Microbiome of the first stool and overweight at age 3 years: A prospective cohort study

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Summary

Background: Several reports have revealed that the first-pass meconium hosts a diverse microbiome, but its clinical significance is not known.

Objective: We designed a prospective population-based cohort study to evaluate whether the meconium microbiome predicts subsequent growth in children.

Methods: The study comprised 212 consecutive newborns with a meconium sample and a follow-up sample at 1 year of age. Trained nurses measured the children for weight and length using standardized techniques. We used next-generation sequencing of bacterial 16S rRNA gene and machine-learning approach for the analysis.

Results: The children with overweight at 3 years of age differed in their meconium microbiome from those with normal weight, having a higher proportion of Bacteroidetes phylum (29% vs 15%, \(P = .013\)). Using the machine-learning approach, the gut microbiome at birth predicted subsequent overweight with area under the curve 0.70 (SD 0.04). A lower proportion of \textit{Staphylococcus} at birth was associated with greater length/height at 1 year (\(\beta = −.68, P = .029\)) and 2 years of age (\(\beta = −.74, P = .030\)).

Conclusions: The microbiome of the first-pass meconium predicted subsequent overweight at the age of 3 years. The association between the gut microbiome and overweight appears to start already during pregnancy and at birth.

KEYWORDS

16S rRNA, childhood obesity, intestinal microbiome, machine learning, next-generation sequencing

1 | INTRODUCTION

Childhood obesity is an increasing problem all over the world.\(^1\) In 2013, more than 20% of both boys and girls in the developed countries were overweight or obese, and the prevalence of these conditions has been increasing in developing countries as well.\(^2\)

Children with obesity have an increased risk of obesity in later life and also an increased risk of hypertension, dyslipidemia and type-2 diabetes.\(^3\)

Antibiotic exposure in infancy and maternal use of antimicrobials during pregnancy have been suggested as factors promoting obesity in children.\(^4,5\) Earlier prospective studies of infants have shown...
intestinal microbiomes at 10 days and 2 years of age to be associated with obesity at the age of 12 years, and the gut microbiome at 3 months with body mass index (BMI) at the age of 5 to 6 years. The use of antimicrobials during pregnancy, maternal BMI before pregnancy, weight gain during pregnancy and the maternal microbiome have been associated with the composition of the intestinal microbiome in early infancy. Several reports have demonstrated a diverse microbiome in the first stool after birth, the first-pass meconium, formed in utero before birth. In our previous study of the same cohort, immediate perinatal factors, such as the mode of delivery or antibiotics during delivery, or the sampling time, did not clearly affect the microbial composition of the meconium. The novel concept of foetal microbiome has been suggested to explain such findings. This idea is still controversial and the clinical significance of the microbiome present in the first stool is not well understood.

Because childhood overweight has earlier been associated with maternal factors during pregnancy, which in turn affect the composition of the first-pass meconium microbiome formed before birth, we set out to study whether alterations in the meconium microbiome predict later overweight in children. After collecting the first-pass meconium and 1-year samples for microbiome analysis, we followed up the growth of children until 3 years of age in a prospective, population-based study.

2 | METHODS

2.1 | Study design

The cohort investigated in this prospective population-based study was the same as we have reported on earlier when considering the maternal influence on the microbial composition of the first-pass meconium. In the present instance, however, the first stool collected after birth was subjected to next-generation sequencing of the bacterial 16S gene and examined together with a follow-up stool sample taken at 1 year of age and growth data compiled up to 3 years of age in order to evaluate the risk of becoming overweight within that time.

2.2 | Population

We recruited consecutive, term and near-term infants (>35 gestational weeks) born in the Central Finland Central Hospital in Jyväskylä, Finland, which serves as the sole primary delivery hospital for a population of 250,000 inhabitants with about 3000 births annually. The families of all 312 infants born between February 3, 2014 and March 13, 2014 were invited to participate in the study. The parents received an information letter while in the maternity ward, and altogether 218 infants whose families gave their informed consent were enrolled in the study. The first-pass meconium was collected from 212 children. The research plan was approved by the Ethics Committee of the Central Finland Hospital District, Jyväskylä, Finland.

2.3 | Growth monitoring

Data on growth at 1, 2 and 3 years of age were obtained from the child health clinics, where trained nurses measured the children’s weight and length/height at scheduled visits using standardized techniques. According to the statistics of the National Institute for Health and Welfare in Finland, more than 99% of Finnish infants attend these regular visits to clinics. The nurses measured the infants in a lying position with a length board, and weights were recorded without clothing on a digital baby scale according to the recommendations issued by the National Institute of Health and Welfare, Finland. After 2 years of age, the children were measured for height in a standing position without shoes or socks, and for weight on a personal scale while wearing light clothing. The scales and boards were checked and calibrated regularly. Lengths/heights were rounded to the nearest 0.1 cm and weights to the nearest 0.01 kg. The current Finnish age and gender-specific growth standards were used to transform the length/height measurements into z-scores and weight measurements into weight-for-length percentages at 1 year of age and BMI-for-age (ISO-BMI) at 2 and 3 years. We then categorized the children into two weight classes. Weight was assessed as a percentage of the median weight-for-length at 1 year, for example, weight-for-length +10% means that a child’s weight is 10% more than the median weight of children in the population with the same measured length. Infants with weight-for-length >10% were considered overweight or obese according to national guidelines. At 2 and 3 years of age, the weight measurements were transformed into ISO-BMI, and children with an ISO-BMI over 25 kg/m² were considered overweight or obese, in accordance with the Finnish Current Care Guidelines for Obesity. From children who no longer lived in the area of Central Finland Central hospital, parents reported the latest growth measurements from the child health clinics in a questionnaire at 1 year of age to complete the growth data.

