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Original article

Gut microbiota composition and derived enterotypes are associated with ponderal status in preschool children. Childhood obesity risk assessment longitudinal study (CORALS) cohort

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Abbreviations: BH, Benjamini–Hochberg; BMI, Body Mass Index; CDP, Cytidine Diphosphate; CLR, Centered Log-Ratio; CORALS, Childhood Obesity Risk Assessment Longitudinal Study; CTP, Cytidine Triphosphate; ED, Entner-Doudoroff; FDR, False Discovery Rate; NW, Normal Weight; OB, Obesity; OW, Overweight; PCOA, Principal Coordinates Analysis; PERMANOVA, Permutational Multivariate Analysis of Variance; SCFA, Short-Chain Fatty Acids; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; UTP, Uridine Triphosphate; UW, Underweight.

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SUMMARY

Background and aims: Childhood obesity is a growing public health concern increasingly linked to gut microbiota. We analysed associations between microbiota composition, functionality, and weight status in 1134 children aged 3–6 years from the CORALS cohort.

Methods: The baseline cross-sectional study stratified participants by weight status (underweight, normal weight, overweight, obesity) and performed shotgun metagenomic sequencing of stool samples. Analyses in R assessed alpha/beta diversity, taxonomic composition, enterotypes, and microbial pathways.

Results: Alpha diversity decreased with increasing BMI, particularly in obesity (Shannon adj.P = 0.00301; Simpson adj.P = 0.00158). Beta diversity revealed distinct microbial structures across groups (p = 0.001). Four enterotypes were identified: obesity was associated with Enterotype 3 (Segatella-dominated, p = 0.023), while Enterotype 1 (Alistipes, Akkermansia, Coprococcus) was enriched in underweight/normal weight. Species linked to obesity included higher *Phocaeicola dorei* (adj.P = 0.003) and *Segatella hominis* (adj.P = 0.001), and lower *Longicatena caecimuris* (adj.P = 0.03) and *Blautia parvula* (adj.P = 0.003). Functional analyses showed downregulation of vitamin and nucleotide biosynthesis pathways and reduced carbohydrate metabolism in overweight/obesity.

Conclusions: Gut microbiota composition and functionality are strongly associated with weight status in early childhood, suggesting microbial biomarkers and metabolic pathways relevant to understand early obesity development.

ClinicalTrials.gov ID NCT06317883.

1. Introduction

Childhood obesity is a major public health concern. In 2024, the WHO estimated that 35 million children under 5 had excess weight, and obesity among those aged 5–19 increased from 2 % in 1990 to 8 % in 2022 [1]. In Spain, the 2023 ALADINO study found 36.1 % of children aged 6 to 9 had overweight or obesity [2]. Childhood obesity often persists into adulthood [3] and is associated with significant physical [4,5] and psychological consequences [6]. Growing attention has focused on the gut microbiota in obesity development [7]. At birth, diverse microbial communities, including Archaea, Bacteria, Eukarya and Viruses, colonize the gastrointestinal tract and undergo rapid changes in early life [8]. This community regulates host immunity, supporting overall health, and maintaining metabolic homeostasis [9]. Early-life gut microbiota development is critical for long-term metabolic health [10], and is shaped by maternal, environmental and host-related factors [11,12]. Prenatal influences include maternal diet and weight, placental function, gestational age, and gestational weight gain [13,14]. After birth, delivery mode, infant feeding, food allergies, and the child's diet further shape colonization [15–17], which matures by age 3–5 [18]. Gut microbiota plays a key role in childhood obesity [19], and compositional differences have been linked to distinct weight status profiles [20,21]. Lower alpha diversity, reflecting reduced within-individual microbial diversity, has been linked to excess body weight [22,23]. In contrast, studies on beta diversity indicates distinct gut microbial compositions between individuals with and without obesity [24,25]. A key mechanism by which gut microbiota may influence host energy metabolism is through fermentation of dietary fiber into short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate [26], which stimulate the secretion of peptide YY and glucagon-like peptide-1 [27], hormones that promote satiety and regulate glucose metabolism [28]. Altered SCFA signaling has been implicated in obesity, contributing to impaired appetite regulation and positive energy balance [29]. Recent research has identified gut enterotypes, clusters of microbial communities dominated by specific taxa, which may influence obesity risk and metabolic outcomes. Understanding the relationships between enterotypes, microbial function, and childhood obesity could inform targeted prevention strategies [30]. Despite growing interest, this area remains underexplored, and well-designed studies are needed to clarify these mechanisms specially in childhood [31].

The aim of this study was to evaluate the composition and functionality of the gut microbiota in relation to weight status in children aged 3–6 years. Specifically, this study sought to perform a metagenomic analysis to assess alpha and beta diversity, as well as bacterial species composition, according to weight status categorized by body mass index (BMI) z-scores. The study also aimed to determine enterotype clusters and to conduct a functional analysis of the metagenomic data to identify metabolic pathways that are differentially represented across weight categories.

2. Methods

2.1. Study design

This analysis was conducted using baseline data of the Childhood Obesity Risk Assessment Longitudinal Study (CORALS), which followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. This is an observational, prospective, multicenter cohort study currently ongoing in preschool children, with a planned follow-up period of 10 years (see supplementary material S1 for timeline of CORALS). Participants were children aged 3–6 years recruited in schools from seven Spanish cities whose tutors agreed to their participation: Barcelona, Córdoba, Pamplona, Reus, Santiago de Compostela, Valencia, and Zaragoza. All schools in the area were invited to take part in the study. Participation was voluntary, and the CORALS PIs visited the schools that agreed to participate to inform the families about the study. Eligibility required parental or caregiver consent, attendance at the baseline visit, and completion of various standardized questionnaires evaluating sociodemographic characteristics, leisure-time physical activity, and dietary intake. Exclusion criteria included children from families with anticipated difficulties in participating throughout the study, issues with language comprehension, or unstable residential situations.

Participant recruitment for the CORALS began in May 2019 and finished in June 2022 with a total sample of 1509 children. The study protocol, which followed the Declaration of Helsinki [32] and all ethical legislations, was reviewed and approved by the ethics committee of each recruitment center (Reference numbers: 051/2019; 4155/2019; 2019/18; 9/19; 19/162; 19/27; and 2019/131). Written informed consent was obtained from all parents or supervisors before any study procedure was carried out. CORALS is registered in clinicaltrials.gov (ID: NCT06317883) were all the details of the protocol are depicted.

2.2. Participants and outcomes

Children aged 3–6 years participating in the CORALS cohort were eligible for this analysis. Only baseline data were used, during which clinical, anthropometric, and biological information, including stool samples, was obtained following standardized protocols. Participants were included if they had available stool sample for microbiota analysis and complete anthropometric measurements, specifically weight (kg) and height (m), required to calculate BMI Z-scores. Children missing either of these data were excluded. Eligible participants were identified from the CORALS database and included consecutively. As this is a cross-sectional analysis of baseline data, no follow-up was conducted. The primary exposure for this analysis was weight status category, and the primary outcome was gut microbiota diversity and composition.

2.2.1. Anthropometry measurements

Height and weight were determined using standardized protocols in all sites [33], while BMI z-score was calculated according to the criteria of Cole et al. [34]. These cut-offs define underweight (UW), normal weight (NW), overweight (OW), and obesity (OB) based on age and sex specific BMI values that correspond to adult BMI equivalents of 18.5, 25, and 30 kg/m^2.

2.2.2. Gut microbiota

Stool samples were collected at home using a Sarstedt tube with an integrated spoon and held at −20 °C. Samples were transported to the research facility under cold chain conditions and immediately stored at −80 °C. Regarding shotgun sequencing, DNA extraction from baseline stool samples was performed using the Magpure Stool DNA LQ kit and the NGS library was prepared using the MGIEasy Universal DNA Library Prep Set. Shotgun metagenomics sequencing was performed in a MGI's DNBSEQ-G50TM sequencing platform to generate paired-end short-reads of maximum 150 bp and a minimum sequencing depth of 20M reads at the MGI Tech Co., Ltd. manufacturing facility in Riga (Latvia). For the taxonomic and differential abundance analysis, trimmomatic [35] was used to trim paired end reads, using a sliding window of 20 bp and a quality threshold of 30. Reads shorter than 75 bp were discarded. FastQC [36] and MultiQC [37] were used to assess sequencing quality before and after trimming. Surviving reads were mapped against the GRCh38 human genome assembly using bowtie2 [38], and mapped reads were discarded. The unmapped reads were then classified at the level of species using Kraken2 [39], with confidence set to 0.5, and Bracken [40], using an updated version of the standard Kraken database (https://genomeidx.s3.amazonaws.com/kraken/k2_standard_20220607.tar.gz). For functional annotation of metagenomes, the Humann3 pipeline was used [41] with default options, starting from the trimmed and human-depleted reads. Then, the humann_regroup_table command was used to translate MetaCyc terms into MetaCyc reactions.

2.3. Data analysis

Analyses were performed using R (v4.2.3). To minimize potential confounding, all statistical models were adjusted for age, sex, and study center, which are known to influence gut microbiota composition in early childhood. Potential sources of selection and measurement bias were addressed by including only participants with complete baseline data and by applying standardized procedures for anthropometric measurements, stool collection, DNA extraction, and sequencing. Additionally, we compared key characteristics (sex and weight categories) between children included in the analysis and those without stool samples; no meaningful differences were observed (see supplementary material S2). No formal sample size calculation was performed for this baseline cross-sectional analysis; all available participants with complete data were included.

2.3.1. Quality control and data filtering

Taxa were filtered to remove features with low prevalence and insufficient absolute read counts. To ensure data quality, metadata were screened for missing or incomplete entries; samples with NA values or empty fields in key variables were removed, and categorical variables were harmonized to maintain consistency. In the microbial dataset, missing read values were handled using NA-aware functions during aggregation. Quality control thresholds were applied by retaining only taxa detected in at least 5 % of samples and with a minimum of 20 reads in at least 5 % of the cohort, thereby removing rare or potentially spurious features. Ambiguous classifications (e.g., uncultured, unknown, unassigned, unidentified) were excluded, and only bacterial taxa with valid genus or species level annotations were kept. Finally, metadata completeness and dataset integrity were ensured by verifying the one-to-one correspondence between sample identifiers in the metadata and the microbial abundance matrix and discarding any mismatched samples. Metagenomic taxonomic profiles were filtered to maintain only bacterial taxa at the genus and species levels.

