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Original article

Gut microbiota is associated with metabolic health in children with obesity

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SUMMARY

Background and aims: We aimed to describe and characterize the gut microbiota composition and diversity in children with obesity according to their metabolic health status.
Methods: Anthropometry, Triglycerides, HDL cholesterol, HOMA-IR, and systolic and diastolic blood pressure (SBP, DBP) were evaluated (and z-score calculated) and faecal samples were collected from 191 children with obesity aged from 8 to 14. All children were classified depending on their cardiometabolic status in either a "metabolically healthy" (MHO; n = 106) or "metabolically unhealthy" (MUO; n = 85) group. Differences in gut microbiota taxonomies and diversity between groups (MUO vs MHO) were analysed. Alpha diversity index was calculated as Chao1 and Simpson's index, and β-diversity was calculated as Adonis Bray–Curtis index. Spearman's correlations and logistic regressions were performed to study the association between cardiometabolic health and the microbiota.
Results: Children in the MUO presented significantly lower alpha diversity and richness than those in the MHO group (Chao1 index p = 0.021, Simpson's index p = 0.045, respectively), whereas microbiota β-diversity did not differ by the cardiometabolic health status (Adonis Bray–Curtis, $R^2 = 0.006$; p = 0.155). The MUO group was characterized by lower relative abundances of the genera *Christensenellaceae R7 group* (MHO:1.42% [0.21–2.94]; MUO:0.47% [0.02–1.60], p < 0.004), and *Akkermansia* (MHO:0.26% [0.01–2.19]; MUO:0.01% [0.00–0.36], p < 0.001) and higher relative abundances of *Bacteroides* (MHO:10.6% [4.64–18.5]; MUO:17.0% [7.18–27.4], p = 0.012) genus. After the adjustment by sex, age, and BMI, higher *Akkermansia* (OR: 0.86, CI: 0.75–0.97; p = 0.033), Christensenellaceae R7 group (OR: 0.86, 95% CI: 075–0.98; p = 0.031) and Chao1 index (OR: 0.86, CI: 0.96–1.00; p = 0.023) represented a lower risk of the presence of one or more altered cardiovascular risk factors.
Conclusion: Lower proportions of *Christensenellaceae* and *Akkermansia* and lower diversity and richness seem to be indicators of a metabolic unhealthy status in children with obesity.

1. Background

Obesity has become a major public health problem during the last decades affecting both adults and children. The importance of the obesity pandemic relies on the fact that its relation with cardiovascular disorders is one of the main mortality risk factors worldwide [1]. Childhood obesity tracks to adolescence and adulthood [2] thus, most adolescents with obesity may have a higher risk of metabolic disorders such as insulin resistance, dyslipidaemia, and hypertension in adult life [3,4].

It has been suggested that a combination of genetic and environmental factors and an unhealthy dietary pattern with insufficient physical activity could be the main reasons for the development of obesity and its metabolic comorbidities [5,6]. Recently, other factors such as sleep duration [7], stress [8], poor calcium intake [9], or the gut microbiota composition [10] are

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suggested as new players in the development of obesity and its comorbidities.

There is an emerging interest in the role of gut microbiota in the regulation of metabolic health. Specific bacterial taxa produce short-chain fatty acids (SCFA) by the fermentation of undigested fibre in the gut [11]. SCFA represent an important source of energy for the host [12] and are involved in different metabolic pathways linked to anti-inflammatory effects, appetite control, or regulation of glucose and lipid metabolisms [13].

The presence of an alteration of the gut microbiota composition, known as dysbiosis, has been described both in adults [14,15] and children [16–19] with obesity. Obesity dysbiosis is characterized by the increased levels of *Firmicutes* vs *Bacteroidetes*, a lower bacterial diversity [14,16,19], and lower absorption of SCFA [20]. The consequences of obesity dysbiosis have been linked with the development of metabolic alterations such as dyslipidaemia and insulin resistance or hypertension [21–24].

However, it has been observed that not all the subjects with obesity present co-morbidities; in fact, it has been estimated that only the 47% of the patients with obesity will develop metabolic syndrome, and 10% of the adults with obesity will not develop any of the disorders involved in it [25]. This absence is more often observed in young and physically active patients, and it has been associated with the new concept called "metabolically healthy obesity" (MHO) [26,27]. Recently it has been described that children categorized as MHO present better metabolic profiles than those classified as "metabolically unhealthy obesity" (MUO), including lower systolic and diastolic blood pressure and better lipid profile [28]. MHO has been frequently defined as obesity without metabolic comorbidities and cardiovascular disease, like type 2 diabetes, dyslipidaemia, hypertension, and atherosclerosis [29]. As there were non-standardized criteria to identify children with MHO, in 2018, Damanhoury et all published a consensus-based definition. The consensus concluded that MHO in children should reflect the absence of any cardiometabolic alteration including dyslipidaemia, hypertension, or glucose intolerance [30] like. We hypothesized that the gut microbiota could be one of the triggering factors of metabolic alterations in children with obesity.

