



Applied nutritional investigation

Parent–child microbiota relationships involved in childhood obesity: A CORALS ancillary study



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ABSTRACT

Objectives: Childhood obesity continues to rise worldwide. Family gut microorganisms may be associated with childhood obesity. The aim of the study was to analyze bacterial similarities in fecal microbiota composition between parent–offspring pairs as linked to body weight.

Methods: A total of 146 father/mother and offspring pairs were categorized into four groups according to the weight status of the parent–child pair as follows: group 1, parent and child with normal weight; group 2, parent and child with overweight/obesity; group 3, parent with normal weight and child with overweight/obesity; group 4, parent with overweight/obesity and child with normal weight. Anthropometric measurements and lifestyle assessments were performed in all participants. Microbiota characteristics were determined by 16S ribosomal RNA gene sequencing. Logistic regression models were performed to determine whether the abundance of any bacteria was able to predict childhood obesity. Moreover, receiver operating characteristic curves were fitted to define the relative diagnostic strength of bacterial taxa for the correct identification of childhood obesity.

Results: The absence/abundance of *Catenibacterium mitsuokai*, *Prevotella stercorea*, *Desulfovibrio piger*, *Massili-prevotella massiliensis*, and *Phascolarctobacterium succinatutens* was involved in body weight family associations. A positive relationship between *P. succinatutens* richness from parents and *M. massiliensis* from children was observed with regard to body weight status (odds ratio, 1.14, $P = 0.013$).

Conclusions: This study describes five potential gut bacteria that may be putatively involved in family weight status relationships and appear to be useful for predicting obesity.

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Introduction

Obesity is defined as an excessive or abnormal accumulation of body fat, implying a remarkable health risk [1]. In fact, the rates of overweight and obesity continue to increase worldwide, and it has been estimated that 1 billion people are overweight, including 340 million adolescents and 39 million children [2]. In addition, childhood obesity is associated with chronic diseases, such as type 2 diabetes, cardiovascular disorders, and some types of cancer, which contribute to premature mortality in adulthood [3–6]. In this context, obesity is the result of energy disequilibrium, with the imbalance involving several causative factors, including (epi) genetics, microbiota, and environmental factors [7,8].

The energy imbalance drives an increase in adipose tissue, which is considered, in part, an “acquired” disease because of the strong influence of lifestyle factors on overweight [9]. Considering that obesity is a complex and preventable multifactorial disease, it is essential to analyze determinants at the individual and population level, being associated with excess body weight for height, in order to prevent obesity in both children and adults [10]. In addition to the genetic factors that contribute to determining overweight and obesity, the intestinal microbiota, as well as the role of gene $\times$ microbiota interactions, has been identified as a potentially important mediator in the development of this disease [11].

Mendelian randomization studies support the role of the heritable background concerning obesity and related adiposity genotypes [12]. A transgenerational increase in susceptibility to obesity has been observed following exposure to various environmental factors [13]. Indeed, the human genome is inherited from parents to offspring and remains stable throughout life, in contrast to the epigenome and gut microbiome, in which the bacterial composition that is found at birth exhibits changes over time due to environmental factors such as diet and lifestyle [14,15]. In fact, the composition of the gut microbiota is largely determined by maternal transmission and birth delivery; however, the relationship of the parental gut microbiota and offspring fecal microorganisms with obesity is not yet well studied or established [15].

In this context, numerous factors may influence the composition of the bacterial microbiota from birth and lactation, giving rise to maternal–fetal microbial transmission, in which family dietary patterns exert a notable impact on fecal abundance, thus influencing the onset and evolution of future diseases [16,17]. Furthermore, the intestinal microbiota in early childhood can be affected by the milieu, such as environmental pollutants, dietary factors, or exposure to antibiotics [18,19]. From this perspective, the aim of this research was to analyze the possible association between the composition of the parental and offspring microbiota and the accompanying impacts on and relationships with overweight and obesity.

Key messages

- A possible interaction exists between transgenerational gut microbiota, specifically from parent to child, which is associated with overweight and obesity.
- The relationship of *Catenibacterium mitsuokai*, *Prevotella stercorea*, *Desulfovibrio piger*, *Massiliprevotella massiliensis*, and *Phascolarctobacterium succinatutens* with excess body weight was evidenced.
- These findings are of relevance as part of a pioneering study anticipating adult obesity.

Materials and methods

Study design and population

This cross-sectional study is ancillary to the CORAL study, an ongoing multicenter study in schoolchildren ages 3 to 6 y old with a planned follow-up of 10 y, which aims to identify potential risk factors in the development of childhood obesity (CORALS). To be included in the study, parents or guardians had to sign an informed consent form, attend the inclusion visit, and complete several questionnaires about physical activity, dietary habits, and lifestyle factors. A detailed description of the CORAL study can be found elsewhere [20]. To be enrolled in this study, only those children and their parents who collected samples for microbiota analysis were counted ($n = 68$ from Pamplona, $n = 5$ from Zaragoza).

The relevant ethics committee (CEIm) of each recruitment center—Navarra (CEIm) and Zaragoza (CEIm Aragón)—approved the study protocol, which was conducted following the code of ethics of the World Medical Association (Declaration of Helsinki) as well as the law regarding clinical studies with humans. The CORAL study is registered in ClinicalTrials.gov (NCT06317883).

Study measurements

Body weight (kg) and body composition of the children were assessed with a beam balance, incorporating a bioelectrical impedance system (MC780SMA; Tanita Europe B.V., Amsterdam, the Netherlands), which is based on measurement of the resistance and reactance to electrical currents in the human body. Height (cm) was measured standing, without the participants wearing shoes, using a calibrated portable stadiometer (seca 213; seca, Hamburg, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and categorized as underweight/normal weight or overweight/obesity according to the cutoff points described by Cole and Lobstein [21]. The BMI z score was calculated through the following formula: observed BMI value – median value of the reference population / standard deviation of the reference population [22]. Waist circumference (cm) was measured at the midway point between the lowest rib and the top of the iliac crest using a stretchable measuring tape (Cescorf, Porto Alegre, Brazil). All of these measurements as well as parental weight and height that were self-reported at the beginning of the study followed standardized protocols.

Blood pressure (mm Hg) in children was determined using an automatic validated monitoring device (M3/HEM-705CP-II Intellisense; OMRON Healthcare, Hoofddorp, the Netherlands) three times in each arm, applying a cuff adapted for each child. Blood samples were drawn after 8 h of overnight fasting via venipuncture and processed (15 min, 3500 rpm, 4°C) at the Center for Nutrition Research facilities in the University of Navarra or University of Zaragoza. The biochemical analyses included glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides (TGs), uric acid, alanine aminotransferase, aspartate aminotransferase, urea, creatinine, total protein, bilirubin, gamma-glutamyl transferase, alkaline phosphatase, and calcium, which were processed in an automatic analyzer (Pentra C200; HORIBA ABC Diagnostics, Montpellier, France). Low-density lipoprotein cholesterol levels were calculated using the Friedewald formula [23]: low-density lipoprotein cholesterol = total cholesterol – high-density lipoprotein cholesterol – TG / 5. Insulin was analyzed in serum with enzyme-linked immunosorbent assay kits (Mercodia, Uppsala, Sweden; Immuno Diagnostics, Woburn, MA, USA) in a Triturus autoanalyzer (Grifols SA, Barcelona, Spain). The homeostasis model assessment of insulin resistance (HOMA-IR) was estimated according to the following formula: $HOMA-IR = (\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose } (\text{mmol/L})) / 22.5$ [24]. The TG glucose (TyG) index was computed using the following formula: $TyG = \ln [TG (\text{mg/dL}) \times \text{fasting glucose } (\text{mg/dL}) / 2]$. All of these determinations were analyzed under validated guidelines and protocols.