2.3.2. Alpha and beta diversity

Microbial alpha and beta diversity analyses were performed using the Phyloseq and Vegan packages [42,43]. All diversity analyses were conducted without rarefaction to avoid data loss and preserve statistical power. Alpha diversity was assessed using three commonly used indices: Shannon, Simpson, and Observed richness. To account for differences in sequencing depth, total read count per sample was included as a covariate in all linear models for alpha diversity, together with study center, sex, and age, with weight categories as the main variable of interest. Principal Coordinates Analysis (PCoA) was performed based on Bray–Curtis distance using relative abundances to evaluate beta diversity. Prior to multivariate analysis, homogeneity of group dispersion was assessed and tested by permutation. Permutational Multivariate Analysis of Variance (PERMANOVA) was applied to assess the effect of weight status on overall microbial community structure, while adjusting for covariates.

2.3.3. Enterotype analysis

For the enterotypes, genus-level taxonomic profiles were transformed using centered log-ratio (CLR) normalization via the ALDEx2 package [44]. Euclidean distances were computed on CLR-transformed data (Aitchison distance) to assess inter-sample variation, and Partitioning Around Medoids (PAM) clustering was applied to identify microbial compositional clusters. The optimal number of clusters was determined using the G1 index (clusterSim v0.51-5), which showed a clear maximum at $k = 4$. This indicates that four clusters provide the most robust structure according to this metric, with minimal improvement obtained by increasing k beyond this value (supplementary material S3). Cluster structure was visualized using PCoA. To further identify the bacterial genera driving the differences between enterotypes, we calculated the mean CLR-transformed abundances of each genus per cluster and evaluated both their variability and statistical significance across groups. Genera were first ranked according to their standard deviation of mean abundances across clusters, and significance was assessed using one-way ANOVA followed by false discovery rate (FDR) correction. The top 15 genera showing the highest variability and meeting statistical significance (FDR <0.05) were selected. We also performed a multinomial logistic regression model (multinom from the nnet package, v7.3-18) to explore the association between weight category at baseline and enterotype assignment. The model included weight status, study center, sex, and age as predictors. NW was used as the reference category. Model coefficients and confidence intervals were extracted and visualized to show the effect of each weight category on the probability of belonging to each enterotype.

2.3.4. *Differential abundance and functional analysis*

We also performed a differential abundance analysis using the DESeq2 package to seek for differences in bacterial species between weight status groups [45]. To account for potential confounding factors, the analysis included the above-mentioned covariates. Differential analysis was performed using the Wald test for pairwise contrasts between UW, OW, OB vs NW. Adjusted p-values were computed using the Benjamini–Hochberg (BH) procedure applied independently within each analysis. In DESeq2, BH correction was performed per contrast. Differentially abundant species were considered significant with adjusted p-value <0.05 and base mean > 800. Functional abundance tables were analyzed for associations with weight status using Maaslin2 [46] with CLR normalization. Maaslin2 was run with a linear model including the covariates as fixed effects. Results were filtered for significance using an FDR threshold of $q < 0.05$. The significant functional pathways were then integrated with species-level differential abundance significant results from DESeq2, and only significant species and pathways in both analyses (functional and taxonomic) were retained for combined interpretation.

3. Results

A total of 1151 children were included in the analysis. Initially we had 1509 participants; however, 321 did not provide stool sample and were excluded. In addition, another 37 were excluded because of non-available anthropometric data or poor metadata quality control (see supplementary material S4 for the flowchart of participants). Table 1 presents the general characteristics of the participants.

Table 1
Baseline characteristics of the participants in CORALS cohort.

	n (1151)	%
Sex		
Boys	583	50.6
Girls	568	50.4
Weight status		
Underweight (UW)	116	10.1
Normal weight (NW)	779	67.6
Overweight (OW)	165	14.3
Obesity (OB)	91	7.9

Data on groups were compared using Chi-square tests.

3.1. *Alpha and beta diversity*

Alpha diversity assessed through the Observed Richness, Shannon and Simpson indices across the different body weight status is shown in Fig. 1a. Comparisons between groups were performed using linear models adjusting for total sequencing reads, center, age, and sex. No significant differences were observed for Observed Richness across weight status categories. For the Shannon index, a significant decrease was detected in individuals with OB compared with NW. The Simpson index also showed a significant decrease in OB compared with NW, suggesting a reduction in gut microbial alpha diversity associated with higher body weight. Other comparisons did not reach statistical significance (all adj. p-values >0.05) (supplementary material S5). PERMANOVA analyses confirmed significant differences in gut microbial beta diversity across weight status ($p = 0.001$, $R^2 = 0.005$) in Fig. 1b. Pairwise comparisons between groups also revealed significant compositional differences between NW vs UW ($p = 0.012$, $R^2 = 0.00185$), NW vs OW ($p = 0.019$, $R^2 = 0.00179$), NW vs OB ($p = 0.001$, $R^2 = 0.00325$), UW vs OW ($p = 0.001$, $R^2 = 0.01011$) and UW vs OB ($p = 0.001$, $R^2 = 0.01895$), showing small effect sizes, although statistically significant, implying limited biological relevance. No significant differences between children with OW and OB were observed ($p > 0.05$), suggesting that microbial community composition does not differ substantially between these two-weight status (see supplementary material S6). While the R^2 values indicate that weight status explains a small proportion of overall microbiota variability, the observed differences are consistent and statistically robust, supporting a relationship between body weight and gut microbial composition.

3.2. *Enterotype distribution*

All the participants were classified into microbial clusters based on their gut microbiota composition, and PCoA identified four distinct groups (see supplementary material S3 for cluster selection). A total of 443, 189, 99, and 420 children were assigned to cluster 1, 2, 3, and 4 (Fig. 2a).

Enterotype 1 was predominantly rich in *Alistipes*, *Akkermansia* and *Coprococcus* while showing lower relative abundance of genera such as *Agathobacter*, *Roseburia*, and *Faecalibacterium*. Enterotype 2 was characterized by a consistent enrichment across several genera, including *Akkermansia, Alistipes, Flavonifator, Bacteroides, Parabacteroides* and *Phoecaicola*, alongside a clear underrepresentation of *Copocroccus* and *Segatella*. Enterotype 3 was distinguished by a marked overrepresentation of *Segatella* and a simultaneous depletion of *Alistipes* and *Akkermansia*. Enterotype 4 displayed a microbial profile with abundance values closer to the overall mean, indicating less pronounced deviations compared to the other clusters. Higher relative abundances were observed for *Roseburia*, *Agathobacter, Ruminococcus, Bifidobacterium, Faecalibacterium* and *Blautia* among others, whereas *Segatella* and *Akkermansia* showed moderately lower values (Fig. 2b).

Across the cohort, Enterotype 1 was the most prevalent (38.4 %), followed by Enterotype 4 (36.4 %), Enterotype 2 (16.4 %) and Enterotype 3 (8.6 %). When stratified by body weight status (Fig. 2c), we found different patterns for each enterotype. Thus, in Enterotype 1 (n = 443), 311 children (70.2 %) were NW, 46 (10.4 %) presented UW, 56 (12.6 %) had OW, and 30 (6.7 %) suffered OB. In Enterotype 2 (n = 189), 126 (66.6 %) were NW, 26 (13.7 %) presented UW, 29 (15.3 %) OW, and 8 (4.2 %) OB. Enterotype 3 (n = 99) included 60 children with NW (60.6 %), 6 with UW (6 %), 18 with OW (18.1 %), and 15 with OB (15.2 %). In Enterotype 4 (n = 420), 282 (67.1 %) were classified as NW, 38 (9 %) as UW, 62 (14.8 %) as OW, and 38 (13.5 %) as OB.

