Association between gut microbiota at 3.5 years of age and body mass index at 5 years: Results from two French nationwide birth cohorts

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Abstract

Background/Objectives:

The relations between the gut microbiota and change in body mass index (BMI) or pediatric overweight in early life remain unclear and there is a scarcity of information regarding the preterm population. This study aimed to investigate how the gut microbiota at 3.5 years of age is associated with (1) the later BMI at 5 years, and (2) BMI z-score variations between 2 and 5 years in children from two French nationwide birth cohorts.

Subjects/Methods:

Bacterial 16S rRNA gene sequencing was performed to profile the gut microbiota at 3.5 years of very preterm children (n = 143, EPIPAGE 2 cohort) and late preterm/full-term children (n = 369, ELFE cohort). Predicted metabolic function abundances was computed using PICRUSt2 tool. Children anthropometric measurements were collected at 2 and 5 years through medical exams or retrieved from the child health booklets. Statistical analyses included multivariable linear and logistic regressions, variable selection using random forest, and microbiome regression-based kernel association tests.

Results

The *Firmicutes* to *Bacteroidetes* (F/B) ratio at 3.5 years was positively associated with BMI z-score at 5 years. Several genera were positively associated (*[Eubacterium] hallii group, Fusicatenibacter*, and *[Eubacterium] ventriosum group*) or negatively associated (*Eggerthella, Colidextribacter*, and *Ruminococcaceae CAG-352*) with BMI z-score at 5 years. Some genera were also associated with BMI z-score variations between 2 and 5 years. Predicted metabolic functions including steroid hormone biosynthesis, biotin metabolism, glycosaminoglycan degradation, and amino sugar and nucleotide sugar metabolism were associated with a lower BMI z-score at 5 years. Biosynthesis of unsaturated fatty acids pathway was associated with a higher BMI z-score.

Conclusions

These findings indicate that the gut microbiota at 3.5 years is associated with later BMI during childhood independently of preterm or term birth suggesting that changes in the gut microbiota that may predispose to adult obesity begin in early childhood.

Introduction

In recent decades, childhood obesity has been considered a growing public health concern worldwide that has reached epidemic proportions (1). World Health Organization (WHO) estimated that 39 million

children under 5 years in 2020 and over 340 million children and adolescents aged 5–19 years in 2016 were overweight or obese (2). The pathogenesis of obesity is not fully understood and involved multiple interactive factors that are subjected to both genetic and environmental influences (3). The concept of the Developmental Origins of Health and Disease (DOHaD) states that susceptibility to some major chronic diseases including obesity can be in part programmed during early childhood, which represents a vulnerability window for later life disease risk (4). The gut microbiota has emerged as a potential critical early-life factor able to program long-term health, (5) and the theory that the gut microbiota may play a significant and causal role in obesity has gained ground. First evidences of the involvement of the gut microbiota in obesity has been demonstrated in animal models with differential gut microbial compositions in genetically obese mice versus their lean and wild-types siblings under the same diet (6). Other studies demonstrated an increase in total body fat in germ-free mice subjected to fecal transplantation from obese mice or adult humans (7). Since, studies on human have thrived with no consensus on potential gut microbiota markers of obesity risk (7). A high *Firmicutes* to *Bacteroidetes* (F/B) ratio is often associated to an obese phenotype. However, diverging conclusions (8, 9) still exist in the literature and further studies are needed to consider it as a hallmark of obesity. While efforts have been devoted into studying the gut microbiota in relation to the onset of obesity, data in the children population are limited. Studies in the pediatric population are often performed cross-sectionally, on small sample size, and mainly compare the gut microbiota between normal weight and obese children (7, 10, 11). There are few studies that looked at the longitudinal relationships between gut microbiota and body mass index (BMI) on a continuous scale throughout infancy and childhood (12–16). Regarding the preterm population, data are sporadic with to date, two studies addressing the subject of gut microbiota and overweight/obesity (17, 18). Therefore, there is a need to decipher the relations between the gut microbiota and the development of pediatric obesity and notably in the particular population of preterm children as some studies reported that they are at greater risk of developing childhood obesity (19, 20).

The present study aimed to investigate the associations between the gut microbiota at 3.5 years using different metrics (diversities, gut microbiota genera abundances, F/B ratio, and metabolic pathways) and subsequent BMI z-score at 5 years as well as the change in BMI z-score between 2 and 5 years in children from a preterm and a general French nationwide birth cohorts.

Methods

Study population

Children included in this study are part of two French nationwide birth cohorts launched in 2011: EPIPAGE 2 (Etude Epidémiologique sur les Petits Ages Gestationnels-2), (21) and ELFE (Etude Longitudinale Française depuis l'Enfance) (22). Briefly, EPIPAGE 2 is a nationwide cohort of preterm infants born before 34 weeks of gestational age (GA) recruited in all maternity units in 25 out of the 26 regions in France. An ancillary study of EPIPAGE 2, namely EPIFLORE, allowed the establishment of a collection of stools carried out in a subset of preterm infants born before 32 weeks of gestational age recruited in 24 voluntary neonatal intensive care units (NICUs). ELFE is a nationwide birth cohort, which included

newborns born after 33 weeks of gestational age in 349 randomly selected maternity units in mainland France. For a subset of these children, stool samples were collected at 3.5 years of age in both cohorts. Both cohorts were approved by the relevant ethical committees. Families agreed to participate in these cohorts with informed consent.