Lifestyle and health determinants

To evaluate the adherence of children to the Mediterranean diet, an ad hoc 18-question questionnaire adapted for children was administered to assess if the consumption of nine foods or nutrient groups fit with the recommendations [20]. Parental adherence to the Mediterranean diet was assessed using a validated 14-point screening questionnaire, considering the consumption of nine food groups or nutrients [25].

Physically related lifestyle features were estimated through an ad hoc 13-item questionnaire based on leisure physical activity, including sedentary behavior score [20]. Sleep and screen time was calculated using different questions via a general physical activity questionnaire based on the Outdoor Playtime Checklist and the Outdoor Playtime Recall Questions [26]. Parental physical activity was calculated using the validated Spanish version of the 16-item global physical activity questionnaire developed by the World Health Organization [26,27].

Fecal sample collection and metagenomic data

Parents (mothers/fathers) collected their own fecal samples and their children's fecal samples using the cryotubes of OMNIgene•GUT kits from DNA Genotek (Ottawa, Ontario, Canada) in compliance with the standard guidelines provided by the supplier [28]. Samples were immediately stored at -80°C for further analyses.

Isolation of DNA from fecal samples and bacterial DNA sequencing were performed by Navarra Services and Technologies (Pamplona, Spain). Genomic DNA was isolated to 250 μL of stabilizing liquid from the OMNIgene•GUT-collected samples using MagMAX CORE Nucleic Acid Purification Kit reagents with the MagMAX CORE Mechanical Lysis Module (Thermo Fisher Scientific, Paisley, UK) on the automated extraction platform. Two different hypervariable regions (V3–V4) of the bacterial 16S ribosomal RNA gene were amplified to characterize the phylogeny and taxonomy of the microbial samples. Library preparation was performed according to the standard instructions for the protocol of the 16S V3–V4 Library Preparation Kit for Illumina (Norgen Biotek, Thorold, Ontario, Canada). Sequences were obtained on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Briefly, sequencing consisted of two polymerase chain reactions (PCRs) in which the V3 and V4 regions of the 16S ribosomal RNA gene were amplified, creating an amplicon of approximately 460 base pairs. In the process, two PCRs were carried out. In the first, 12.5 ng of genomic DNA and 16S-F and 16S-R primers were used (16S amplicon PCR forward primer = 5' TCGTCGCGCAGCGTCAGATGTGTAT-AAGAGACAGCCTACGGGNGGCWGCAG; 16S amplicon PCR reverse primer = 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC).

The protocol followed for the first PCR was 95°C for 3 min and 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, as described elsewhere [29]. We used 72°C for 5 min for later refrigeration and held at 4°C . After the cleansing process, 5 mL was taken from the first PCR sample to use for the second PCR. For the second PCR, the protocol followed was 95°C for 3 min and eight cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Finally, we used 72°C for 5 min for later refrigeration and held at 4°C . After each PCR, a cleansing process was carried out to clear the sample of primers.

Microbiome bioinformatics analyses were performed by Navarra Services and Technologies and BatchX (San Jose, CA, USA) using QIIME 2 2023.2, a next-generation microbiome bioinformatics platform [30]. Briefly, raw sequence data were demultiplexed and subsampled before any downstream analysis. Subsampling was set to 2 million reads to obtain the maximum number of informative sequences, avoiding redundant information. A quality filtering step was performed followed by a reduction of sequencing errors via denoising with DADA2 [31,32] to identify all possible amplicon sequence variants (ASVs). The truncation and trimming parameters in DADA2 were set to $-p\text{-trim-left-f } 18$, $-p\text{-trim-left-r } 22$ and $-p\text{-trunc-len-f } 240$, $-p\text{-trunc-len-r } 240$. Illumina quality score binning was considered during data preprocessing and denoising [32]. ASV identification was

performed with QIIME 2, employing classify-sklearn for taxonomic classification [33], used against version 11.5 of the Ribosomal Database Project [34]. Phylogeny was constructed with fasttree2 [35] after the corresponding alignment with mafft [36] using q2-alignment and q2-phylogeny. The abundance matrices were then filtered and normalized at each level of classification: ASV, species, genus, family, order, class, and phylum.

Statistical analyses

Considering the group heterogeneity of the study population and to analyze the possible relationship with the microbiota, the families were studied in parent–child pairs (PCPs). Thus, families with several children, parents, or both gave rise to several pairs. For example, families with both parents and two children resulted in four different PCPs: father–child1, father–child2, mother–child1, and mother–child2. Next, the entire group of PCPs ($N = 146$) was categorized into four groups corresponding to parent–child weight status: 1) normal-weight parent and child ($n = 75$), 2) parent and child with overweight/obesity ($n = 12$), 3) normal-weight parent and child with overweight/obesity ($n = 12$), and 4) parent with overweight/obesity and normal-weight child ($n = 47$).

Microbiota analyses were performed using the MicrobiomeAnalyst web server (<http://www.microbiomeanalyst.ca>). Alpha diversity was evaluated using Shannon and Chao1 indexes and beta diversity was determined using Bray–Curtis index and the permutational multivariate analysis of variance test. Comparative analysis of the microbiota between groups according to weight status was performed by Student's t test. Abundance data were normalized with centered log ratio before statistical treatment. Data normality was first studied using the Shapiro–Wilk test. Variables were expressed as mean (standard deviation) or median (interquartile range) according to their distribution. The mean of group 2 was used to establish the cutoff point for high or low bacterial abundance. Moreover, categorical variables were expressed as percentage and differences were examined using the χ^2 test. Comparisons between groups were performed using the analysis of variance test. Tukey's post hoc test was used for multiple group comparison. Logistic regression analyses were performed to determine whether any bacterial taxa were able to predict childhood obesity. Receiver operating characteristic curves were devised to assess the relative diagnostic strength of the different bacterial taxa for the correct assignment of childhood obesity. We used the area under the curve (AUC) to quantify accuracy. We interpreted an AUC between 0.90 and 0.80 as indicative of a good diagnostic test, an AUC between 0.80 and 0.70 as indicative of a fair diagnostic test, and an AUC between 0.70 and 0.60 as indicative of a poor diagnostic test. All P values were two-tailed and considered statistically significant if $P < 0.05$. The software used for the statistical analysis was Stata 12.1 (StataCorp, College Station, TX, USA), the manual of which was used as support.

