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ARTICLE

Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children

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Abstract Obesity and overweight are health problems of multifactorial etiology, which may include changes in the microbiome. In Mexico, more than 30 % of the child population between 5 and 11 years of age suffer from being overweight or are obese, which makes it a public health issue in progress. The purpose of this work was to measure the short-chain fatty acid concentration by high-performance liquid chromatography (HPLC), and to characterize the bacterial diversity by ion torrent semiconductor sequencing, of 16S rDNA libraries prepared from stools collected from a sample of well-characterized Mexican children for normal weight, overweight, and obese conditions by anthropometric and biochemical criteria. We found that triglyceride levels are increased in overweight and obese children, who presented altered propionic and butyric acid concentrations in feces. In addition, although the colon microbiota did not show a clear bacterial dysbiosis among the three conditions, the abundance of some particular bacteria was changed with respect to normal controls. We conclude from our results that the imbalance in the abundance of at least nine different bacteria as well as altered short-chain fatty acid concentration in feces is associated to the overweight and obese conditions of Mexican children.

Abbreviations

SCFAs	Short-chain fatty acids
NGS	Next-generation sequencing
nt	Nucleotides

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Introduction

Obesity is a multifactorial metabolic disease associated with a high risk to develop other chronic maladies [1]. The problem of obesity used to be limited to developed countries; however, this is no longer the case, as the obesity epidemic is now a global issue [2]. The worldwide prevalence of obesity has increased over the past three decades [3], and Mexico is now just behind the United States in experiencing the worst epidemic of this disease in the world [4]. The complications caused by overweight and obesity are the fifth leading cause of all worldwide deaths, with more than 3 million annual deaths attributed to them [5]. Almost 70 % of the adult Mexican population has excessive body weight [6], and according

to the last Mexican National Survey of Health 2012, more than 30 % of the child population between 5 and 11 years of age presents problems of overweight or obesity, which makes it an issue of public health [7].

Obesity is a process that generally starts in childhood or adolescence, and is set up by an imbalance between energy intake and energy expenditure. This disorder is also an important risk factor for the development of chronic diseases, such as type 2 diabetes and cardiovascular disease [8–10]. The basis that leads to the development of obesity shows that in addition to the genetic component of the human genome, in many cases, there is a clear influence of the human microbiome [11]. The microbiome is the full set of genes in the genomes of all microbes that live in the human body, and whose expression has an influence on its systemic function [12]. Recent reports in the mice model have shown that overweight and obesity are associated with a particular type of bacteria that inhabits the digestive tract; other studies in adult humans have shown that variations in the relative abundance of two phyla, *Firmicutes* and *Bacteroidetes*, are related to the condition of the accumulation of body fat [13].

The digestive tract is the largest immunological organ in the adult, with approximately 100 trillion bacteria living in it [14]; however, usually, there are no health problems, since this organ sustains a balanced mutualistic relationship with the commensal bacteria [15]. The proper association between bacteria and the wall of the digestive tract occurs through the interaction of bacterial structural components and metabolites, with specialized receptors in the gut [16]. The short-chain fatty acids (SCFAs), products of the anaerobic fermentation by some species of bacteria, have been used traditionally as a therapy for colitis and ulcerative colitis, due to their anti-inflammatory effect [17]. It is presumed that inhibition of the NFκB factor is involved in its mechanism of action [18]; thus, these metabolites would be related to signal transduction pathways, with an influence on the systemic inflammation state [19].

Obesity is a chronic inflammatory process of low intensity of multifactorial etiology, where a large proportion of cases might be due to a dysfunction in the genetic expression of the microbiome, affecting the systemic signaling in the human body. The microbiota colonizes the human body at birth, and there is a succession in the bacterial diversity with an abundance of lactobacilli or staphylococci according to the method of birth, to enterobacteria in about the first month, which moves to bifidobacteria and *Bacteroides* before the sixth month, and, finally, to an abundance of *Bacteroidetes* and *Firmicutes* from the age of 2 years [20].