Data collection and processing

Anthropometric measurements (height and weight) were collected during clinical examinations at 2 and 5 years or retrieved from health professional records in the child health booklet. Age- and sex-specific BMI z-scores were computed at both 2 and 5 years according to the WHO growth references and overweight and obesity at 5 years were defined using the WHO standard cut-offs (23). Categories were as follows: thinness (BMI z-score < -2), normal (BMI z-score ≥ -2 to < 1), overweight (BMI z-score $\geq +1$ to $< +2$), and obese (BMI z-score $\geq +2$). Given the low prevalence of the extreme categories children in our study population (Table 1), thin and normal children were pooled in a “non-Overweight/Obese” (non-Ow/Ob) group and overweight and obese children were pooled in an “Overweight/Obese” (Ow/Ob) group. For a subgroup of ELFE children with available data, BMI z-score around 3.5 years (median age, 39.6 months, interquartile range [IQR] 37.4–41.8 months) was computed.

Table 1
Characteristics of the 512 included children.

Characteristics	**Total (N = 512)**	***missing values* (N)**
Maternal age (years)		9 (1.8%)
< 25	27 (5.3%)	
[25–35[	364 (71.1%)	
≥ 35	112 (21.9%)	
Household income		36 (7.0%)
< 1500€	11 (2.1%)	
[1500–4000€[	305 (59.6%)	
>=4000€	160 (31.3%)	
Maternal level of education		3 (0.6%)
< 12 years	25 (4.9%)	
12 years (High schoo)l	102 (19.9%)	
13–14 years	127 (24.8%)	
≥ 15 years	255 (49.8%)	
Mother born in France		
No	36 (7.0%)	
Yes	476 (93.0%)	
Maternal prepregnancy BMI		13 (2.5%)
Underweight	34 (6.6%)	
Normal	346 (67.6%)	
Overweight	88 (17.2%)	
Obese	31 (6.1%)	
Gestational age (gestational weeks categories)		3 (0.6%)
24–26	12 (2.3%)	
27–32	131 (25.6%)	

Continuous variables are given as mean (sd). Distributions in categorical variable are given in numbers of events (percentage). Number of missing data are displayed if any.

*Overweight/obesity (Ow/Ob) status at 5 years of age, defined by age and sex-specific BMI z-score based on the WHO standard cut-offs.

Characteristics	Total (N = 512)	*missing values* (N)
33–37	9 (1.8%)	
≥ 37	357 (69.7%)	
Delivery mode		5 (1.0%)
Cesarean	163 (31.8%)	
Vaginal	344 (67.2%)	
Sex		
Boys	280 (54.7%)	
Girls	232 (45.3%)	
Human milk consumption		53 (10.4%)
No	87 (17.0%)	
Yes	372 (72.7%)	
Age at 5 years		
Mean (SD)	5.07 (0.465)	
BMI z-score 5 years		
Mean (SD)	-0.149 (0.945)	
Child weight status at 5 years categories*		
Thinness	18 (3.5%)	
Normal	439 (85.7%)	
Overweight	51 (10.0%)	
Obese	4 (0.8%)	
Child weight status at 5 years*		
Non-Overweight/Obese	457 (89.3%)	
Overweight/Obese	55 (10.7%)	
Continuous variables are given as mean (sd). Distributions in categorical variable are given in numbers of events (percentage). Number of missing data are displayed if any.		
*Overweight/obesity (Ow/Ob) status at 5 years of age, defined by age and sex-specific BMI z-score based on the WHO standard cut-offs.		

For both cohorts, covariates were collected from medical and obstetrical records, by interviews, or self-administrated questionnaires. Maternal information included maternal age, country of birth, maternal

education, household income, and prepregnancy BMI (Table 1). For children, information regarding gestational age, delivery mode, and human milk consumption was collected. From available data on human milk consumption in both cohorts, an “human milk consumption” variable was created referring to any human milk intake whatever the source (own mother or donor), the mode (bottle or breast), and exclusively or not.

For the ELFE cohort only, information regarding the age at complementary feeding and child diet at 2 years of age was also available: mothers were asked about usual children’s diet of their children using 20 questions food frequency questionnaire. Using a Principal Component Analysis (PCA), we identified two dietary patterns; “unhealthy” and “healthy” presented in Table S1.