Table 1
General features and biochemical markers of the child population according to group distribution^c

Variable	Group 1 [†] ($n = 75$)	Group 2 [‡] ($n = 12$)	Group 3 [§] ($n = 12$)	Group 4 [¶] ($n = 47$)	P value [▽]
Child general characteristics					
Sex, %, boys/girls	47.1/55.1	7.4/9.0	10.3/6.4	35.3/24.5	0.654
Age, y	4.2 (1.0)	4.3 (1.3)	5.0 (0.7)	4.1 (0.9)	0.042 ^{b,f}
Weight, kg	17.7 (15.8, 19.6)	22.9 (20.3, 28.1)	23.6 (20.7, 28.1)	17.6 (15.6, 19.1)	< 0.001 ^{a,b,e,f}
Height, cm	107.5 (100.0, 111.0)	109.5 (101.6, 121.7)	114.2 (107.3, 122.8)	106.5 (99.7, 111.0)	0.002 ^{b,f}
BMI z score	0.1 (−0.5, −0.8)	2.3 (1.9, 2.6)	1.6 (1.5, 2.0)	0.0 (−0.5, −0.8)	< 0.001 ^{a,b,e,f}
Waist circumference, cm	54.0 (51.5, 56.0)	60.7 (58.8, 67.3)	59.7 (58.5, 62.5)	53.3 (50.1, 56.7)	< 0.001 ^{a,b,e,f}
Body fat mass, kg	3.8 (3.6, 4.2)	6.4 (5.3, 7.7)	6.1 (5.2, 7.1)	3.9 (3.3, 4.9)	< 0.001 ^{a,b,e,f}
Muscle mass, kg	13.2 (11.6, 14.4)	15.0 (14.2, 19.7)	16.3 (14.5, 19.4)	13.0 (11.4, 14.0)	< 0.001 ^{a,b,e,f}
Total body water, kg	10.2 (9.1, 11.3)	11.7 (11.0, 15.2)	12.6 (11.3, 15.0)	10.2 (9.0, 10.8)	< 0.001 ^{a,b,e,f}
Clinical blood biochemical markers					
Insulin, $\mu\text{U/mL}$	2.3 (1.1, 3.6)	8.4 (5.7, 10.8)	4.5 (3.8, 6.4)	2.2 (1.3, 3.8)	< 0.001 ^{a,b,d,e,f}
HOMA-IR	0.5 (0.3, 0.7)	2.1 (1.3, 2.5)	0.9 (0.8, 1.4)	0.5 (0.3, 0.8)	< 0.001 ^{a,b,d,e,f}
Triglycerides, mg/dL	47.0 (42.0, 58.0)	64.0 (51.0, 107.0)	51.0 (41.0, 64.0)	48.0 (43.0, 60.0)	< 0.001 ^{a,d,e}
TyG	4.2 (4.1, 4.3)	4.3 (4.2, 4.6)	4.2 (4.1, 4.3)	4.2 (4.1, 4.3)	0.001 ^{a,d,e}

BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; TyG, triglyceride glucose index.

Variables are shown as mean (SD) or median (IQR) according to the distribution; categorical variables are shown as percentage and compared by χ^2 test.

[†]Normal-weight parent and children.

[‡]Parent and children with excess body weight.

[§]Normal-weight parent and children with excess body weight.

[¶]Parent with excess body weight and normal-weight children.

[▽] P value of the comparison among the four groups.

^aDifference among groups 1–2.

^bDifference among groups 1–3.

^cDifference among groups 1–4.

^dDifference among groups 2–3.

^eDifference among groups 2–4.

^fDifference among groups 3–4.

Results

Baseline characteristics of the child study population are reported in Table 1 according to the weight status of the PCP (group 1, parent and child with normal weight; group 2, parent and child with excess body weight; group 3, parent with normal weight and child with excess body weight; group 4, parent with excess body weight and child with normal weight). According to the statistical analysis, the group of parents and offsprings with excess body weight showed higher levels of TGs ($P < 0.001$), TyG index ($P = 0.001$), insulin and HOMA-IR values ($P < 0.001$). Supplementary Table 1 shows that systolic ($P < 0.001$) and diastolic ($P = 0.0028$) blood pressure was lower in children with normal weight than in children with excess body weight, whereas heart rate ($P < 0.001$) was higher in normal-weight children. Other biochemical data are also available in Supplementary Table 1. Adherence to a Mediterranean diet ($P = 0.042$) and active lifestyle score ($P = 0.002$) were significantly lower in children in group 2 (Table 2). Both body weight and parental BMI were lower in groups with normal-weight parents (groups 1 and 3). In addition, significant differences were found among the four groups according to the sex of the parents ($P = 0.001$), where it was noted that the parents with the highest percentage of overweight or obesity were men. By contrast, in groups 1 and 3, most of the normal-weight parents were women.

After screening the fecal microbiota of the participants, significant differences were found in the abundance of 26 bacterial species of both individuals of the PCPs among the four groups (Supplementary Fig. 1). Twenty-two of these species were found to

cluster in areas of high or low abundance. A cutoff point based on the mean of group 2 was established to separate the areas of high and low abundance of each bacterial species, and the percentage of PCPs with both members of the pair in each area was calculated. In this way, five taxa were selected for presenting in group 2, with a high percentage of cases with both members of the PCP in the high area and little or no percentage in the rest of the groups. After studying these bacterial species, it was observed that five were overexpressed in group 2 (PCP with excess body weight) compared with the rest of the groups. We found that the presence of a high abundance of these taxa (*C. mitsuokai*, *P. stercora*, *D. piger*, *M. massiliensis*, or *P. succinatutens*) was much more frequent (72.7%) in both components of the PCP of the overweight or obesity group (group 2) than the rest of the PCP groups (1.3%, 15.3%, and 6.5% in groups 1, 3, and 4, respectively) (Fig. 1).

When studying the differences between the five more abundant bacterial species of the children in group 2 and the other groups, we identified significant differences in *C. mitsuokai* ($P = 0.002$), *M. massiliensis* ($P = 0.005$), and *P. succinatutens* ($P = 0.036$). Regarding parental bacteria, differences between groups were found in all of the parents, with much higher abundance in the parents of group 2 (Table 3). The alpha and beta diversity of the samples was also analyzed. No significant differences were found in Chao1 and Shannon indexes of alpha diversity, whereas beta diversity between groups was shown to be statistically significant ($P < 0.001$), especially between groups in which children were overweight and groups in which they were not. No differences were observed in the ratio of Firmicutes to Bacteroidetes between groups.

Table 2
General, anthropometric, and lifestyle characteristics of parents according to group distribution