The reports investigating the composition of the gut microbiota according to the metabolic health status of the population with obesity are scarce in adults [31,32] and to our knowledge, there are only two recent works in small samples of children with obesity (overall 20 of 65 children with obesity) investigating this association [33,34]. Even the two studies associated a lower diversity to the metabolic unhealthy obesity condition, there is no specific taxonomic that could be associated with the onset of metabolic alteration.

Therefore, our study aimed to describe the gut microbiota composition and diversity in children with obesity and to characterize the differences in the gut microbiota according to their metabolic health status.

2. Methods

2.1. Study design and participants

This was a cross-sectional observational study, secondary to a randomized clustered clinical trial on a motivational intervention to treat children with obesity. To perform the present study, we used the baseline anthropometry and biochemistry data along with the microbiota composition of the participants enrolled in the OBEMAT2.0 clinical trial [35].

Data from 315 children with obesity (170 males; 145 females) aged 8 to 14 were obtained from the clinical trial OBEMAT2.0 at baseline. Children were recruited from June 2016 to March 2018 from primary health care centres belonging to the "Camp de Tarragona" healthcare area. Obesity was considered according to BMI values equal to or higher than 97th percentile from Hernandez et al. according to the National Clinical Practice Guidelines [36]. The visit was conducted at hospital Universitari de Tarragona Joan XXIII and Hospital Universitari Sant Joan de Reus between June 2016 and March 2018, where participants of the Obemat2.0 trial were invited to take part in a voluntary faecal collection; 191 of the participants accepted and brought a sample to the centre.

2.2. Anthropometrical and biochemical measurements

Bodyweight was evaluated using a digital scale (SECA 769) with a precision of 50 g in underwear. Height was measured by a wall-mounted stadiometer (SECA 216) with 0.1 cm of precision. The waist was measured as the mid-point between the iliac crest and lower rib with a Holtain waist circumference non-extensible tape with a precision of 1 mm [37]. Body mass index (BMI) was calculated as weight over squared height (kg/m^2). BMI was converted into z scores using the World Health Organization 2007 reference data [38].

A trained nurse extracted a blood sample from participants in fasting conditions. High-density lipoproteins cholesterol (HDL) (mg/dL), triglycerides (mg/dL), and glucose (mg/dL) were measured in the respective laboratories of local study centres using routine clinical diagnostic enzymatic methods, and insulin (μIU/ml) was quantified by immunoradiometric assays. The Homeostasis Model Assessment of Insulin resistance (HOMA-IR) was calculated as HOMA-IR = (Inulin mIU x Glucose (mmol/L)/22.5 as previously reported [39].

Trained study personnel measured systolic (SBP) and diastolic blood pressure (DBP) (mmHg) at least 20 min after arriving at the study centre. Blood pressure was assessed in duplicate (with a time slot of 5 min between measures) using a Dinamap Pro 100 device on the left arm, while the child remained to sit down with the arm laying comfortably. The mean value of the two duplicate measures was then calculated.

Biochemical parameters, SBP, and DBP were standardized as z-scores using the Stavnsbo references [40].

2.3. Faecal DNA collection and extraction

The study participants were instructed to self-collect a faecal sample and then store it at -20 °C until the visit. Participants were provided with tubes, isolation bags, and icy patches to transport the samples to the clinic, where the faecal samples were stored at -80 °C until their analysis.

DNA was extracted from approximately 200 mg of stool samples using the MagAttract PowerSoil DNA kit (Qiagen, Venlo, Netherlands) for KingFisher Duo Primer Purification System (Thermo Fischer Scientific Inc, Waltham, MA, USA) following the manufacturer's protocol at the ICTS infrastructure with the equipment of the Centre for Omic Sciences (COS), Joint Unit of the Universitat Rovira i Virgili and Eurecat.

2.4. Sequencing and bioinformatics analysis

DNA libraries were obtained following the 16srDNA GENE Metagenomic Sequencing Library Preparation Illumina protocol (Cod. 15044233 Rev. A). The gene-specific sequences were targeting the variable V3 and V4 regions. The primers were selected according to Klindworth et al., 2019 [41]. Microbial genomic DNA (5 ng/μl in 10 mM Tris pH 8.5) was used to initiate the protocol. The

multiplexing step was performed using Nextera XT Index Kit (FC-131-1096) (Illumina, San Diego, CA, USA). One μl of the PCR product was run on a Bioanalyzer DNA 1000 chip to verify the size (the expected size on a Bioanalyzer trace was ~550 bp). The libraries were sequenced using a 2 × 300pb paired-end run (MiSeq Reagent kit v3 (MS-102-3001)) on a MiSeq- Illumina Sequencer (FISABIO sequencing service, Valencia, Spain) according to the manufacturer's instructions. The quality assessment was performed using prinseq-lite program [42] and sequences were selected with a minimum length of 50. Sequence data were analysed using quiime 2 pipelines by Boylen et al. [43]. The metataxonomic analyses were performed using some quiime2 plugins. Denoising, paired-ends joining and chimera depletion was performed starting from paired ends data using the DADA2 pipeline [44]. Taxonomic assignment was conducted using the Silva v138 database with the addition of the specie level classification by the same database [45]. Taxa representing less than 0.01% of the reads across all the samples were filtered.