Samples collection, DNA extraction, sequencing, data processing

The stool samples collection, DNA extraction, sequencing, and data processing procedures were performed as previously described (24). Briefly, DNA extraction was performed according to the International Human Microbiome Standards operating procedure, (25) and the sequencing was performed using Illumina MiSeq technology (V3, 2x 250 bp) targeting the V3-V4 primers (V3fwd: TACGGRAGGCAGCAG, V4rev: TACCAGGGTATCTAAT) regions of the 16S bacterial rRNA gene. To adjust for the influence of uneven sampling depth, each sample was rarefied to 9258 reads and rarefied data were used for all downstream analyses unless stated otherwise.

Furthermore, we performed a quantitative PCR (qPCR) targeting specifically *A. muciniphila* (see supplementary materials).

Gut microbiota profiling and functional pathways

We used different metrics to describe the gut microbiota. Alpha diversity was estimated using Chao1 estimate (richness), and Shannon index (evenness). Beta diversity was assessed by computing dissimilarity matrices using Bray-Curtis and both Unweighted and Weighted Unifrac distances. We calculated the F/B ratio by dividing the relative abundance of *Firmicutes* by that of *Bacteroidetes.* Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways were inferred using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (26). More information is provided in supplementary materials.

Sample selection

Stools were collected and processed for 208 EPIFLORE and 630 ELFE children. We previously demonstrated that twin children shared a more similar gut microbiota (24). Hence, we randomly excluded one of the twins from a related pair or two children from triplets to assess as much variability as possible in the gut microbiota of children at 3.5 years of age, leading to the non-inclusion of 40 children (EPIFLORE, n = 36; ELFE, n = 4) (Figure S1). Among the remaining 798 children, we selected only those with BMI z-scores available at both ages, 2 years (median age, 24.4 months, [IQR] 24.0-24.7 months) and

5 years (median age, 63.3 months, [IQR] 57.3–65.0 months). The analyses were performed on 512 children (EPIFLORE, n = 143; ELFE, n = 369) (Figure S1).

Statistical Analyses

Main analyses

Associations between BMI z-score at 5 years, alpha diversity, F/B ratio, and *A. muciniphila* relative abundance assessed through qPCR were tested by multivariable linear regressions and associations with weight status at 5 years were assessed by multivariable logistic regressions. Models were adjusted for the following confounders identified with a Directed Acyclic Graph (DAG) (Figure S2); maternal prepregnancy BMI, maternal country of birth, gestational age (continuous), delivery mode, age, sex and human milk consumption. As our primary outcome (BMI z-score) is continuous, we used Microbiome Regression-Based Kernel Association Tests (MiRKAT) (27) using the Bray-Curtis, unweighted and weighted UniFrac distance matrices to investigate the relationship between the overall gut microbiota composition at 3.5 years and BMI z-score at 5 year.

Using VSURF (Variable Selection using Random Forests) for variable selection (28) (see supplementary materials), we intended to find a set of specific genera/KEGG pathways associated with BMI z-score at 5 years. In order to assist in the interpretation (29) and specifically to determine whether any of these selected genera/KEGG pathways showed a linear association with BMI z-score or weight status, we performed multivariable linear and logistic regressions, controlled for the potential confounding variables (maternal prepregnancy BMI, maternal country of birth, gestational age, delivery mode, age, sex and human milk consumption). Multiple testing corrections were not applied to these results since regression models were employed as a subsequent step to assist in the interpretation of the random forest results rather than as a tool for discovery applied to all features.

The random forest-based algorithm for missing data imputation from the missForest (30) function of the randomForest R package was used to impute missing values in covariates (Table 1).

Complementary and sensitivity analyses

As complementary analyses, we re-runed all the multivariable models adjusting for BMI z-score at 2 years of age to assess the associations between the different gut microbiota metrics and the change in BMI z-scores between 2 and 5 years of age accounting for baseline BMI z-score at age 2. As sensitivity analyses, we repeated all primary analyses on complete cases (children without missing values for covariates).

All analyses were performed using the R software version 4.1.2 (R Foundation).

Results

Participants characteristics

Characteristics of the sample are described in Table 1. Almost 50% of mothers had at least 15 years of education, 93% were born in France and 23% were overweight or obese. Concerning the children, 55% were boys, 30% were born preterm. At 5 years, nearly, 11% of children were Ow/Ob and the median [IQR] age- and sex-specific BMI z-score at this age was − 0.16 [-0.74, 0.48] (Table 1). The Ow/Ob children represented around 8% and 12% in the EPIFLORE and ELFE cohorts respectively.

Children gut microbiota, BMI z-score prediction and childhood overweight/obesity at 5 years of age

Figure S3 shows the children gut microbiota composition at both phylum and genus levels. There was no interaction between gestational age and alpha diversity or F/B ratio index for 5-years BMI z-score or weight status (Table S2). Alpha diversity (Chao1 estimate and Shannon index) was neither associated with BMI z-score nor with the weight status in both unadjusted and adjusted multivariate analyses (Table 2).

Table 2
Associations between the BMI z-score, weight status at 5 years and children gut microbiota alpha diversity measurements and F/B ratio at 3.5 years of age.