Variable	Group 1 † (n = 75)	Group 2 ‡ (n = 12)	Group 3 § (n = 12)	Group 4 ¶ (n = 47)	P value ∇
Child lifestyle factors					
Mediterranean diet, points	11.2 (2.19)	9.5 (1.4)	11.1 (1.9)	11.5 (2.2)	0.042 ^e
Physical activity, min/wk	60.0 (50.0, 90.0)	30.0 (0.0, 60.0)	52.5 (15.0, 80.0)	90.0 (30.0, 120.0)	0.079
Active lifestyle, points	9.0 (8.0, 10.0)	7.0 (7.0, 8.0)	8.0 (8.0, 9.5)	8.0 (7.0, 9.0)	0.002 ^{a,c}
Screen time/weekday, %	63.6/47.8	9.1/8.0	6.1/8.9	21.2/35.4	0.364
Screen time/weekend d, %	80.0/50.4	0.0/8.5	0.0/8.5	20.0/32.6	0.858
Sleep time, h/weekday	10.3 (10.0, 11.0)	10.0 (10.0, 10.2)	10.2 (9.0, 10.7)	10.0 (10.0, 10.3)	0.062
Sleep time, h/weekend d	10.3 (10.0, 11.0)	10.7 (10.0, 11.0)	9.5 (7.7, 10.3)	10.0 (10.0, 10.3)	< 0.001 ^{b,d,f}
Family characteristics					
Parent sex, %					0.001 ^{a,b}
Male	34.7	75.0	33.3	68.1	
Female	65.3	25.0	66.7	31.9	
Parent age, y	40.4 (4.4)	41.9 (5.7)	41.3 (4.3)	41.9 (4.7)	0.328
Father weight, kg	74.0 (72.0, 76.0)	92.0 (87.0, 105.0)	74.5 (65.5, 84.0)	91.0 (85.0, 96.0)	< 0.001 ^{a,c,d,f}
Mother weight, kg	57.1 (53.0, 64.0)	87.0 (76.0, 90.0)	67.0 (59.0, 72.5)	78.5 (70.0, 83.0)	< 0.001 ^{a,c,d,e}
Father BMI, kg/m ²	23.2 (23.0, 23.8)	29.8 (27.1, 31.4)	23.4 (22.6, 23.9)	28.2 (26.0, 29.2)	< 0.001 ^{a,c,e,f}
Mother BMI, kg/m ²	21.3 (20.1, 22.5)	31.1 (27.9, 36.2)	21.9 (20.5, 24.1)	27.5 (26.0, 31.3)	< 0.001 ^{a,c,d}
Parent MedDiet, points	9.1 (1.6)	8.8 (2.5)	9.3 (2.3)	8.5 (1.6)	0.265
Physical activity, MET, min/wk	1360 (600, 2880)	1620 (330, 2520)	3320 (1040, 7920)	1320 (360, 3840)	0.235

BMI, body mass index; IQR, interquartile range; MedDiet, Mediterranean diet; MET, metabolic equivalent of task.

Variables are shown as mean (SD) or median (IQR) according to the distribution; categorical variables are shown as percentage and compared by χ^2 test.

† Normal-weight parent and children.

‡ Parent and children with excess body weight.

§ Normal-weight parent and children with excess body weight.

¶ Parent with excess body weight and normal-weight children.

∇ P value of the comparison among the four groups.

^a Difference among groups 1–2.

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^f Difference among groups 3–4.

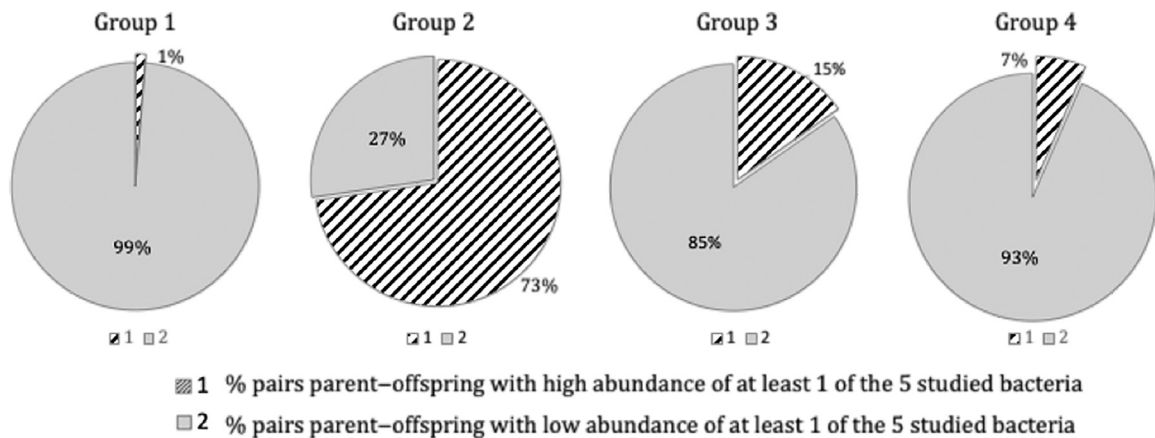


Fig. 1. Percentage of parent–child pairs in which both members have a high abundance of *Catenibacterium mitsuokai*, *Prevotella stercorea*, *Desulfovibrio piger*, *Massiliprevotella massiliensis*, or *Phascolarctobacterium succinatutens* by group. Group 1 = normal-weight parent and children, group 2 = parent and children with excess body weight, group 3 = normal-weight parent and children with excess body weight, group 4 = parent with excess body weight and normal-weight children.

Multivariable logistic regression models adjusted by child and parent sex and parent weight status were performed to evaluate the relationship of gut microbiota with childhood obesity (Table 4). Model 1 showed a latent risk of childhood obesity based on parental information, finding that parent *P. succinatutens* ($P = 0.031$) was statistically significant. By contrast, the second model was based on the child's parameters, revealing that child *M. massiliensis* ($P = 0.047$) with an R^2 of 0.16 for the fitted model showed an association with childhood obesity. In parallel, the third regression model established for the change in childhood obesity demonstrated a significant interaction between these two bacterial species ($P = 0.013$).

In addition, simple linear regression analyses of the interaction are plotted in Figure 2. The analysis of the modifying effect

revealed that when the child had a low abundance of *M. massiliensis*, regardless of the parent's *P. succinatutens*, the risk of obesity was low. By contrast, when the abundance of this bacterium rose in the child, if *P. succinatutens* of the parent also increased, the risk of childhood obesity was higher. A receiver operating characteristic curve adjusted by child sex, parent sex, parent weight status, and parent and child adherence to a Mediterranean diet was built to determine the predictability of childhood obesity. AUC was estimated as high as 0.86 (Fig. 3).

Discussion

This pioneering study is among the first to investigate the heritability/transfer of microbiota from parents (mothers and fathers)

Table 3
Selected bacterial (NRA) and alpha diversity of children in the different groups

