al., 2018
20	Cholestenone	385.3487	9.76708	Alfano et al., 2019
21	Hydroxytetradecenoylcarnitine (C14:1-OH)	386.2899	5.568466	Robinson et al., 2018
22	Hexadecadienoylcarnitine (C16:2)	396.31	5.9437513	Robinson et al., 2018
23	Hexadecenoylcarnitine (C16:1)	398.3264	6.1093335	Robinson et al., 2018
24	Hydroxyhexadecadienoylcarnitine (C16:1-OH)	412.3045	5.749766	Robinson et al., 2018
25	LysoPC(16:1)	494.325	6.817081	Robinson et al., 2018
26	LysoPC(18:3)	518.3216	6.7819257	Robinson et al., 2018
27	LysoPC(18:1)	522.3555	6.979925	Robinson et al., 2018
28	LysoPC(20:2)	548.3681	7.141414	Robinson et al., 2018
29	LysoPC(20:4)	563.3141	6.8930106	(Alfano et al., 2019; Robinson et al., 2018),
30	LysoPC(22:6)	568.3409	6.88448	Robinson et al., 2018
31	LysoPC(22:5)	570.3551	7.206504	Robinson et al., 2018
32	Diacylglycerol (C34:2)	615.4959	9.762555	Robinson et al., 2018
33	Diacylglycerol (C36:4)	639.4946	9.408274	Robinson et al., 2018
34	Diacylglycerol (C36:3)	641.5112	9.9381895	Robinson et al., 2018
35	PC(30:0)	706.541	8.492703	(Alfano et al., 2019; Robinson et al., 2018),
36	PC(32:0)	734.57	8.960004	Robinson et al., 2018
37	PC(34:2)	758.5747	8.684198	Robinson et al., 2018
38	PlasmalogenPC(36:4) or PC(O-36:5)	766.5815	8.858829	Alfano et al., 2019; Robinson et al., 2018
39	PlasmalogenPC(36:3) or PC(O-36:4)	768.5883	9.189	(Alfano et al., 2019; Robinson et al., 2018),
40	PC(36:4)	782.5722	9.57233	(Alfano et al., 2019; Robinson et al., 2018),
41	PC(36:4) isomer	793.5614	8.628368	Robinson et al., 2018
42	Plasmalogen PC(38:4) or PC(O-38:5)	794.6046	9.77853	(Alfano et al., 2019; Robinson et al., 2018),
43	PC(38:4)	810.6053	9.168946	Robinson et al., 2018

Table S5: Logistic regression odds ratio per standard deviation (95% CI) for rapid growth at twelve months for Model 1 and 2** for the birthweight related metabolites.*

num	Metabolite	Model 1*			Model 2**		
		Odd ratio (95%CIs)	p-value	False discovery rate	Odd ratio (95%CIs)	p-value	False discovery rate
1	Butyrylcarnitine/Isobutyrylcarnitine (C4:0)	1.231 (0.982,1.543)	7.09E-02	1.42E-01	1.133 (0.855,1.502)	3.84E-01	5.82E-01
2	Decanoylcarnitine (C10:0)	1.193 (0.945,1.506)	1.37E-01	2.37E-01	1.204 (0.915,1.584)	1.85E-01	3.95E-01
3	Decenoylcarnitine (C10:1)	1.405 (1.106,1.785)	5.39E-03	2.32E-02	1.435 (1.087,1.893)	1.07E-02	1.18E-01
4	Dodecanoylcarnitine (C12:0)	1.140 (0.907,1.432)	2.61E-01	3.96E-01	1.195 (0.912,1.566)	1.96E-01	3.95E-01
5	Dodecenoylcarnitine (C12:1)	1.121 (0.896,1.403)	3.17E-01	4.65E-01	1.151 (0.882,1.502)	3.02E-01	5.10E-01
6	Hexadecadienoylcarnitine (C16:2)	1.247 (0.998,1.557)	5.21E-02	1.15E-01	1.138 (0.874,1.482)	3.36E-01	5.27E-01
7	Hexadecenoylcarnitine (C16:1)	1.068 (0.852,1.338)	5.68E-01	6.75E-01	1.060 (0.807,1.391)	6.75E-01	7.56E-01
8	Hexenoylcarnitine (C6:1)	1.261 (0.984,1.617)	6.73E-02	1.41E-01	1.088 (0.808,1.465)	5.77E-01	7.27E-01
9	Hydroxyhexadecadienoylcarnitine (C16:1-OH)	1.180 (0.950,1.466)	1.35E-01	2.37E-01	1.167 (0.901,1.512)	2.41E-01	4.61E-01
10	Hydroxytetradecenoylcarnitine (C14:1-OH)	1.088 (0.868,1.364)	4.63E-01	6.17E-01	1.071 (0.816,1.405)	6.21E-01	7.39E-01
11	Octanoylcarnitine (C8:0)	1.155 (0.918,1.454)	2.18E-01	3.42E-01	1.073 (0.822,1.400)	6.05E-01	7.39E-01
12	Tetradecadiencarnitine (C14:2)	1.325 (1.059,1.658)	1.39E-02	4.37E-02	1.315 (1.013,1.706)	3.97E-02	2.01E-01
13	Tetradecanoylcarnitine (C14:0)	1.034 (0.816,1.311)	7.80E-01	8.37E-01	0.991 (0.745,1.318)	9.50E-01	9.50E-01
14	Tetradecenoylcarnitine (C14:1)	1.058 (0.845,1.324)	6.26E-01	7.25E-01	1.048 (0.804,1.366)	7.28E-01	7.81E-01
15	Leucine	1.045 (0.821,1.329)	7.20E-01	7.92E-01	0.902 (0.678,1.200)	4.79E-01	6.40E-01
16	Tryptophan	0.923 (0.736,1.158)	4.89E-01	6.25E-01	0.947 (0.725,1.236)	6.87E-01	7.56E-01
17	Sphingosine	0.942 (0.718,1.236)	6.65E-01	7.50E-01	0.788 (0.605,1.028)	7.94E-02	2.91E-01
18	Docosahexaenoic acid	1.292 (1.005,1.660)	4.58E-02	1.06E-01	1.456 (1.109,1.911)	6.78E-03	9.95E-02
19	Diacylglycerol (C34:2)	1.033 (0.778,1.372)	8.20E-01	8.39E-01	1.135 (0.829,1.554)	4.30E-01	6.10E-01
20	Diacylglycerol (C36:3)	1.084 (0.832,1.413)	5.48E-01	6.70E-01	1.273 (0.961,1.688)	9.30E-02	3.11E-01
21	Diacylglycerol (C36:4)	1.123 (0.880,1.432)	3.51E-01	4.98E-01	1.376 (1.054,1.796)	1.91E-02	1.40E-01
22	LysoPC(16:1)	1.004 (0.803,1.256)	9.69E-01	9.69E-01	0.798 (0.608,1.046)	1.02E-01	3.11E-01
23	LysoPC(18:1)	1.185 (0.946,1.484)	1.40E-01	2.37E-01	0.939 (0.717,1.229)	6.45E-01	7.47E-01
24	LysoPC(18:3)	1.090 (0.871,1.365)	4.53E-01	6.17E-01	0.963 (0.732,1.266)	7.86E-01	8.24E-01
25	LysoPC(20:2)	1.303 (1.012,1.677)	3.97E-02	9.71E-02	1.081 (0.822,1.420)	5.78E-01	7.27E-01
26	LysoPC(20:4)	1.435 (1.106,1.861)	6.52E-03	2.39E-02	1.282 (0.949,1.733)	1.06E-01	3.11E-01
27	LysoPC(22:5)	0.971 (0.759,1.243)	8.16E-01	8.39E-01	0.757 (0.564,1.016)	6.33E-02	2.53E-01
28	LysoPC(22:6)	0.862 (0.682,1.090)	2.14E-01	3.42E-01	0.857 (0.657,1.118)	2.55E-01	4.67E-01
29	PC(30:0)	1.312 (1.046,1.647)	1.89E-02	5.55E-02	0.987 (0.749,1.302)	9.29E-01	9.50E-01
30	PC(32:0)	1.396 (1.112,1.753)	4.06E-03	2.20E-02	1.148 (0.885,1.488)	2.99E-01	5.10E-01
31	PC(34:2)	1.542 (1.222,1.946)	2.62E-04	3.84E-03	1.456 (1.132,1.872)	3.46E-03	7.62E-02
32	PC(36:4)	1.415 (1.125,1.779)	2.99E-03	2.19E-02	1.119 (0.856,1.462)	4.11E-01	6.03E-01
33	PC(36:4) isomer	1.417 (1.114,1.802)	4.49E-03	2.20E-02	1.297 (0.988,1.702)	6.06E-02	2.53E-01
34	PC(38:4)	1.274 (1.019,1.593)	3.33E-02	9.15E-02	1.197 (0.928,1.544)	1.67E-01	3.86E-01
35	Plasmalogen PC(38:4) or PC(O-38:5)	1.379 (1.097,1.732)	5.80E-03	2.32E-02	1.099 (0.846,1.429)	4.80E-01	6.40E-01
36	PlasmalogenPC(36:3) or PC(O-36:4)	1.431 (1.142,1.792)	1.84E-03	1.62E-02	1.218 (0.943,1.572)	1.31E-01	3.19E-01
37	PlasmalogenPC(36:4) or PC(O-36:5)	1.484 (1.180,1.868)	7.52E-04	8.27E-03	1.317 (1.011,1.716)	4.11E-02	2.01E-01
38	Cholestenone	2.064 (1.594,2.673)	3.98E-08	1.75E-06	1.755 (1.236,2.491)	1.66E-03	3.32E-03
39	Cholesterol	1.535 (1.220,1.930)	2.49E-04	3.84E-03	1.229 (0.941,1.606)	1.30E-01	3.19E-01
40	Progesterone	1.402 (1.092,1.800)	7.97E-03	2.70E-02	1.428 (1.051,1.940)	2.26E-02	1.42E-01
41	Indolelactic acid	1.300 (1.013,1.667)	3.89E-02	9.71E-02	1.255 (0.938,1.679)	1.26E-01	3.19E-01
42	Methoxykynurenic acid	1.084 (0.859,1.367)	4.98E-01	6.25E-01	1.150 (0.867,1.524)	3.33E-01	5.27E-01
43	Retinol	0.792 (0.603,1.041)	9.41E-02	1.80E-01	0.820 (0.606,1.109)	1.97E-01	3.95E-01

**** Model 1 (adjusted for sex and age of child at outcome measurement, ethnicity and we used a random effects model by cohort)***

*****Model 2 (Model 1 adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery)***

Table S6: All metabolomic features significantly associated (FDR 5%) with overweight/obesity at early childhood. In case of more than one feature per compound were detected, the feature with highest intensity is written in bold.