N = 512	BMI z-score 5 years				Weight status (Ow/Ob vs non-Ow/Ob)			
	Beta[c] (95% CI)	p-value	Beta† (95% CI)	p-value	OR[c] (95% CI)	p-value	OR† (95% CI)	p-value
Chao1 estimate OTU level	0.0004 (-0.0006, 0.001)	0.40	0.0001 (-0.0009, 0.001)	0.51	0.99 (0.99, 1.003	0.67	0.99 (0.99, 1.002)	0.42
Chao1 estimate Genus level	0.003 (-0.003, 0.009)	0.35	0.002 (-0.0041, 0.01)	0.52	0.99 (0.97, 1.01)	0.56	0.99 (0.97, 1.01)	0.51
Shannon index OTU level	0.18 (-0.03, 0.39)	0.093	0.12 (-0.09, 0.33)	0.27	0.85 (0.42, 1.75)	0.65	0.71 (0.34, 1.53)	0.38
Shannon index Genus level	0.22 (0.003, 0.44)	**0.047**	0.16 (-0.06, 0.38)	0.15	0.97 (0.46, 2.07)	0.94	0.86 (0.40, 1.85)	0.69
F/B ratio	0.13 (0.03, 0.22)	**0.010**	0.10 (0.0004, 0.20)	**0.049**	1.29 (0.96, 1.69)	0.084	1.30 (0.93, 1.76)	0.12
Beta coefficient and 95% confidence interval (CI) from multivariable linear regressions.								
Odds ratio and 95% confidence interval (CI) from multivariable logistic regressions. The reference for the models is the non-Ow/Ob category defined by age and sex-specific BMI z-score WHO standard cut-offs.								
F/B: *Firmicutes/Bacteroidetes*; Ow/Ob; Overweight/Obese								
[c]Crude estimates of univariate models								
†Models are adjusted for maternal prepregnancy BMI, maternal country of birth, gestational age, delivery mode, age, sex and human milk consumption.								
P-values in bold denote a statistical significance for p-values ≤ 0.05.								

The F/B ratio was significantly associated with 5-years BMI z-score in the multi-adjusted model and the Odds ratio (OR) for Ow/Ob was 1.30 [0.93–1.76] (Table 2). *A. muciniphila* relative abundance quantified by qPCR was not associated with neither BMI z-score nor with weight status at 5 years (Table S3, Figure S4).

The overall gut microbiota assessed by the beta diversity was significantly associated with the 5-years BMI z-score in unadjusted analysis according to the 3 dissimilarity distances used at both genus and OTU levels (Table S4). At the OTU level, when adjusted for confounding factors, trends were still observed

only for taxonomic phylogeny (unweight and weighted UniFrac), without reaching significance. At the genus level, the overall gut microbiota composition assessed by the beta diversity was significantly associated with the 5-years BMI z-score according to Bray-Curtis dissimilarity distance (Table S4). When stratified by cohort, some associations were still observed for ELFE children but not for EPIFLORE children (Table S5).

The random forests analysis selected 15 genera as the most highly related to BMI z-score (Fig. 1). Among the 15 genera, 6 of them showed a linear relationship with BMI z-score. Greater abundances of *[Eubacterium] hallii group, Fusicatenibacter*, and *[Eubacterium] ventriosum group* were associated with a higher BMI z-score while greater abundances of *Eggerthella, Colidextribacter*, and *Ruminococcaceae CAG-352* were associated with a lower BMI z-score (Fig. 1). Greater abundance of *Ruminococcaceae CAG-352* was also associated with lower probability of overweight/obesity at 5 years. There was an absence of interaction with gestational age for any of these associations (Table S2).

Functional capabilities, BMI z-score prediction and childhood overweight/obesity at 5 years of age

The random forests approach selected 17 KEGG pathways as the most highly related to BMI z-score at 5 years (Fig. 2) ; 5 of them showed a linear relationship with BMI z-score. Greater abundances of steroid hormone biosynthesis, biotin metabolism, glycosaminoglycan degradation, and amino sugar and nucleotide sugar metabolism pathways were associated with a lower BMI z-score. On the contrary, a greater abundance of biosynthesis of unsaturated fatty acids pathway was associated with a higher BMI z-score. There was an absence of interaction with gestational age for any of these associations (Table S2).

Complementary and sensitivity analyses

We adjusted all our previous models for BMI z-score at 2 years to assess if the gut microbiota was associated with BMI z-score change between 2 and 5 years (Tables S4 and S6, Figs. 1 and 2). We found 3 genera that were significantly associated with the variation of BMI z-scores between 2 and 5 years as follows (Fig. 1): *[Eubacterium] hallii group* and *Fusicatenibacter* were positively associated with the BMI z-score; *Ruminococcaceae CAG-352* was negatively associated with the BMI z-score variation meaning this genus was associated with a lower BMI increase between 2 and 5 years (Fig. 1). The diversity measurements, the F/B ratio and KEGG pathways were not significantly associated with the variation of BMI z-scores between 2 and 5 years (Tables S4 and S6; Fig. 2). The association between the 3.5 years gut microbiota characteristics and 2-years BMI z-score are shown in Tables S7 and S8. Most of the associations found at 5 years were already present at 2 years.