Variable	Group 1 [†] (n = 75)	Group 2 [‡] (n = 12)	Group 3 [§] (n = 12)	Group 4 [¶] (n = 47)	P value [▽]
Child species					
<i>Catenibacterium mitsuokai</i> , NRA	0.5 (−0.7, 1.4)	1.0 (−0.3, 5.0)	0.1 (−0.6, 0.9)	0.1 (−1.8, 0.9)	0.002 ^{a,d,e}
<i>Prevotella stercorea</i> , NRA	0.8 (−0.6, 1.7)	1.8 (−0.4, 6.3)	1.2 (0.2, 2.0)	0.9 (−0.3, 1.6)	0.196
<i>Desulfovibrio piger</i> , NRA	−1.3 (−3.6, −0.6)	−1.0 (−3.7, 3.6)	−2.7 (−3.5, −0.6)	−1.1 (−3.8, −0.2)	0.367
<i>Massiliprevotella massiliensis</i> , NRA	−1.1 (−3.5, −0.3)	0.0 (−0.5, 3.2)	−0.4 (−1.4, 0.3)	−0.9 (−3.6, 0.2)	0.005 ^{a,e}
<i>Phascolarctobacterium succinatutens</i> , NRA	0.1 (−1.1, 0.8)	0.7 (−0.6, 1.0)	1.0 (0.1, 1.2)	−0.1 (−3.5, 0.7)	0.036
Parent species					
<i>Catenibacterium mitsuokai</i> , NRA	0.0 (−1.5, 0.9)	3.2 (−1.6, 6.9)	−0.4 (−2.6, 0.9)	0.1 (−2.2, 4.9)	0.019 ^{a,d}
<i>Prevotella stercorea</i> , NRA	0.9 (−0.1, 1.9)	4.0 (0.9, 8.1)	1.2 (−0.2, 2.6)	1.0 (−0.2, 1.9)	0.002 ^{a,e}
<i>Desulfovibrio piger</i> , NRA	−1.3 (−3.7, 0.0)	5.0 (−2.9, 5.2)	−1.8 (−3.6, −0.7)	−1.5 (−3.2, −0.0)	0.017 ^{a,d,e}
<i>Massiliprevotella massiliensis</i> , NRA	−2.2 (−3.9, −0.4)	3.2 (−0.3, 6.3)	−0.7 (−3.0, 0.3)	−0.6 (−3.5, −0.2)	< 0.001 ^{a,d,e}
<i>Phascolarctobacterium succinatutens</i> , NRA	−0.1 (−3.5, −0.2)	1.4 (0.3, 6.4)	1.3 (−0.3, 5.2)	−0.1 (−2.8, 0.8)	0.008 ^a
Diversity					
Shannon	3.6	3.7	3.6	3.5	0.319
Chao1	222.4 (2.7)	226.9 (3.5)	218.0 (2.1)	223.2 (2.5)	0.647
Firmicutes/Bacteroidetes	1.1 (1.0, 1.1)	1.1 (1.0, 1.1)	1.0 (1.0, 1.1)	1.1 (1.0, 1.2)	0.134

IQR, interquartile range; NRA, normalized relative abundance; SE, standard error.

Variables are shown as median (IQR) according to the distribution; diversity data are shown as mean (SE).

[†]Normal-weight parent and children.

[‡]Parent and children with excess body weight.

[§]Normal-weight parent and children with excess body weight.

[¶]Parent with excess body weight and normal-weight children.

[▽]P value of the comparison among the four groups.

^aDifference among groups 1–2.

^bDifference among groups 1–3.

^cDifference among groups 1–4.

^dDifference among groups 2–3.

^eDifference among groups 2–4.^fDifference among groups 3–4.

Table 4

Logistic regression model based on childhood obesity as dependent variable

Variable	Childhood obesity		
	OR (95% CI)	P value	Pseudo R ²
Model 1		0.029	0.14
Parent adherence to MedDiet, score 0–14 points	1.12 (0.84, 1.49)	0.451	
Parent <i>Catenibacterium mitsuokai</i>	0.94 (0.79, 1.12)	0.497	
Parent <i>Prevotella stercorea</i>	1.15 (0.97, 1.36)	0.110	
Parent <i>Desulfovibrio piger</i>	1.03 (0.90, 1.19)	0.650	
Parent <i>Massiliprevotella massiliensis</i>	1.09 (0.94, 1.27)	0.250	
Parent <i>Phascolarctobacterium succinatutens</i>	1.19 (1.01, 1.39)	0.031	
Model 2		0.016	0.16
Child adherence to MedDiet, score 0–14 points	0.76 (0.61, 0.95)	0.018	
Child <i>Catenibacterium mitsuokai</i>	1.06 (0.77, 1.47)	0.711	
Child <i>Prevotella stercorea</i>	0.91 (0.72, 1.15)	0.446	
Child <i>Desulfovibrio piger</i>	0.98 (0.80, 1.20)	0.826	
Child <i>Massiliprevotella massiliensis</i>	1.27 (1.00, 1.62)	0.047	
Child <i>Phascolarctobacterium succinatutens</i>	1.26 (0.99, 1.61)	0.059	
Model 3		< 0.001	0.28
Parent adherence to MedDiet, score 0–14 points	1.29 (0.96, 1.73)	0.090	
Child adherence to MedDiet, score 0–14 points	0.62 (0.47, 0.82)	0.001	
Parent <i>Phascolarctobacterium succinatutens</i> /child <i>Massiliprevotella massiliensis</i>	1.14 (1.03, 1.27)	0.013	

CI, confidence interval; MedDiet, Mediterranean diet; OR, odds ratio.

All models adjusted by child sex, parent sex, and parent weight status; OR represents changes in outcomes with regard to the onset of childhood obesity in the study population.

to children as putatively involved in obesity onset. In this context, an association between *P. succinatutens* of the parent and *M. massiliensis* of the child was identified, whose emergence was associated with childhood obesity. Interestingly, as the abundance of both bacteria in fecal stools is higher, the risk of presenting childhood obesity is greater, whereas if parental bacterial richness is low, the risk of having excess body weight is less in the offspring.

Furthermore, the abundance of *C. mitsuokai*, *P. stercorea*, *D. piger*, *M. massiliensis*, or *P. succinatutens* in both PCP members was higher in group 2 (parent and child with excess body weight) compared with the other groups. Notably, an association between *P. succinatutens* and body weight has been established in human adults, with a decrease in relative abundance following weight loss intervention [37]. In 2017, a new species of *Massiliprevotella* called *M. massiliensis* was reported, which is closely related to *P. stercorea* [38]. This genus presented a higher relative abundance in the

group of children with obesity compared with the control group [39]. These findings are in agreement with the outcomes of our study, in which the parent–child group with excess body weight showed a higher abundance of these bacterial microorganisms.

Although no significant differences were found among the four groups, the relative abundance of *D. piger* is higher in cohorts of children with non-alcoholic fatty liver disease as well as adults with obesity [40,41]. The present study found a clear increase in the relative abundance of *C. mitsuokai* in the parent–child group with excess body weight. Obesity-related differences at the species level have been identified in several investigations [42]. Thus, *C. mitsuokai* is associated with dysbiosis of the gut microbiome caused by intake of a high-fat diet and sugar and an unhealthy fasting lipid profile [43]. In any case, all of the species that were selected in the present analysis have been associated with obesity risk in previous studies.

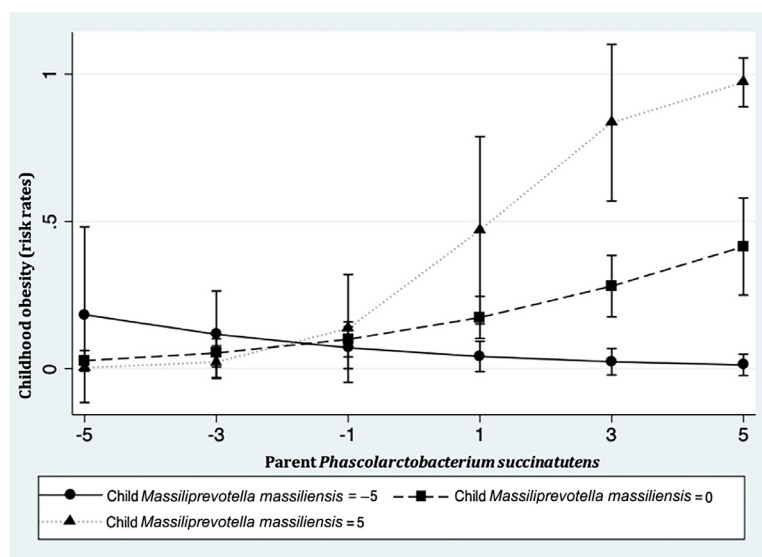


Fig. 2. Effect on childhood obesity of the change in parent *Phascolarctobacterium succinatutens* and child *Massiliprevotella massiliensis* adjusted by child sex, parent sex, parent weight status, and adherence to Mediterranean diet.