2.5. Cardiometabolic health

To assess the children's cardiometabolic risk, we created a continuous cardiometabolic risk score (Cmet Risk) based on Eisenmann et al. [46]. This score was the sum of the standardized SBP, DBP triglycerides, HOMA-IR, and HDL cholesterol z-scores, this last one multiplied by -1 (as HDL cholesterol is inversely related to cardiometabolic risk). A higher score was indicative of a less favourable cardiometabolic profile.

Al the participants included in the study were classified by their metabolically status in children with metabolically healthy obesity (MHO) and children with metabolically unhealthy obesity (MUO). The parameters included in this classification were Triglycerides, HDL, SBP, DBP, and HOMA-IR. We considered a child as belonging to the MUO if at least one of the parameters previously described was over ≥1.5SD of the z-score (or ≤ 1.5SD for HDL).

2.6. Statistical analysis

Descriptive data were reported as the median and interquartile range for continuous variables and as a percentage for categorical variables. For testing differences in health outcome parameters, the relative abundance of bacteria taxa, and alpha diversity between MUO and MHO groups, Mann–Whitney U-test was performed. Only bacteria with a P-value < 0.01 were considered significantly different.

Spearman's rank correlations were used to find associations between the alpha diversity index and the cardiovascular risk factors and the metabolic risk score as a continuous variable.

Beta diversity analysis was conducted by permutational multivariate analysis to assess the effect of the metabolic status on the gut microbial composition at amplicon sequence variant (ASV) level (Adonis test) using Bray–Curtis distance in the MicrobiomeAnalyst online platform [47]. The clustering of the samples according to the metabolic status was performed by principal component analysis at the ASV level.

Associations between the cardiometabolic risk factors (as continuous variables) and the bacteria with at least 0.01% of abundance were assessed using Spearman's rank correlation. The bacteria that were significantly associated with metabolic risk score or the cardiometabolic risk factors and the alpha diversity indexes were included in logistic regression models performed in R version 4.1 [48]. These models were adjusted for age, sex, and BMI (z-score). Results were expressed by odds ratio (OR) and 95% confidential interval (95% IC).

2.7. Ethics

The study followed the rules of the Declaration of Helsinki [49] and was approved by the ethics committees responsible for the activity of all the involved study centres. All parents or legal guardians signed the informed consent before study enrolment and children aged 12 years or above signed informed assent to participate in the study.

3. Results

3.1. Description of the population

One hundred ninety-one participants of the Obemat 2.0 trial were classified by their metabolically status. Characteristics of the study participants are described in Table 1. The median age of all the participants was 10 [9.0–12.0] years and did not significantly differ between groups. Almost half (45%) of all the participants presented MUO. Significant differences for all the cardiometabolic risk factors were found between the MHO and the MUO group, including the z-BMI ($p = 0.003$) and waist ($p < 0.001$). Table S1 shows the number of participants that presented each altered cardiovascular risk factor.

3.2. Differences in microbiota composition and diversity depending on cardiometabolic risk

In terms of alpha diversity, the MUO group presented lower richness than the MHO group measured as Chao1 ($p = 0.021$), ACE ($p = 0.020$), and Simpson ($p = 0.045$) indices (Fig. 1A).

Assuming an alpha risk of 0.05 and beta risk of 0.33, a difference between groups (MHO and MUO) of 6.6 has a statistical power of 67%.

Studying each metabolic risk factor independently, those children with altered triglycerides levels presented lower alpha diversity than those with normal levels ($p = 0.027$ in Chao 1 and $p = 0.047$ in Shannon index) (Table S2). A similar trend was observed in children with altered SBP compared to children with not altered SBP ($p = 0.035$, Chao1 index). No differences in microbial diversity were found between children with other metabolic alterations.

Despite the overall microbiota β-diversity was not significantly affected by cardiometabolic status (Adonis Bray–Curtis, $R^2 = 0.006$; $p = 0.155$) (Fig. 1B), differences in specific taxa were found between groups. Relative abundance of the most abundant phylum according to the metabolic condition of the participants is presented in Fig. 2A. Firmicutes were the most abundant taxa in both groups followed by Bacteroidota, both represented 90% of all bacteria. Higher relative abundances of Firmicutes ($p = 0.002$) and Verrucomicrobia ($p = 0.001$) phyla were found in the MHO group compared to MUO, which showed enrichment in Bacteroidetes phylum ($p = 0.002$). Descriptive data are shown in Table S3.