For the subgroup of ELFE children with available BMI z-score at 3.5 years, consistent strength and direction of associations were found in cross-sectional analyses even after adding age at complementary feeding and dietary patterns as potential confounders. Nonetheless, the overall gut microbiota

composition and some associations such as those with *Colidextribacter* or biotin metabolism and amino sugar and nucleotide sugar metabolism pathways were no longer significant (Tables S9 and S10).

The results of the complete-case analyses yielded consistent findings overall (Tables S11 and S12).

Discussion

Our study identified associations between children gut microbiota composition at 3.5 years and later childhood BMI at age 5 as well as changes in BMI between 2 and 5 years. At 5 years, we found a positive association between the F/B ratio and BMI z-score. We also reported that several genera were associated with BMI z-score. Additionally, gut microbiota-associated metabolic functions including steroid hormone biosynthesis, biotin metabolism, glycosaminoglycan degradation, and amino sugar and nucleotide sugar metabolism were negatively associated with BMI z-score, while a positive association was found with the biosynthesis of unsaturated fatty acids metabolism. These associations were independent of preterm or term birth.

The higher F/B ratio observed in children with higher BMI z-score at 5 years is consistent with previous studies reporting a higher F/B ratio in obese versus control groups in Kazakh (aged 7–13 years) and Belgian (aged 6–16 years) children populations (31, 32). A review including studies with children between birth and 13 years supports the changes in *Firmicutes* and *Bacteroidetes* levels as a potential significant indicator for childhood obesity (33). However, a cross-sectional study including Korean children between age 5–13 found that obese versus normal-weight children had lower relative abundance of *Bacteroidetes* but not *Firmicutes* (34). Furthermore, a combined systematic review and meta-analysis including children between 2–18 years proposed that F/B ratio have some effects on childhood obesity but no clear trend could be identified (35). Most of the previous research on gut microbiota and BMI has compared obese and lean individuals in groups, whereas in the present study, we modeled BMI as a continuous variable. We found a positive association between F/B ratio and BMI z-score suggesting that the observed association may gradually occur as F/B ratio increases and is not limited to extreme values of BMI. Furthermore, F/B ratio was also positively associated with BMI z-score at 2 years but was not associated with the BMI variation between 2 and 5 years. Therefore, our results suggest that F/B ratio is a characteristic of the gut microbiota associated with BMI as early as 2 years of age but do not predict a higher increase of BMI between 2 and 5 years. However, a study has reported no relationship between F/B ratio and BMI z-score throughout childhood in a Dutch children population (12). Of note, this study only took into account birth weight as a potential covariate. The F/B ratio is widely accepted as a biomarker of intestinal homeostasis. The increased F/B ratio and the change of body composition could reflect an increased capacity to ferment dietary polysaccharides to short-chain fatty acids (SCFA)(36). SCFAs produced by the gut microbiota (acetate, propionate, and butyrate) are known to play an important role in gut barrier functioning and appetite regulation (37); however, their role in the pathogenesis of obesity is still subject to controversies (38–40). Nonetheless, given the high heterogeneity in the literature in both children and adults, it is unclear whether F/B is associated with obesity, especially in the pediatric

population due to the extremely diverse age distribution of the population in existing studies and the inherent plasticity of the gut microbiota during this period, making the issue even more complex.

We found higher abundances of *[Eubacterium] hallii group, Fusicatenibacter*, and *[Eubacterium] ventriosum group* genera associated with higher BMI z-score at age 5. This is in agreement with data reporting a positive association between BMI and *Eubacterium hallii* in a Dutch children population aged 9–18 months (16) or with obesity status in Korean children aged 7–18 years (41). Different studies also found positive associations between obesity status (42, 43) or BMI z-score (41) and *Fusicatenibacter* abundance in children populations. Regarding *[Eubacterium] ventriosum group*, we did not find in the literature previous data in children. However, there are studies in adults that found an association between *Eubacterium ventriosum* and obesity status (44) or higher BMI (45). Our study also highlighted 3 genera negatively associated with BMI z-score, i.e., *Eggerthella, Colidextribacter*, and *Ruminococcaceae CAG-352*. Regarding *Eggerthella* genus, a combined systematic review and meta-analysis revealed a significantly lower relative proportion of *Eggerthella* in obese versus non-obese adults (9) but no studies linked this genus and BMI or obesity status during childhood. To the best of our knowledge, no literature is available regarding obesity and both *Colidextribacter* and *Ruminococcaceae CAG-352*. More information is required to establish clearly how these genera could be associated with obesity. Our study also revealed two genera associated with increase BMI z-score variations between 2 and 5 years: *[Eubacterium] hallii group* and *Fusicatenibacter*. Interestingly, these genera belong to the *Lachnospiraceae* family (*Firmicutes* phylum), known to be high producers of SCFAs (46). These genera through their metabolites, may be able to positively influence an acceleration of the increase in BMI during early childhood which is in accordance with previous data reporting a positive association between *Firmicutes* species richness and childhood overweight and obesity (47).