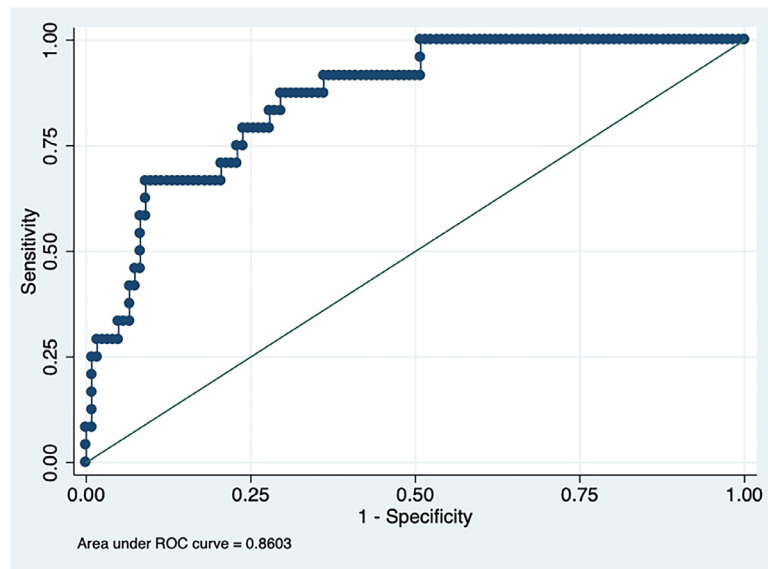


Fig. 3. ROC curve of logistic regression of analysis for the interaction regression model of childhood obesity of parent *Phascolarctobacterium succinatutens* and child *Massiliprevotella massiliensis* adjusted by child sex, parent sex, parent weight status, and adherence to Mediterranean diet. ROC, receiver operating characteristic.

Despite the clear and stable microbial transfer from mothers to children (around 50% of the shared strains) reported by Valles-Colomer et al. [15], there are no studies to date that consider the father factor in the heritability of the intestinal microbiota [44]. Valles-Colomer et al., established a relationship of the oral microbiota in cohabiting individuals. In this context, Knoop et al. [45] demonstrated that shared family environment was the most important predictor of overall microbiome similarity.

In the groups of parents with excess body weight, most were men (group 2, 72.7%, group 4, 69.6%), whereas in the groups of normal-weight parents, women accounted for the highest percentage (group 1, 65.8%, group 3, 61.5%). These data are of interest because worldwide obesity is more prevalent in women than in men [46]; however, this study suggests that excess body weight is better transmitted to children when it comes from fathers, but sex-linked factors such as dietary intake, physical (in)activity, and sleep time cannot be discarded.

Moreover, contrary to our data, some previous studies with a higher number of participants demonstrated that children with excess adiposity showed higher blood pressure and heart rate [47,48], which is an important predictor of hypertension in adulthood. In any case, as expected, TG, TyG, insulin, and HOMA-IR levels, as markers of insulin resistance, were higher in overweight than normal-weight children. Interestingly, this increase was even more significant when the parent was also overweight or obese. These data are consistent with early literature [49–51]. However, in this study, it was not possible to detect a significant increase in glucose or dyslipidemia [50,52], only trends.

As widely described, there is a beneficial association between Mediterranean diet adherence in both children and adults and maintenance of a healthy body weight [53,54]. Our results revealed that both children and parents with overweight or obesity have a lower adherence to this dietary pattern. Moreover, Goswami et al. [55] recently described a relationship between physical activity and body weight in children, which is in accordance with our results, showing that children with excess body weight have a less active lifestyle; however, it can also be concluded that this is a consequence rather than a cause [56]. These pioneering findings may be helpful for future treatments for obesity with better precision with respect to dietary interventions.

Furthermore, regarding dietary patterns and obesity, a meta-analysis evidenced an inverse association between dairy consumption and childhood obesity [57]. Along these lines, it has been suggested that consumption of ultra-processed foods carries an increased risk of developing obesity during childhood [58]. Thus, more studies are needed to evaluate the role of heritability of microbiota and diet in childhood obesity and their mutual interaction.

This study has some limitations. The sample size is relatively small for a study of this type, as often found in the literature. No multiple testing correction was done in order to counteract multiple testing problem. In addition, the Mediterranean diet adherence questionnaires are not validated for children of this age group, which makes possible the presence of biases. Moreover, type I and type II errors cannot be discarded. Nevertheless, the plausibility of the findings supports our research because all microorganisms have been found to be related to obesity. Finally, the nature of 16S sequencing limits taxonomic profiling to genus-level resolution, as the primers used for amplification bind to regions not conserved across all bacteria, not allowing differentiation between closely related bacteria at species level. This limitation in taxonomic identification also reduces the possibility of inferring the functionality of the microbiome. A strength is that outcomes cover some gaps in the literature with putative value.

Conclusions

The present study suggests a possible interaction between transgenerational gut microbiota, specifically from parent to child, which is associated with overweight and obesity status. These findings are of relevance as part of a pioneering study anticipating adult obesity. Despite being a study in a very specific population, it is of great interest, being one of the first studies in which this interaction has been investigated. Indeed, it has been evidenced that the relationships between a group of bacteria are associated with excess body weight depending on parent–child obesity status. Further research providing support for future hypothesis-driven investigations is needed in these family groups for prevention of the obesity epidemic.

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Declaration of competing interest

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CRediT authorship contribution statement

Begoña de Cuevillas: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Jose I. Riezu-Boj:** Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **Fermín I. Milagro:** Writing – review & editing, Resources, Investigation. **Sergio Galera Alquegui:** Writing – review & editing, Formal analysis, Data curation. **Nancy Babio:** Writing – review & editing, Project administration, Funding acquisition. **Belén Pastor-Villaescusa:** Writing – review & editing, Methodology, Investigation. **Mercedes Gil-Campos:** Writing – review & editing, Project administration, Funding acquisition. **Rosaura Leis:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation. **Pilar De Miguel-Etayo:** Writing – review & editing, Methodology, Investigation. **Luis A. Moreno:** Writing – review & editing, Project administration, Methodology, Funding acquisition. **Jordi Salas-Salvadó:** Writing – review & editing, Methodology, Funding acquisition. **J. Alfredo Martínez:** Writing – original draft, Supervision, Resources, Investigation, Conceptualization. **Santiago Navas-Carretero:** Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Supplementary materials