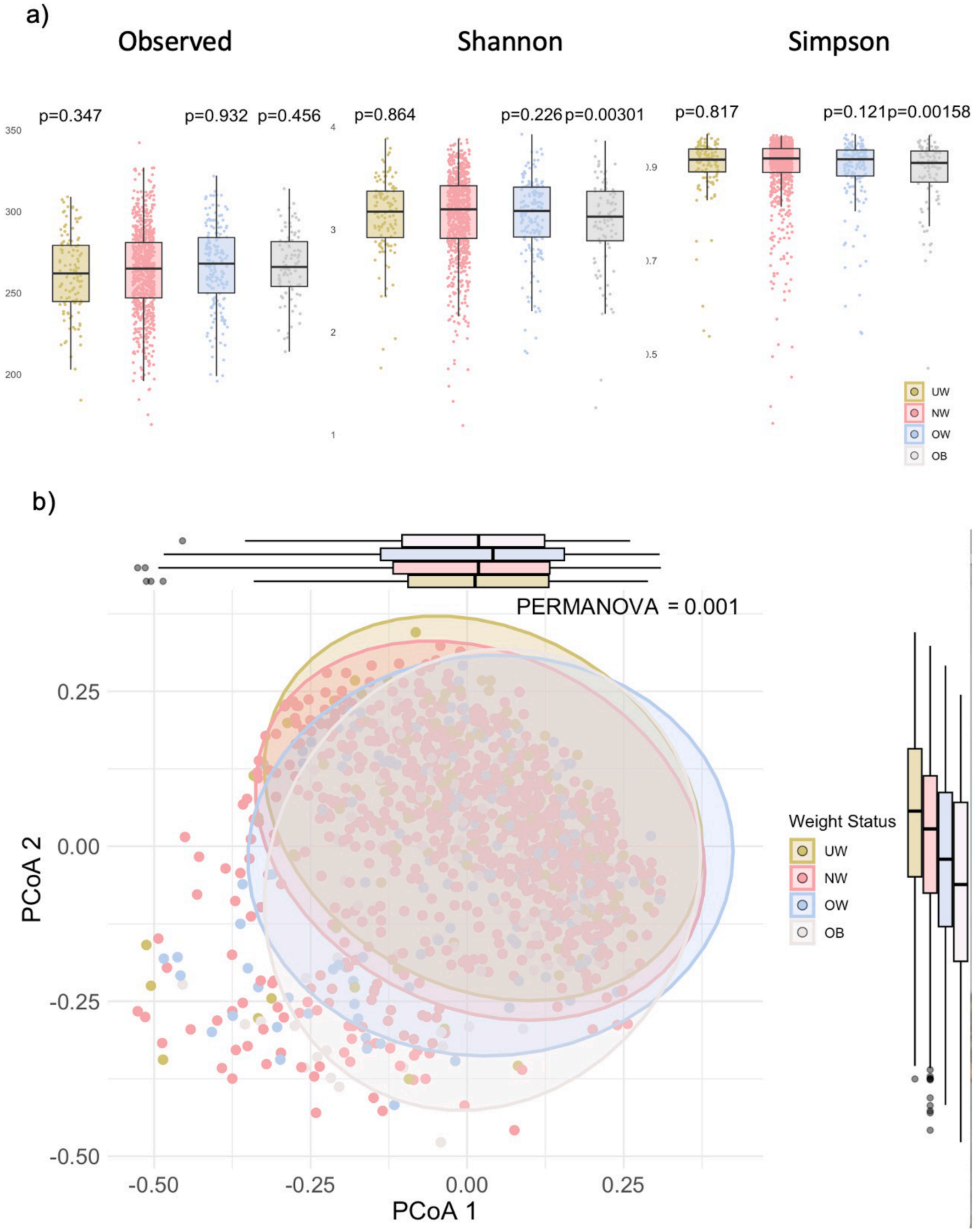

Fig. 1. Alpha and beta diversity across weight status categories in children from Corals cohort.
1a) Alpha diversity indices (Observed richness, Shannon, and Simpson) across weight status categories. Pairwise comparisons were performed using linear models adjusting for total sequencing reads, center, age, and sex. Adjusted p-values <0.05 were considered statistically significant. Points represent individual samples; boxplots show the distribution per group. 1b) Beta-diversity differences between participant's obesity categories group. Principal coordinates analysis plot based on Bray–Curtis distance. Abbreviations: UW: underweight; NW: normal weight; OW: overweight; OB: obesity.

Multinomial regression models were used to assess the association between weight status and cluster classification, using NW as the reference (Fig. 2d). The only significant association observed was for children with OB, who had slightly higher odds of belonging to cluster 3 ($\beta = 0.80$, SE = 0.36, 95 % CI: 0.09–1.51, $p = 0.026$). No other significant associations were found (supplementary material S7). It should be noted that enterotypes in early childhood are likely to represent gradients in microbial composition rather than discrete, stable clusters, due to the instability of the microbiome still under development at this age. Therefore, these associations should be interpreted with caution, as they may reflect tendencies rather than fixed patterns.

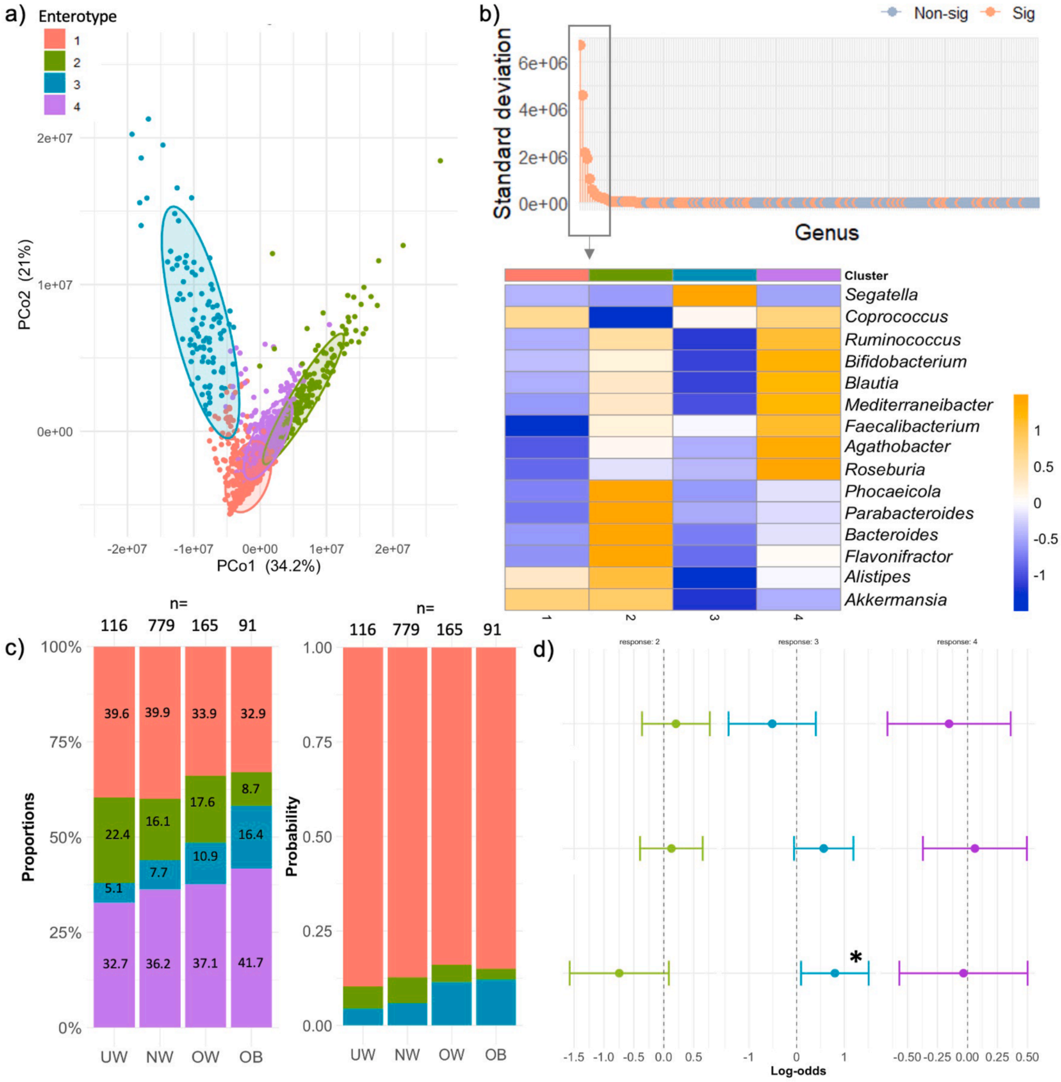

Fig. 2. Analysis of enterotype distribution and weight status in CORALS cohort.
2a) Principal Coordinates Analysis (PCoA) plot based on Bray–Curtis distance. Ellipses indicate 95 % confidence intervals for each cluster. 2b) Top 15 bacterial genera with the highest variability: the lollipop plot shows the standard deviation of each genus, while the heatmap displays their standardized relative abundance (z-score) across clusters. 2c) Proportion of samples assigned to each cluster according to weight status (left) and probability of membership to each cluster for each weight status (right). 2d) Log-odds estimates and their confidence intervals for the association between weight status and enterotype. Significant associations ($p < 0.05$) are indicated with an *. Abbreviations: UW: underweight; NW: normal weight; OW: overweight; OB: obesity.

3.3. Differences in bacterial species between weight categories

We performed a DESeq2 analysis at the species level to identify compositional differences across weight categories. Pairwise comparisons were conducted with the NW group as the reference (supplementary material – S8), adjusting for study center, sex, and age. Overall, the taxa exhibiting significant intergroup differences ($p < 0.05$) indicate distinct microbiome composition across weight categories (Fig. 3). Compared to NW children, 34 species differed in the UW group, 36 in the OW group, and 26 in the OB group, with 23, 24, and 14 species being unique to each category, respectively (Fig. 4a). Figure 4b highlights the relative abundance of four species associated with OB status. *Phocaeicola dorei* (adj.P = 0.006) and *Segatella hominis* (adj.P = 0.001) were significantly more abundant in the OB group compared to NW, whereas *Longicatena caecimuris* (adj.P = 0.03) and *Blautia parvula* (adj.P = 0.003) decreased in children with OB. The potential associations of the differential abundance of specific species between groups are further discussed. A complete Venn Diagram can be seen on supplementary material S9.

3.4. Functional analysis

Functional analysis was conducted using weight status as the primary comparison groups. Differential abundance analysis of metabolic pathways revealed distinct profiles associated with children's weight status (UW, OW, OB) compared to those with NW (Fig. 5 and supplementary material S10). In the OB group, 13 pathways differed significantly, with 11 pathways showing lower abundance and 2 pathways higher abundance compared to NW. The depleted pathways were mainly related to vitamin metabolism, nucleotide biosynthesis, and carbohydrate degradation. Specifically, pyridoxal 5′-phosphate biosynthesis I and the super pathway of pyridoxal 5′-phosphate biosynthesis and salvage were notably reduced. Several nucleotide-related pathways involved in dephosphorylation, pyrimidine biosynthesis (PWY-7282) and the anhydromuropeptides recycling pathway were also reduced. In addition, reduced abundances were observed in carbohydrate metabolism pathways, including chitin derivatives degradation II, glucose and xylose degradation, superpathway of fucose and