Of note, findings have reported a negative link between *A. municiphila* and obesity (48), including in children (49). However, other studies did not report such an association (8, 33). In the present study, our results did not find a significant association between *A. municiphila* and with Z-score BMI nor weight status at 5 years.

A recent review of the literature presents the evidence on how dietary fatty acids can modulate gut microbiota composition and obesity (50). In mice model, an omega-6-polyunsaturated fatty acid (PUFA)-rich dietary intake is associated with metabolic dysbiosis such as obesity (50). In a human randomized, controlled, cross-over study, it increased serum triglyceride levels, favored the accumulation of fat in adipose tissue and stimulated the inflammatory processes (51). The western high fat diet is rich in omega-6 PUFA but poor in omega-3 PUFA (52) and a higher adherence to a western dietary pattern have been associated with childhood overweight and obesity (53). Over stimulation of gut microbiota taxa involved in omega-6-PUFA metabolism may explain the positive association between the biosynthesis of unsaturated fatty acids KEGG pathway and BMI z-score at 5 years that we observed. This is consistent with the positive association that we found cross-sectionally at 3.5 years between BMI z-score and this specific pathway.

Children who were characterized with higher BMI z-score were also characterized with reduce levels of steroid hormone biosynthesis metabolic pathway. This is in line with data on children or adolescents, finding an enrichment of the steroid hormone biosynthesis pathway in normal weight children compared to obese ones (34, 54, 55). Steroid hormones have been shown to have direct effects in the metabolism, accumulation and distribution of adipose tissues and obesity with notably a tendency to increase central obesity with a decrease in sex steroid hormones during aging (56). Nonetheless, the interplay between gut microbiota, steroid hormones and the development of obesity still need further investigations, particularly during the childhood period.

Regarding the lower functional abundance of predicted biotin metabolism pathways in children with higher BMI z-score at 5 years, only one study on Korean children between 5 and 13 years also reported that the biotin metabolism pathway was enriched in normal weight children compared to obese ones (34). Biotin/vitamin B7 is a micronutrient that are derived mostly from food but also, to a lesser amount, through gut microbiota synthesis and is involved in numerous activities implicated in the host's energy metabolism and adipose tissues homeostasis as coenzymes or cofactors (57, 58). The data from the the MetaCardis cohort (n = 1500 adult subjects) have reported a decrease in human genes expression involved in the biosynthesis and uptake of biotin, a reduction of microbial biotin producers in fecal microbiota of participants with severe obesity and suboptimal circulating biotin levels associated with severe obesity (59).

Additionally, the glycosaminoglycan degradation and amino sugar and nucleotide sugar metabolism pathways were negatively associated with BMI z-score at 5 years. Glycosaminoglycans (GAGs) are amino sugar-containing polysaccharides and are part of proteoglycans, forming the extracellular matrices of almost all mammalian tissues. Besides, they can be a source of nutriments, or be degraded by providing in a variety of metabolites involved in glucose, cholesterol, and lipids metabolism for gut microorganisms. Of note, previously, the glycosaminoglycan degradation and amino sugar and nucleotide sugar metabolism pathways were negatively associated with obesity in children (34, 55) and adults (60) respectively.

This study has some limitations including the use of the 16S rRNA gene sequencing approach limiting the description of bacterial composition. Shotgun metagenomic sequencing approach would have provided additional information regarding microbiome functional profiles and significance at the species level of the microbiome. Furthermore, gut microbiota metrics were assessed at genus level or higher taxonomic rank. Even though our study is limited by the lack at of repeated assessments of the children gut microbiota throughout the first years of life, it provides information on relationships between gut microbiota and pediatric overweight/obesity in a context of few longitudinal studies published on this topic (12–15).

Major strengths of this study include the recruitment of a large population of French preschool children from two well described nationwide birth cohorts providing an accurate description of anthropometric measurements and assessment of relevant confounding factors. The longitudinal design through

anthropometric measurements collected at both 2 and 5 years allowed us to assess the associations between the gut microbiota and the BMI z-score variation in this specific age range. Inclusion of a large sample of preterm children rarely studied at this age add substantial novelty information regarding this particular population. Use of robust statistics and carefully adjusted models taking into account potential confounding in the relation between gut microbiota and development of pediatric overweight/obesity is another strength of this study.