The gut microbiota participates in the saccharification of undigested polysaccharides to easily absorbable monosaccharides, and activation of lipoprotein lipase by direct action on the villus epithelium [21], playing a role in nutrient acquisition and energy regulation by the host [22]. It has been postulated that gut microbiota contributes to obesity, by increasing energy harvesting from diet, and modulating through its metabolites, the host metabolic pathways involved in lipid metabolism and energy regulating homoeostasis [23]. To date, the main reported metabolic products of colon microbiota are acetic, propionic, and butyric acids or SCFAs [24], which can be utilized for de novo lipid or glucose synthesis [25, 26]. Alteration in the levels of SCFAs in obesity might be associated to bacterial dysbiosis in the colon, making it essential to explore the diversity of bacterial communities and the SCFAs level to comprehend its role in the development of this disease.

Culture-independent techniques to study colon microbiota have advanced very much in recent years [27]. Currently, colon microbiota profiling can be done by 16S rDNA fingerprinting using fecal microbial DNA. The reported results show that prominent phyla in the distal colon microbiota are *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* [28]. In this work, we characterized the microbial diversity found in the distal colon of overweight and obese Mexican children, and compared it to the diversity found in normal weight children by metagenomics analysis. Likewise, we measured the concentration of SCFAs in feces from the studied subjects, to characterize the metabolic activity of the bacterial community.

Materials and methods

Selection of study subjects

A total of 190 unrelated children, 9–11 years old (81 normal, 29 overweight, and 80 obese), were selected from a public primary school in the Ecatepec borough in the Greater Mexico City area. Children were classified into three groups using anthropometric, body mass index (BMI), and biochemical profile studies. Selected children were healthy and had not received any antibiotics in the immediately previous 3-month period. Informed consent was signed by parents and children in accordance with the Helsinki Declaration revised in 2000. The research protocol was approved by the Local Ethical Committee Board of Health from the Instituto Mexicano del Seguro Social R-2011–1402 1402–10, Mexico City.

Clinical evaluation

Children were weighed using a scale and measured using a stadiometer. The BMI was calculated and classified based on the World Health Organization (WHO) norms [29]. According to this, for ages ranging from 2 to 20 years, normal children have a BMI between the 10th and 85th percentiles, overweight above the 85th and up to 95th percentiles, while obese children have a BMI greater than the 95th percentile. The waist was measured at the midpoint between the lower rib and iliac crest.

Dietary diversity assessment

Diversity in the diet was estimated using a 7-days recall [30]. Information was collected from children in our database supervised by their parents. A set of seven foods/food groups diversity indicators were selected as follows: (1) starchy staples; (2) legumes; (3) dairy; (4) meat; (5) vitamin A-rich fruits and vegetables; (6) other fruits and vegetables or fruit juices; and (7) foods made with oil, fat, or butter. Foods/food groups that the child consumed ≥3 days in the previous week received a score of 1, and those that the child consumed <3 days in the past week were scored 0. A final score was calculated for each child by summing the values of all the consumed groups; thus, a score of 7 was the maximum possible value for each individual.

Biochemical studies

Two blood samples were taken with 12-h fasting, in a tube with EDTA and in a Vacutainer rapid serum tube. Glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were measured in mg/dL using an ILab 350 System [31].

Sample collection and processing

Fecal samples were collected aseptically in a sterile stool container from normal weight, overweight, and obese children. Samples were transported to the laboratory using frozen ice packs, aliquoted, and immediately stored at −70 °C until further processing. For SCFA analysis, fecal samples were dried to constant weight.

DNA extraction from feces

DNA was extracted from 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen). The quantity of purified DNA was measured at 260/280 absorbance using a NanoDrop Lite Spectrophotometer (Thermo Scientific), and the quality was evaluated by electrophoretic fractionation in 0.5 % agarose gels.