Compound	*m/z*	Rt(min)	Annotation	Estimate	Std Error	t-value	p-value*
5	**129.0025**	**0.4939376**	**Unidentified (U1)**	**-0.661**	**0.185**	**-3.571**	**3.55E-04**
5	86.99288	0.4942084	Unidentified (U1)	-0.627	0.179	-3.512	4.44E-04
6	**196.9619**	**0.5246815**	**Unidentified (U2)**	**0.832**	**0.219**	**3.794**	**1.48E-04**
6	253.9104	0.5244932	Unidentified (U2)	0.762	0.214	3.565	3.64E-04
7	514.878	0.5776492	Unidentified (U3)	-0.698	0.170	-4.115	3.88E-05
7	582.8643	0.5770611	Unidentified (U3)	-0.677	0.165	-4.111	3.94E-05
7	446.8882	0.577687	Unidentified (U3)	-0.676	0.165	-4.100	4.14E-05
7	700.8185	0.5779851	Unidentified (U3)	-0.767	0.189	-4.065	4.81E-05
7	378.9011	0.5771529	Unidentified (U3)	-0.683	0.168	-4.063	4.85E-05
7	726.8223	0.5750447	Unidentified (U3)	-0.720	0.179	-4.032	5.53E-05
7	760.8202	0.5745219	Unidentified (U3)	-0.684	0.170	-4.020	5.82E-05
7	650.8544	0.5757769	Unidentified (U3)	-0.612	0.154	-3.981	6.85E-05
7	692.8324	0.5753962	Unidentified (U3)	-0.723	0.182	-3.973	7.11E-05
7	718.8356	0.5748507	Unidentified (U3)	-0.574	0.147	-3.914	9.09E-05
7	**242.9253**	**0.5751633**	**Unidentified (U3)**	**-0.696**	**0.178**	**-3.906**	**9.39E-05**
7	312.9127	0.5741116	Unidentified (U3)	-0.714	0.183	-3.903	9.49E-05
7	108.9488	0.5738347	Unidentified (U3)	-0.724	0.187	-3.866	1.11E-04
7	106.9512	0.5741819	Unidentified (U3)	-0.713	0.187	-3.821	1.33E-04
7	310.9139	0.5743221	Unidentified (U3)	-0.689	0.180	-3.819	1.34E-04
7	658.8351	0.5753322	Unidentified (U3)	-0.704	0.185	-3.809	1.40E-04
7	176.937	0.5720361	Unidentified (U3)	-0.716	0.189	-3.796	1.47E-04
7	174.9394	0.5723583	Unidentified (U3)	-0.707	0.187	-3.771	1.62E-04
7	828.8043	0.5720578	Unidentified (U3)	-0.645	0.175	-3.696	2.19E-04
7	870.7891	0.5769692	Unidentified (U3)	-0.710	0.196	-3.629	2.84E-04
7	624.843	0.5741374	Unidentified (U3)	-0.726	0.200	-3.620	2.94E-04
7	598.8356	0.5796612	Unidentified (U3)	-0.705	0.195	-3.620	2.95E-04
7	462.8653	0.5798938	Unidentified (U3)	-0.695	0.194	-3.581	3.42E-04
7	394.8761	0.5797012	Unidentified (U3)	-0.695	0.195	-3.571	3.56E-04
7	530.8525	0.579978	Unidentified (U3)	-0.694	0.195	-3.565	3.63E-04
7	666.8233	0.5794433	Unidentified (U3)	-0.712	0.200	-3.557	3.75E-04
7	258.9015	0.5785645	Unidentified (U3)	-0.682	0.193	-3.530	4.15E-04
7	794.8107	0.5737721	Unidentified (U3)	-0.611	0.173	-3.529	4.16E-04
7	734.812	0.5787313	Unidentified (U3)	-0.696	0.197	-3.527	4.21E-04
7	326.8868	0.5771512	Unidentified (U3)	-0.686	0.196	-3.494	4.76E-04
8	154.0264	0.6849625	Unidentified (U4)	-0.702	0.172	-4.088	4.35E-05
9	169.134	0.6985534	Unidentified (U5)	-0.759	0.206	-3.687	2.27E-04
10	209.1159	6.164805	Unidentified (U7)	-0.671	0.181	-3.705	2.12E-04
13	443.4095	8.544215	Unidentified (U9)	0.893	0.221	4.046	5.21E-05
13	**460.4366**	**8.543666**	**Unidentified (U9)**	**1.001**	**0.262**	**3.822**	**1.32E-04**
14	**72.08108**	**0.8007007**	**Valine**	**-0.611**	**0.163**	**-3.748**	**1.78E-04**
14	249.0292	0.8028366	Valine	-0.670	0.181	-3.694	2.21E-04

*Model was adjusted for child's sex and age at outcome measurement and ethnicity. We used a random effects model by cohort

Table S7: Logistic regression odds ratio per standard deviation (95% CI) for overweight/obesity in early childhood for Model 1 and 2** for the birthweight related metabolites.*

num	Metabolite	Model 1*			Model 2**		
		Odd ratio (95%CIs)	p-value	False discovery rate	Odd ratio (95%CIs)	p-value	False discovery rate
1	Butyrylcarnitine/Isobutyrylcarnitine (C4:0)	0.777 (0.570,1.058)	1.09E-01	6.43E-01	0.734 (0.498,1.081)	1.17E-01	3.58E-01
2	Decanoylcarnitine (C10:0)	0.912 (0.653,1.274)	5.89E-01	8.36E-01	0.868 (0.581,1.296)	4.88E-01	7.67E-01
3	Decenoylcarnitine (C10:1)	1.193 (0.858,1.659)	2.93E-01	7.70E-01	1.155 (0.769,1.734)	4.88E-01	7.67E-01
4	Dodecanoylcarnitine (C12:0)	0.906 (0.646,1.272)	5.70E-01	8.36E-01	0.834 (0.553,1.258)	3.86E-01	7.04E-01
5	Dodecenoylcarnitine (C12:1)	0.902 (0.658,1.235)	5.20E-01	8.36E-01	0.716 (0.476,1.077)	1.09E-01	3.58E-01
6	Hexadecadienoylcarnitine (C16:2)	0.941 (0.701,1.263)	6.86E-01	8.39E-01	0.928 (0.649,1.326)	6.80E-01	9.07E-01
7	Hexadecenoylcarnitine (C16:1)	0.772 (0.544,1.094)	1.46E-01	6.43E-01	0.627 (0.400,0.984)	4.23E-02	3.12E-01
8	Hexenoylcarnitine (C6:1)	0.764 (0.530,1.102)	1.50E-01	6.43E-01	0.613 (0.382,0.984)	4.26E-02	3.12E-01
9	Hydroxyhexadecadienoylcarnitine (C16:1-OH)	1.033 (0.787,1.356)	8.14E-01	9.01E-01	1.040 (0.746,1.449)	8.18E-01	9.66E-01
10	Hydroxytetradecenoylcarnitine (C14:1-OH)	1.096 (0.801,1.498)	5.68E-01	8.36E-01	1.053 (0.711,1.559)	7.96E-01	9.66E-01
11	Octanoylcarnitine (C8:0)	0.963 (0.695,1.333)	8.19E-01	9.01E-01	0.936 (0.643,1.363)	7.31E-01	9.28E-01
12	Tetradecadiencarnitine (C14:2)	1.004 (0.744,1.355)	9.79E-01	9.79E-01	0.940 (0.653,1.352)	7.39E-01	9.28E-01
13	Tetradecanoylcarnitine (C14:0)	0.880 (0.638,1.214)	4.37E-01	8.36E-01	0.776 (0.531,1.133)	1.88E-01	4.22E-01
14	Tetradecenoylcarnitine (C14:1)	0.907 (0.657,1.251)	5.51E-01	8.36E-01	0.779 (0.523,1.160)	2.19E-01	4.37E-01
15	Leucine	0.658 (0.460,0.941)	2.17E-02	4.78E-01	0.469 (0.293,0.751)	1.61E-03	4.90E-02
16	Tryptophan	0.728 (0.529,1.002)	5.11E-02	6.28E-01	0.720 (0.496,1.045)	8.38E-02	3.58E-01
17	Sphingosine	0.821 (0.552,1.221)	3.29E-01	7.70E-01	0.742 (0.507,1.086)	1.25E-01	3.58E-01
18	Docosahexaenoic acid	0.610 (0.412,0.903)	1.35E-02	4.78E-01	0.619 (0.395,0.970)	3.63E-02	3.12E-01
19	Diacylglycerol (C34:2)	0.835 (0.537,1.300)	4.25E-01	8.36E-01	0.640 (0.372,1.100)	1.07E-01	3.58E-01
20	Diacylglycerol (C36:3)	0.920 (0.618,1.368)	6.79E-01	8.39E-01	0.990 (0.654,1.498)	9.63E-01	9.93E-01
21	Diacylglycerol (C36:4)	1.146 (0.820,1.601)	4.24E-01	8.36E-01	1.182 (0.801,1.742)	4.00E-01	7.04E-01
22	LysoPC(16:1)	1.213 (0.880,1.671)	2.38E-01	7.50E-01	1.154 (0.789,1.688)	4.59E-01	7.67E-01
23	LysoPC(18:1)	1.117 (0.813,1.536)	4.94E-01	8.36E-01	1.118 (0.764,1.636)	5.65E-01	7.94E-01
24	LysoPC(18:3)	1.253 (0.917,1.713)	1.56E-01	6.43E-01	1.263 (0.875,1.821)	2.12E-01	4.37E-01
25	LysoPC(20:2)	1.022 (0.704,1.484)	9.09E-01	9.46E-01	1.126 (0.784,1.618)	5.20E-01	7.88E-01
26	LysoPC(20:4)	0.959 (0.689,1.334)	8.03E-01	9.01E-01	0.752 (0.520,1.088)	1.30E-01	3.58E-01
27	LysoPC(22:5)	1.102 (0.759,1.601)	6.10E-01	8.38E-01	0.957 (0.593,1.545)	8.58E-01	9.68E-01
28	LysoPC(22:6)	0.928 (0.664,1.297)	6.61E-01	8.39E-01	0.993 (0.706,1.399)	9.70E-01	9.93E-01
29	PC(30:0)	0.968 (0.702,1.335)	8.42E-01	9.04E-01	1.043 (0.702,1.551)	8.34E-01	9.66E-01
30	PC(32:0)	1.015 (0.746,1.381)	9.25E-01	9.46E-01	1.115 (0.760,1.637)	5.78E-01	7.94E-01
31	PC(34:2)	1.068 (0.775,1.472)	6.87E-01	8.39E-01	1.271 (0.887,1.821)	1.92E-01	4.22E-01
32	PC(36:4)	0.905 (0.661,1.238)	5.31E-01	8.36E-01	0.981 (0.680,1.414)	9.17E-01	9.84E-01
33	PC(36:4) isomer	0.829 (0.616,1.115)	2.14E-01	7.24E-01	0.750 (0.527,1.067)	1.10E-01	3.58E-01
34	PC(38:4)	0.810 (0.594,1.104)	1.81E-01	6.65E-01	0.717 (0.486,1.057)	9.34E-02	3.58E-01
35	Plasmalogen PC(38:4) or PC(O-38:5)	0.795 (0.576,1.096)	1.61E-01	6.43E-01	0.762 (0.522,1.115)	1.61E-01	4.18E-01
36	PlasmalogenPC(36:3) or PC(O-36:4)	0.855 (0.623,1.173)	3.33E-01	7.70E-01	0.809 (0.556,1.178)	2.69E-01	5.15E-01
37	PlasmalogenPC(36:4) or PC(O-36:5)	0.796 (0.582,1.088)	1.53E-01	6.43E-01	0.764 (0.519,1.125)	1.73E-01	4.22E-01
38	Cholestenone	1.312 (0.942,1.826)	1.08E-01	6.43E-01	1.343 (0.923,1.953)	1.23E-01	3.58E-01
39	Cholesterol	0.919 (0.675,1.250)	5.89E-01	8.36E-01	0.999 (0.691,1.445)	9.97E-01	9.97E-01
40	Progesterone	0.732 (0.531,1.009)	5.71E-02	6.28E-01	0.590 (0.383,0.910)	1.71E-02	2.81E-01
41	Indolelactic acid	0.854 (0.629,1.160)	3.13E-01	7.70E-01	0.657 (0.462,0.934)	1.92E-02	2.81E-01
42	Methoxykynurenic acid	1.185 (0.843,1.667)	3.29E-01	7.70E-01	0.977 (0.656,1.456)	9.10E-01	9.84E-01
43	Retinol	0.866 (0.590,1.271)	4.62E-01	8.36E-01	0.731 (0.488,1.094)	1.27E-01	3.58E-01