2.4 | Data collection and microbiome analyses

We performed the microbiome analyses blinded for the growth data. We have previously reported in detail on the storage, DNA extraction, quantification, cycling conditions and analyses of the first-pass meconium samples. Briefly, all the families who were enrolled in the study completed a questionnaire of maternal medical history including the information about gestational diabetes and consumption of antimicrobials during pregnancy. The nurses completed a questionnaire of pregnancy and birth. The first-pass meconium samples were collected from the diapers by the midwives. The faecal samples were put into two sample tubes and they were immediately cooled and kept at refrigerator temperature for less than 24 h and then frozen at −22 °C before processing at University of Oulu, Finland. Similar diapers and sample tubes were used throughout the study. DNA was extracted from the follow-up stool samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germantown, Maryland) and primers F519 and R926 were used to amplify a portion of the 16S rRNA gene.
The follow-up stool samples were collected at 1 year of age. The families were sent two sample tubes and the faecal samples were mailed to the University of Oulu, Finland, for microbiome analyses. The samples were stored at −20 °C before processing. We used the same protocols for faecal DNA extraction and amplification of bacterial 16S rRNA genes as for meconium samples. In addition, the families completed a detailed follow-up questionnaire at 1 year of age including information about breastfeeding, formula feeding and introduction to solid foods.

To characterize the microbiome of the follow-up stool samples, we used the Ion Torrent PGM system to sequence the V4-V5 hypervariable regions of the 16S rRNA gene and then processed and analysed the 16S rRNA gene sequences with QIIME 1.9.0. The sequences were then binned according to sample-specific barcodes using the QIIME split_libraries.py tool, after which the barcode and primer sequences were trimmed and filtered for quality using the default parameters. Chimeric sequences were removed with the USEARCH quality filtering tool in QIIME using the Greengenes reference database. The final dataset comprised 1.71 million readings from the follow-up samples after filtering, the median being 17,582 readings per sample. We clustered the sequences into operational taxonomic units (OTUs) with a similarity of threshold of 97% based on the differences between the bacterial DNA sequences. Rarefaction curves for the OTU counts were calculated using the QIIME package, and phylogenetic trees were formed from NAST-trimmed aligned sequences in FastTree2. We then used QIIME to conduct the rarefaction, relative abundance and core microbiome analyses. To estimate the alpha diversity of the microbiome, we used the Shannon-Weaver, Simpson and Chao1 indices. The raw Ion Torrent data have been deposited in NCBI-SRA with the accession number -SRP069890.

### 2.5 Statistical analysis and machine-learning analysis

We compared the mean proportions of the selected bacterial phyla and genera and the mean bacterial diversity indices for the meconium and 1-year microbiomes between the two weight groups at 1, 2 and 3 years of age, and used the Mann-Whitney U test to assess the differences in microbiome composition between the children in the different weight groups. We have earlier reported that 19 of the 212 meconium samples did not amplify sufficiently, that is, the number of readings was <1000 per sample. Meconium samples that did not amplify sufficiently were coded as zero for analyses of relative abundances, and they were not included in the analyses for bacterial diversity indices and the number of OTUs. The statistical significance level for analyses performed on the basis of pre-existing hypotheses was 5% (P = .05), and the bacterial groups chosen for examination were ones that have been reported to be associated with obesity, such as the phyla Bacteroidetes and Firmicutes, and the genera Bacteroides, Staphylococcus and Clostridium, or associated with antimicrobial exposure, such as the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria.

Factor analysis with varimax rotation was used to identify pattern of correlations within selected bacterial phyla and genera. Regression scores of factors with eigenvalues greater than one and duration of breastfeeding were employed in multiple regression analyses to examine the associations with subsequent ISO-BMI and weight. We also used linear regression analysis of the faecal microbiome obtained at birth, after adjustment for mode of delivery, to predict subsequent growth in length/height. SPSS 26 software (SPSS Inc., Chicago, Illinois) was used for these analyses.

For the machine learning analysis, weighted Random Forest classifiers were trained on the relative abundance tables to classify the meconium samples into those representing children with obesity and those with normal weight at birth and at 1 year of age. All bacterial genera and species that were found in significant amounts in the faecal sample were used for the machine learning analyses. The models were validated and built using a repeated nested cross-validation approach in Scikit-learn. Receiver operating characteristic area under the curve (ROC AUC) was chosen as the performance metric. ROC-curve describes the ability of the algorithm to discriminate between children with normal weight and overweight. The nested cross-validation was repeated 40 times and the resulting ROC curves were averaged. The approach was tested against random chance with a permutation test score implemented in Scikit-learn in each iteration, and the resulting P values were combined using Fisher’s method. Visualizations were obtained with the Matplotlib package. Dummy Classifiers from Scikit-learn were used to represent the random chance baseline in these visualizations.

### RESULTS

#### 3.1 Faecal samples and growth data

A first-pass meconium sample was obtained from 212 infants, and a follow-up faecal sample from 96 of these. The growth data obtained at scheduled visits to health centre clinics were obtained for 186 children at 1 year of age, 144 at 2 years and 91 at 3 years (Figure 1). There were no significant differences between the weight groups, nor

**FIGURE 1:** Study design
were the results affected by the mode of delivery, the mother's educational level, gestational diabetes or antimicrobial exposure (Table 1). We did not find any associations between the meconium microbiome and the child's birth weight. A total of 31 (17%) of the 185 children with growth data available were overweight at 1 year of age, 26 (18%) out of 144 at 2 years and 17 (19%) out of 90 at 3 years, which is in accordance with the prevalence of childhood obesity in Finland.35 From 1 year of age to the latest measurement point, in total of 108 subjects (74%) remained with normal weight status, 14 remained with overweight or obese weight status (9.6%), 16 (11%) changed from normal weight category to overweight/obese and 8 (5.5%) from overweight/obese to normal weight category.