Construction of the 16S rDNA library

For each fecal DNA sample, an amplicon of approximately 263 bp including the V3 polymorphic region of the bacterial 16S rDNA was amplified using a sense V3-341F primer containing a particular 12-bp Golay barcode [32], an A adapter for massive sequencing in Ion Torrent PGM (Life Technologies), and an antisense V3-518R containing Truncated P1 (TrP1) adapters [33]. The complete list of primers used in this study is reported in Table S1 of the Supplementary Material. The thermocycler program was 5 min at 95 °C; 25 cycles of 15 s at 94 °C, 15 s at 62 °C, and 15 s at 72 °C; followed by 10 min at 72 °C. Amplification was carried out using a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems).

Ion torrent semiconductor DNA sequencing

For sequencing, equivalent amounts of amplicons (10 μg each), were combined in groups of 50 individuals, regardless of their normal, overweight, or obese phenotype. Each pool of 16S rDNA V3 libraries were fractionated by electrophoresis in 2 % agarose gels, cut, and purified using the Wizard SV Gel and PCR Clean-Up System (Promega). The DNA concentration of each library was measured by the NanoDrop Lite Spectrophotometer (Thermo Scientific). From the concentration and the average size of each amplicon library, the amount of DNA fragments per microliter was calculated using an Agilent 2100 Bioanalyzer, and libraries for each run were diluted to 26 pM prior to clonal amplification. Emulsion polymerase chain reaction (PCR) was carried out using the Ion OneTouch™ 200 Template Kit v2 DL (Life Technologies), according to the manufacturer's instructions. Amplicon enrichment with ion spheres was done using Ion OneTouch ES. The sequencing was done using the Ion 316 Chip Kit v2 and the Ion Torrent PGM System. After sequencing, reads were filtered by the PGM software to remove low quality and polyclonal sequences. During this process, sequences matching the 3′ adapter were automatically trimmed and filtered.

Analysis of sequenced data for microbial diversity

Ion Torrent PGM software, Torrent Suite v4.0.2, was used to demultiplex the sequenced data based on their barcodes in normal, overweight, and obese phenotypes. Poor quality reads were eliminated from the datasets, i.e., quality score ≤20, containing homopolymers >6, length <200 nt, and containing errors in primers or barcodes. Filtered data were exported as FASTQ files. Demultiplexed sequencing data were analyzed using QIIME software v1.8.0 pipeline [34]. FASTQ files were converted into FASTA files, and all demultiplexed files were concatenated into a single file. Closed reference operational taxonomic units (OTUs) were determined at the 97 % similarity level using the UCLUST algorithm [35]. Chimeras were detected and removed from the datasets using the ChimeraSlayer [36]. Sequence alignments were done against the Greengenes core set [37].

Statistical analysis

The anthropometric characteristics were statistically analyzed using Chi-square, analysis of variance (ANOVA), and Kruskal–Wallis one-way ANOVA. Microbial diversity was assessed through beta diversity. The beta diversity analysis was calculated using UniFrac analysis [38], using a phylogenetic tree computed with the FastTree program and rarefied

OTUs tables in biom format as input. The abundance of the bacterial groups at different taxonomic levels (phylum, family, and genus) was separately explored by principal component analysis (PCA) and unweighted pair group method with arithmetic mean (UPGMA) clustering.

Measurement of SCFAs concentration by HPLC

Fecal samples to be analyzed for SCFAs content were dried to constant weight and subsequently processed using the solid-phase extraction method to analyze via high-performance liquid chromatography (HPLC; Agilent Technologies 1260). 100 mg of dried feces were suspended completely in 1 mL of deionized water by vigorous vortexing at maximum speed for 5 min. The suspension was centrifuged at 15,800 rcf for 5 min, the supernatant was transferred to a fresh tube, and the pH was adjusted to 6 using 0.1 M HCl. This solution was passed through activated C-18 max 100 mg/1 mL GracePure™ Reversed-Phase SPE Columns. The SCFAs were eluted using 1 mL 100 % absolute ethanol and analyzed via HPLC [39], using 0.1 M Glycine-HCl as the mobile phase. Methimazole at 10 mM was used as the internal standard.