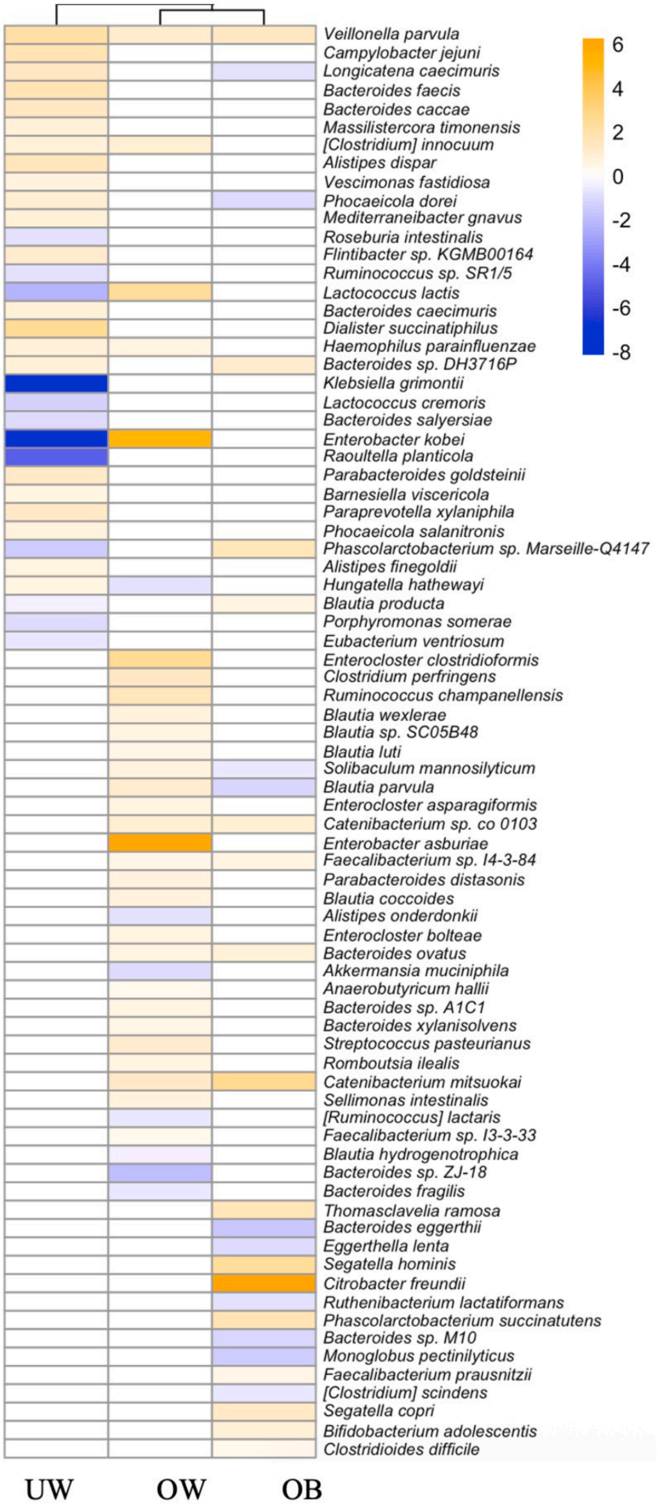

Fig. 3. Differential abundance of bacterial species by weight status in the CORALS cohort.

Heatmap showing the $\log_2$ fold change in the relative abundance of bacterial species across weight status groups: UW, OW, and OB, compared to NW. Only species with an adjusted p-value <0.05 (Benjamini-Hochberg corrected) are shown. Yellow indicates higher expression ($\log_2FC > 0$), and blue indicates lower expression ($\log_2FC < 0$). Abbreviations: UW: underweight; OW: overweight; OB: obesity.

rhamnose degradation, the Entner-Doudoroff (ED) pathway and anaerobic sucrose degradation. A lipid metabolism pathway, cytidine diphosphate (CDP) diacylglycerol biosynthesis II, also showed lower abundance in the OB group. Conversely, 2 pathways were significantly increased; the super pathway of polyamine biosynthesis II and uridine triphosphate (UTP) and cytidine triphosphate (CTP) dephosphorylation I. In children with OW, 9 pathways were significantly lower compared to the NW group. Most differences were observed in pathways involved in nucleotide metabolism, particularly purine and pyrimidine biosynthesis (PWY-7197, PWY0-162, PWY-841, PWY-7282). Pathways specifically involved in the biosynthesis of adenosine nucleotides were reduced (PWY-7229, PWY-6126). As in the OB group, the pyridoxal 5′-phosphate biosynthesis pathways (PYRIDOXSYN-PWY, PWY0-845) were also depleted. Finally, children in the UW group showed significant increase in four pathways: menaquinol-8 biosynthesis III, 1,4-dihydroxy-6-naphthoate biosynthesis II, adenosine nucleotides degradation II and pyridoxal 5-phosphate biosynthesis I.

We further explored which bacterial species, identified as significant in the DESeq2 analysis, were associated with the differentially abundant metabolic pathways. One species showed a significant association. In the OB group, *Faecalibacterium prausnitzii* was positively associated with the UTP and CTP dephosphorylation I pathway (PWY-7185) (coef = 0.21; q-value = 0.02).

4. Discussion

Based on the current findings, this study indicates that ponderal status is associated with differences in gut microbiota composition and functionality, resulting in distinct enterotypes or microbial clusters, among preschool children included from CORALS cohort.

4.1. Alpha and beta diversity

Regarding alpha diversity, the results establish that children with OB showed reduced diversity compared to those with NW. This finding is consistent with previous studies in children reporting obesity-associated alterations in gut microbiota diversity [47–49]. Children with OB exhibited lower alpha diversity, a pattern that has been previously observed in studies describing altered gut microbiota profiles in this population and associations with markers of low-grade systemic inflammation and metabolic risk [50]. Importantly, this reduction was not driven by changes in microbial richness, defined as the number of different taxa present, as richness-based metrics (Observed index) were comparable across weight categories. Instead, differences in alpha diversity were explained by alterations in microbial evenness, which reflects how evenly taxa are distributed in terms of relative abundance. Lower Shannon and Simpson indices in children with obesity indicate reduced microbial evenness, reflecting increased dominance of specific taxa rather than a reduction in the total number of microbial taxa [22,23]. Regarding beta diversity, differences in gut microbiota composition were observed across weight categories, particularly between UW and children with excess weight, while differences between OW and OB were less pronounced. This pattern of differences in microbiota composition in children with different ponderal status has also been reported in other studies, with children of similar age from East Asia [51,52].

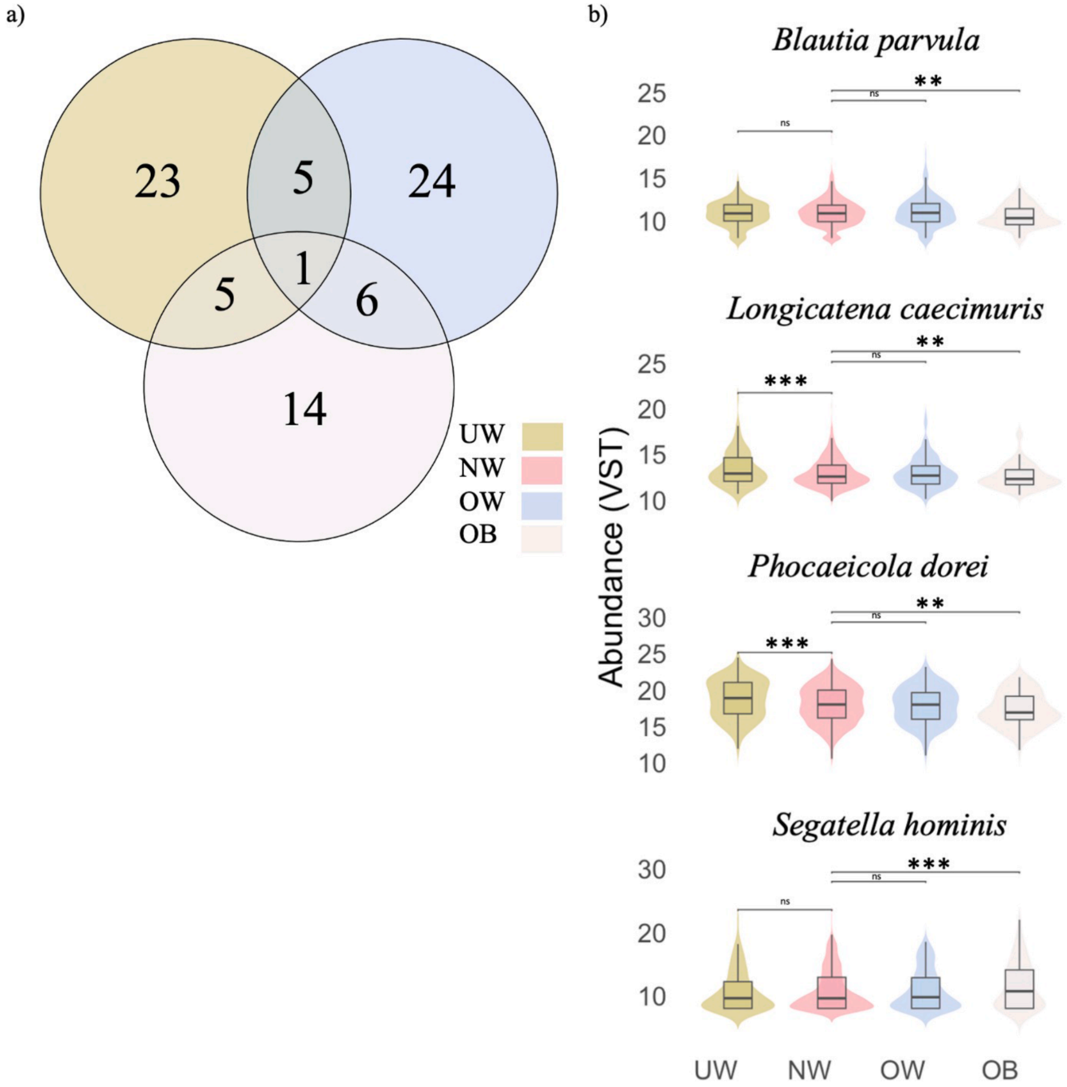

Fig. 4. Venn Diagram and relative abundance of species in the CORALS cohort.
4a) Venn Diagram displaying the bacterial species that are significantly different across weight status. 4b) Relative abundance of four obesity-associated species. Abbreviations: UW: underweight; OW: overweight; OB: obesity.

These findings consistently indicate that weight status is associated with differences in gut microbiota composition, particularly when comparing UW or NW individuals with those living with OW or OB.