### 3.2 The intestinal microbiome and the risk of being overweight

The newborn infants, whose mothers had gestational diabetes, had higher abundance of phylum Actinobacteria (1.0% [SD 1.8] vs 0.82% [SD 2.8], \(P = .01\)) and phylum Bacteroidetes (20% [SD 25] vs 14% [SD 20], \(P = .11\)) in meconium, but the difference in Bacteroidetes was not statistically significant.

### Table 1 Prenatal and perinatal characteristics of children with normal weight and overweight at 2 and 3 years of age

| Child's weight at 2 years of age | Child's weight at 3 years<sup>a</sup> |
|---------------------------------|-----------------------------------|
| Normal weight N = 118 (82%)   | Overweight N = 26 (18%)           | Normal weight N = 73 (81%)   | Overweight N = 17 (19%) |
| Gender                          |                                   |                                |                        |
| Male, n (%)                     | 54 (46)                           | 20 (77)                        | 31 (43)                | 11 (65)                           |
| Female, n (%)                   | 64 (54)                           | 6 (23)<sup>*</sup>             | 42 (58)                | 6 (35)                            |
| Mode of delivery                |                                   |                                |                        |                                  |
| Vaginal, n (%)                  | 97 (82)                           | 19 (73)                        | 59 (81)                | 13 (76)                           |
| Caesarean section, n (%)        | 21 (18)                           | 7 (27)                         | 14 (19)                | 4 (24)                            |
| Mother's education level        |                                   |                                |                        |                                  |
| Elementary school, n (%)        | 4 (3)                             | 2 (8)                          | 2 (3)                  | 0 (0)                             |
| Senior high school, n (%)       | 11 (9)                            | 1 (4)                          | 6 (8)                  | 1 (6)                             |
| Vocational school, n (%)        | 37 (31)                           | 6 (23)                         | 20 (27)                | 5 (30)                            |
| Polytechnic, n (%)              | 35 (30)                           | 10 (38)                        | 24 (33)                | 8 (47)                            |
| University, n (%)               | 31 (26)                           | 7 (27)                         | 21 (29)                | 3 (18)                            |
| Mother's gestational diabetes   |                                   |                                |                        |                                  |
| Yes, n (%)                      | 18 (15)                           | 6 (23)                         | 9 (12)                 | 5 (29)                            |
| No, n (%)                       | 100 (85)                          | 20 (77)                        | 64 (88)                | 12 (71)                           |
| Antibiotics during pregnancy    |                                   |                                |                        |                                  |
| Yes, n (%)                      | 18 (15)                           | 3 (12)                         | 10 (14)                | 0 (0)                             |
| No, n (%)                       | 100 (85)                          | 23 (88)                        | 63 (86)                | 17 (100)                          |
| Antimicrobials during delivery<sup>b</sup> |   |                                |                        |                                  |
| Yes, n (%)                      | 36 (31)                           | 8 (31)                         | 28 (38)                | 7 (41)                            |
| No, n (%)                       | 82 (69)                           | 18 (69)                        | 45 (62)                | 10 (59)                           |
| Birthweight, mean g (SD)        | 3466 (480)                        | 3925 (422)**                  | 3481 (474)             | 3604 (468)                        |
| Any breastfeeding<sup>c</sup>, n (%) | 93 (98)                           | 18 (100)                      | 59 (100)               | 12 (100)                          |
| Duration of breastfeeding<sup>c</sup>, mean months (range) | 9.0 (0.25-12)                | 7.8 (0.5-11.5)               | 9.0 (1.0-12.0)         | 9.7 (0.5-12)                      |
| Vitamin D supplementation<sup>c</sup>, n (%) | 92 (97)                           | 16 (89)                        | 58 (98)                | 12 (100)                          |

<sup>a</sup>Percentage of the children with growth data available (N = 186 at 1 year, N = 144 at 2 years and N = 91 at 3 years), weight was not recorded for one child at age 1 year and height was not recorded for one child at 3 years of age.

<sup>b</sup>Cefuroxime (n = 22), penicillin (n = 20) and piperacillin-tazobactam (n = 2).

<sup>c</sup>Information about breastfeeding and vitamin D supplementation was acquired using a questionnaire at 1 year of age and was available for 113 children at age 2 years and 71 at age 3 years.

<sup>d</sup>The National D-vitamin supplementation recommendation for children aged 2 weeks to 2 years in the Nordic Nutrition Recommendations (2012) is 10 μg daily year round.