Results

Triglycerides levels are increased in Mexican overweight and obese children

A total of 190 children aged 9 to 11 years were selected from the database and classified into normal weight (n=81), overweight (n=29), and obese (n=80) phenotypes based on WHO norms. The anthropometric data showed a significant difference in weight and height; the statistical analysis indicated that the BMI was increased in overweight and obese children with respect to normal (p<0.001). The biochemical studies revealed that the bloodstream triglycerides levels were significantly elevated in overweight and obese children (p=0.0001). The fasting glucose levels were below 100 mg/dL in the normal, overweight, and obese children, and there was no difference among them (p=0.192); likewise, there was no difference in the LDL (p=0.246) and HDL levels (p=0.104). Moreover, although the concentration of bloodstream cholesterol was above 170 mg/dL for overweight and obese children, the difference with respect to normal was not significant (p=0.194) (Table 1). We explored the dietary diversity in all children using a 7-days recall study as described in the Materials and methods section, without finding a significant difference among the three groups that could explain the increase in triglycerides levels we observed (Table 2).

Overweight and obese children present altered propionic and butyric concentration in feces

The SCFA concentration (butyric, propionic, and acetic) was measured in feces by HPLC as described in the Materials and methods section. We found that feces collected from overweight and obese children contained a significantly lower butyric acid concentration compared to normal weight children (p=0.023) (Fig. 1). In the case of propionic acid, the feces of obese children had a significantly lower concentration in comparison to overweight (p=0.025) children, and at the same time, the concentration of propionic acid in obese children was lower than the concentration found in normal children (p=0.048). Conversely, there was no difference in the concentration of acetic acid among the three groups (Fig. 1).

Colon microbiota in Mexican overweight and obese children does not show a clear imbalance in the bacterial diversity

We next characterized the relative abundance of dominant bacterial phyla in feces collected from normal, overweight, and obese Mexican children. The bacterial diversity of 16S rDNA libraries was determined by ion semiconductor DNA sequencing, and the data were processed as described in the Materials and methods section. The results showed a lower abundance of the *Proteobacteria* phylum in overweight (8 %) and obese (4 %) children compared to the normal control (10 %) children, and a slight increase in the abundance of *Firmicutes* in overweight (52 %) and obese (50 %) children compared to normal weight (46 %); however, the differences were not of statistical significance. There was also no difference in the percentage of abundance of the *Actinobacteria* and *Bacteroidetes* phyla among the groups (Fig. 2).

The abundance of particular bacteria is altered in overweight and obese Mexican children with respect to normal controls

When we examined for particular genera or families whose abundance changed in association with the phenotypic condition, we found that two genera and one family increased in overweight and obese children: the genus *Faecalibacterium* sp. (p=0.042) (Fig. 3b), the family Lachnospiraceae (p=0.018) (Fig. 3d), and the genus *Roseburia* sp. (p=0.015) (Fig. 3i). In addition, the analysis of the OTUs data showed a decrease in the genus *Succinivibrio* sp. (p=0.003) (Fig. 3a), the genus *Erwinia* sp. (p=0.003) (Fig. 3e), and the genus *Oscillospira* sp. (p=0.024) (Fig. 3g); however, the abundance of the last genus was lower in the overweight phenotype compared to the obese phenotype. Besides, the genus *Blautia* sp. (p=0.036) (Fig. 3c), the genus *Coprococcus* sp. (p=0.023) (Fig. 3f), and the family Enterobacteriaceae (p=0.030) (Fig. 3h) were clearly increased in the overweight phenotype.