At lower taxonomical levels (Fig. 2B-E), participants with metabolic alterations (MUO) presented a higher relative abundance of the order of Bacteroidales ($p = 0.010$) and the genus of Bacteroides ($p = 0.012$). The relative abundance of the Christensenellaceae family and its lower taxonomic level Christensenellaceae R-7 group ($p = 0.004$), was more than twice higher in the MHO than in the MUO group (MHO:1.42 [0.21–2.94]; MUO:0.47 [0.02–1.60]; $p = 0.004$). The *Akkermansia* genus and the higher taxonomic levels were also under-expressed in the MUO group (MHO: 0.26% [0.01–2.19]; MUO: 0.01% [0.00–0.36]; $p = 0.001$) (Table S3).

Indeed, the relative abundance of the Christensenellaceae family and the genus *Christensenellaceae_R-7_group* significantly

Table 1
Characteristics of the study participants separated by their cardio-metabolic status.

	Whole Sample (n = 191)	MHO (n = 106)	MUO (n = 85)	P-Value
	median [IQR]	median [IQR]	median [IQR]	
Gender (male/female)	110/81	60/46	57/35	0.872
Age (y)	10.0 [9.00; 12.0]	10.0 [9.00; 11.0]	11.0 [9.00; 12.0]	0.102
BMI z-score	2.54 [2.29; 2.83]	2.45 [2.22; 2.75]	2.64 [2.36; 3.00]	0.003
Waist z-sore	2.05 [1.63; 2.41]	1.86 [1.60; 2.21]	2.24 [1.87; 2.65]	<0.001
Triglycerides z-score	0.31 [−0.23; 1.10]	−0.03 [−0.45; 0.41]	0.79 [0.26; 1.89]	<0.001
HDL z-score	−0.62 [−0.97; −0.05]	−0.35 [−0.82; 0.27]	−0.84 [−1.16; −0.48]	<0.001
SBP z-score	0.47 [−0.10; 1.10]	0.29 [−0.15; 0.67]	0.82 [−0.05; 1.86]	<0.001
DBP z-score	−0.13 [−0.61; 0.44]	−0.38 [−0.71; 0.24]	0.26 [−0.31; 0.83]	<0.001
HOMA-IR z-score	0.50 [0.05; 1.25]	0.25 [−0.05; 0.62]	1.24 [0.45; 2.25]	<0.001
Cardiometabolic Risk z-score	2.24 [0.27; 3.95]	0.63 [−0.74; 2.02]	4.13 [2.84; 6.07]	<0.001

MHO: metabolically healthy obesity group; MUO metabolically unhealthy obesity group. IQR: interquartile range. BMI: body mass index. HDL: High Density Lipoproteins cholesterol. SBP: systolic blood pressure. DBP: diastolic blood pressure. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance. P-value calculated by Mann Withney's U-test.

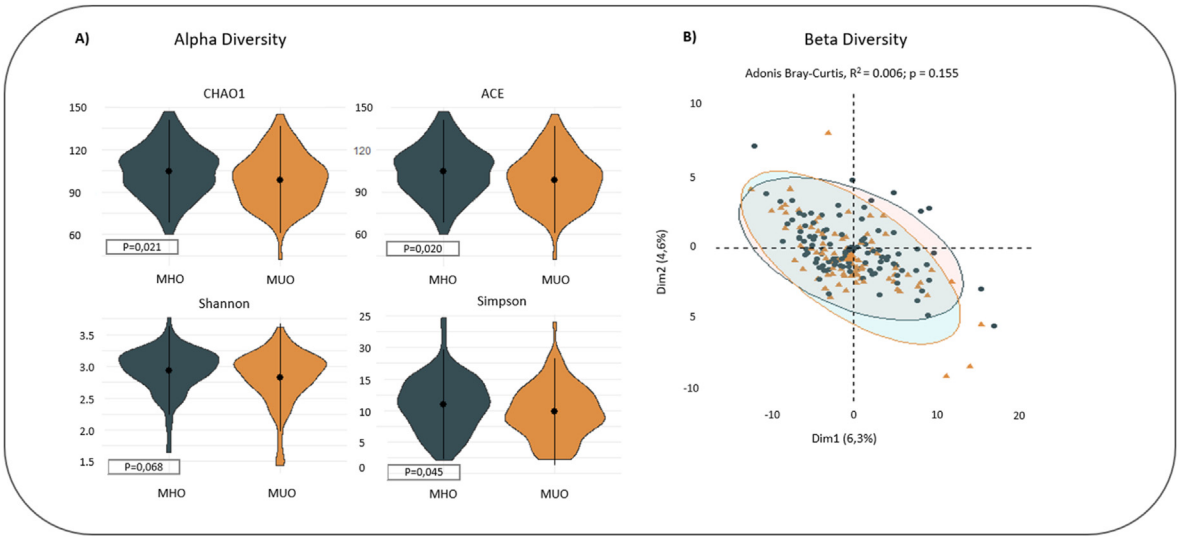

Fig. 1. Characterization of beta-diversity and alpha diversity of gut microbiome of participants in the Metabolically Healthy Obesity and Metabolically Unhealthy Obesity (MHO and MUO) groups. A) Alpha diversity index. Violin plots represent the differences at the genus level among children with MHO and MUO using the Mann–Whitney U test B). The clustering of the samples according to the metabolic status was performed by principal component analysis at the amplicon sequence variable, ASV, level.