4.2. Enterotypes

Our findings highlight an association between gut microbiota composition and body weight status in childhood, particularly through the identification of distinct enterotypes or microbial clusters, with children classified as OB more frequently observed in Enterotype 3, dominated by *Segatella (*formerly *Prevotella*) and reduced in *Akkermansia* and *Alistipes*. *Akkermansia* and *Alistipes* have previously been associated with anti-inflammatory properties, gut barrier integrity, and better metabolic profiles in other studies [53,54]. Enterotype 1 was more prevalent in children with UW and NW compared to OW and OB, indicating differences in microbial composition across groups. Interestingly, Enterotype 3, showed a gradual increase from UW to OB groups. Previous studies in children have also reported associations between *Prevotella* and OB [55]. *Prevotella* has been commonly linked with dietary patterns high in carbohydrates, mainly from grains and simple sugars [56] whereas an inverse correlation between dietary fibre intake and *Prevotella* enterotype has been described [57]. When comparing these results with the commonly reported Arumugam enterotypes, our Enterotype 3 is similar to their *Prevotella* enterotype [58]. Although literature on enterotypes in children is limited, previous studies have typically identified the *Bacteroides* dominated enterotype (as components of cluster 2 in our classification) as more frequent in children with metabolically favorable profiles [59].

However, Enterotype 3 was observed more frequently in children with OB in our cohort, adding descriptive insight into microbial composition across weight categories. The lack of significant associations for Enterotype 4 is also noteworthy, given its richness in genera traditionally considered beneficial (*Ruminococcus*, *Bifidobacterium, Akkermansia, Blautia*). *Ruminococcus* and related genera are well-known producers of SCFAs [60], particularly butyrate, which has been associated with anti-inflammatory properties [61] and stimulation of enteroendocrine hormones such as GLP-1 [62]. Several studies have shown that individuals with OB exhibit elevated levels of SCFAs [63]. SCFAs are also

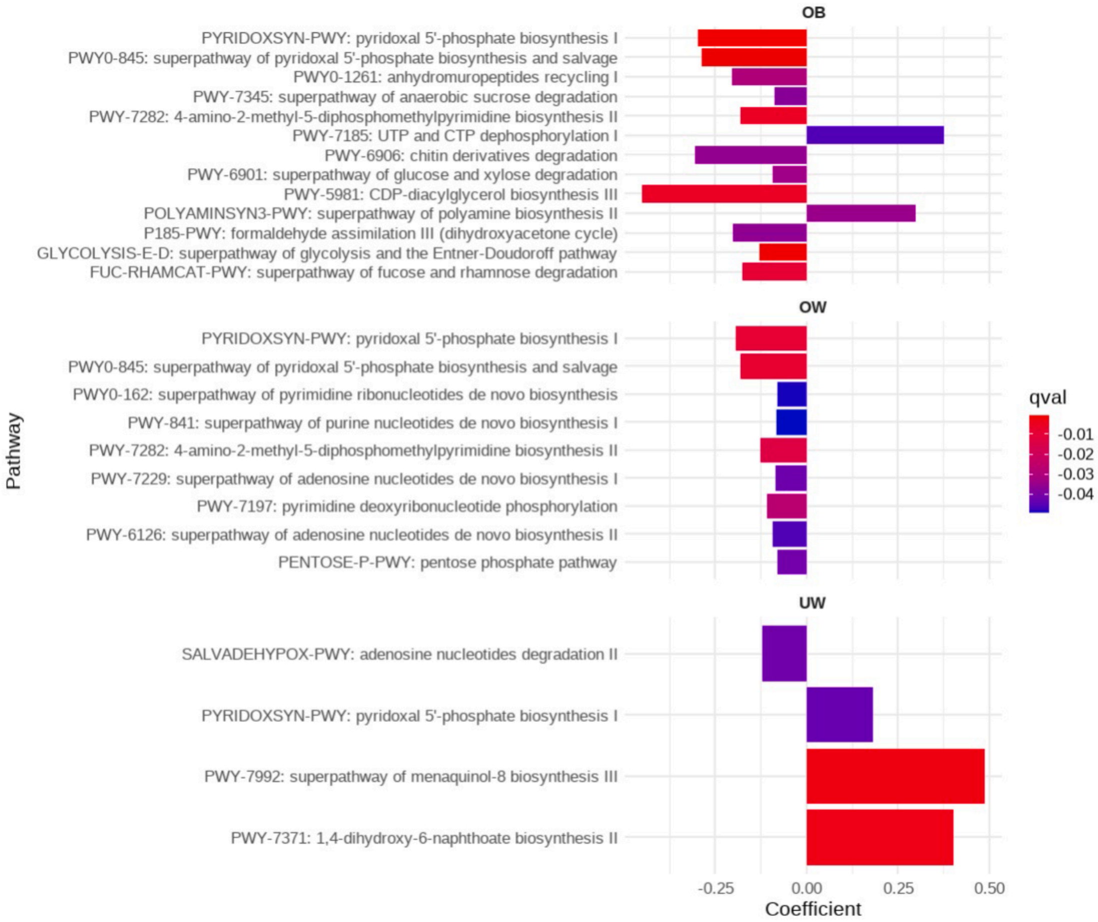

Fig. 5. Functional analysis associated with weight status in CORALS cohort.
Differential abundance of microbial metabolic pathways by weight status. Significantly different pathways (q < 0.05) in children with OB, OW or UW compared to those with NW. Bars represent effect sizes (regression coefficients), with positive values indicating increased abundance and negative values indicating depletion. Color intensity reflects statistical significance (q-value). Abbreviations: UW: underweight; OW: overweight; OB: obesity.

involved in higher energy extraction from the diet, suggesting that their effects may not always be protective regarding weight status. Nonetheless, SCFAs are rapidly utilized by host tissues which could partly explain lack of consistency in the correlations between their quantities and the metabolic phenotype across different studies. *Ruminococcus* has been associated with a Westernized dietary pattern [64]. Given its great efficiency in digesting simple carbohydrates, this enterotype may contribute to weight gain [65], consistent with our results, as children with OW and OB had higher proportions of cluster 4. These observations indicate that the presence of these genera alone does not fully explain differences in weight status and that their relative contributions may vary depending on the specificities of the microbial community and host factors. Although each enterotype is defined by a predominant genus allowing stratification, the approach also overlooks other taxa that may substantially contribute to the observed associations with body weight, while also highlighting the potential influence of environmental and lifestyle related factors.

Enterotype distribution differed across weight categories, indicating compositional differences associated with weight status. It is well established that gut microbiota composition varies significantly with age, and early childhood represents a key period of its maturation. During this stage, the microbiome is highly dynamic, less stable than in adults, and continues to change throughout the early years [66]. In addition, gut microbiota composition in early childhood is influenced by multiple factors beyond adiposity, including dietary intake and infant feeding, antibiotic exposure, infections, lifestyle, or socioeconomic conditions, which may shape microbial trajectories [10,12]. Given this, the findings identified in our study should be interpreted as descriptive compositional differences across weight categories rather than evidence of causal or stable metabolic profiles. As such, enterotype classification should be interpreted as an oversimplification of the gut microbiota's complexity. Previously described enterotypes may only become clearly distinguishable after 6 years of age, when the gut microbiota begins to stabilize [19]. Longitudinal studies in children aged 3–6 years integrating dietary, clinical, and lifestyle cofounders are needed to better understand enterotype and gut microbiota development and its implications for metabolic health [30,31].

4.3. Species distribution in weight categories

The differences in microbial abundances indicate associations between certain species and body weight status during infancy [67]. Of note is that species-level associations observed in children aged 3–6 years may not fully align with findings from studies in older children or adults as gut microbiota is still under development. In our cohort, the increase in several taxa in UW children, including *Phocaeicola dorei, Bacteroides caecimuris,* and *Alistipes dispar*, all belonging to the phylum *Bacteroidota*, are consistent with previous research suggesting a higher prevalence of

Bacteroidota associated with undernutrition [68]. *Klebsiella grimontii*, a species that decreased in UW and *Klebsiella michiganensis,* are emerging pathogens recognized for their potential to cause infections [69,70]. *Phocaeicola dorei* appears to colonize the gut after delivery and become more abundant after the introduction of solid foods, but its positive association with undernourishment remains unclear [71]. In addition, *Phocaeicola dorei* showed a decrease from UW to NW, OW and OB groups. This pattern aligns with previous studies reporting higher abundance of this species in children with lower BMI and associations with lower prevalence of OB and atherosclerosis [72], consistent with a possible association with favourable metabolic profiles. *Longicatena caecimuris* exhibited a decreasing trend from UW to NW and from NW to OB. This finding contrasts with some reports associating members of the Erysipelotrichaceae family with excess body weight and metabolic disturbances [73]. The observed reduction may reflect species specific roles within this family, suggesting that *Longicatena caecimuris* could be linked to a healthier metabolic profile. However, further studies are needed to clarify its function in humans, especially in children, given that the gut microbiota in this age group is still maturing and species level associations may differ.

The OW group showed mixed results. The observed increases in *Enterobacter kobei* and *Enterobacter asburiae*, both members of the *Enterobacteriaceae* family are notable, as these bacteria are classified as opportunistic pathogens causing a range of infections, including urinary tract, respiratory, and bloodstream infections [74], which may be relevant in pediatric populations including children with OW. *Ruminococcus champanellensis* was also more abundant in OW children. To our knowledge, there is no literature linking this species to pediatric metabolic alterations. However, other species within the same genus, such as *Ruminococcus torques*, have been associated with metabolic dysfunction-associated pediatric fatty liver disease in other studies [75]. However, in our cohort we only observed an association with OW status. Additionally, *Escherichia coli*, previously associated with excess body weight in children [76], was not found to be more abundant in excess body weight in our cohort. Additionally, the marked reduction of *Akkermansia muciniphila* in the OW group aligns with the widely reported association with metabolic health [77,78]. Its lower abundance in children with OW could be explained by the role attributed to this species in improving some metabolic features (excess weight, gut barrier dysfunction or low-grade inflammation) in preclinical studies, but causality cannot be inferred in our study in children.