<sup>*</sup>P < .05 (Chi-square test); **P < .001 (t test).
## TABLE 2
Microbiome of the first-pass meconium, that is, the first stool after birth, and subsequent overweight

| Meconium microbiome | Weight at 1 year | | Weight at 2 years | | Weight at 3 years | |
|---------------------|------------------|------------------|------------------|------------------|------------------|
|                     | Normal weightN = 154 (83%) | overweightN = 31 (17%) | P valuea | Normal weightN = 118 (82%) | overweight N = 26 (18%) | P value |
| Chao1 (SD) | 321 (223) | 337 (216) | .52 | 319 (224) | 364 (221) | .44 |
| Shannon (SD) | 5.6 (1.6) | 5.4 (1.8) | .99 | 5.5 (1.6) | 5.6 (1.7) | .89 |
| Simpson (SD) | 0.91 (0.10) | 0.90 (0.13) | .71 | 0.91 (0.11) | 0.90 (0.10) | .88 |
| OTUsb (SD) | 185 (123) | 160 (135) | .30 | 178 (123) | 185 (143) | .98 |
| Phyla | Mean relative abundance % (SD)c | Mean relative abundance % (SD) | Mean relative abundance % (SD) | |
| Actinobacteria | 0.91 (2.4) | 0.26 (0.59) | .02 | 0.70 (1.7) | 0.49 (1.2) | .59 |
| Bacteroidetes | 15 (21) | 15 (22) | .37 | 15 (22) | 18 (22) | .55 |
| Firmicutes | 44 (32) | 40 (34) | .54 | 41 (33) | 47 (36) | .45 |
| Proteobacteria | 31 (35) | 21 (28) | .10 | 32 (35) | 18 (30) | .04 |
| Genera | | | | | | |
| Bacteroides spp. | 12 (20) | 12 (19) | .76 | 12 (20) | 15 (20) | .39 |
| Bifidobacterium spp. | 0.00 (0.02) | 0.01 (0.02) | .20 | 0.00 (0.02) | 0.01 (0.02) | .08 |
| Clostridium spp. | 1.2 (9.5) | 0.0 (0.02) | .45 | 1.4 (10.7) | NA d | .37 |
| Enterococcus spp. | 4.6 (19) | 4.7 (17) | .40 | 4.1 (18) | 6.2 (22) | .26 |
| Faecalibacterium spp. | 14 (2.4) | 0.99 (20) | .51 | 1.3 (2.4) | 1.6 (2.5) | .52 |
| Lachnospira spp. | 1.3 (2.1) | 0.99 (1.8) | .55 | 1.2 (2.1) | 1.7 (2.3) | .13 |
| Lactobacillus spp. | 4.2 (14) | 5.9 (19) | .38 | 5.1 (16) | 1.4 (2.0) | .97 |
| Staphylococcus spp. | 15 (26) | 12 (23) | .39 | 11 (23) | 18 (31) | .82 |
| Streptococcus spp. | 5.7 (11) | 2.4 (4.1) | .22 | 4.8 (9.2) | 4.0 (13) | .21 |
| Species | | | | | | |
| Bacteroides fragilis | 2.8 (7.9) | 3.6 (9.0) | .96 | 2.9 (8.8) | 4.3 (9.8) | .47 |

Abbreviation: OTUs, operational taxonomic units.

aMann-Whitney U test was used for the comparisons.
bOperational taxonomic unit.
cSamples with fewer than 1000 readings were coded as zero for relative abundances.
dDNA of Bifidobacterium spp. and Clostridium spp. was not detected in these groups.
At the phylum level, the proportion of the phylum Bacteroidetes was higher in the univariate analysis (29% [SD 22] vs 15% [SD 22], \( P = .013 \)) in the meconium of the children with overweight at 3 years of age than in those with normal weight. Correspondingly, the children with overweight at 3 years of age had a lower proportion of the phylum Proteobacteria at birth (19% [SD 27] vs 35% [35], \( P = .07 \)), although the difference was not statistically significant (Table 2). The proportion of the phylum Actinobacteria in the meconium was lower in the children who were overweight at 1 year (0.26% [SD 0.59] vs 0.91% [SD 2.4], \( P = .02 \); Table 2), whereas that of Proteobacteria was lower in the children who were overweight at 2 years of age than in the children of normal weight (18% [SD 30] vs 32% [SD 35], \( P = .04 \); Table 2).

At the genus level, the proportion of the genus Bacteroides was higher (25% [SD 21] vs 12% [SD 21], \( P = .01 \)) and that of Enterococcus was lower (0.08% [SD 0.30] vs 4.8% [SD 19], \( P = .008 \)) in the meconium of the children with subsequent overweight at 3 years of age. Most notably, the children who were overweight at 3 years of age had higher proportions of Bacteroides fragilis at birth (6.8% [SD 12] vs 3.4% [SD 11], \( P = .047 \); Table 2).

There were no differences in the proportions of major phyla in the gut microbiome at 1 year of age between children who were overweight and those with normal weight at 1, 2 and 3 years of age. At the genus level, children who were overweight at 1 year of age had lower proportions of Lactobacillus (0.11% [SD 0.16] vs 0.33% [SD 1.5], \( P = .04 \)) in the faecal sample at 1 year of age than those children with normal weight (Supporting Information Table S1).

We used statistical factor analysis to investigate the associations of correlated microbiome features in meconium with subsequent weight (Supporting Information Table S2). In linear regression analysis adjusted for the duration of breastfeeding, the factor with a low relative abundance of Actinobacteria at birth was associated with a higher weight in kilograms at the age of 3 years (\( \beta = .26, 95\% \text{ CI} [0.03 \text{ to } 0.90], P = .04 \)). The factor with a high abundance of Lactobacillus at birth was associated with a lower weight at the age of 3 years (\( \beta = -.20, 95\% \text{ CI} [-0.58 \text{ to } 0.05], P = .10 \)), but the association was not statistically significant.

### 3.3 Predicting overweight using a machine learning approach

Using a machine learning approach, the faecal microbiome at birth predicted overweight at the age of 3 years with AUC 0.70 [SD 0.04], \( P \text{ value <.001} \) (Figure 2A). The 1-year microbiome did not predict subsequent overweight, however (AUC 0.58 [SD 0.07], \( P > .05 \); Figure 2B). The most important microbial features for machine-learning analysis in the faecal microbiome at birth were genera Bacteroides, Staphylococcus, Lactobacillus, Streptococcus, Blautia, Enterococcus, Tepidimonas and Ralstonia and families Comamonadaceae, Rikenellaceae, Bradyrhizobiaceae and Xanthomonadaceae.