differed between children with normal and high serum triglycerides concentrations (1.46 [0.41–2.89] vs. 0.64 [0.14–1.71]; p = 0.029). Children with altered triglycerides presented as well higher abundances of *Coprococcus* (0.72 [0.36–1.14]; 0.99 [0.54–1.74]; p = 0.045) and less *Bacteroides dorei* (0.37 [0.00–1.76]; 0.13 (0.00–0.39); p = 0.029). Children with altered HOMA-IR presented higher levels of *Bacteroides* (5.12 [1.86–10.5]; 7.6 [3.19–13.1; p 0.036), and *Alistipes* (3.75 [1.26–6.65]; 5.30 [2.90–9.34]; p = 0.042) genera in comparison with those participants without this alteration. The same trend was observed in those participants with altered SBP, who showed higher abundances of *Bacteroides* genus, and lower abundances of Christensenellacea R-7 group (Table S4).

3.3. Associations of some microbial taxa with the cardiometabolic health

Figure 3 represents the associations between some bacterial taxa, alpha diversity, and cardiometabolic risk factors. An inverse correlation of the Chao1 index with the metabolic risk score (Rho = −0.15; p = 0.03) and triglycerides (Rho = −0.15; p = 0.03) and a direct correlation with HDL (Rho 0.15; p = 0.04) was observed. The association with the metabolic risk score and the alpha diversity index remained significant after the adjustment by sex, age, and BMI (Table S5).

The correlation analyses revealed that there were several bacteria associated with the cardiometabolic risk score. The metabolic risk score was positively associated with Bacteroidota (Rho = 0.15; p = 0.03) and Proteobacteria (Rho = 0.14; p = 0.042) phyla and inversely associated with Firmicutes (Rho = 0.14; p = 0.047). These trends represent the associations that we found into the corresponding lower taxonomic levels Bacteroidales order (Rho = 0.15; p = 0.03), Clostridia (Rho = −0.15; p = 0.033), and Gammaproteobacteria (Rho = 0.16; p = 0.026) classes. Moreover, the Verrucomicrobiae class was also negatively associated with the metabolic risk score (Rho = −0.15; p = 0.041).

In lower taxonomic levels, we observed how *Christensenellaceae R7-group* (Rho = −0.18; p = 0.01) and *Akkermansia* genus (Rho = −0.15; p = 0.045) were negatively associated with the metabolic risk score. *Christensenellaceae R-7 group* also was associated inversely with the triglyceride levels (Rho = −0.15; p = 0.04) (Fig. 3).

Although we have not observed a significant association between the BMI and the diversity, the abundance of the *Christensenellaceae R7-group* was inversely associated with the BMI of our participants (Rho = −0.15; p = 0.04).