Regarding children with OB, characterized by a more distinct BMI, several taxa were more abundant in this group, including *Clostridium difficile, Faecalibacterium* spp., and *Thomasclavelia ramosa*, which have been previously reported to be more prevalent in children with OB or higher BMI in observational studies [79,80]. We observed that *Faecalibacterium prausnitzii* and *Faecalibacterium* sp. *I4-3-84* were increased in OB, consistent with existing literature [81,82]. The first species has been consistently linked to beneficial health outcomes in various studies, such as protection against depression and a lower prevalence of OB [83,84]. Regarding the *Blautia* genus, *Blautia producta* was increased in OB, whereas *Blautia parvula* was reduced. Some studies have found that species such as *Blautia luti* may have beneficial anti-inflammatory properties [85], which contrast our findings as *Blautia luti* increased in the OW group. Although *Faecalibacterium prausnitzii* and *Blautia luti* have been associated with metabolic health in adults, their increased abundance in children with excess weight in our cohort may reflect age specific microbial dynamics within the microbiome, highlighting that species level effects may not translate directly from adults to young children. However, when considering the genus level abundance across enterotypes, we observed an overall reduction of *Blautia* in OB. This suggests that, despite the increase of certain species, the total abundance of the genus is decreased, highlighting that not all *Blautia* species contribute equally and may have different roles. Shared increases in *Blautia parvula, Catenibacterium mitsuokai, Bacteroides ovatus* and *Faecalibacterium* sp. *I4-3-84* were observed in both OW and OB groups, suggesting an association with higher weight status, consistent with findings from two French pediatric cohorts [86]. *Segatella hominis* was found to be stable in UW, NW and OW, but increased in OB, in line with the observed enrichment of the *Segatella* genus in the OB-associated enterotype, cluster 3. Previous studies have reported associations between *Segatella* and OB, being more abundant in children with higher BMI [87]. *Citrobacter freundii,* increased in children with OB, has been reported to be more abundant in children with higher fasting insulin levels, consistent with observational associations in OB [88]. Finally, the marked increase of *Catenibacterium mitsukoae* is consistent with previous literature. It belongs to the phylum *Bacillota*, which has been reported to be more abundant in individuals with obesity [89], in line with the overall tendency of this phylum to be increased in obesity phenotypes. These findings reveal differences in microbial abundances across weight categories, suggesting associations with body weight, but should be interpreted cautiously given the observational design and the ongoing maturation of the gut microbiota in early childhood. It is important to note that during early childhood, gut microbial composition is strongly shaped by factors such as dietary patterns, physical activity, mode of delivery, antibiotic exposure, infectious episodes, and socioeconomic conditions [12]. These factors may modulate species-level associations independently of body weight and should be considered in future studies designed to better clarify their relative contributions.

4.4. Functional analysis

To date, studies exploring the functional profiles of the gut microbiota in children with OW or OB remain scarce, highlighting the need for more research in this population. Our findings contribute to this understudied field; however, given the cross-sectional design, all interpretations must be considered associative rather than mechanistic and further investigations are needed to validate these observations. In our cohort, children with OW and OB showed lower relative abundance of pyridoxal 5′-phosphate biosynthesis pathways, and children with UW an increase in this pathway, indicating an association with weight status. Pyridoxal 5′-phosphate is the biologically active form of vitamin B6 which is involved in amino acid, lipid, and carbohydrate metabolism, as well as in immune modulation [90–92]. Previous studies have linked vitamin B6 deficiency to metabolic dysfunction and increased inflammation, which are frequently observed in individuals with obesity [93]. Regarding nucleotide pathways, both OW and OB groups showed a reduction in purine and pyrimidine nucleotide biosynthesis microbial pathways. These nucleotides are essential for DNA and RNA synthesis in microbes and the host's cells, including intestinal epithelium and immune cells, supporting tissue renewal and gut homeostasis [94]. These processes are described in the literature, but our results do not allow conclusions about whether these processes are affected. Our observations are consistent with previous studies reporting higher microbial nucleotide biosynthesis pathway abundance in lean individuals compared to those with obesity [95]. In rapidly renewing tissues like muscle, particularly during growth, nucleotide demand increases to support regeneration [96]. In our cohort, adenosine microbial biosynthesis pathways were reduced in OW children.

These findings represent an association between microbial nucleotide metabolism and weight status; however, more studies are needed to determine whether these associations have any implication for growth or metabolism. The observed depletion of microbial carbohydrate metabolism pathways in the OB group including chitin derivatives degradation, glucose and xylose degradation, anaerobic sucrose degradation, and the ED pathways is notable. The decrease in microbial pathways involved in glucose and xylose degradation in children with OB may indicate differences in the relative abundance of routes involved in the degradation of simple sugars. Consequently, more glucose may be available for host absorption, potentially increasing systemic energy availability and contributing to adipogenesis when energy intake exceeds expenditure, as shown in other articles [97]. Nonetheless, our results reflect variation in pathway abundance and do not imply reduced functional capacity. Interestingly, the ED pathway, which has been reported to be responsible for catabolizing glucose into pyruvate through a distinct set of enzymes different from those used in glycolysis or the pentose phosphate pathway [98], is also reduced in children with OB. This pathway is particularly active in certain Gram-negative bacteria [98]. As with the general glucose degradation pathway, a reduction in conversion of glucose to pyruvate may be associated with a lower microbial production of key metabolic intermediates such as SCFA. At the same time, more glucose may remain available for host absorption and lipogenesis. Alterations in microbial carbohydrate metabolism may be associated with early-life features of an obesogenic metabolic profile. However, SCFA production or glucose availability cannot be inferred from cross-sectional pathway abundance data and thus, these differences should be interpreted strictly as exploratory associations. Moving to lipid metabolism, the CDP diacylglycerol biosynthesis pathway III (PWY-5981), was decreased in OB. It is involved in phosphatidylglycerol synthesis [99]. Given the link between excessive phospholipid biosynthesis and OB risk in adults [100], the lower abundance observed in our cohort may reflect age-specific differences in microbial lipid metabolism rather than a causal effect. Lipids remain essential for children's growth and brain development [101], and variations in microbial lipid pathways may be associated with differences in lipid availability. However, the biological relevance of these observed differences remains unclear. Regarding bacterial species, *Faecalibacterium prausnitzii* was associated with the UTP and CTP dephosphorylation I pathway, which was also more abundant in children with OB. Altogether, these findings suggest that children with OB exhibit the most pronounced differences in microbial functional pathway abundance, particularly in carbohydrate and vitamin pathways, whereas OW children show more subtle shifts primarily in nucleotide metabolism. In contrast, UW children displayed minimal pathway alterations. All findings should be interpreted as associations derived from a cross-sectional analysis. Moreover, functional pathways inferred from metagenomic data reflect potential microbial metabolic capacity rather than actual functional activity and are likely influenced by microbial community structure or dietary factors; therefore, no causal or mechanistic interpretations can be made.

Despite the valuable insights provided by our study, several important limitations should be acknowledged. First, the cross-sectional design prevents causal inference between gut microbiota composition and obesity development in early childhood. While we observed associations between certain microbial features and weight categories, we cannot determine whether these patterns contribute to obesity or are a consequence of it. Second, although the overall sample size was large, the groups were highly heterogeneous in clinical characteristics, which may introduce variability and affect the interpretation of microbiota differences. However, this heterogeneity may enhance the external validity of our findings, reflecting the diversity seen in real-world pediatric populations. Third, we used BMI as a standardized, age and sex adjusted measure of adiposity; however, BMI does not capture fat distribution or body composition nuances in young children. While it remains the most feasible tool for large scale studies, this limitation should be considered when interpreting associations with microbial composition. Importantly, our models did not include several factors known to influence gut microbiota in early childhood, such as diet, antibiotic exposure, recent infections, socioeconomic status, mode of delivery, breastfeeding history, and probiotic use. Their omission may have contributed to residual confounding, potentially influencing the observed associations between microbial features and BMI z-score. In spite of that, our study provides insightful research into microbiota patterns in an understudied age group, highlighting associations that can guide future research. We emphasize that these results should be interpreted with caution and that longitudinal studies including these variables are needed to infer the direction of causality. Moreover, uncertainty in enterotype classification due to variations of gut composition with age and generally low effect sizes (PERMANOVA R^2), common in microbiota research, should be considered avoiding overinterpretation of the results and the potential clinical relevance. Another key consideration is that the gut microbiota of children aged 3–6 years is still developing. Ongoing maturation and dynamic shifts in composition add variability to the observed patterns. This, together with the limited number of studies in this age group, restricts generalization and prevents highlighting results as fully conclusive. Observed microbial patterns may continue to evolve over time, emphasizing the need for longitudinal studies to track microbiota trajectories and their long-term impact on metabolic health. Finally, despite these limitations, our study gives meaningful information on gut microbiota in an understudied pediatric population. By identifying associations with early-life weight status, our findings lay the groundwork for future longitudinal research to clarify causal relationships, explore the role of modifiable environmental factors, and investigate potential microbiota-targeted strategies for childhood obesity prevention.

5. Conclusions

Our findings indicate that childhood weight status, particularly obesity, in children from 3 to 6 years is associated with reduced gut microbiota alpha and altered beta diversity, as well as significant shifts in taxonomic composition. Enterotype distribution varies according to weight status, with a higher prevalence of *Segatella* and a reduction in *Akkermansia/Alistipes* profiles among children with obesity. In addition, alterations in several bacterial species highlight their potential role in weight status. Functional analyses further reveal that the gut microbiota of children with obesity is especially characterized by distinct patterns in nucleotide and carbohydrate metabolism. Together, these results underscore the gut microbiota's relevance not only as a biomarker of pediatric weight status but also as a potential modulator of metabolic health. Further research is needed to validate and expand upon these findings and to explore the potential of microbiota-targeted strategies for obesity prevention and management in children.

Author contributions

Conceptualization: NV-B, MC, MG-C, NB, LAM, JS-S, SN-C. Data curation: CM-M, PM-C, YS. Formal analysis: NV-B, CM-M, PM-C, YS, SN-C. Funding acquisition: MG-C, NB, LAM, JS-S, YS, SN-C. Investigation: NV-B, CM-M, AME, CC-C, AH-C, MLM-B, JMJ-C, RV-C, JP-D, IR-D, BP-V. Methodology: MC, NV-B, CM-M, JAM, MG-C, RL, NB, LAM, CMA, MLM-B, PM-C, JMJ-C, JS-S, YS, SN-C. Project administrations: MG-C, RL, NB, DC, CMA, JS-S, SN-C. Resources: MG-C, NB, LAM, RL, DC, JS-S, SN-C. Supervision: MC, SN-C. Validation: PM-C, SN-C. Writing – original draft: NV-B. Writing – review and editing: CM-M, MC, JAM, MG-C, RL, MB, LAM, DC, AME, CMA, CC-C, RP-L, AH-C, MLM-B, PM-C, JMJ-C, RV-C, JP-D, IR-DT, BP-V, MJT-A, JS-S, YS, SN-C. All individuals who meet the authorship criteria have been included as authors, and no eligible contributor has been excluded.