Table 1 Clinical characteristics of children in the study

Characteristics	Normal	Overweight	Obese	*p*-Value
Number of children	*n*=81 (42.6 %)	*n*=29 (15.3 %)	*n*=80 (42.1 %)	nd
Female (%)	57.3	62.1	52.5	0.641[a]
Age (years)	9.85±0.83	9.86±0.92	10.04±0.85	0.352[b]
Anthropometric				
Weight (kg)	31.68±5.10	38.13±3.94	46.48±6.94	<0.001[c]
Height (cm)	1.37±0.06	1.39±0.06	1.41±0.06	0.020[b]
BMI (kg/m^2)	16.77±1.74	19.82±1.02	23.40±2.78	<0.001[c]
BMI (percentile)	51.08±25.47	89.12±2.89	97.82±3.67	<0.001[c]
BMI	<85 pc	≥85 pc and ≤95 pc	>95 pc	nd
Metabolic factors				
Fasting glucose (mg/dL)	96.09±10.33	96.57±7.73	93.79±8.25	0.192[b]
Triglycerides (mg/dL)	97.83±38.36	111.76±43.41	134.18±49.47	0.0001[b]
Total cholesterol (mg/dL)	166.70±27.32	175.28±25.15	174.95±36.72	0.194[b]
HDL (mg/dL)	51.94±10.45	49.53±9.60	48.41±11.06	0.104[b]
LDL (mg/dL)	95.22±23.41	103.44±22.14	99.68±34.64	0.246[c]

BMI body mass index; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein; *pc* percentile; *nd* not determined

Data are means ± standard deviation. *p*-Values were calculated according to: [a] Chi-square test, [b] ANOVA test for equal variances, and [c] Kruskal–Wallis test for different variances. $p<0.05$ is considered statistically significant

Discussion

In this work, we studied well-characterized individuals selected from a cohort investigating overweight and obesity in Mexican children, measuring their anthropometric characteristics, biochemical profiles and the concentration of SCFAs, and the bacterial diversity of the microbiota found in stool samples.

In recent years, the impact of colon microbiota in human health has been widely recognized, and current reports mainly focus on exploring the association of colon microbiota to obesity development [40, 41]. It has been reported that the relative abundance of two phyla, *Bacteroidetes* and *Firmicutes*, play a vital role in the development of obesity and type 2 diabetes [42, 43]. These two bacterial phyla include members capable of producing SCFAs from otherwise undigested carbohydrate fibers in the distal colon, thus providing additional energy from diet. It is believed that some diseases and health disorders are associated to an imbalance in the microbial community, obesity being a well-known example [44].

The colonic epithelium receives about 70 % of its energy from SCFAs, mainly from butyric acid [45]. Propionic acid, on the other hand, is a precursor for protein synthesis, gluconeogenesis, and liponeogenesis in the liver [46, 47]. Acetic acid, another SCFA, is a substrate for cholesterol synthesis [48], as well as a suppressor of appetite through a central hypothalamic mechanism [49]. In our work, the overweight and obese children showed altered propionic and butyric acid concentrations, with the butyrate concentration in feces being significantly reduced in obese children in comparison to normal weight children. The decrease in SCFAs concentration observed in feces might be explained, for instance, by a general dysbiosis in the microbial population causing a lower production or a higher mucosal absorption. Although we did not observe a significant dysbiosis in the bacterial population in feces in overweight and obese children (Fig. 2a), the abundance of some key members for SCFA production can be compromised. It has been reported that SCFAs levels are predominantly linked to changes in the distal colon bacterial diversity [50, 51]. Additionally, an increase in SCFAs

Table 2 Dietary diversity of the study children by phenotypic classification

	Mean diversity score (range 0–7)	% with low diversity, 0–2 food groups	% with middle diversity, 3–4 food groups	% with high diversity, 5–7 food groups
Normal	2.8	41.3	39.1	19.6
Overweight	2.5	53.3	40.0	6.7
Obese	2.8	44.7	36.2	19.1