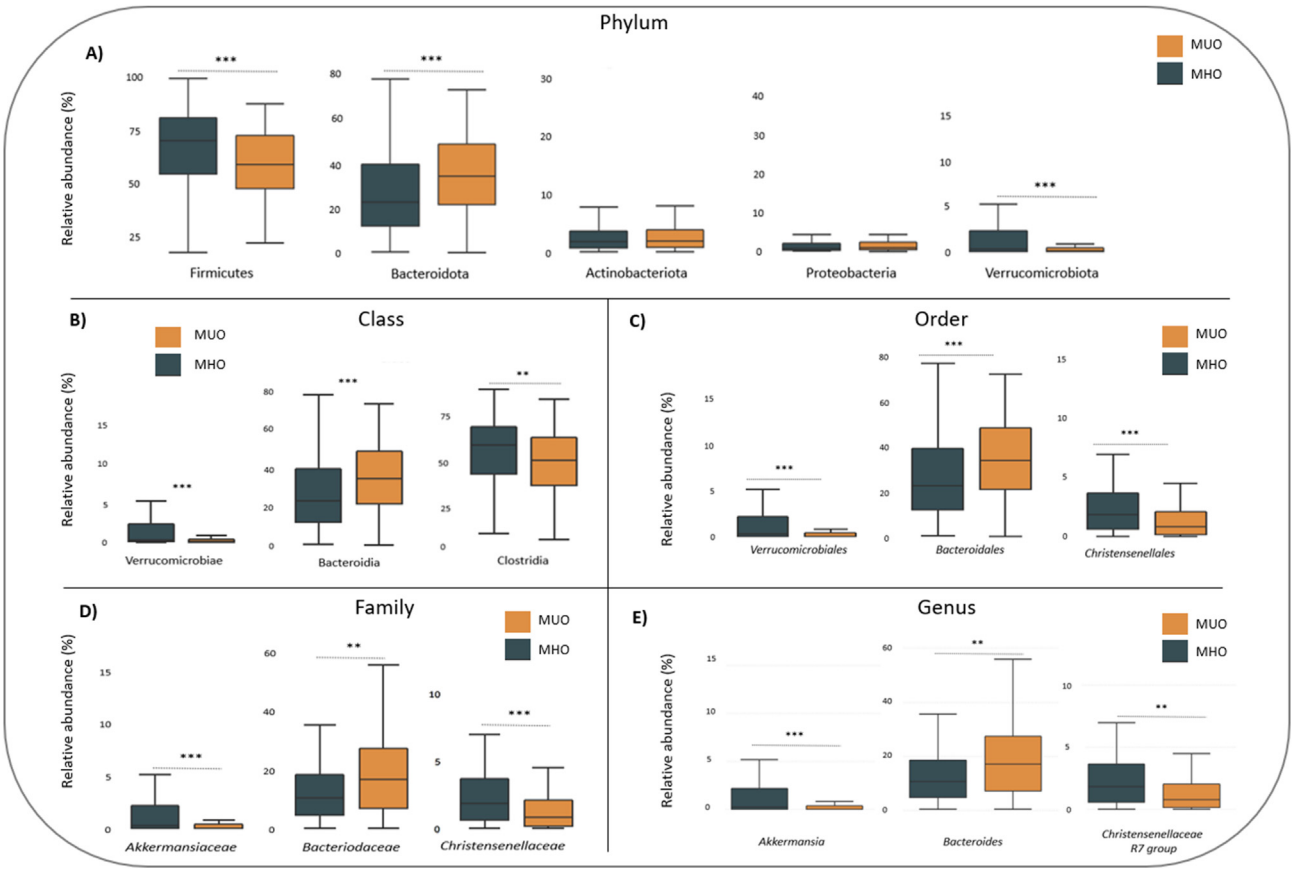

Fig. 2. Box plot representation of the differences at Phylum, Class, Order, Family, and genus levels between the MUO and the MUO group. Mann Whitney U test was used to calculate the significance. **p < 0.05; ***p < 0.01.

Logistic regression models of different bacteria on MHO vs. MUO adjusted by sex, BMI, and age were performed (Table 2). BMI was the variable that most affected the cardiovascular risk. However, we observed that the association of Bacteroidales, *Christensenellaceae R-7,* and *Akkermansia* were consistent after the adjustment. The greater presence of *Akkermansia* and *Christensenellaceae R7* was associated with having a lower risk of presenting any alteration in one or more of the different cardiometabolic risk factors. The greater presence of Bacteroidales was associated with a higher risk of being classified in the MUO group.

4. Discussion

Our study reveals that children with metabolic unhealthy obesity were characterized by lower diversity and lower relative abundance of Christensenellales (*Christensenellaceae R-7*) and Verrucomicrobiales *(Akkermansia)* than those without any cardiometabolic alteration. Furthermore, children with metabolic unhealthy obesity showed, at the same time, an enrichment of *Bacteroides*. To the best of our knowledge, this is the work with the higher number of participants with obesity that have studied the association of the gut microbiota with metabolic health in children.

We observed that the most abundant phyla in the overall sample were Firmicutes and Bacteroidetes, consistently with what was previously described [18,19,50]. Our participants presented lower proportions of Bacteroidetes in comparison with Firmicutes, which might be explained by the obesity condition. Moreover, we have detected specific characteristics of the microbiota profile depending on the metabolic health group. Supporting the literature [33,34] we have observed that the MUO group presented lower diversity than the MHO group. Rampelli et al. observed how a worse lifestyle was associated with low diversity and a worse inflammatory profile which could trigger the development of obesity and its comorbidities. Moreover, low microbial diversity is considered one of the main markers of intestinal dysbiosis and it has been associated with several alterations such as higher insulin resistance, adiposity, dyslipidaemia, and worse inflammatory profile [33,51].

When we looked into detail the microbiota profile of children with MUO and MHO. We observed that children with MUO presented a higher proportion of Bacteroides and children with MHO higher proportion of Christensenellaceae and Akkermansia. Consistently, the diversity was inversely associated with the abundance of Bacteroides and directly with Akkermansia and Christensenellace.

Consistently with previous works, the group with MUO presented a higher abundance of the *Bacteroides* genus and upper taxonomic levels in comparison with the MHO [34]. It has been proposed *Bacteroides* genus as a biomarker of a western diet, rich in fat and protein [52]. Actually, Some *Bacteroides* spp. would be especially efficient readapting their metabolism to different gut environments [53].

Christensenella genera have been reported as health-related bacteria in adulthood [54,55]. Christenesenellaceae family was consistently found to be increased in healthy populations compared to disease groups in several cohort studies [33,56,57]. Some authors have previously observed that Christensenellaceae were depleted in participants with metabolic syndrome [58,59] and inversely correlated with metabolic risk in adult populations [60].