**** Model 1 (adjusted for child sex and age at outcome measurement, ethnicity and we used a random effects model by cohort),***

*****Model 2 (Model 1 adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery)***

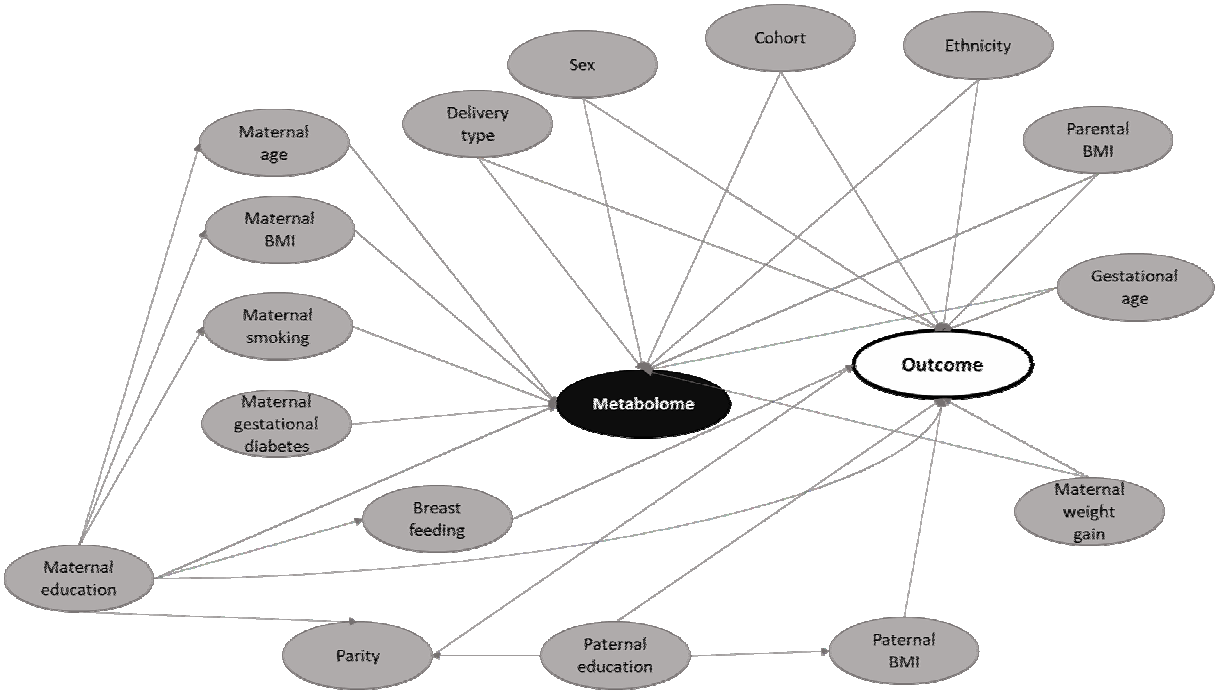


Figure S1: Directed acyclical graph (DAG) to visualise assumptions regarding covariates, metabolome and outcome. Covariates are coloured gray, metabolome black and outcome white. The examining outcomes are: 1) rapid growth at 1st year of age and 2) overweight/obesity in early childhood.

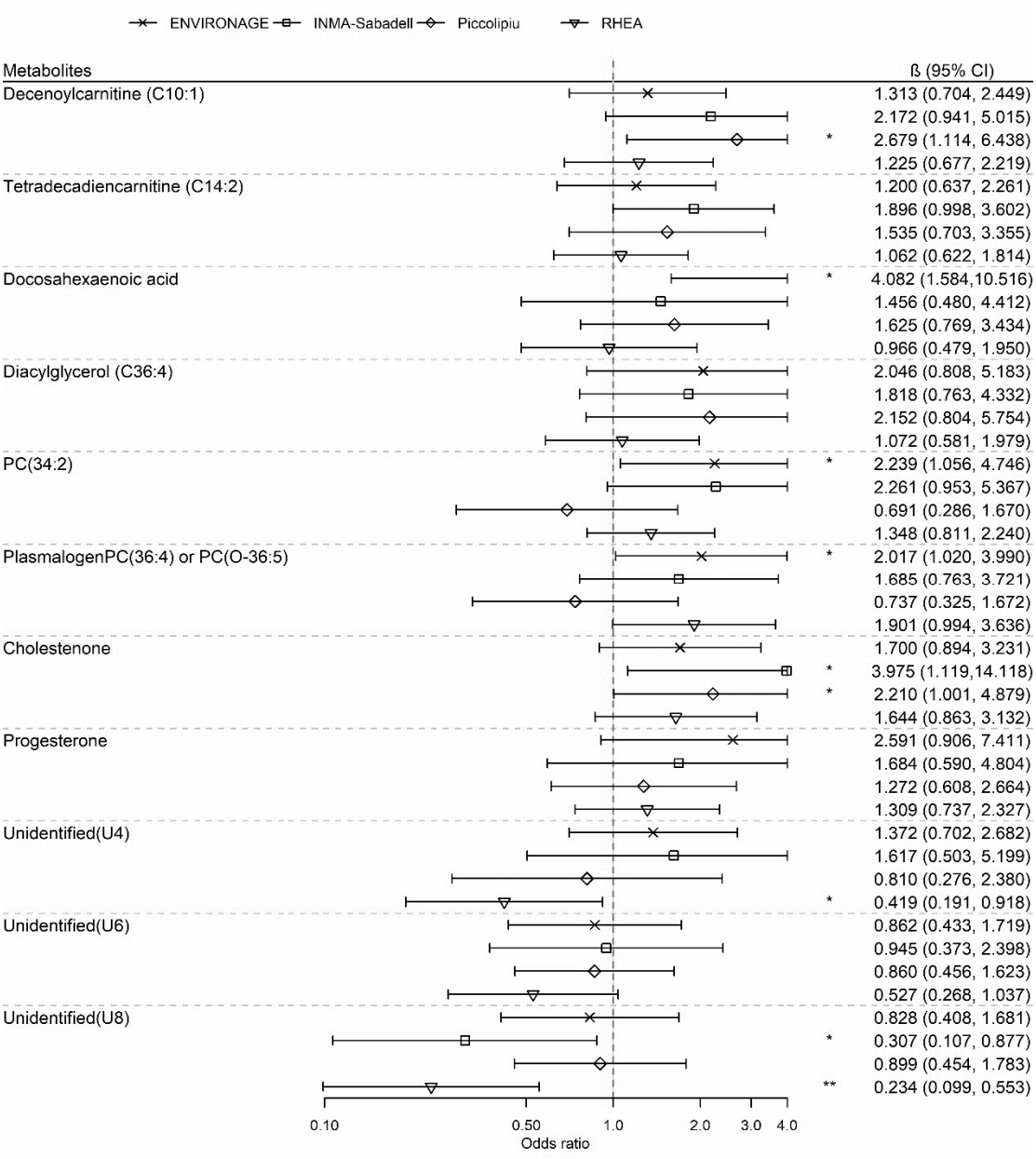


*Figure S2: Logistic regression odds ratio per standard deviation (95% CI) for rapid growth for Model 2 (adjusted for child sex and age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by cohort for the 8 between the 6 nominal statistically significant birthweight related metabolites and the 4 associated with rapid growth at 12 months. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

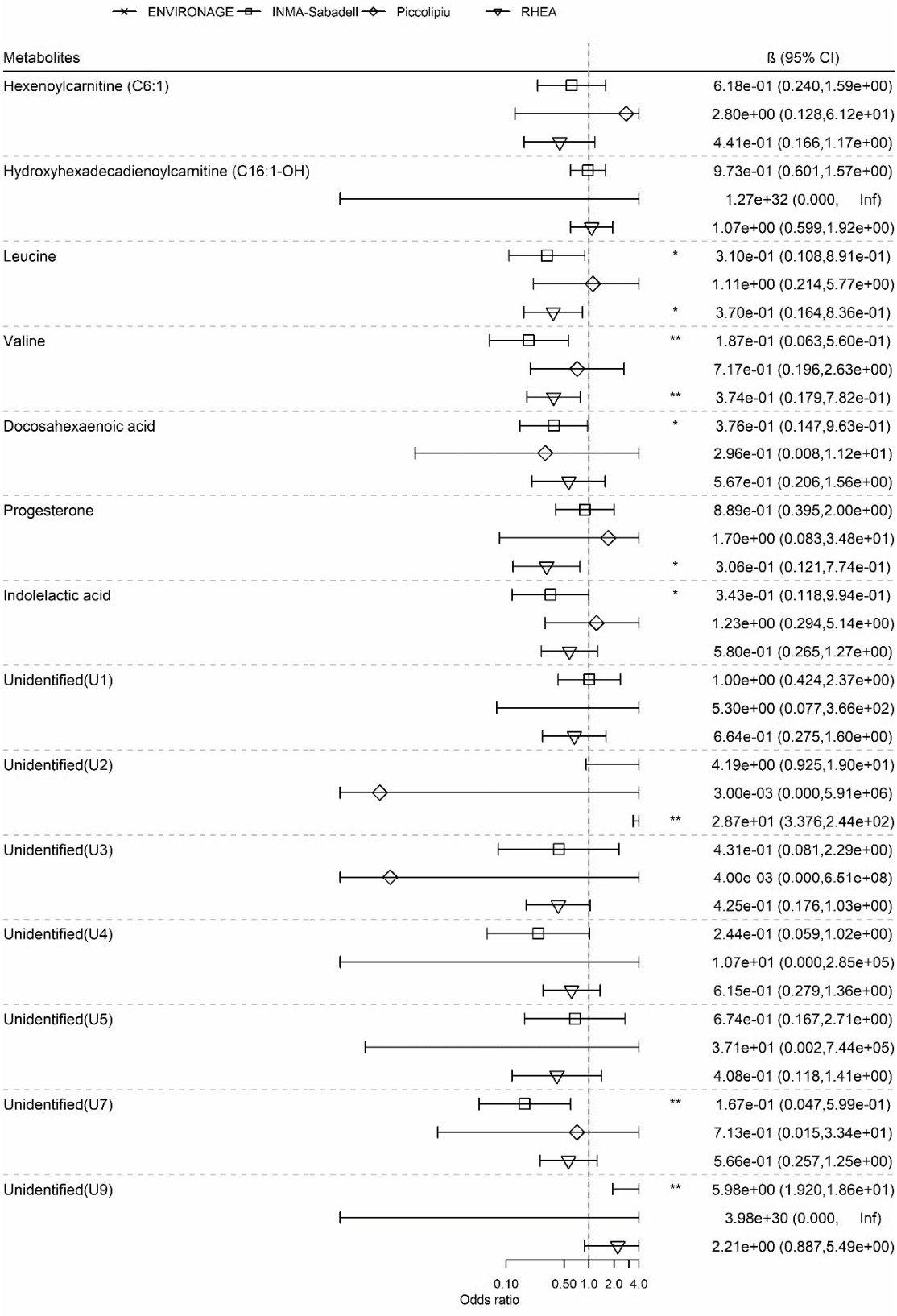


Figure S3: Logistic regression odds ratio per standard deviation (95% CI) for overweight/obesity in early childhood for Model 2 (adjusted for child sex and age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by cohort for the 6 nominal statistically significant birthweight related metabolites and the 8