Food groups: (1) starchy staples; (2) legumes; (3) dairy; (4) meat; (5) vitamin A-rich fruits and vegetables; (6) other fruits and vegetables or fruit juices; and (7) foods made with oil, fat, or butter [30]

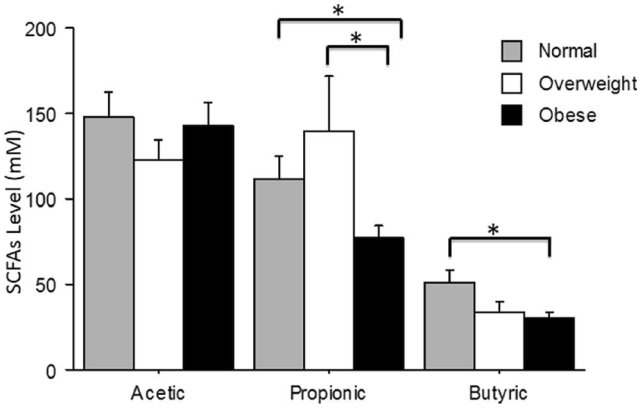

Fig. 1 Short-chain fatty acid (SCFA) concentration in feces. The SCFA concentration in feces was measured as described in the Materials and methods section. Data are shown as: normal weight children, *gray bars*; overweight children, *white bars*; and obese children, *black bars*. The *error bars* represent mean ± standard error. The *asterisks* indicate statistically significant differences in data ($p<0.05$). *y*-axis, SCFA concentration in mM; *x*-axis, SCFA and phenotypic categories

absorption, occurring in parallel, could explain the increase in triglycerides levels observed in the overweight and obese children in our study.

The current concept supports that the microbial biome in the gut is somehow stable, and the chromosomal genetic inheritance in the individual selects the gut microbial diversity, although environmental factors may fine-tune the diversity [52]. In such a sense, it has been reported that the human gut microbiome may be altered by long- or short-term diet changes, regardless of whether it is a herbivorous or carnivorous regimen. Particularly, an animal-based diet stimulates the gut microbiota to induce the secretion of inflammatory molecules by the host, which could be associated to disorders such as obesity and metabolic syndrome [53].

The next-generation sequencing (NGS) technology has greatly advanced the studies on human microbiota. The data on bacterial diversity characterized in bacterial ribosomal V3-16S rDNA libraries by massive sequencing in our work showed that the phylum *Proteobacteria* was relatively more abundant in normal weight children in comparison to overweight children, and more abundant in overweight than in obese children. Moreover, the phylum *Firmicutes* was less abundant in normal weight than overweight and obese children. It is remarkable to find that the phylum *Bacteroidetes* was decreased in overweight but increased in obese children in comparison to normal weight children. It cannot be concluded from our data whether the differences in the proportion of the mentioned phyla for each phenotypic category are the result of the low intensity inflammatory condition occurring in the gut of overweight and obese children or if changes in the bacterial communities play a role in the development of this condition. It could also be argued that a particular diet regime in the overweight and obese Mexican individuals may favor, for instance, the disappearance of key members of the phylum *Proteobacteria*, whose functional activity producing metabolites is related to maintaining a healthy gut. However, we did not find a significant difference in the diet diversity as indicated by the 7-days dietary diversity study performed in this work. Our study is one of the first reported metagenomics characterizations of the gut microbial communities in Mexican children, and the reported abundances may be related to the particular genetic background found in Mexicans.

We did not find a clear general dysbiosis in the bacterial communities, but a closer view of our sequencing data revealed that the abundance of particular bacteria was changed in overweight and obese children compared to the normal controls. In this manner, when we look at the genera level, the proteobacteria *Succinivibrio* sp. was more abundant in normal weight children. It has been proved that *Succinivibrio* sp. plays a role in regulating the energy produced by SCFAs in swine [54]; one possibility is that a decrease in the abundance of this genus deregulates the SCFAs production in the gut, reducing particularly the production of butyrate. In addition, we found a greater abundance of *Erwinia* sp., a well-known phytopathogen in normal weight compared to overweight and obese children. It has been reported that this bacteria can produce ketoaldonic acid from sugars [55], which is a precursor for SCFAs. In the case of the genus *Oscillospira* sp., a metagenomics study carried out on monozygotic twins revealed that the BMI negatively correlated with the abundance of this genus [56], which is in agreement with our results (Fig. 3g). We found that *Oscillospira* sp. is more abundant in lean children with a lower BMI (Table 1).