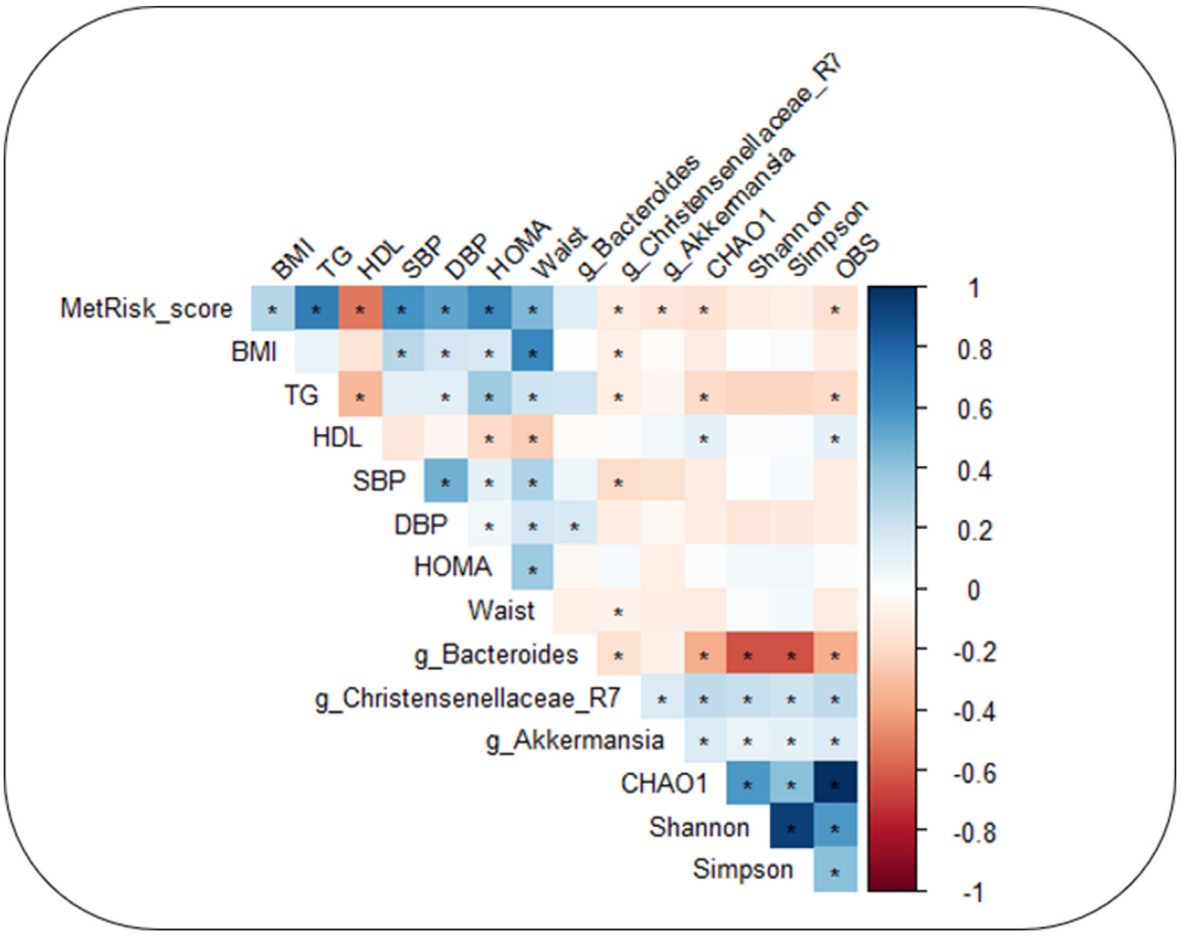

Fig. 3. Paired correlations between bacteria, alpha diversity, and cardiometabolic risk factors. The heatmap represents Spearman's rank correlation coefficients between the different parameters. Positive or negative correlations are represented in blue and red respectively. BMI: Body mass index, TG: triglycerides HDL: high-density lipoproteins, SBP: systolic blood pressure, DBP diastolic blood pressure. HOMA insulin resistance index, MetRisk_score: cardiometabolic risk score. Cardiovascular factors are expressed as z-score. * Indicates Spearman's correlation p value < 0.05.

Table 2
Logistic regression models of bacterial abundances with effect on the presence of one or more altered metabolic risk factors (being classified as Metabolically Healthy Obesity).

Predictors	Bacteroides model			Christensenellaceae model			Akkermansia model			Mixed model		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Intercept	0.00	0.00–0.01	**<0.001**	0.00	0.00–0.05	**<0.001**	0.00	0.00–0.07	**0.001**	0.00	0.00–0.03	**<0.001**
Age (years)	1.38	1.11–1.72	**0.004**	1.34	1.08–1.66	**0.008**	1.29	1.04–1.61	**0.022**	1.30	1.05–1.64	0.019
Sex [girl]	1.30	0.68–2.50	0.431	1.40	0.74–2.68	0.307	1.31	0.69–2.50	0.406	1.43	0.74–2.80	0.297
BMI	4.50	2.05–10.45	**<0.001**	3.86	1.79–8.79	**0.001**	3.76	1.75–8.51	**0.001**	4.36	1.96–10.25	**<0.001**
Bacteroides	1.03	1.01–1.05	**0.001**							1.03	1.00–1.05	**0.023**
Christensenellaceae_R-7				0.86	0.75–0.98	**0.031**				0.99	0.83–1.18	0.878
Akkermansia							0.86	0.74–0.97	**0.033**	1.00	0.84–1.19	0.965
Interaction (Akkermansia*Christensenellacea_R-7)										0.90	0.78–0.99	0.076
Observations	191			191			191			191		
R^2 Tjur	0.131			0.102			0.108			0.160		