*associated with overweight/obesity in early childhood. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

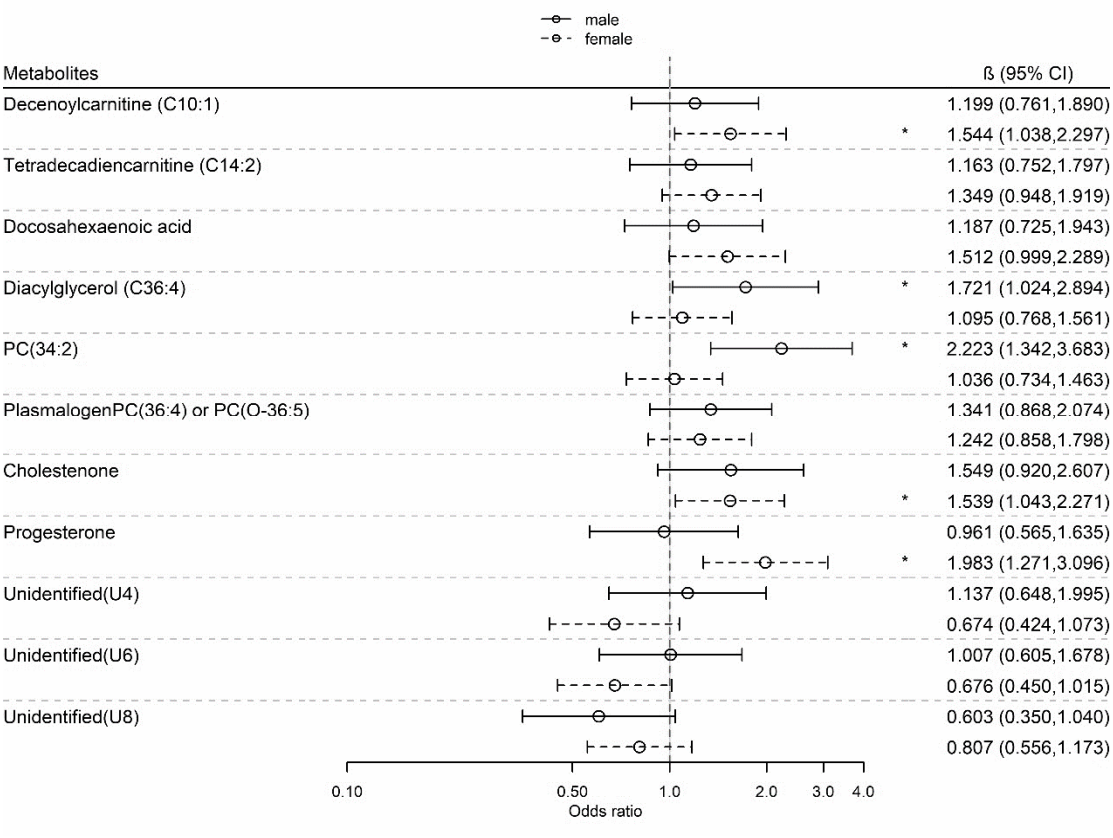


*Figure S4: Logistic regression odds ratio per standard deviation (95% CI) for rapid growth for Model 2 (adjusted for child sex, age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by sex for the 7 nominal statistically significant birthweight related metabolites and the 4 associated with rapid growth at 12 months. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

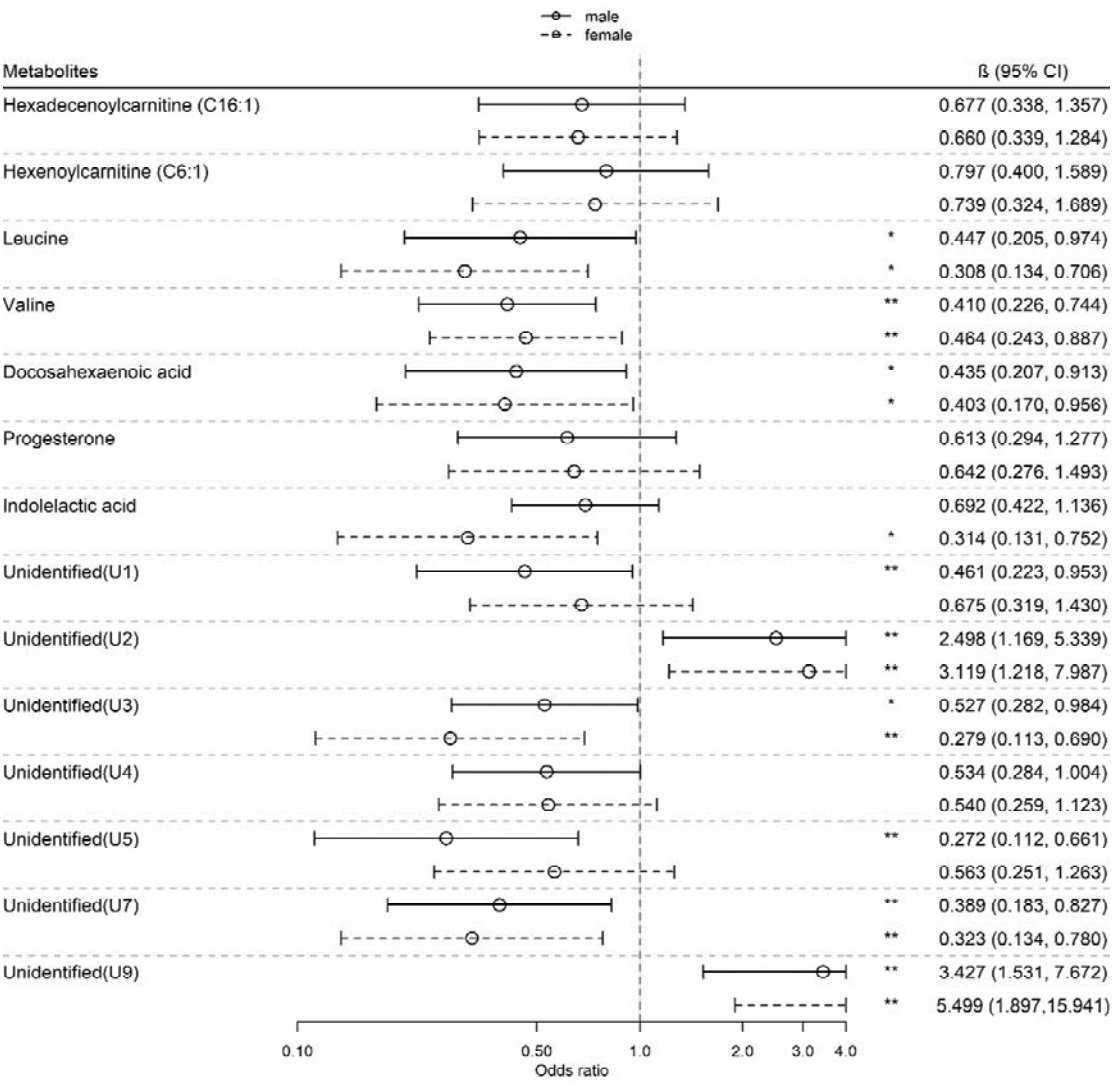


*Figure S5: Logistic regression odds ratio per standard deviation (95% CI) for overweight/obesity in early childhood for Model 2 (adjusted for child sex, age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by sex for the 6 nominal statistically significant birthweight related metabolites and the 8 associated with overweight/obesity in early childhood. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

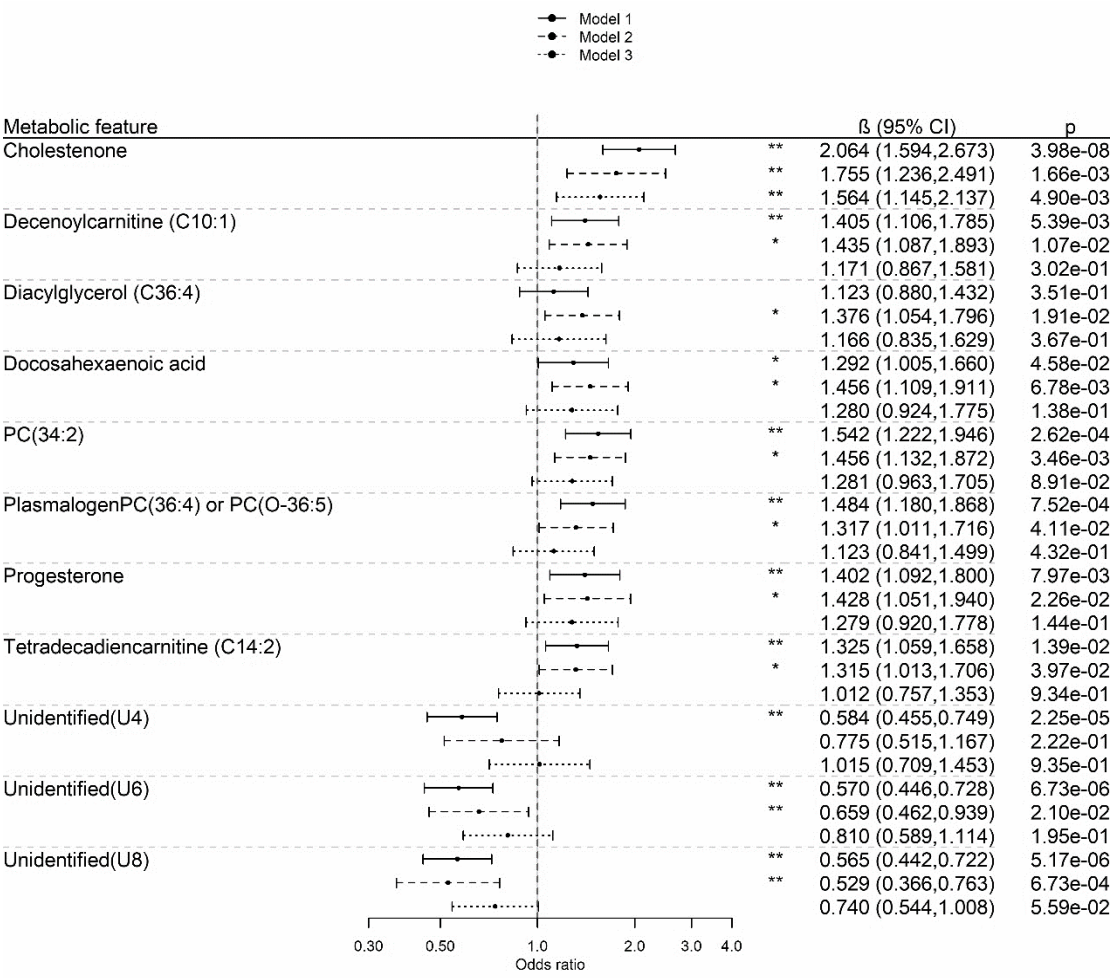


*Figure S6: Logistic regression odds ratio per standard deviation (95% CI) for rapid growth at twelve months for Model 1 (adjusted for child sex and age at outcome measurement, ethnicity and we used a random effects model by cohort), Model 2 (Model 1 adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) and Model 3 (Model 2 adjusted for birthweight) for the 7 nominal statistically significant birthweight related metabolites and the 4 associated with rapid growth at twelve months. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