For the phylum *Firmicutes*, *Faecalibacterium* sp. was the most abundant genus among obese children in our study. This genus has a high capacity for energy extraction from undigested carbohydrate fibers in the distal colon; a similar result has been reported in obese Indian children [57]. We also observed an increase in the abundance of *Roseburia* sp., a saccharolytic, butyric acid-producing bacteria, among overweight and obese children in our study. A decrease in the abundance of *Roseburia* sp. was observed in parallel to a reduction in body weight of obese Spanish adolescents [58]. It was reported that the family Lachnospiraceae became more abundant along with an increase in body weight of mice fed with high-fat diet in comparison to low-fat diet [59]. This result agrees with our findings. In another work, the family Lachnospiraceae colonizing germ-free *ob*/*ob* mice induced an increase in the blood glucose concentration and an increase in the liver and mesenteric adipose weight, suggesting that this bacteria has a role in the development of obesity and diabetes [60].

In overweight children, we found a significant increase in the abundance of *Blautia* sp., *Coprococcus* sp., and the family Enterobacteriaceae. The family Enterobacteriaceae includes Gram-negative bacteria whose lipid A portion of the lipopolysaccharide induces

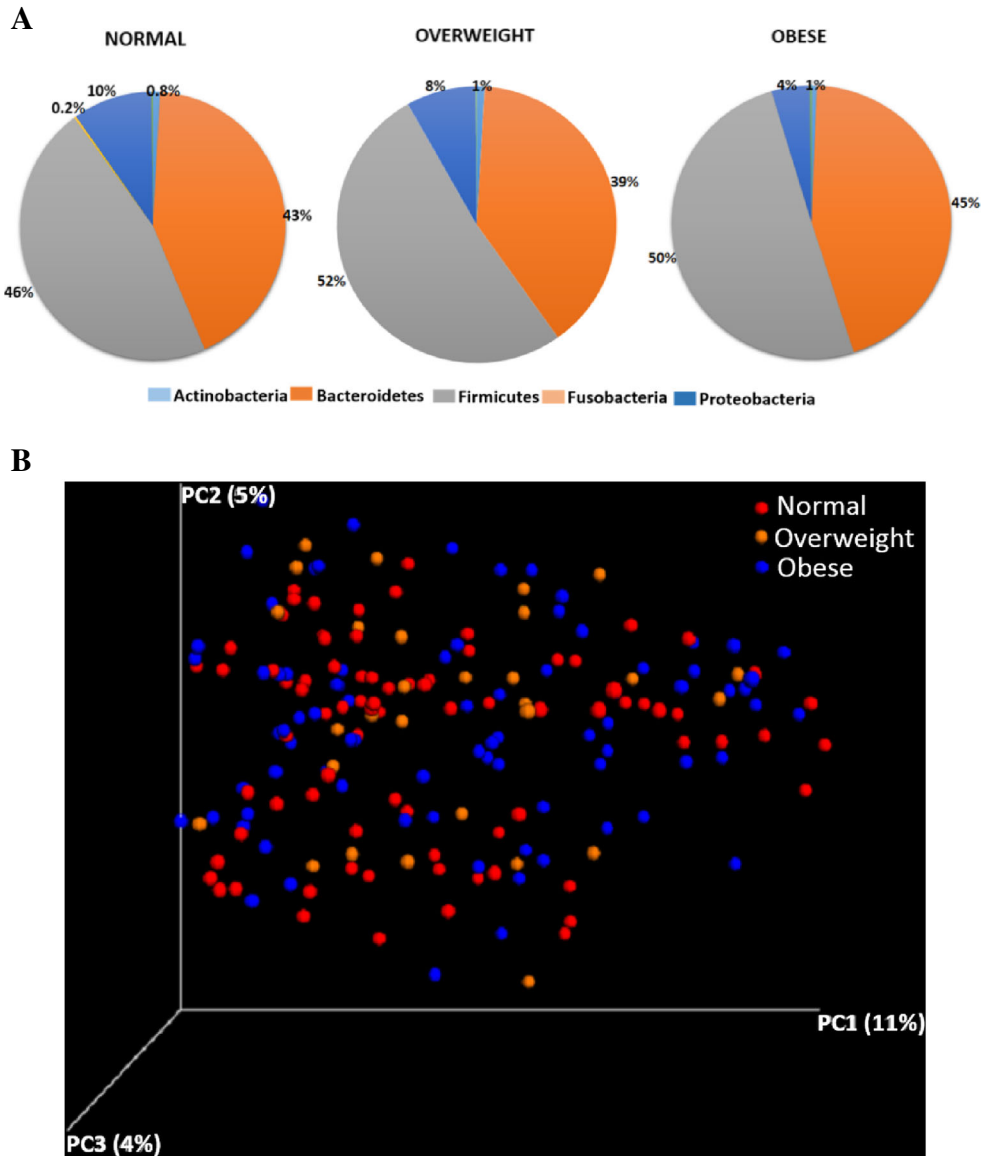

Fig. 2 Relative abundance of dominant bacterial phyla in normal, overweight, and obese Mexican children. **a** Combined distribution of bacteria at the phylum level present in feces collected from normal weight (n=81), overweight (n=29), and obese (n=80) Mexican children. The bacterial diversity of 16S rDNA libraries was determined by ion semiconductor DNA sequencing and data processed as described in the Materials and methods section. Each pie diagram represents the diversity of the phenotypic category indicated at the top. The phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria* phyla are indicated by the different colors, as shown at the bottom of the charts. The relative abundance (%) of phyla in each category is indicated beside each color. **b** Unweighted UniFrac analyses were used to calculate distances between samples obtained from normal, overweight, and obese children, and three-dimensional scatter plots were generated by using principal coordinates analysis (PCoA)