In fact, Fun J et al., observed that high levels of this family were associated with low triglyceride levels and elevated high-density lipoproteins [61]. Regarding the works performed in children, it has been observed that Christensenellaceae is associated with healthy metabolic status [56]. Yuan X et al. concluded that MHO and normal-weight children showed higher levels in comparison with those MUO children [33]. Our data add evidence in children to support the hypothesis that higher proportions of Christensenellaceae could prevent the presence of metabolic alterations. It has been suggested that the relative abundance of this family is one of most associated with the host genetic variations [62] and it is commonly cited among the heritable components of the gut microbiota [63]. This, among others, considered heritable families, has been inversely associated with visceral fat and other metabolic alterations [64] suggesting a potential role of host genes to modify the relationship between their microbiome and metabolic health. Although the mechanisms are still not clearly understood, our data support the hypothesis suggesting an important role of the

Chirstensenellaceae family in the prevention of the onset of the comorbidities linked to obesity.

On the other hand, our sample of children with MUO was characterized by almost inexistent relative abundances of the genus *Akkermansia*. This genus has been associated with leanness in previous works, both in adults and children [65–68]. Beyond this, in our sample of children with obesity, lower abundances of *Akkermansia* were associated with higher metabolic risk scores and higher risk of developing one or more cardiovascular risk factors independently of body mass index.

Although our results and previous works showed associations between Christensenellaceae and *Akkermansia* and metabolic health, further investigation is needed to understand the mechanisms underlying the link between them and the development of obesity-related comorbidities. A possible mechanistic link between *Akkermansia* and metabolic health could be that associating *Akkermansia muciniphyla* spp. with acetate levels, which are known to present an important role in the prevention of weight gain because of its capacity to stimulate the release of anorexigenic peptides and its anti-inflammatory activity [69]. Regarding Christensenellaceae, it has been reported that some species are involved in bile acids metabolism [70]. Bile acids have been proposed as mediators of the interaction between gut microbiota and the host metabolism since they are important signalling molecules that could regulate several systemic functions involved in energy metabolism [71].

We cannot attribute the health effects of the microbiota to a single taxa. Our results make us hypothesize that the increased growth of *Akkermansia* and Christensenellaceae may limit the *Bacteroides'* overgrowth, and this is reflected in the higher diversity indexes. The combination of these different factors may partly explain the onset of cardiometabolic alterations in obesity. Further investigation is needed to unravel the mechanisms involved in the associations and how diet could contribute to the growth of specific bacterial taxa.

A limitation of our study is that we cannot establish a cause-effect relationship. Within this field of research, one of the already recognized challenges nowadays is to elucidate if the observed changes in the gut microbiota are the cause or the effect of the obesity-related comorbidities. To date, there are scarce pieces of evidence supporting causation in humans. However, there are methods like faecal microbiome transplants that have shown that the microbiome itself can modulate cardiometabolic health. Concretely, faecal microbiome transplants have been effective at improving glucose metabolism [72–74].

Our work could have been limited by the relatively young age of our population, given that alterations were expected to be mild. For this reason, the fact that we found differences in gut microbiota in children with metabolic unhealthy obesity provides relevance and power to our findings. The large sample of children with obesity and the fact that the detected associations between microbiota and cardiometabolic health were consistent with those previously proposed in adults, are in turn strengths of our work.

Efforts should be made to transfer these results to clinical practise. For example, the detection of certain strains and/or the evaluation of the gut microbiota diversity could potentially provide tools to tailoring interventions in children with obesity.

5. Conclusions

We found differences in the microbiota of children with healthy and unhealthy obesity, suggesting that a certain degree of dysbiosis could link obesity and cardiometabolic health in childhood. Low proportions of *Christensenellaceae*_R-7 and *Akkermansia,* higher proportions of *Bacteroides,* and lower bacterial diversity seem to be indicators of a metabolic unhealthy status in children with obesity. Further research is needed to transfer this knowledge to the prevention of the onset of comorbidities associated with obesity.

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Author's contributions

MA and VL conceived the stud. MA performed analyses. MSR, VL, and NF supported analyses. RC and JE and VL obtained resources and supervised. MA drafted the manuscript. JE, NF, RC-M, MS-R, AF, GC, and VL reviewed, edited, and agreed with the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest. The funding source had no role in the design of this study and will have no role during its execution, analyses, interpretation of the data, or in any decision to submit results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.06.007.

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