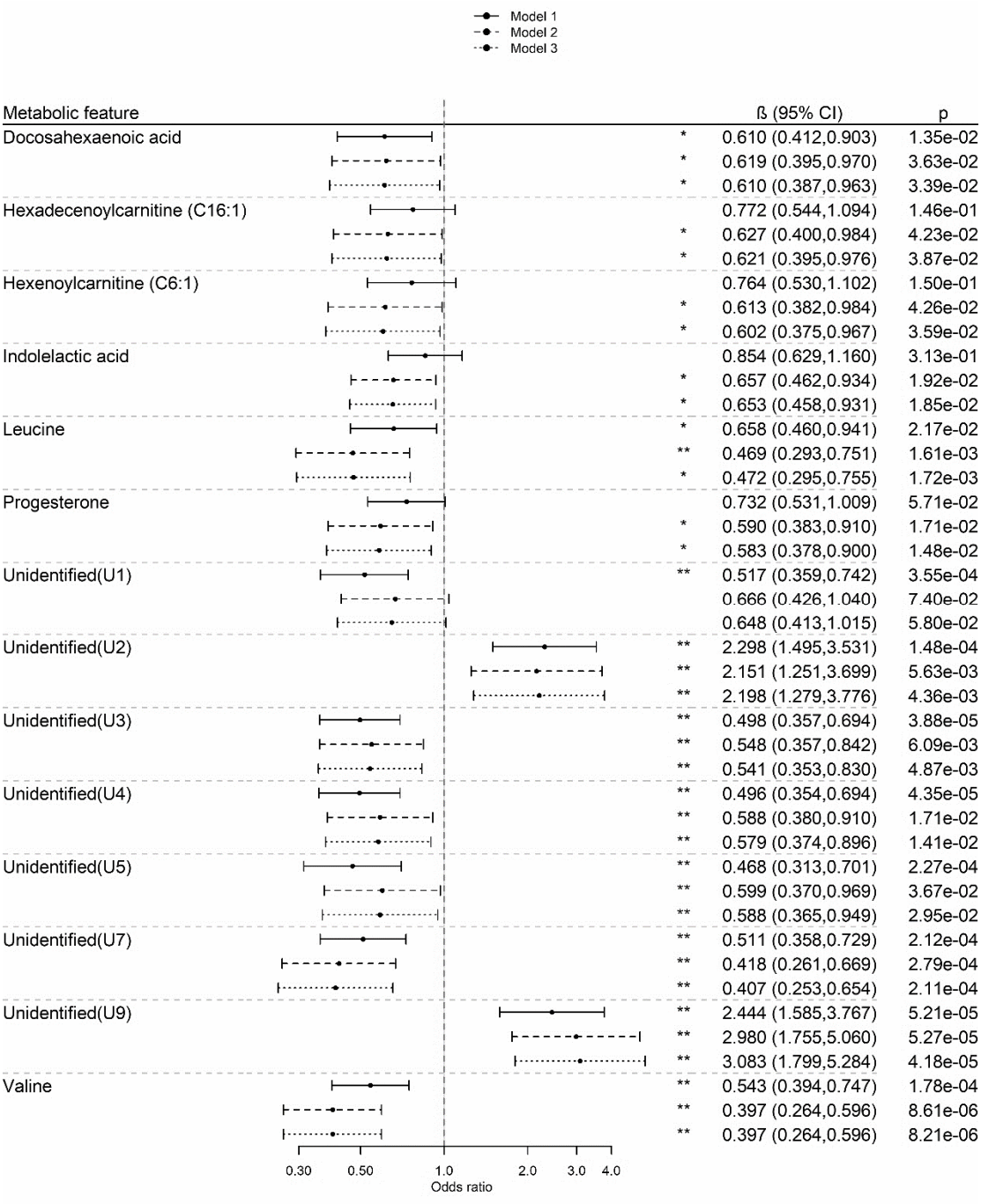


*Figure S7: Logistic regression odds ratio per standard deviation (95% CI) for overweight/obesity in early childhood for Model 1 (adjusted for child sex and age at outcome measurement, ethnicity and we used a random effects model by cohort), Model 2 (Model 1 adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) and Model 3 (Model 2 adjusted for birthweight) for the 6 nominal statistically significant birthweight related metabolites and the 8 associated with overweight/obesity in early childhood. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

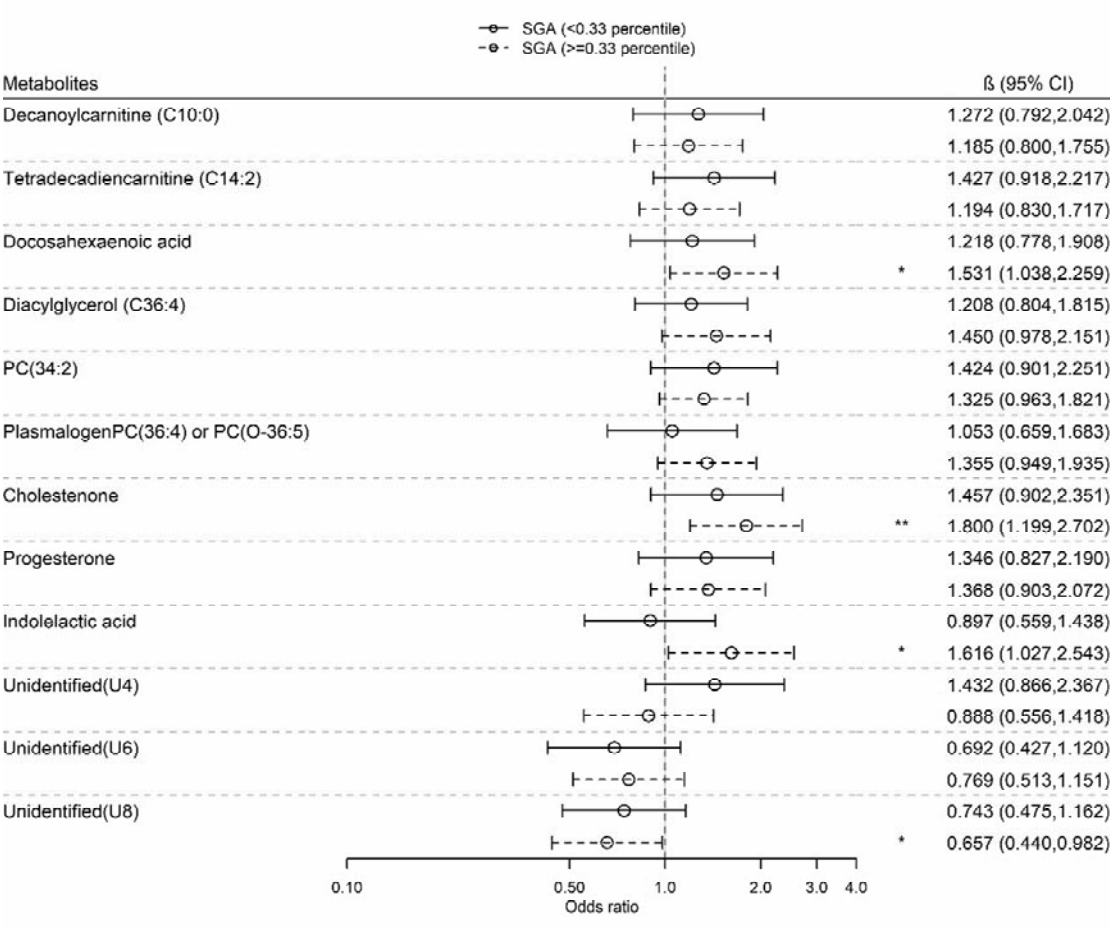


*Figure S8: Logistic regression odds ratio per standard deviation (95% CI) for rapid growth for Model 2 (adjusted for child sex, age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by Small of Gestational Age (SGA) for the 7 nominal statistically significant birthweight related metabolites and the 4 associated with rapid growth at 12 months. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