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References

- [1] World Health Organization. Obesity. Available at: https://www.who.int/health-topics/obesity#tab=tab_1. Accessed March 9, 2023.
- [2] World Health Organization. World Obesity Day 2022—accelerating action to stop obesity. Available at: <https://www.who.int/news/item/04-03-2022-world-obesity-day-2022-accelerating-action-to-stop-obesity>. Accessed March 9, 2023.
- [3] Piché ME, Tchernof A, Després JP. Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res* 2020;126:1477–500. <https://doi.org/10.1161/circresaha.120.316101>.
- [4] Horeish A, Tsur AM, Bardugo A, Twig G. Adolescent and childhood obesity and excess morbidity and mortality in young adulthood—a systematic review. *Curr Obes Rep* 2021;10:301–10. <https://doi.org/10.1007/S13679-021-00439-9>.
- [5] Agagündüz D, Icer MA, Yesildemir O, Koçak T, Kocayigit E, Capasso R. The roles of dietary lipids and lipodomics in gut–brain axis in type 2 diabetes mellitus. *J Transl Med* 2023;21:240. <https://doi.org/10.1186/S12967-023-04088-5>.
- [6] Abenavoli L, Scarpellini E, Colica C, Boccuto L, Salehi B, Sharifi-Rad J, et al. Gut microbiota and obesity: a role for probiotics. *Nutrients* 2019;11:2690. <https://doi.org/10.3390/nu11112690>.
- [7] McAllister EJ, Dhurandhar NV, Keith SW, Aronne LJ, Barger J, Baskin M, et al. Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* 2009;49:868. <https://doi.org/10.1080/10408390903372599>.
- [8] González-Muniesa P, Martínez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, et al. Obesity. *Nat Rev Dis Primers* 2017;3:1–18. <https://doi.org/10.1038/nrdp.2017.34>.
- [9] Jehan S, Zizi F, Pandi-Perumal SR, McFarlane SI, Jean-Louis G, Myers AK. Energy imbalance: obesity, associated comorbidities, prevention, management and public health implications. *Adv Obes Weight Manag Control* 2020;10:146. <https://doi.org/10.15406/aowmc.2020.10.00321>.
- [10] World Health Organization. WHO European regional obesity report 2022. Available at: <https://www.who.int/europe/publications/i/item/9789289057738>. Accessed April 2024.
- [11] Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, Milagro FI, Martínez JA. Diet, gut microbiota, and obesity: links with host genetics and epigenetics and potential applications. *Adv Nutr* 2019;10(suppl 1):S17–30. <https://doi.org/10.1093/advances/nmy078>.
- [12] Bouchard C. Genetics of obesity: what we have learned over decades of research. *Obesity (Silver Spring)* 2021;29:802–20. <https://doi.org/10.1002/oby.23116>.
- [13] King SE, Skinner MK. Epigenetic transgenerational inheritance of obesity susceptibility. *Trends Endocrinol Metab* 2020;31:478. <https://doi.org/10.1016/j.tem.2020.02.009>.
- [14] Chen L, Wang D, Garmaeva S, Kurilshikov A, Vich Vila A, Gacesa R, et al. The long-term genetic stability and individual specificity of the human gut microbiome. *Cell* 2021;184:2302–2315.e12. <https://doi.org/10.1016/j.cell.2021.03.024>.
- [15] Valles-Colomer M, Blanco-Míguez A, Manghi P, Asnicar F, Dubois L, Golzato D, et al. The person-to-person transmission landscape of the gut and oral microbiomes. *Nature* 2023;614:125–35. <https://doi.org/10.1038/s41586-022-05620-1>.
- [16] 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* 2021;23(suppl 1):e13394. <https://doi.org/10.1111/obr.13394>.
- [17] Vandenplas Y, Carnielli VP, Książyk J, Luna MS, Migacheva N, Mosselmans JM, et al. Factors affecting early-life intestinal microbiota development. *Nutrition* 2020;78:110812. <https://doi.org/10.1016/j.nut.2020.110812>.
- [18] Naspolini NF, Meyer A, Moreira JC, Sun H, Froes-Asmus CIR, Dominguez-Bello MG. Environmental pollutant exposure associated with altered early-life gut microbiome: results from a birth cohort study. *Environ Res* 2022;205:112545. <https://doi.org/10.1016/j.envres.2021.112545>.
- [19] Melo NcdO, Cuevas-Sierra A, Fernández-Cruz E, de la O V, Martínez JA. Fecal microbiota composition as a metagenomic biomarker of dietary intake. *Int J Mol Sci* 2023;24:4918. <https://doi.org/10.3390/ijms24054918>.
- [20] Garcidueñas-Fimbres TE, Paz-Graniel I, Gómez-Martínez C, Jurado-Castro JM, Leis R, Escribano J, Moreno LA, Navas-Carretero S, Portoles O, Pérez-Vega KA, Gil-Campos M, López-Rubio A, Rey-Reñones C, De Miguel-Etayo P, Martínez JA, Flores-Rojas K, Vázquez-Cobela R, Luque V, Miguel-Berges ML, Pastor-Villaescusa B, Llorente-Cantarero FJ, Salas-Salvadó J, Babio N. Childhood Obesity Risk Assessment Longitudinal Study (CORALS) study investigators. Associations Between Eating Speed, Diet Quality, Adiposity, and Cardiometabolic Risk Factors. *J Pediatr* 2023;252:31–9. <https://doi.org/10.1016/j.jpeds.2022.08.024>.
- [21] Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012;7:284–94. <https://doi.org/10.1111/j.2047-6310.2012.00064.x>.
- [22] Martínez-Millana A, Hulst JM, Boon M, Witters P, Fernandez-Llatas C, Asseiceira I, et al. Optimisation of children z-score calculation based on new statistical techniques. *PLoS One* 2018;13:e0208362. <https://doi.org/10.1371/journal.pone.0208362>.
- [23] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502. <https://doi.org/10.1093/clinchem/18.6.499>.
- [24] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9. <https://doi.org/10.1007/bf00280883>.
- [25] Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. *Int J Epidemiol* 2019;48:387–388o. <https://doi.org/10.1093/ije/dyy225>.
- [26] Verbeest V, De Henauw S, Bammann K, Barba G, Hadjigeorgiou C, Eiben G, et al. Are context-specific measures of parental-reported physical activity and sedentary behaviour associated with accelerometer data in 2–9-year-old European children? *Public Health Nutr* 2015;18:860–8. <https://doi.org/10.1017/S136898001400086X>.
- [27] De La Cámara MA, Higuera-Fresnillo S, Cabanas-Sánchez V, Sadarangani KP, Martínez-Gómez D, Veiga OL. Criterion validity of the sedentary behavior question from the global physical activity questionnaire in older adults. *J Phys Act Health* 2020;17:2–12. <https://doi.org/10.1123/jpah.2019-0145>.