### 3.4 The intestinal microbiome and growth in length/height

In the linear regression model adjusted for mode of delivery, a higher abundance of the genus Enterococcus (\( \beta = .89, 95\% \text{ CI} [0.05 \text{ to } 1.7], P = .038 \)) and a lower abundance of the genus Staphylococcus (\( \beta = -.68, 95\% \text{ CI} [-1.3 \text{ to } -0.7], P = .029 \)) at birth were associated with a higher length-for-age at 1 year of age (Table 3). A lower proportion of Staphylococcus (\( \beta = -.74, 95\% \text{ CI} [-1.4 \text{ to } -0.7], P = .030 \)) at birth was also associated with a greater length/height at 2 years of age (Table 3). The microbiome at 1 year of age was not associated with growth in length/height at 1, 2 or 3 years (Supporting Information Table S3). Using machine-learning approach, the faecal microbiome at birth did not predict the growth in length at 1 year of age (AUC 0.48 [SD 0.04], \( P = .99 \)).

![Figure 2](image-url)  
**Figure 2** Classifier performances predicting obesity at 3 years from the meconium (a) and 1-year stool microbiomes (b)
TABLE 3  Associations of microbiome of the first-pass meconium and length/height at 1, 2 and 3 years of age

| Meconium microbiome | Length-for-age at 1 year | Height-for-age at 2 year | Height-for-age at 3 year |
|---------------------|--------------------------|--------------------------|--------------------------|
|                     | Beta (95% CI)             | P value                  | Beta (95% CI)             | P value                  | Beta (95% CI)             | P value                  |
| Chao1 (SD)          | 0.00 (0.00 to 0.001)      | .44                      | 0.00 (−0.001 to 0.001)    | .52                      | 0.00 (−0.001 to 0.001)    | .75                      |
| Shannon (SD)        | 0.03 (−0.08 to 0.14)      | .61                      | 0.03 (−0.08 to 0.14)      | .60                      | −0.01 (−0.16 to 0.13)     | .86                      |
| Simpson (SD)        | −0.07 (−1.7 to 1.5)       | .93                      | −0.04 (−1.7 to 1.6)       | .96                      | 0.79 (−2.9 to 1.3)        | .47                      |
| OTUs (SD)           | 0.00 (−0.001 to 0.002)    | .59                      | 0.00 (−0.001 to 0.002)    | .48                      | 0.00 (−0.001 to 0.002)    | .66                      |

Phyla

| Phyla              | Value of beta coefficient | P value | Value of beta coefficient | P value | Value of beta coefficient | P value |
|--------------------|---------------------------|---------|---------------------------|---------|---------------------------|---------|
| Actinobacteria     | −1.4 (−8.5 to 5.7)        | .70     | −4.8 (−14.9 to 5.3)       | .34     | 0.50 (−12 to 13)          | .94     |
| Bacteroidetes      | 0.32 (−0.42 to 1.1)       | .40     | 0.25 (−0.51 to 0.99)      | .52     | 0.38 (−0.55 to 1.3)       | .42     |
| Firmicutes         | 0.06 (−0.44 to 0.55)      | .82     | 0.06 (−0.43 to 0.55)      | .81     | 0.30 (−0.41 to 1.0)       | .41     |
| Proteobacteria     | −0.30 (−0.77 to 0.17)     | .21     | −0.20 (−0.68 to 0.27)     | .40     | −0.49 (−1.1 to 0.12)      | .11     |

Genera

| Genera              | Value of beta coefficient | P value | Value of beta coefficient | P value | Value of beta coefficient | P value |
|---------------------|---------------------------|---------|---------------------------|---------|---------------------------|---------|
| Bacteroides spp.    | 0.43 (−0.36 to 1.3)       | .28     | 0.44 (−0.38 to 1.3)       | .29     | 0.49 (−0.51 to 1.5)       | .33     |
| Bifidobacterium spp.| −80 (−873 to 713)         | .84     | 531 (−426 to 1488)       | .28     | 1062 (−1318 to 3442)     | .38     |
| Clostridium spp.    | −0.66 (−2.5 to 1.2)       | .48     | 0.08 (−1.59 to 1.75)     | .92     | −177 (−1269 to 915)      | .75     |
| Enterococcus spp.   | 0.89 (0.05 to 1.7)        | .038    | 0.58 (−0.29 to 1.5)      | .19     | 0.54 (−0.66 to 1.7)      | .37     |
| Faecalibacterium spp.| 4.3 (−2.5 to 11.1)     | .21     | 4.2 (−2.7 to 11)         | .23     | 4.8 (−3.7 to 13)         | .27     |
| Lachnospira spp.    | 4.2 (−3.6 to 11.9)        | .29     | 4.7 (−2.9 to 12.4)       | .22     | 3.2 (−5.8 to 12.3)       | .48     |
| Lactobacillus spp.  | 0.04 (−1.1 to 1.1)        | .95     | −0.07 (1.2 to 1.1)       | .90     | −0.53 (−1.9 to 0.86)     | .45     |
| Staphylococcus spp. | −0.68 (−1.3 to −0.7)      | .029    | −0.74 (−1.4 to −0.07)    | .03     | −0.62 (−1.5 to 0.3)      | .19     |
| Streptococcus spp.  | −0.005 (−1.5 to 1.5)      | .99     | 0.28 (−1.4 to 1.9)       | .74     | 2.1 (−0.03 to 4.2)       | .054    |

Species

| Species             | Value of beta coefficient | P value | Value of beta coefficient | P value | Value of beta coefficient | P value |
|---------------------|---------------------------|---------|---------------------------|---------|---------------------------|---------|
| Bacteroides fragilis| 0.98 (−0.99 to 2.9)       | .33     | 0.91 (0.92 to 2.7)        | .33     | 0.43 (−7.5 to 8.4)        | .92     |

*aNegative beta coefficient values indicate inverse association, and positive beta values indicate positive correlation between relative abundance and height. Value of beta coefficient reflects the effect size of the association between relative abundance of bacterial taxa and length or height.
*bOperational taxonomic unit.