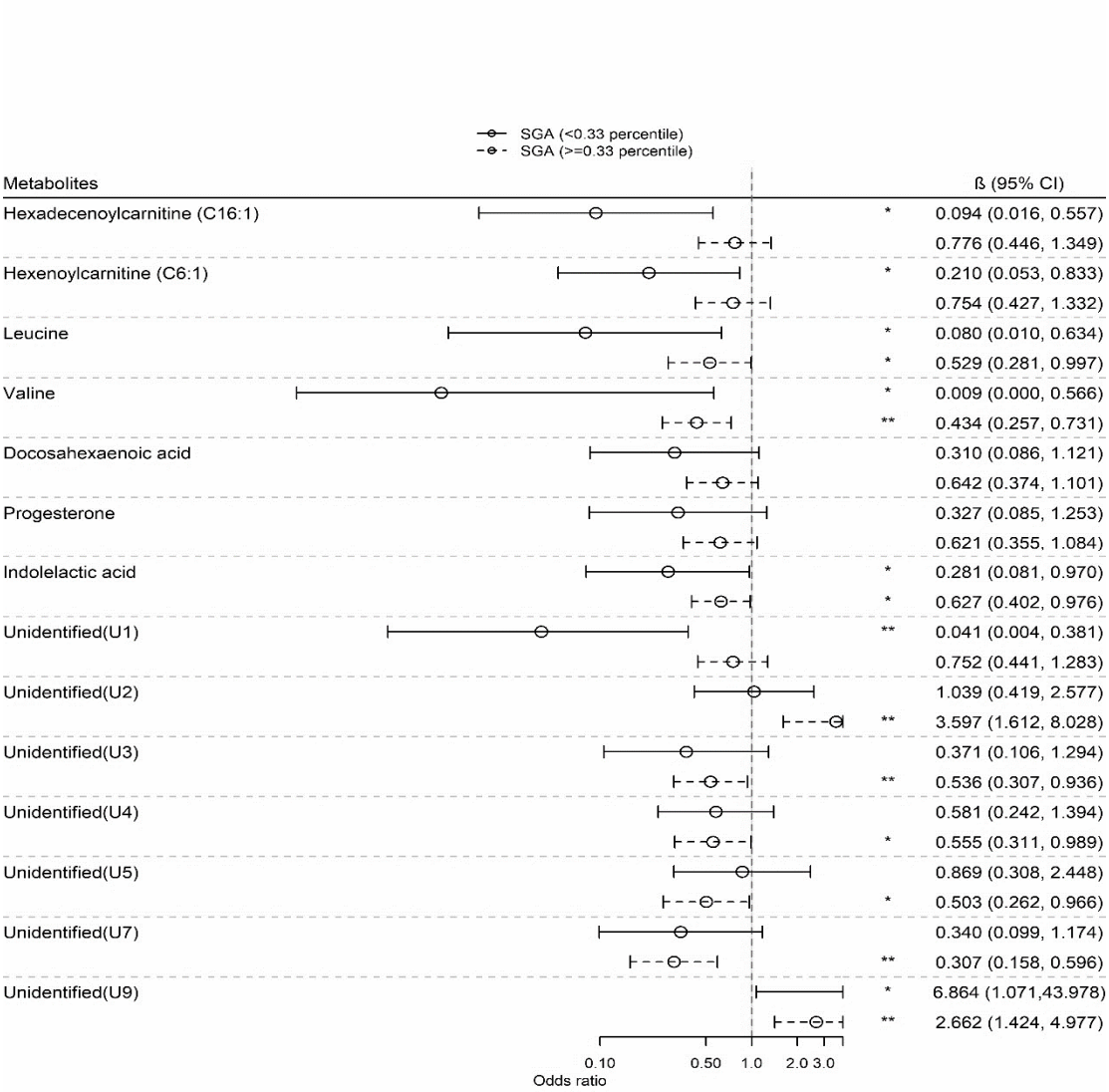


*Figure S9: Logistic regression odds ratio per standard deviation (95% CI) for overweight/obesity in early childhood for Model 2 (adjusted for child sex, age at outcome measurement, ethnicity and we used a random effects model by cohort adjusted for maternal BMI, paternal BMI, gestational age, weight gained during pregnancy, paternal education, passive and active smoking status during pregnancy, parity, and mode of delivery) stratified by Small of Gestational Age (SGA) for the 6 nominal statistically significant birthweight related metabolites and the 8 associated with overweight/obesity in early childhood. Where * is P< 0.05 and ** is FDR<0.05. Bars show 95% confidence intervals.*

1. Study population

The ENVIRONAGE cohort recruits since 2010, and the sampling of this specific study population occurred between 2014 and 2015 in Belgium. Women were recruited when they arrived at the South-East-Limburg Hospital in Gent Follow-up anthropometric data collection for children is available up to two years of age. ENVIRONAGE study was approved by the ethical committees of Hasselt University and Hospital East-Limburg, Genk, Belgium. The INMA cohort is a network of birth cohorts in Spain that recruited pregnant women from the first trimester at public primary health care centers or hospitals in Sabadell from July 2004 to July 2006. Follow-up anthropometric data measurements, samples and surveys of the participating children have been collected until 16 years of age. INMA study was approved by the Ethical Committee of the Municipal Institute of Medical Investigation. The Piccolipiu study recruited women giving birth between 2011 and 2013 at selected hospitals in five Italian cities, Turin, Trieste, Viareggio, Florence, Rome. Children included in STOP were selected from the Turin center. Follow-up anthropometric data collection surveys occurred at 6, 12 and 24 months after the delivery and then when the children turned 4 and 6 years with direct measurements at a clinical visit. For Piccolipiu study, Ethical approvals have been obtained from the Ethics Committees of the Local Health Unit Roma E (management center), of the Istituto Superiore di Sanità (National Institute of Public Health), and of each local center. The Rhea cohort enrolled women during the first trimester of pregnancy at public primary health care centres or hospitals in Heraklion, Greece, between 2007 and 2008. Follow-up anthropometric measurements, samples and surveys for the participants are available up to 11 years. Follow-up anthropometric measurements, samples and surveys for the participants are available up to 11 years. Rhea was approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece. For all studies, informed consent was given by all participants.

Venipuncture was used for collecting blood samples of cord vessels before the placenta was delivered. Samples were processed into either plasma (Environage, Piccolipiu) or serum (Rhea, INMA) as previously described[1]. Cohort inclusion criteria and further protocols can be found in the respective cohort references. Samples were selected from each cohort on the basis of biomaterial and data availability[2-5]. Selected samples were shipped to the International Agency for Research on Cancer, Lyon, France for metabolomics analysis.

Family lifestyle factors were collected from mothers through an interview by trained fieldworkers and medical history for each family transferred from hospital records[1].

Regarding maternal diet during pregnancy (Table S1-S2), in the INMA cohort, an adapted version of Willett's questionnaire[6] was developed and validated for the Spanish population[7].A Food Frequency Questionnaire (FFQ) was administered by trained interviewers during the 3rd trimester. The questionnaire consisted of question related to the frequency that a participant had consumed specific types of food[8].The questionnaire had nine possible intake food categories, ranging from 'never or less than once per month' to '6 or more times per day'. The average daily food consumption calculated based on the overall intake frequency for each food item intake for each participant. In the RHEA cohort was developed a semi-quantitative questionnaire, containing 250 food items[8]. The participants were asked about both the frequency of consumption and the average portion size. The exact frequency of consumption was given per day, per week and/or per month, depending on the food item. The intake frequency for each food item was converted to the average daily intake for each participant. In the ENVIRONAGE cohort information on the maternal diet during the pregnancy was derived from the questionnaire filled out after delivery, including questions on the consumption of soft drinks, fish, fruit, and vegetable intake. Participants were asked for the frequency of average portion consumption per day and/or per week, depending on the food item. In the Piccolipiu cohort an FFQ for 13 items based on other questionnaires, but not ad hoc validated, was used.

2. Untargeted metabolomics

Cord blood samples were prepared by protein precipitation and analyzed in randomized order as a single uninterrupted batch with a UHPLC-QTOF-MS system consisting of a 1290 Binary LC, a Jet Stream electrospray ionization source, and a 6550 QTOF mass spectrometer (Agilent Technologies). Details of the analysis have been described earlier Robinson, Keski-Rahkonen *et al* [1]. In short, 30 µL of the sample was mixed with 200 µL of acetonitrile and filtered with 0.2 µm polypropene well plate filters, and the analysis was performed on a reversed phase column using a 13-minute methanol-water gradient. The mass spectrometer was operated in positive polarity with a mass range of 50-1000 Da. Preprocessing of the acquired data was carried out using Agilent's recursive feature finding workflow as described earlier in detail [1]. Briefly, a molecular feature extraction algorithm was used to find singly charged proton adducts, which were filtered by detection frequency and peak size into a target list of features, which were extracted from the raw data using a find-by-ion algorithm with a matching tolerance for the mass and retention time at ±10 ppm and ±0.04 min. Peak areas were used as a measurement of feature intensity. Metabolic features present in <60% of the samples were removed and data were log-transformed. Missing values were imputed leaving 4714 features for analysis using imputeLCMD R package [9]. For identification of the features discovered in the present study, mass-to-charge ratios (m/z) were searched in the Human Metabolome Database [10] and METLIN [11], using ions [M+H]+, [M-H2O+H]+ and [M+Na]+, with 15 ppm molecular weight tolerance. Identity of the candidate metabolites was confirmed by reanalysis of representative samples together with pure chemical standards and comparing retention times and MS/MS spectra. When standards were not available, MS/MS spectra were acquired when possible and compared against those in public databases (www.mzcloud.org, METLIN). Level of identification was defined as proposed by Sumner, Amberg *et al* [12]. Chromatograms and mass spectra of all identified compounds are provided in the Supporting Information.

3. Random Forest and model evaluation for optimism

A bootstrap method of 1000 repetitions was advocated to quantify optimism and evaluate the generalization of the model. In this analysis, we had two dependent variables to examine. The first dependent variable was the rapid growth at twelve months of age and the second in early childhood as it was defined in the main text. We used three different sets of independent variables for each of the outcomes: 1) traditional risk factors (cohort, ethnicity, maternal BMI, paternal BMI, gestational age, maternal weight gained during pregnancy, paternal education, maternal passive and active smoking status during pregnancy, parity and mode of delivery), 2)significantly associated metabolites from the MWAS analysis, and 3) significantly associated metabolites in combination with traditional risk factors. Al the models were adjusted for age and gender. A Random Forest classification model of 250 trees was trained on the relevant training set using Scikit-learn default parameters [13].

For all the bootstrapped models, we use a training set (random 80% of the total observations) to determine the optimum probability threshold, and the performance was evaluated on the relevant test set (remaining 20% of the total observations) for the cohorts that remained to the sample. The performance of all the models was assessed through receiver operating characteristic (ROC curve), and we estimate the bootstrapped 95% confidence intervals.

To further evaluate the predictive model, we performed a leave-one-out analysis by repeating the modelling process on a combined data set with one cohort out. We carried out this evaluation step following the above-mentioned methodology.

The results showed that the rapid growth prediction model trained using only traditional risk factors and exhibited a moderate predictive ability of an AUROC value of 0.69 (bootstrap

95% confidence interval (CI): 0.62, 0.77)). Adding the four metabolites (cholestenone, U2, U4, and U8) identified in the MWAS analysis into the prediction model, increased the AUROC to 0.77 (bootstrap 95% confidence interval (CI): 0.71, 0.83)) (Table S8). For overweight, using traditional risk factors alone, the AUROC was 0.69 (bootstrap 95% confidence interval (CI): 0.63, 0.75)), while a model using only the eight metabolites, Valine, U1, U2, U3, U4, U5, U7 and U9, identified in the MWAS analysis had an AUROC of 0.76 (bootstrap 95% confidence interval (CI): 0.69, 0.81)). The combined traditional risk factor and metabolite model was strongly predictive of overweight with an AUROC of 0.82 (bootstrap 95% confidence interval (CI): 0.79, 0.85)) (Table S8).

Table S8: Summary of rapid growth and 12 months of age and overweight/obesity in childhood. Average AUROC across 1000 bootstrapped test sets for all the cohorts.

Model*		Rapid growth at 12 months of age			Overweight/obesity in early childhood		
		Average AUROC	Lower 95%CI	Upper 95%CI	Average AUROC	Lower 95%CI	Upper 95%CI
1	Questionnaires**	0.69	0.62	0.77	0.69	0.63	0.75
2	Metabolomics***	0.72	0.64	0.81	0.76	0.69	0.81
3	Metabolomics and questionnaires****	0.77	0.71	0.83	0.82	0.79	0.85

*All the models were adjusted for age and sex.
**Multivariate analysis for cohort, ethnicity, maternal BMI, paternal BMI, gestational age, maternal weight gained during pregnancy, paternal education, maternal passive and active smoking status during pregnancy, parity, and mode of delivery.
Multivariate analysis of rapid growth at 12 months of age for Cholestenone, U4, U6 and U8 and of overweight/obesity in early childhood for Valine, U1, U2, U3, U4, U5, U7 and U9* Multivariate model using the covariates of model 1 and 2.

Table S9: Summary of rapid growth and 12 months of age and overweight/obesity in childhood. Average ROC and CI95% across 1000 bootstrapped test sets using and leave-cohort-out approach.