- [28] Le François B, Bouevitch A, Lynch D, Doukhanine E, Iwasior R. Beyond bacteria: characterization and analysis of the mycobiome and virome in human gut samples. 2018. DNA Genotek Inc (Ottawa). URL: <https://www.dnagenotek.com/ROW/support/scientific-posters/omnigene-gut>. Last accessed: April 2024
- [29] Martí M, Spreckels JE, Jenmalm MC, Abrahamsson T. A protocol for characterization of extremely preterm infant gut microbiota in double-blind clinical trials. STAR Protoc 2021;2:100652. <https://doi.org/10.1016/j.xpro.2021.100652>.
- [30] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852–7. <https://doi.org/10.1038/s41587-019-0209-9>.
- [31] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3. <https://doi.org/10.1038/nmeth.3869>.
- [32] Fritz MHY, Leinonen R, Cochrane G, Birney E. Efficient storage of high throughput DNA sequencing data using reference-based compression. Genome Res 2011;21:734–40. <https://doi.org/10.1101/gr.114819.110>.
- [33] Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 2018;6:1–17. <https://doi.org/10.1186/s40168-018-0470-z/tables/3>.
- [34] Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 2014;42:D633–42. <https://doi.org/10.1093/nar/gkt1244>.
- [35] Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 2010;5:e9490. <https://doi.org/10.1371/journal.pone.0009490>.
- [36] Katoh K, Misawa K, Kuma KI, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 2002;30:3059–66. <https://doi.org/10.1093/nar/gkf436>.
- [37] Cuevas-Sierra A, Romo-Hualde A, Aranaz P, Goni L, Cuervo M, Martínez JA, et al. Diet- and sex-related changes of gut microbiota composition and functional profiles after 4 months of weight loss intervention. Eur J Nutr 2021;60:3279–301. <https://doi.org/10.1007/S00394-021-02508-0>.
- [38] Ndongo S, Cadoret F, Dubourg G, Delerac J, Fournier PE, Raoult D, et al. 'Collinsella phocaensis' sp. nov., 'Clostridium merdae' sp. nov., 'Sutterella massiliensis' sp. nov., 'Sutterella timonensis' sp. nov., 'Enorma phocaensis' sp. nov., 'Mallihella massiliensis' gen. nov., sp. nov., 'Mordavella massiliensis' gen. nov., sp. nov. and 'Massiliprevotella massiliensis' gen. nov., sp. nov., 9 new species isolated from fresh stool samples of healthy French patients. New Microbes New Infect 2017;17:89–95. <https://doi.org/10.1016/j.nmni.2017.02.005>.
- [39] Gao X, Jia R, Xie L, Kuang L, Feng L, Wan C. A study of the correlation between obesity and intestinal flora in school-age children. Sci Rep 2018;8:14511. <https://doi.org/10.1038/s41598-018-32730-6>.
- [40] Lin YC, Lin HF, Wu CC, Chen CL, Ni YH. Pathogenic effects of *Desulfovibrio* in the gut on fatty liver in diet-induced obese mice and children with obesity. J Gastroenterol 2022;57:913–25. <https://doi.org/10.1007/S00535-022-01909-0/figures/7>.
- [41] Palmas V, Pisanu S, Madau V, Casula E, Deledda A, Cusano R, et al. Gut microbiota markers associated with obesity and overweight in Italian adults. Sci Rep 2021;11:5532. <https://doi.org/10.1038/s41598-021-84928-w>.
- [42] Sanmiguel C, Gupta A, Mayer EA. Gut microbiome and obesity: a plausible explanation for obesity. Curr Obes Rep 2015;4:250. <https://doi.org/10.1007/S13679-015-0152-0>.
- [43] Dubé MP, Park SY, Ross H, Love TMT, Morris SR, Lee HY. Daily HIV pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate-emtricitabine reduced *Streptococcus* and increased Erysipelotrichaceae in rectal microbiota. Sci Rep 2018;8:15212. <https://doi.org/10.1038/s41598-018-33524-6>.
- [44] Hemmati M, Kashanipoor S, Mazaheri P, Alibabaei F, Babaeizad A, Asli S, et al. Importance of gut microbiota metabolites in the development of cardiovascular diseases (CVD). Life Sci 2023;329:121947. <https://doi.org/10.1016/j.lfs.2023.121947>.
- [45] Knoop KA, Holtz LR, Newberry RD. Inherited nongenetic influences on the gut microbiome and immune system. Birth Defects Res 2018;110:1494. <https://doi.org/10.1002/bdr2.1436>.
- [46] Cooper AJ, Gupta SR, Moustafa AF, Chao AM. Sex/gender differences in obesity prevalence, comorbidities, and treatment. Curr Obes Rep 2021;10:458–66. <https://doi.org/10.1007/s13679-021-00453-x/tables/1>.
- [47] Murphy MO, Huang H, Bauer JA, Schadler A, Makhoul M, Clasey JL, et al. Impact of pediatric obesity on diurnal blood pressure assessment and cardiovascular risk markers. Front Pediatr 2021;9:596142. <https://doi.org/10.3389/fped.2021.596142/full>.
- [48] Turcanu S, Gusetu G, Ciobanu DM, Istratoaie S, Rosu R, Alexandru MI, et al. Body size influences heart rate in children aged 6 to 18 years old. Medicine (Baltimore) 2023;102:e32602. <https://doi.org/10.1097/md.00000000000032602>.
- [49] Calcatera V, Verduci E, Vandoni M, Rossi V, Fiore G, Massini G, et al. The effect of healthy lifestyle strategies on the management of insulin resistance in children and adolescents with obesity: a narrative review. Nutrients 2022;14:4692. <https://doi.org/10.3390/nu14214692>.
- [50] Brzeziński M, Metelska P, Mysliwiec M, Szlagaty-Sidorkiewicz A. Lipid disorders in children living with overweight and obesity—large cohort study from Poland. Lipids Health Dis 2020;19:47. <https://doi.org/10.1186/s12944-020-01218-6>.
- [51] Zhou J, Bai L, Tong L, Jia L, Ding W. Association of the triglyceride-glucose index with weight-adjusted appendicular lean mass in Chinese adolescents aged 12–18 years old. Sci Rep 2022;12:11160. <https://doi.org/10.1038/S41598-022-15012-0>.
- [52] Weiss R, Magge SN, Santoro N, Giannini C, Boston R, Holder T, et al. Glucose effectiveness in obese children: relation to degree of obesity and dysglycemia. Diabetes Care 2015;38:689–95. <https://doi.org/10.2337/dc14-2183/-/dc1>.
- [53] Muscogiuri G, Verde L, Sulu C, Katsiki N, Hassapidou M, Frias-Toral E, et al. Mediterranean diet and obesity-related disorders: what is the evidence? Curr Obes Rep 2022;11:287. <https://doi.org/10.1007/s13679-022-00481-1>.
- [54] Lassale C, Fitó M, Morales-Suárez-Varela M, Moya A, Gómez SF, Schröder H. Mediterranean diet and adiposity in children and adolescents: a systematic review. Obes Rev 2022;23:e13381. <https://doi.org/10.1111/obr.13381>.
- [55] Goswami N, Trozic I, Fredriksen MV, Fredriksen PM. The effect of physical activity intervention and nutritional habits on anthropometric measures in elementary school children: the Health Oriented Pedagogical Project (HOPP). Int J Obes (Lond) 2021;45:1677–86. <https://doi.org/10.1038/s41366-021-00830-5>.
- [56] Safaei M, Sundararajan EA, Driss M, Boulila W, Shapi'i A. A systematic literature review on obesity: understanding the causes & consequences of obesity and reviewing various machine learning approaches used to predict obesity. Comput Biol Med 2021;136:104754. <https://doi.org/10.1016/j.combiomed.2021.104754>.
- [57] Babio N, Becerra-Tomás N, Nishi SK, López-González L, Paz-Graniel I, García-Gavilán J, et al. Total dairy consumption in relation to overweight and obesity in children and adolescents: a systematic review and meta-analysis. Obes Rev 2022;23:e13400. <https://doi.org/10.1111/obr.13400>.
- [58] Neri D, Steele EM, Khandpur N, Cediell G, Zapata ME, Rauber F, et al. Ultraprocessed food consumption and dietary nutrient profiles associated with obesity: a multicountry study of children and adolescents. Obes Rev 2022;23:e13387. <https://doi.org/10.1111/obr.13387>.