Ethic committee references

19/27; 4155/2019; 2019/18; 051/2019; 2019/131; 9/19; 19/162. Written informed consent was obtained from all parents or supervisors before any study-related procedures were carried out. In addition, CORALS is registered in clinicaltrials.gov (ID: NCT06317883).

Data sharing statement

The datasets generated and analyzed in this study cannot be made publicly available due to data protection regulations and ethical considerations. Sharing this information could compromise participants' consent, as they agreed for their data to be used solely by the original research team. However, collaboration on data analyses can be requested by sending a letter to the CORALS Steering Committee (estudiocoral@corals.es). The request will be forwarded to all members of the Committee for their review and deliberation.

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Declaration of Generative AI and AI-assisted technologies

The authors declare that they did not use any Generative AI or AI assisted technologies during the writing process.

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Declaration of interests

The authors report there are no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2025.106558.

References

[1] World Health Organization. WHO. Fact sheets. Overweight and obesity [cited 2025 May 22]; Available from: https://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight.

[2] Agencia Española de Seguridad Alimentaria y Nutrición, Ministerio de Derechos Sociales C y A. Estudio ALADINO 2023: Estudio sobre Alimentación. Actividad Física, Desarrollo Infantil y Obesidad en España 2023. 2023.

[3] Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev 2016 Feb 23;17(2):95–107.

[4] de Lamas C, Kalén A, Anguita-Ruiz A, Pérez-Ferreirós A, Picáns-Leis R, Flores K, et al. Progression of metabolic syndrome and associated cardiometabolic risk factors from prepuberty to puberty in children: the PUBMEP study. Front Endocrinol 2022;13:1082684.

[5] González-Gil EM, Anguita-Ruiz A, Kalén A, De Las Lamas Perez C, Rupérez AI, Vázquez-Cobela R, et al. Longitudinal associations between cardiovascular biomarkers and metabolic syndrome during puberty: the PUBMEP study. Eur J Pediatr 2023 Jan;182(1):419–29.

[6] Lam BCC, Lim AYL, Chan SL, Yum MPS, Koh NSY, Finkelstein EA. The impact of obesity: a narrative review. Singap Med J 2023 Mar;64(3):163–71.

[7] Geng J, Ni Q, Sun W, Li L, Feng X. The links between gut microbiota and obesity and obesity related diseases. Biomed Pharmacother 2022 Mar;147:112678.

[8] Ogunrinola GA, Oyewale JO, Oshamika OO, Olasehinde GI. The human microbiome and its impacts on health. Internet J Microbiol 2020 Jun 12;2020:1–7.

[9] Luikham S, Bhattacharyya J. The gut microbiome, human nutrition, and immunity: visualizing the future. In: Microbiome, immunity, digestive health and nutrition. Elsevier; 2022. p. 41–51.

[10] Yang J, Wu J, Li Y, Zhang Y, Cho WC, Ju X, et al. Gut bacteria formation and influencing factors. FEMS Microbiol Ecol 2021 Mar 31;97(4).

[11] Yao Y, Cai X, Ye Y, Wang F, Chen F, Zheng C. The role of microbiota in infant health: from early life to adulthood. Front Immunol 2021;12:708472.

[12] Jeong S. Factors influencing development of the infant microbiota: from prenatal period to early infancy. Clin Exp Pediatr 2022 Sep;65(9):439–47.

[13] Basak S, Mallick R, Navya Sree B, Duttaroy A. Placental epigenome impacts fetal development: effects of maternal nutrients and Gut Microbiota. Nutrients 2024 Jun 13;16(12):1860.

[14] Barrientos G, Ronchi F, Conrad ML. Nutrition during pregnancy: influence on the gut microbiome and fetal development. Am J Reprod Immunol 2024 Jan 11;91(1).

[15] Herrera-Quintana L, Vázquez-Lorente H, Hinojosa-Nogueira D, Plaza-Diaz J. Relationship between infant feeding and the microbiome: implications for allergies and food intolerances. Children 2024 Aug 22;11(8):1030.

[16] Noles DL, Matzeller KL, Frank DN, Krebs NF, Tang M. Complementary feeding and infant gut Microbiota: a narrative review. Nutrients 2025 Feb 20;17(5):743.

[17] Laursen MF. Gut microbiota development: influence of diet from infancy to toddlerhood. Ann Nutr Metab 2021;77(Suppl. 3):21–34.

[18] Dogra S, Chung C, Wang D, Sakwinska O, Colombo Mottaz S, Sprenger N. Nurturing the early life gut microbiome and immune maturation for long term health. Microorganisms 2021 Oct 7;9(10):2110.

[19] Pan Y, Jiao FY. Link between childhood obesity and gut microbiota. World J Gastroenterol 2024 Aug 14;30(30):3560–3.

[20] de Cuevillas B, Milagro FI, Tur JA, Gil-Campos M, de Miguel-Etayo P, Martínez JA, et al. Fecal microbiota relationships with childhood obesity: a scoping comprehensive review. Obes Rev 2022 Jan 15;23(S1).

[21] Nóbrega R, Costa CFFA, Cerqueira Ó, Inês A, Carrola JS, Gonçalves C. Association between gut microbiota and pediatric obesity: a systematic review. Nutrition 2025 Jun 18;140:112875.

[22] Thukral AK. A review on measurement of alpha diversity in biology. Agric Res J 2017;54(1):1.

[23] Liu BN, Liu XT, Liang ZH, Wang JH. Gut microbiota in obesity. World J Gastroenterol 2021 Jul 7;27(25):3837–50.

[24] Pinart M, Dötsch A, Schlicht K, Laudes M, Bouwman J, Forslund SK, et al. Gut microbiome composition in Obese and non-obese persons: a systematic review and meta-analysis. Nutrients 2021 Dec 21;14(1).

[25] Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol 2007 Mar;73(5):1576–85.

[26] Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, et al. Short-chain fatty-acid-producing bacteria: key components of the human Gut Microbiota. Nutrients 2023 May 6;15(9).

[27] Psichas A, Sleeth ML, Murphy KG, Brooks L, Bewick GA, Hanyaloglu AC, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. Int J Obes 2015 Mar;39(3):424–9.

[28] De Silva A, Bloom SR. Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity. Gut Liver 2012 Jan;6(1):10–20.

[29] Huber H, Schieren A, Holst JJ, Simon MC. Dietary impact on fasting and stimulated GLP-1 secretion in different metabolic conditions – a narrative review. Am J Clin Nutr 2024 Mar;119(3):599–627.

[30] Corbin KD, Igudesman D, Smith SR, Zengler K, Krajmalnik-Brown R. Targeting the Gut Microbiota's role in host energy absorption with precision nutrition interventions for the prevention and treatment of obesity. Nutr Rev 2025 Apr;15.

[31] Sehgal K, Khanna S. Gut microbiota: a target for intervention in obesity. Expet Rev Gastroenterol Hepatol 2021 Oct 3;15(10):1169–79.

[32] World Medical Association Declaration of Helsinki. JAMA 2025 Jan 7;333(1):71.

[33] Larruy-García A, Miguel-Berges ML, Torre IR, Pastor-Villaescusa B, Leis R, Babio N, et al. Cross-sectional associations between Mediterranean diet and body composition in preschool children. CORAL Study Pediatr Obes 2025 Jul 21;20(7).

[34] Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 2012 Aug 19;7(4):284–94.

[35] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014 Aug 1;30(15):2114–20.

[36] Andrews S. KFSPABLKCWS. FastQC. Babraham. 2012. UK.

[37] Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 2016 Oct 1;32(19):3047–8.

[38] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 2012 Apr 4;9(4):357–9.

[39] Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol 2019 Nov 28;20(1):257.

[40] Lu J, Breitwieser FP, Thielen P, Salzberg SL. Bracken: estimating species abundance in metagenomics data. PeerJ Comput Sci 2017 Jan 2;3:e104.

[41] Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife 2021 May 4;10.

[42] McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013 Apr 22;8(4):e61217.

[43] Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, et al. Vegan: community ecology package. CRAN: Contributed Packages; 2001.

[44] Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2014 Dec 5;2(1):15.

[45] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014 Dec 5;15(12):550.

[46] Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol 2021 Nov 16;17(11):e1009442.

[47] Joseph N, Clayton JB, Hoops SL, Linhardt CA, Mohd Hashim A, Mohd Yusof BN, et al. Alteration of the gut microbiome in normal and overweight school children from Selangor with *lactobacillus* fermented milk administration. Evol Bioinf Online 2020 Jan 23:16.

[48] Li XM, Lv Q, Chen YJ, Yan LB, Xiong X. Association between childhood obesity and gut microbiota: 16S rRNA gene sequencing-based cohort study. World J Gastroenterol 2024 Apr 28;30(16):2249–57.

[49] Ma T, Wu Z, Lin J, Shan C, Abasijiang A, Zhao J. Characterization of the oral and gut microbiome in children with obesity aged 3 to 5 years. Front Cell Infect Microbiol 2023 Mar 29:13.

[50] Koller AM, Săsăran MO, Mărginean CO. The role of Gut Microbiota in pediatric obesity and metabolic disorders: insights from a comprehensive review. Nutrients 2025 May 30;17(11):1883.

[51] Ma T, Wu Z, Lin J, Shan C, Abasijiang A, Zhao J. Characterization of the oral and gut microbiome in children with obesity aged 3 to 5 years. Front Cell Infect Microbiol 2023 Mar 29:13.

[52] Chen X, Zhang D, Sun H, Jiang F, Shen Y, Wei P, et al. Characterization of the gut microbiota in Chinese children with overweight and obesity using 16S rRNA gene sequencing. PeerJ 2021 Jun 8;9:e11439.

[53] Rodrigues VF, Elias-Oliveira J, Pereira ÍS, Pereira JA, Barbosa SC, Machado MSG, et al. Akkermansia muciniphila and gut immune System: a good friendship that attenuates inflammatory bowel disease, obesity, and diabetes. Front Immunol 2022 Jul 7;13.