Model*		Validation cohort	Rapid growth at 12 months of age			Overweight/obesity in early childhood		
			Average AUROC	Lower 95%CI	Upper 95%CI	Average AUROC	Lower 95%CI	Upper 95%CI
1	Questionnaires**	ENVIRONAGE	0.72	0.68	0.74	-	-	-
		Piccolipiu	0.74	0.69	0.79	0.79	0.75	0.83
		RHEA	0.61	0.57	0.66	0.63	0.61	0.65
		INMA-Sabadell	0.80	0.77	0.83	0.68	0.64	0.72
2	Metabolomics***	ENVIRONAGE	0.64	0.61	0.67	-	-	-
		Piccolipiu	0.68	0.64	0.72	0.62	0.59	0.73
		RHEA	0.74	0.73	0.75	0.75	0.71	0.78
		INMA-Sabadell	0.70	0.67	0.73	0.62	0.58	0.64
3	Metabolomics and questionnaires****	ENVIRONAGE	0.70	0.67	0.74	-	-	-
		Piccolipiu	0.81	0.76	0.83	0.64	0.60	0.68
		RHEA	0.65	0.61	0.68	0.79	0.74	0.83

		INMA-Sabadell	0.82	0.79	0.85	0.71	0.68	0.74

*All the models were adjusted for age and sex.
**Multivariate analysis for ethnicity, maternal BMI, paternal BM, gestational age, maternal weight gained during pregnancy, paternal education, maternal passive and active smoking status during pregnancy, parity, and mode of delivery.
***Multivariate analysis of rapid growth at 12 months of age for Cholestenone, U4, U6 and U8 and of overweight/obesity in early childhood for Valine, U1, U2, U3, U4, U5, U7 and U9
****Multivariate model using the covariates of model 1 and 2.

The results of the leave one cohort out analysis (Table S9) for rapid growth showed an improvement in predictive performance upon addition of metabolites for Piccolipiu, Rhea and INMA as validation cohorts. For overweight, the leave one cohort out analysis (Table S9) showed an improvement in predictive performance upon addition of metabolites for Rhea and INMA as validation cohorts. These differences in predictive performance across cohorts may reflect the heterogeneity of the metabolic profiles we observed in each cohort and also, for overweight models, the lower age range and proportion of overweight cases in the Piccolipiu cohort (Figure S10).

The statistical analyses were performed using R ('The R Project for Statistical Computing') software environment (v3.5.2) and Python 3.6.

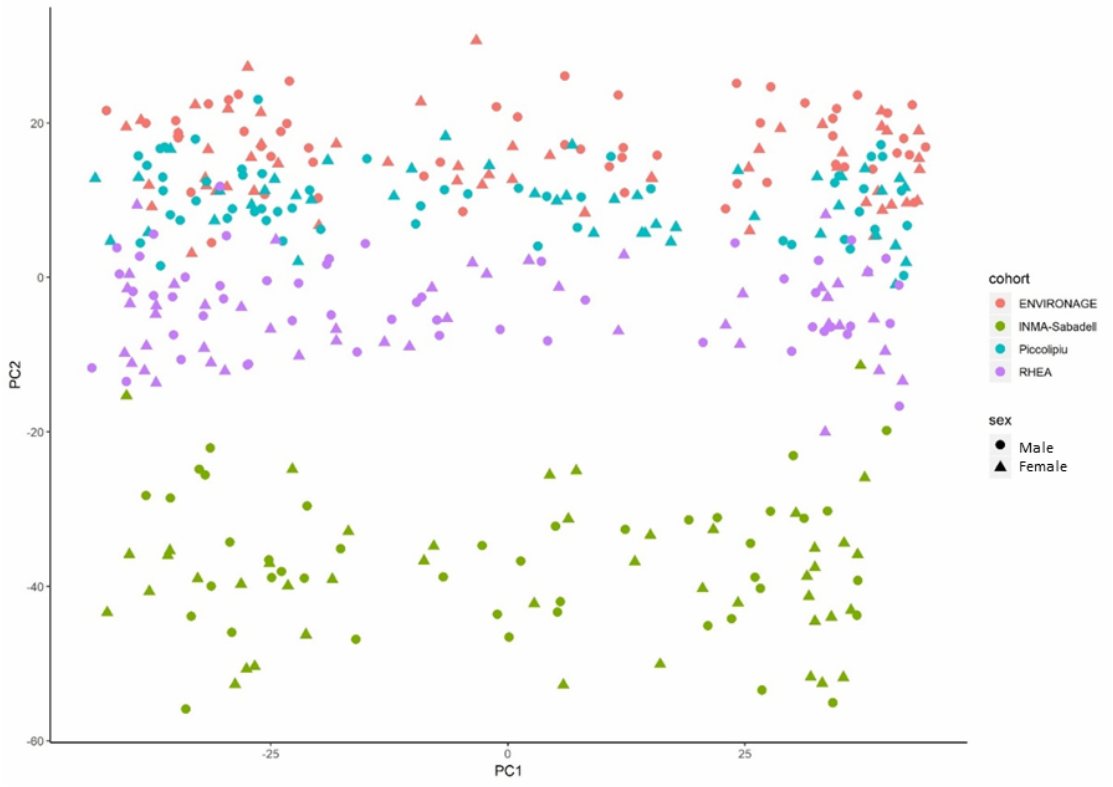


Figure S10: PCA analysis of the whole metabolome and scatter plot of first two principal components, coloured by cohort.

4. Metabolic pathway enrichment analysis

We performed pathway enrichment analysis using Mummichog (version: 2.3.3-20200213, default metabolic human model MFN_1.10.4.). Mummichog is a bioinformatics Python-based platform that infers and categorizes functional biological activity using directly the output from mass spectrometry [14]. The algorithm searches tentative compound lists from metabolite reference databases against an integrated model of human metabolism to identify functional activity. Fisher's exact tests are used to infer p-values, which are adjusted for type I error through a pathway permutation procedure. Likelihood of pathway enrichment across significant features is compared to pathways identified across the entire compound set in a reference list (the entire metabolome dataset), considering the probability of mapping the significant metabolic features to pathways. Mummichog parameters were set to match against ions included in the 'positive mode' setting at ±8 ppm mass tolerance ("M+H[1+]" and "M+Na[1+]").

Mummichog assigned tentative annotations to 405 of the 4714 features as significant ($P<0.05$) for rapid growth in 12 months (Supporting information 2) and to 613 of the 4714 for overweight/obesity in early childhood (Supporting information 3). Mummichog reference feature list was mapped to 627 Empirical Compounds which 69 were statistically significant for rapid growth in 12 months and 78 statistically significant for overweight/obesity in early childhood. According Mummichog, Empirical Compounds are putative metabolites as measured by Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). These putative metabolites can contain a mixture of enantiomers, stereoisomers, and positional isomers that are not resolved by the instruments[15].

The results showed that the three enriched pathways with overlap size ≥4 for rapid growth in infancy were "Androgen and estrogen biosynthesis and metabolism", "C21-steroid hormone biosynthesis and metabolism" and "Urea cycle/amino group metabolism" (Table S10) and

enriched pathways with overlap size ≥4 for overweight/obesity in early childhood were "Valine, leucine and isoleucine degradation", "Biopterin metabolism" and "Glycine, serine, alanine and threonine metabolism"(Table S11).

Additionally, to further validate the pathways proposed by mummichog, we carried out a manual curation of the metabolite identities assigned by mummichog. For the compounds previously identified by the laboratory and for which pure chemical standards were available, retention times were compared to exclude false mummichog annotations.

The results of this manual validation lend support for the correctness of following mummichog-predicted pathways: for rapid growth, "C21-steroid hormone biosynthesis and metabolism", "androgen and estrogen biosynthesis and metabolism", and "Urea cycle/amino group metabolism" retained overlap sizes of 13,12 and 5, respectively after excluding the false metabolite annotations (Table S10). For overweight, "Glycine, serine, alanine and threonine metabolism" retained an overlap size of 5, although the statistical support was weak (p = 0.05 before manual exclusion) (Table S11).

Table S10: Mummichog analysis statistically significant pathways for rapid growth at 12 months of age.

Pathways	Overlap size[A]	Pathway size[B]	p-value[C]	Overlap empirical compounds[E]
C21-steroid hormone biosynthesis and metabolism	15	58	8e-05	E285, E479, E151[D], E423, E487[D], E124, E181, E309, E386, E539, E219, E382, E379, E416, E36
Androgen and estrogen biosynthesis and metabolism	12	30	8e-05	E285, E386, E36, E423, E124, E416, E309, E219, E539, E463, E209, E382
Urea cycle/amino group metabolism	8	34	0.007	E288[D], E387, E94, E57[D], E488[D], E98, E548[D], E37[D]

A Pathway size is number of detected Empirical Compounds for each pathway.
B Overlap size is number of significant Empirical Compounds.
C Empirical p-values are estimated by permutation test.
D This empirical compound has not been identified in the manual identification.

E Details on empirical compounds are available in supporting information 2.

Table S11: Mummichog analysis statistically significant pathways for overweight/obesity in early childhood.

Pathways	Overlap size[A]	Pathway size[B]	p-value[C]	Overlap empirical compounds[E]
Valine, leucine and isoleucine degradation	6	13	0.0006	E34, E17, E239, E350[D], E549[D], E180[D]
Biopterin metabolism	4	9	0.005	E484[D], E601, E290[D], E175
Glycine, serine, alanine and threonine metabolism	8	42	0.050	E17, E350[D], E449, E3, E407, E549[D], E180[D], E394

A Pathway size is number of detected Empirical Compounds for each pathway.
B Overlap size is number of significant Empirical Compounds.
C Empirical p-values are estimated by permutation test.
D This empirical compound has not been identified in the manual identification.
E Details on empirical compounds are available in supporting information 3.

5. Modelling of weight and height growth trajectories

Patterns of growth across childhood follow a complex pattern (growth is non-linear). We used a two-step approach to estimate growth curves for participating cohorts. First, we identified for each cohort the best fitting fractional polynomials of age and constructed sex- and age- specific weight and height growth curves[16]. Briefly, a series of models were carried out for each cohort in which age was raised to a large number of combinations of powers (each of the following single powers, plus each combination of two powers: −2, −1, −0.5, 0, 0.5, 1, 2, 3, where a power of zero is the log function), resulting in a wide range of possible weight and height curves[17]. Then we used mixed-effects linear regression models with the previously identified fractional polynomials of age, including a random intercept for child and random age slopes. Such models allow for individual variation in growth curves within each cohort, and use all available data from all the eligible children under a missing at random assumption[18]. Predicted weight and height values within each cohort were estimated for the exact age of 12months for the cohorts' individuals.

We used the WHO growth charts to monitor child growth [19, 20]. These charts are growth standards based on data collected from selected communities worldwide. The use of WHO standards allows for growth assessment of children independent of ethnicity and socioeconomic status, thus, permitting international comparisons. These charts have been

adopted in a growing number of countries in Europe and other parts of the world [21], and endorsed by international bodies such as the United Nations Standing Committee on Nutrition [22] and International Pediatric Association [23].

The selected models are available in Table S12 and the performance of the models are presented in Figure S11-S14.

Table S12: Comparison of prediction concordance from different fractional polynomial powers for sex-specific weight and height in participating cohorts.

			Boys										Girls									
			Weight					Height					Weight					Height				
Cohort	N	n	Powers		rho_c*	Difference		Powers		rho_c*	Difference		Powers		rho_c*	Difference		Powers		rho_c*	Difference	
						Mean	(SD)				Mean	(SD)				Mean	(SD)				Mean	(SD)
ENVIRONAGE	108	1104	-2	0.5	0.994	-0.001	(0.477)	-2	0.5	0.998	-0.011	(1.134)	0	0	0.996	-0.001	(0.408)	-1	0.5	0.998	-0.006	(1.048)
INMA-Sabadell	404	3149	0.5	3	0.997	-0.000	(0.592)	0	1	0.998	-0.000	(1.415)	0.5	3	0.997	0.000	(0.506)	0.5	3	0.998	-0.000	(1.417)
PICCOLIPIU	99	943	0	0	0.991	-0.000	(0.359)	0	0	0.989	-0.000	(1.537)	0.5	1	0.992	0.000	(0.300)	0	0	0.989	-0.000	(1.456)
RHEA	1092	21045	0	0.5	0.989	-0.000	(0.858)	0.5	0.5	0.997	0.000	(1.530)	0.5	3	0.996	-0.000	(0.502)	0.5	1	0.997	0.000	(1.384)

* ***rho$_c$***: concordance correlation coefficient.