Conclusion

Our data showed that some gut microbiota characteristics at 3.5 years are associated with later BMI-z-score at 5 years independently of preterm or term birth. These modifications might even begin as early as 2 years of age. A higher F/B ratio was associated with a higher BMI z-score and several genera abundance, and their associated metabolic pathways were observed as predictors of later BMI z-score. Differences in gut microbiota composition associated with obesity in adults are observed in childhood suggesting that alterations in the gut microbiota that may lead to adult obesity occur in early childhood. Further large longitudinal studies characterizing the gut microbiota throughout childhood and gathering crucial confounding factors are warranted to understand when the switch to an obese-like gut microbiota takes place and better understand the etiology of obesity.

Declarations

Author Contribution J-CR, M-JB, M-AC, P-YA and JA were responsible for data collection. GT, M-JB, M-AC and JA designed the research. GT and JD analyzed the data. GT drafted the manuscript and J-CR, M-JB, M-AC, P-YA, JA and JD contributed to writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing Interests The authors declare no competing interests.

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Figures

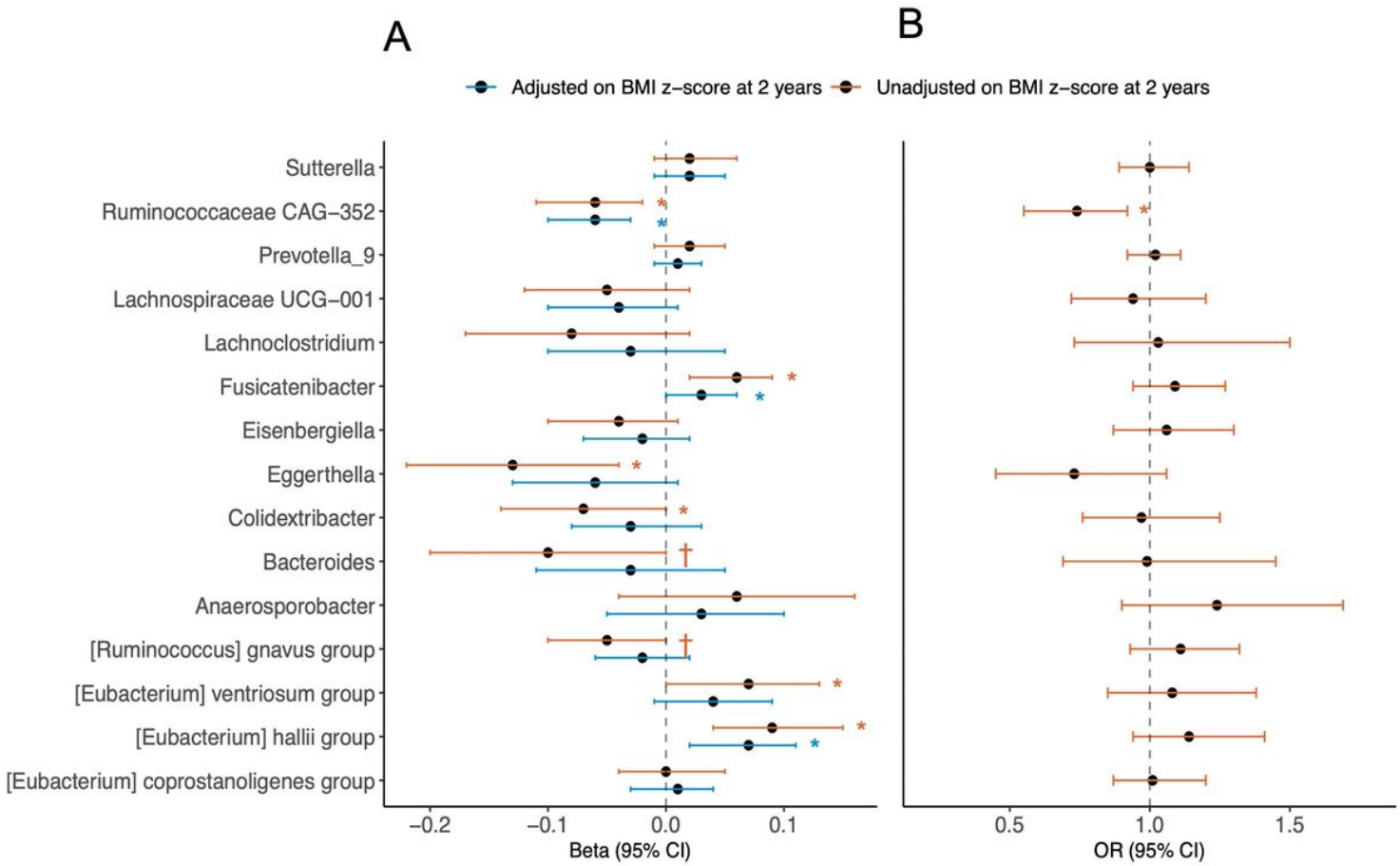

Figure 1

Gut microbiota genera at 3.5 years of age associated with BMI z-score and weight status at 5 years of age.

(A) multivariable linear and (B) multivariable logistic regression models**.**

The reference for the models is the non-Ow/Ob category defined by age and sex-specific BMI z-score WHO standard cut-offs.