[54] Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. The genus alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol 2020 Jun 9:11.

[55] Gong J, Zhang Q, Hu R, Yang X, Fang C, Yao L, et al. Effects of Prevotella copri on insulin, gut microbiota and bile acids. Gut Microbes 2024;16(1):2340487.

[56] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science (1979) 2011 Oct 7;334(6052):105–8.

[57] Zhong H, Penders J, Shi Z, Ren H, Cai K, Fang C, et al. Impact of early events and lifestyle on the gut microbiota and metabolic phenotypes in young school-age children. Microbiome 2019 Dec 4;7(1):2.

[58] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011 May 12;473(7346):174–80.

[59] Alcazar M, Escribano J, Ferré N, Closa-Monasterolo R, Selma-Royo M, Feliu A, et al. Gut microbiota is associated with metabolic health in children with obesity. Clinical Nutrition 2022 Aug;41(8):1680–8.

[60] Xie J, Li L, Dai T, Qi X, Wang Y, Zheng T, et al. Short-chain fatty acids produced by ruminococcaceae mediate α-Linolenic acid promote intestinal stem cells proliferation. Mol Nutr Food Res 2022 Jan 17;66(1).

[61] Liu H, Wang J, He T, Becker S, Zhang G, Li D, et al. Butyrate: a double-edged sword for health? Adv Nutr 2018 Jan;9(1):21–9.

[62] Sanz Y, Cryan JF, Deschasaux-Tanguy M, Elinav E, Lambrecht R, Veiga P. The gut microbiome connects nutrition and human health. Nat Rev Gastroenterol Hepatol 2025 Aug;22(8):534–55.

[63] Kim KN, Yao Y, Ju SY. Short chain fatty acids and fecal microbiota abundance in humans with obesity: a systematic review and meta-analysis. Nutrients 2019 Oct 18;11(10):2512.

[64] Davis EC, Dinsmoor AM, Wang M, Donovan SM. Microbiome composition in pediatric populations from birth to adolescence: impact of diet and prebiotic and probiotic interventions. Dig Dis Sci 2020 Mar 31;65(3):706–22.

[65] Knights D, Ward TL, McKinlay CE, Miller H, Gonzalez A, McDonald D, et al. Rethinking "Enterotypes.". Cell Host Microbe 2014 Oct;16(4):433–7.

[66] Ronan V, Yeasin R, Claud EC. Childhood development and the microbiome—the intestinal microbiota in maintenance of health and development of disease during childhood development. Gastroenterology 2021 Jan;160(2):495–506.

[67] Krajmalnik-Brown R, Ilhan Z, Kang D, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract 2012 Apr 24;27(2):201–14.

[68] Iddrisu I, Monteagudo-Mera A, Poveda C, Pyle S, Shahzad M, Andrews S, et al. Malnutrition and Gut Microbiota in children. Nutrients 2021 Aug 8;13(8):2727.

[69] Xu P, Zhang D, Zhuo W, Zhou L, Du Y, Zhang P, et al. Characterization of a highly virulent Klebsiella michiganensis strain isolated from a preterm infant with sepsis. Infect Drug Resist 2024 Nov;17:4973–83.

[70] Campos-Madueno EI, Mauri C, Meroni E, Paseiro PP, Consonni A, Luzzaro F, et al. Simultaneous gut colonization by Klebsiella grimontii and Escherichia coli co-possessing the blaKPC-3-carrying pQil plasmid. Eur J Clin Microbiol Infect Dis 2022 Jul 28;41(7):1087–91.

[71] Wang S, Zeng S, Egan M, Cherry P, Strain C, Morais E, et al. Metagenomic analysis of mother-infant gut microbiome reveals global distinct and shared microbial signatures. Gut Microbes 2021 Jan 1;13(1).

[72] Nakashima H, Shinohara R, Emoto T, Saito Y, Yoshida N, Hirata K ichi, et al. Phocaeicola dorei and Phocaeicola vulgatus protect against atherosclerosis by regulating gut immunity. J Atherosclerosis Thromb 2025;65460.

[73] Kaakoush NO. Insights into the role of Erysipelotrichaceae in the human host. Front Cell Infect Microbiol 2015 Nov 20:5.

[74] Sanders WE, Sanders CC. Enterobacter spp.: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 1997 Apr;10(2):220–41.

[75] Du L, Zhang K, Liang L, Yang Y, Lu D, Zhou Y, et al. Multi-omics analyses of the gut microbiota and metabolites in children with metabolic dysfunction-associated steatotic liver disease. mSystems 2025 Apr 22;10(4).

[76] Gao X, Jia R, Xie L, Kuang L, Feng L, Wan C. Obesity in school-aged children and its correlation with Gut E.coli and Bifidobacteria: a case–control study. BMC Pediatr 2015 Dec 30;15(1):64.

[77] Rodrigues VF, Elias-Oliveira J, Pereira ÍS, Pereira JA, Barbosa SC, Machado MSG, et al. Akkermansia muciniphila and gut immune system: a good friendship that attenuates inflammatory bowel disease, obesity, and diabetes. Front Immunol 2022 Jul 7;13.

[78] Yan J, Sheng L, Li H. *Akkermansia muciniphila* : is it the Holy Grail for ameliorating metabolic diseases? Gut Microbes 2021 Jan 1;13(1).

[79] Leung J, Burke B, Ford D, Garvin G, Korn C, Sulis C, et al. Possible association between obesity and *Clostridium difficile* infection. Emerg Infect Dis 2013 Nov;19(11):1791–8.

[80] Woting A, Pfeiffer N, Loh G, Klaus S, Blaut M. Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. mBio 2014 Oct 31;5(5).

[81] Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AMS, Ramakrishna BS. Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children. Br J Nutr 2010 Feb 14;103(3):335–8.

[82] Riva A, Borgo F, Lassandro C, Verduci E, Morace G, Borghi E, et al. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in *Firmicutes* populations. Environ Microbiol 2017 Jan 22;19(1):95–105.

[83] Hao Z, Wang W, Guo R, Liu H. Faecalibacterium prausnitzii (ATCC 27766) has preventive and therapeutic effects on chronic unpredictable mild stress-induced depression-like and anxiety-like behavior in rats. Psychoneuroendocrinology 2019 Jun;104:132–42.

[84] Leylabadlo HE, Ghotaslou R, Feizabadi MM, Farajnia S, Moaddab SY, Ganbarov K, et al. The critical role of Faecalibacterium prausnitzii in human health: an overview. Microb Pathog 2020 Dec;149:104344.

[85] Benítez-Páez A, Gómez del Pugar EM, López-Almela I, Moya-Pérez Á, Codoñer-Franch P, Sanz Y. Depletion of *Blautia* species in the microbiota of Obese children relates to intestinal inflammation and metabolic phenotype worsening. mSystems 2020 Apr 28;5(2).

[86] Toubon G, Butel MJ, Rozé JC, Delannoy J, Ancel PY, Aires J, et al. Association between gut microbiota at 3.5 years of age and body mass index at 5 years: results from two French nationwide birth cohorts. Int J Obes 2024 Apr 15;48(4):503–11.

[87] Hu HJ, Park SG, Jang HB, Choi MK, Park KH, Kang JH, et al. Correction: obesity alters the microbial community profile in Korean adolescents. PLoS One 2015 Sep 4;10(9):e0138015.

[88] Liu L, Li M, Qin Y, Liu Y, Li M, Lian B, et al. Childhood obesity and insulin resistance is correlated with gut microbiome serum protein: an integrated metagenomic and proteomic analysis. Sci Rep 2025 Jul 1;15(1):21436.

[89] Cho KY. Association of gut microbiota with obesity in children and adolescents. Clin Exp Pediatr 2023 Apr 15;66(4):148–54.

[90] Parra M, Stahl S, Hellmann H. Vitamin B6 and its role in cell metabolism and physiology. Cells 2018 Jul 22;7(7):84.

[91] Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. Front Nutr 2019 Apr 17;6.

[92] Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. Front Nutr 2019 Apr 17;6.

[93] Aasheim ET, Hofsø D, Hjelmesæth J, Birkeland KI, Bøhmer T. Vitamin status in morbidly obese patients: a cross-sectional study. Am J Clin Nutr 2008 Feb;87(2):362–9.

[94] Li M, Liu B, Li R, Yang P, Leng P, Huang Y. Exploration of the link between gut microbiota and purinergic signalling. Purinergic Signal 2023 Mar 19;19(1):315–27.

[95] Chanda D, De D. Meta-analysis reveals obesity associated gut microbial alteration patterns and reproducible contributors of functional shift. Gut Microbes 2024 Dec 31;16(1).

[96] Uauy R, Quan R, Gil A. Role of nucleotides in intestinal development and repair: implications for infant nutrition. J Nutr 1994 Aug;124:1436S–41S.

[97] Schwarz JM, Clearfield M, Mulligan K. Conversion of Sugar to Fat: is Hepatic de Novo Lipogenesis Leading to Metabolic Syndrome and Associated Chronic Diseases? J Osteopath Med 2017 Aug 1;117(8):520–7.

[98] Sokatch JR. Aerobic metabolism of carbohydrates. In: Bacterial physiology and metabolism. Elsevier; 1969. p. 112–30.

[99] National Center for Biotechnology Information. PubChem pathway summary for pathway PWY-5667, CDP-diacylglycerol biosynthesisSource: BioCyc. 2025 [cited 2025 Jun 12]; Available from: https://pubchem.ncbi.nlm.nih.gov/pathway/BioCyc:HUMAN_PWY-5667.

[100] Sharma NK, Langberg KA, Mondal AK, Das SK. Phospholipid biosynthesis genes and susceptibility to obesity: analysis of expression and polymorphisms. PLoS One 2013 May 28;8(5):e65303.

[101] Teoh OH, Lin TP, Abrahamse-Berkeveld M, Winokan A, Chong YS, Yap F, et al. An infant formula with large, milk phospholipid-coated lipid droplets supports adequate growth and is well-tolerated in healthy, term Asian infants: a randomized, controlled double- clinical trial. Nutrients 2022 Feb 1;14(3):634.