3.5  Breastfeeding and intestinal microbiome at 1 year of age

The faecal sample at 1 year of age and dietary status was available from 94 children. The relative abundance of Lactobacillus spp. was higher in children who were exclusive breastfed until 4 months of age (N = 63) (0.47% [SD 1.8] vs 0.11% [SD 0.29], P = .83) than those who were not, but the difference was not statistically significant. There was no difference in the relative abundance of Bifidobacterium spp. with respect to feeding status at 4 months of age (0.02% [SD 0.04] vs 0.05% [SD 0.17], P = .49).

4  DISCUSSION

In this prospective population-based cohort study, the microbiome of the first-pass meconium predicted the risk of being overweight at the age of 3 years, but the intestinal microbiome at 1 year of age was not clearly associated with the risk of being overweight in early childhood. Increased abundances of the phylum Bacteroidetes, the genus Bacteroides and specifically B. fragilis in the meconium were associated with overweight at the age of 3 years. Bacteroides and Staphylococcus aureus have been reported to show higher frequencies during pregnancy in women with overweight compared with women normal weight, and a higher frequency of Bacteroides has been shown to correlate with excessive weight gain during pregnancy. High levels of B. fragilis and low levels of Staphylococcus in infants aged between 3 weeks and 1 year have been associated with higher BMI at preschool age. In a Finnish study, the proportion of Bacteroides in the intestinal microbiome at 3 months of age was associated with the child’s BMI at 5 to 6 years in the group of children with minimal lifetime antibiotic exposure, whereas in the large prospective KOALA birth cohort study with conventional and anthroposophical lifestyle subcohorts, it was seen that gut colonization with B. fragilis at the age of 1 month was associated with higher BMI at a later age in the children in the conventional subcohort who had been following a low-fibre diet. In a Swedish case-control study of 20 children with obesity and 20 children with normal weight at age 4 to 5 years, there was no significant difference in B. fragilis occurrence. In a cross-sectional study of children aged 6 to 12 years, the relative abundance of Bacteroides eggerthii has been reported to be higher in children with obesity. Furthermore, in the present study, the factor with lower relative abundance of phylum Actinobacteria was negatively associated with weight at 3 years of age which is consistent with previous
It has been suggested that the microbiome of the first-pass meconium could serve as a proxy for the foetal microbiota. Interestingly, the biodiversity of the home environment was found to influence the composition of the meconium microbiome in our earlier study with the same cohort. The composition of the first-pass meconium could thus reflect the maternal influence on the child, as maternal prenatal factors such as antimicrobial exposure during pregnancy and maternal obesity are known to alter the microbiome composition of the newborn. Maternal obesity is associated with an increased risk of the children showing high birth weight and developing overweight or obesity and metabolic syndrome. It is still unclear whether the present results reflect intrauterine colonization or postnatal colonization. Furthermore, DNA-based PCR does not necessarily indicate the presence of viable, metabolically active bacteria. However, the composition of meconium microbiome has been associated with maternal prenatal factors in several studies. Interestingly, Alam et al recently reported the detection of bacterial DNA in faetal lung in a high-quality study suggesting that infant microbiome could be acquired already in utero.

Several mechanisms have been suggested to be involved in microbiome-induced obesity. In mice, high-fat diet increases the proportion of bacterial lipopolysaccharides (LPS) in the gut microbiota, and LPS may initiate metabolic disorders including adipose tissue weight gain. The intestinal microbiome has the capacity to ferment otherwise indigestible carbohydrates into short-chain fatty acids (SCFAs). Short-chain fatty acids may increase intestinal energy harvesting, but they can also increase energy consumption, contribute to improved insulin sensitivity, improve intestinal barrier function and protect from LPS induced inflammation. Short-chain fatty acids, in particular acetate, are also known to regulate the levels of gut satiety hormone such as Peptide YY and GLP-1, and thereby regulate food intake. In our study, the relative abundance of Enterococcus was lower in meconium microbiome in children with overweight at 3 years of age, but not at 1 and 2 years of age. A recent study showed that infant-gut originated Lactobacillus and Enterococcus strains with probiotic attributes modulated gut microbiome in mice and increased higher SCFA production in the intestine, suggesting that Enterococcus spp. could prevent obesity through SCFA production. The reason why the difference was found only at 3 years of age is unclear, but it could be that during gut maturation, different species might play different roles in the regulation of growth.

The secular trend of increasing childhood stature noted in developed countries is a well-characterized phenomenon, which is not fully understood. To our knowledge, there are no earlier studies investigating the role of gut microbiota on length/height in children; only the child's use of antibiotics has been reported to be associated with increased height. We found here, however, that a higher proportion of Staphylococcus spp. in the meconium was associated with smaller heights-for-age at 1 and 2 years, even when adjusted for mode of delivery. Staphylococcus is an early colonizer of the infant gut, and its abundance could reflect a slower colonization process in the newborn after birth. Alternatively, a more abundant, diverse microbiome is formed before birth and the observed relative difference in Staphylococcus spp. could be an indicator of other differences in bacterial taxa that have more influence on the child's growth.