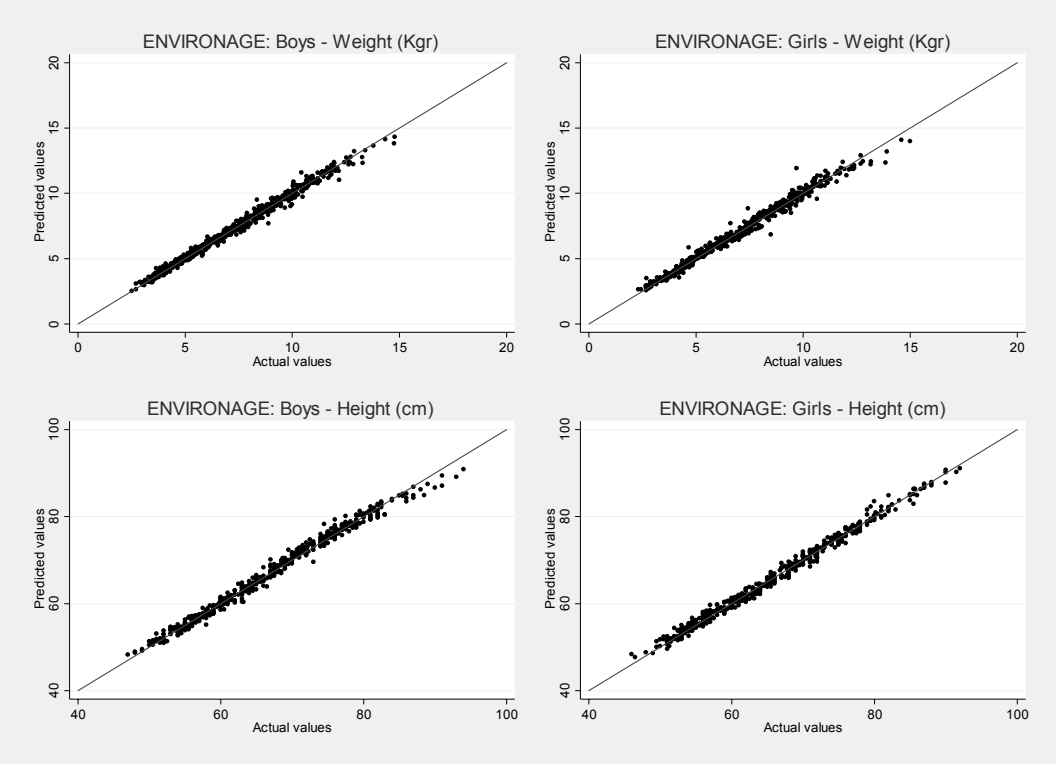
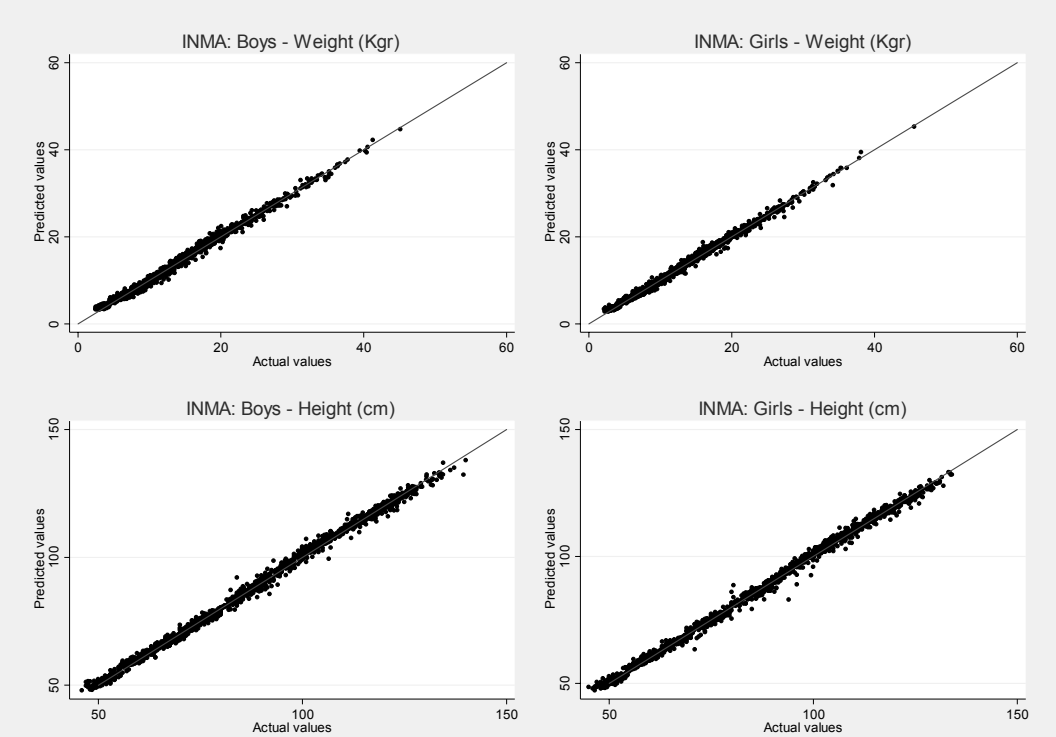


Figure S11: Actual vs Predicted values of weight and height in participating ENVIRONAGE cohort.



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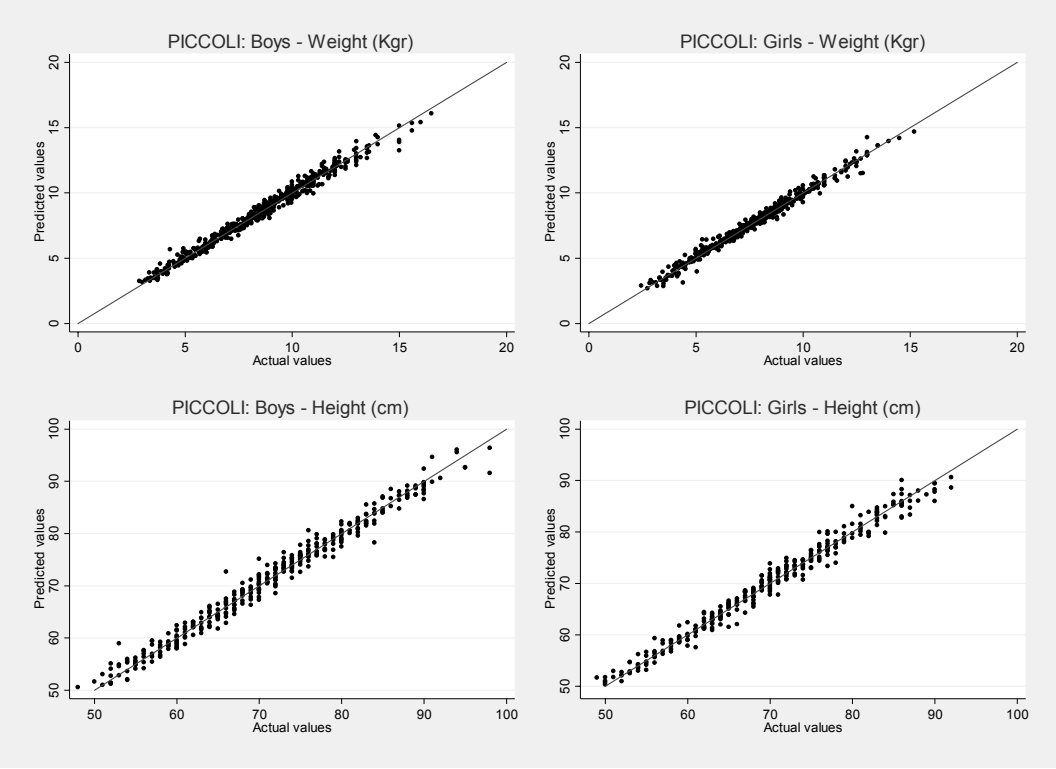
421 *Figure S12: Actual vs Predicted values of weight and height in participating INMA cohort.*



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423 *Figure S13: Actual vs Predicted values of weight and height in participating Piccolipiu cohort.*

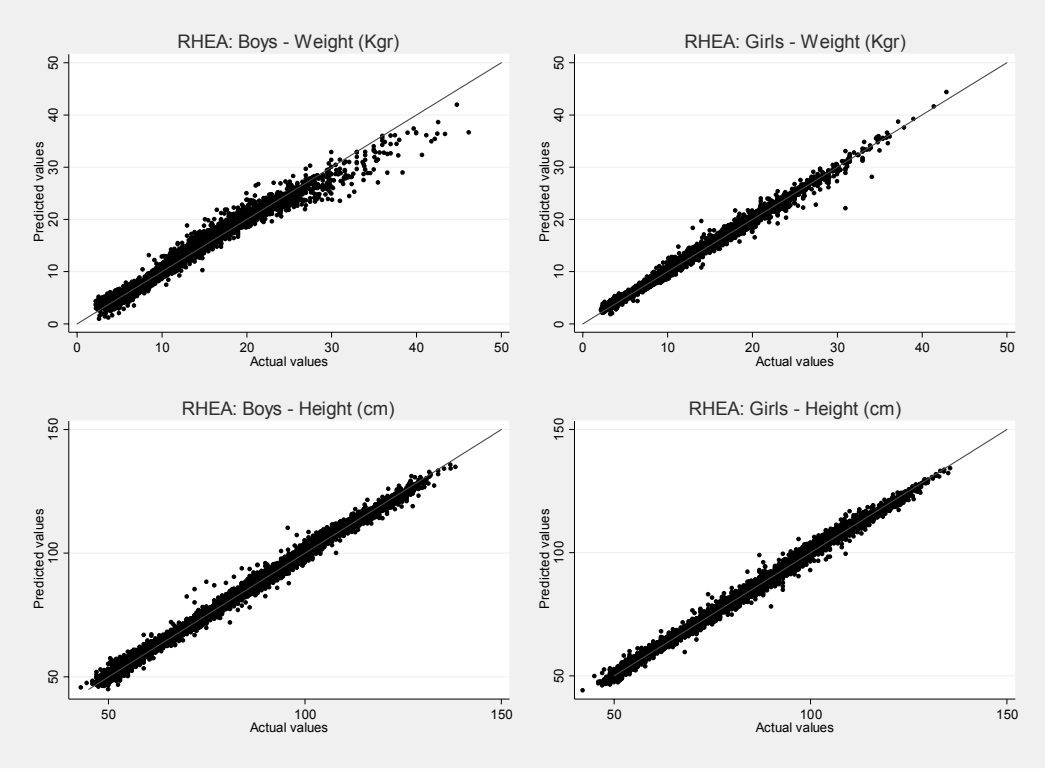

Figure S14: Actual vs Predicted values of weight and height in participating RHEA cohort.

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