Models are adjusted for maternal prepregnancy BMI, maternal country of birth, gestational age, delivery mode, age, sex and human milk consumption.

Color of the bars indicated whether the models were adjusted or not on BMI z-score at 2 years.

Abbreviations: CI, Confidence Interval; BMI, Body Mass Index; Ow/Ob; Overweight/Obese

* P-value ≤ 0.05

† P-value ≤ 0.10

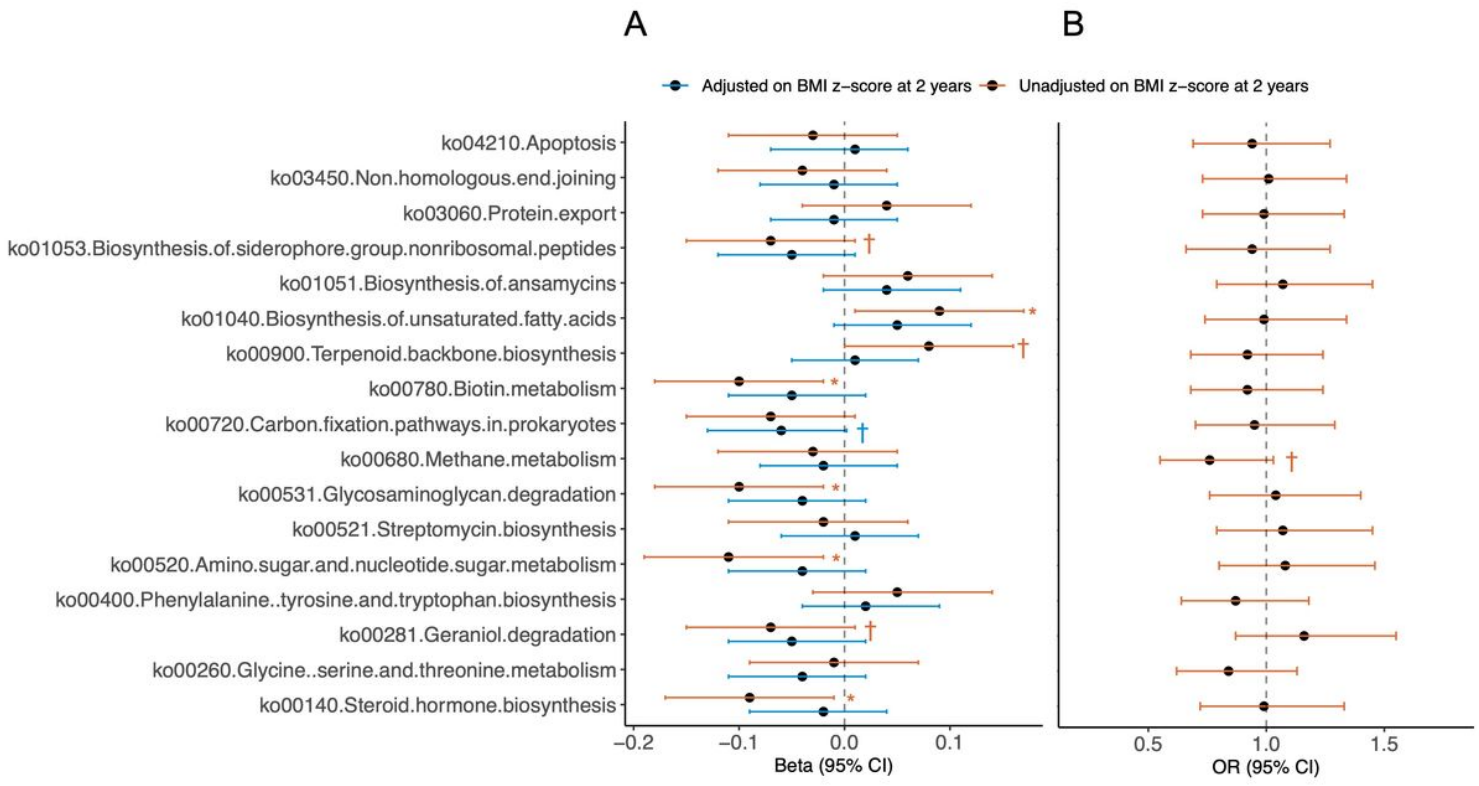

Figure 2

KEGG metabolic pathways of 3.5 years gut microbiota associated with BMI z-score and weight status at 5 years of age.

(A) multivariable linear (A) and (B) multivariable logistic regression models**.**

The reference for the models is the non-Ow/Ob category defined by age and sex-specific BMI z-score WHO standard cut-offs.

Models are adjusted for maternal prepregnancy BMI, maternal country of birth, gestational age, delivery mode, age, sex and human milk consumption.

Color of the bars indicated whether the models were adjusted or not on BMI z-score at 2 years.

Abbreviations: CI, Confidence Interval; BMI, Body Mass Index; Ow/Ob; Overweight/Obese

* P-value ≤ 0.05

† P-value ≤ 0.10

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