Our study was not designed to evaluate the impact of breastfeeding on subsequent overweight since the first-pass meconium was formed in utero before breastfeeding was begun. Yet, breastfeeding may further influence the risk of overweight after birth. In the study population, exclusive breastfeeding was recommended until 4 to 6 months of age and most babies begin to eat solid foods as a complement to breastfeeding or formula feeding at 4 to 6 months of age according to national guidelines.

The strength of our study lies in the prospective, population-based design for investigating the microbiome of the first-pass meconium, as this enabled us to evaluate the effect of the meconium microbiome on subsequent growth. The growth data were of high quality and were obtained using national guidelines and reference databases. We then used machine-based learning analysis to test the results with additional tools besides the conventional forms of statistical analysis. The main benefit of machine learning analysis is that it uses all the microbiome data to compose a classifier algorithm, thereby avoiding conclusions based solely on univariate analyses and it can reveal intrinsic differences between study groups. Machine learning methods have been widely used in microbiome studies because of their ability to tackle highly dimensional and noisy data while revealing important variables. As we used a population-based birth cohort, the sample size was limited regarding overweight subjects, and thus our cohort did not allow us to perform a multivariate analysis on the effect of antimicrobials before and after birth on the microbiome and on growth. In addition, we are not able to conclude from our cohort whether the association between the meconium microbiome and subsequent overweight was a direct part of the pathogenic pathway leading to obesity in children or merely a surrogate marker reflecting maternal factors and the microbial environment during pregnancy. One intriguing result, however, was that the microbial composition of the meconium predicted later obesity in these children.

In conclusion, we show that the microbiome of the first-pass meconium, formed during pregnancy, was associated with later overweight in the same children at the age of 3 years. Our results emphasize the importance of investigating maternal and prenatal factors when considering the pathogenesis of paediatric obesity.

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.
AUTHOR CONTRIBUTIONS

K.K., collected the clinical data, performed and interpreted the data analyses and wrote the first draft of the manuscript. M.R., created the overall study design, drafted the data collection questionnaire and spreadsheet and planned the data analyses. P.V., performed the machine learning analysis. N.P., wrote the research plan, organized the collecting of stool samples and interpreted and analysed both the microbiome data and the clinical data. J.S., helped to develop and write the research plan, organized the statistical data analyses and interpreted the data. M.V.T., performed the 16sRNA analyses and bioinformatic analyses and was responsible for the quality of the work in the research laboratory. P.K., performed the 16sRNA analyses and bioinformatic analyses. M.O., organized the statistical data analyses and interpreted the data. T.P., planned and performed all the statistical analyses that involved combining the microbiome and clinical data. All the authors have revised the manuscript for intellectual content, have approved the final manuscript as submitted and have agreed to be accountable for all aspects of the work.

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REFERENCES

1. de Onis M, Blossner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr. 2010;92(5):1257-1264.
2. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the global burden of disease study 2013. Lancet. 2014;384(9945):766-781.
3. Dalla Valle M, Laatikainen T, Kalliokoski T, Nykanen P, Jaaskelainen J. Childhood obesity in specialist care—searching for a healthy obese child. Ann Med. 2015;47(8):639-654.
4. Saari A, Virta LJ, Sankilampi U, Dunkel L, Saxen H. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. Pediatrics. 2015;135(4):617-626.
5. Mueller NT, Whyatt R, Hoepner L, et al. Prenatal exposure to antibiotics, Cesarean section and risk of childhood obesity. Int J Obes (Lond). 2015;39(4):665-670.
6. Stanislawski MA, Dabelea D, Wagner BD, et al. Gut microbiota in the first 2 years of life and the association with body mass index at age 12 in a norwegian birth cohort. MBio. 2018;9(5):e01751-18. https://doi.org/10.1128/mBio.01751-18.
7. Korpela K, Zijlmans MA, Kuitemun M, et al. Childhood BMI in relation to microbiota in infancy and lifetime antibiotic use. Microbiome. 2017;5(1):26.
8. Nagacka A, Salazar N, Suarez M, et al. Impact of intrapartum antimicrobial prophylaxis on the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. Microbiome. 2017;5(1):93-93.
9. Collado MC, Isolauri E, Laitinen K, Salminen S. Effect of mother’s weight on infant’s microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. Am J Clin Nutr. 2010;92(5):1023-1030.
10. Romano-Keeler J, Weitkamp JH. Maternal influences on fetal microbiobial colonization and immune development. Pediatr Res. 2015;77(1-2):189-195.
11. Gosalbes MJ, Llop S, Valles Y, Moya A, Ballester F, Francino MP. Meconium microbiota types dominated by lactic acid or enteric bacteria are microbiologically associated with maternal eczema and respiratory problems in infants. Clin Exp Allergy. 2013;43(2):198-211.
12. Jimenez E, Fernandez L, Marin ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by Cesarean section. Curr Microbiol. 2005;51(4):270-274.
13. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014;6(237):237ra65.
14. Tapiainen T, Paalanne N, Teješvı MV, et al. Maternal influence on the fetal microbiome in a population-based study of the first-pass meconium. Pediatr Res. 2018;84(3):371-379.
15. Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48-44.
16. de Goffau MC, Lager S, Sovio U, et al. Human placenta has no microbiome but can contain potential pathogens. Nature. 2019;572(7769):329-334.
17. National Institute for Health and Welfare. Primary Health Care. Children, Young People and Families; 2013. https://thl.fi/fi/web/ lapsen-ja-vanhemman-ymparistomuutokset/fakomatka/ lapsen-ja-vanhemman-ymparistomuutokset/fakomatka/be65c2c5-60ce-4502-b0bc-370382f51f5f.html. Accessed December 3, 2018.
18. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann Med. 2011;43(3):235-248.
19. Sankilampi U, Hannila ML, Saari A, Gissler M, Dunkel L. New population-based references for birth weight, length, and head circumference in singletons and twins from 23 to 43 gestation weeks. Ann Med. 2013;45(5-6):446-454.
20. Working group set up by the Finnish Medical Society Duodecim and the Finnish Pediatric Society. Obesity (children). Current Care Guidelines. Obesity (Children). Current Care Guidelines. 2012. www.kaypahoito.fi. Accessed September 19, 2018.
21. Angelakis E, Armougom F, Millon M, Raoult D. The relationship between gut microbiota and weight gain in humans. Future Microbiol. 2012;7(1):91-109.
22. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-2200.
23. Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5(3):e9490.
24. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012;488(7413):621-626.
25. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007;1(1):56-66.
26. Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in finnish preschool children. Nat Commun. 2016;7:10410.
27. Scheepers LE, Penders J, Mbakwa CA, Thijs C, Mommers M, Arts IC. The intestinal microbiota composition and weight development in children; the KOALA birth cohort study. Int J Obes (Lond). 2015;39(1):16-25.
28. Tun HM, Bridgman SL, Chari R, et al. Roles of birth mode and infant gut microbiota in intergenerational transmission of overweight and...
29. Forbes JD, Azad MB, Vehling L, et al. Association of exposure to formula in the hospital and subsequent infant feeding practices with gut microbiota and risk of overweight in the first year of life. JAMA Pediatr. 2018;172(4):368-377.

30. Rodriguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015;26:26050. https://doi.org/10.3402/mehd.v26i26050.

31. Chen C, Liaw A, Breiman L. Using random forest to learn imbalanced data (Report 666). Berkeley, CA: University of California, Berkeley; 2004.

32. Breiman L. Random forests. Mach Learn. 2001;45(1):5-32.

33. Pedregosa F, Varoquaux G, Gramfort A, et al. Scikit-learn: machine learning in python. J Mach Learn Res. 2011;12:2825-2830.

34. Hunter JD. Matplotlib: a 2D graphics environment. Comput Sci Eng. 2007;9(3):90-95. https://doi.org/10.1109/MCSE.2007.55.

35. Vuorela N, Saha MT, Salo M. Prevalence of overweight and obesity in 5- and 12-year-old Finnish children in 1986 and 2006. Acta Paediatr. 2009;98(3):507-512. https://doi.org/10.1111/j.1651-2227.2008.01110.x.

36. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr. 2008;88(4):894-899.

37. Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN. Intestinal microflora and body mass index during the first three years of life: an observational study. Gut Pathog. 2011;3(1):8-8.

38. Karlsson CL, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with norrer disease. Acta Paediatr. 2019;14(4):e12480. https://doi.org/10.1111/ijpo.12480.

39. Lopez-Contreras BE, Moran-Ramos S, Villarruel-Vazquez R, et al. Composition of gut microbiota in obese and normal-weight Mexican school-age children and its association with metabolic traits. Pediatr Obes. 2018;13(6):381-388. https://doi.org/10.1111/ijpo.12262.

40. Bai J, Hu Y, Bruner DW. Composition of gut microbiota and its association with body mass index and lifestyle factors in a cohort of 7-18 years old children from the American gut project. Pediatr Obes. 2019;14(4):e12480. https://doi.org/10.1111/ijpo.12480.

41. Walker RW, Clemente JC, Peter I, Loos RJF. The prenatal gut microbiome: are we colonized with bacteria in utero? Pediatr Obes. 2017;12:3-17. https://doi.org/10.1111/ijpo.12217.

42. Kuperman AA, Koren O. Antibiotic use during pregnancy: how bad is it? BMC Med. 2016;14(1):91.

43. Gohir W, Ratcliffe EM, Sloboda DM. Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk. Pediatr Res. 2015;77(1–2):196-204.

44. Al Alam D, Danopoulos S, Grubbs B, et al. Human fetal lungs harbor a microbiome signature. Am J Respir Crit Care Med. 2020;201(8):1002–1006. https://doi.org/10.1164/rcrm.201911-2127LE.

45. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-1772.

46. Macfarlane G, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc. 2003;62(1):67-72.

47. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nutr Endocrinol. 2015;11(10):577-591. https://doi.org/10.1038/nrendo.2015.128.

48. Hernandez MAG, Canfora EE, Jocken JWE, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients. 2019;11(8):1943. https://doi.org/10.3390/nu11081943.

49. Nagpal R, Wang S, Ahmadi S, et al. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mouse and human gut microbiome. Sci Rep. 2018;8(1):12649-12644. https://doi.org/10.1038/s41598-018-30114-4.

50. Lamkaer A, Attrup Schroder S, Schmidt IM, Horby Jorgensen M, Fleischer MK. Secular change in adult stature has come to a halt in northern Europe and Italy. Acta Paediatr. 2006;95(6):754-755.

51. Gough EK, Moodie EE, Prendergast AJ, et al. The impact of antibiotics on growth in children in low and middle income countries: systematic review and meta-analysis of randomised controlled trials. BMJ. 2014;348:g2267.

52. Salminen S, Endo A, Isolauri E, Scalabrini D. Early gut colonization with lactobacilli and staphylococcus in infants: the hygiene hypothesis extended. J Pediatr Gastroenterol Nutr. 2016;62(1):80-86.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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