proinflammatory activity in the host [61]; *Blautia* sp. was reported to induce high-fat diet obesity in a gnotobiotic rat model [62] and *Coprococcus* sp. was also reported to be more abundant in rats fed a high-cholesterol diet [63]. The proliferation of these bacteria might favor the sustained low-intensity inflammatory condition of the gut, an important trait occurring in overweight and obese children. The increase of *Coprococcus* sp. may also be the explanation for the rise in propionic acid concentration found in feces obtained from overweight children (Fig. 1); it has been revealed that this genus produces abundant propionic acid in the human gut [64]. Even though *Coprococcus* sp. is abundant in obese children (Fig. 3f), the decrease in propionic acid concentration may be the consequence of proliferation of another bacteria in the gut, which transforms this acid to a different metabolite.

From this study, we conclude that, in Mexican obese children, the distal colon microbiota has a higher abundance of *Faecalibacterium* sp., Lachnospiraceae, and *Roseburia* sp. from the *Firmicutes* phylum. It is believed these bacteria extract a great extent of energy from undigested fiber, affecting positively the energy balance. Thus, it is plausible that this effect in the energy balance, along with other factors such as chromosomal inheritance in children, environment, and diet, will shape together to develop obesity. On the other hand, in normal weight children, there are commensal bacteria such as *Oscillospira* sp. and *Succinivibrio* sp. helping to regulate the energy balance.

A study on the role of gut microbiota in obese preschool children in Sweden reported a significant increase in the family Enterobacteriaceae and a significant decrease in *Desulfovibrio* sp. and *Akkermansia* sp. in comparison to

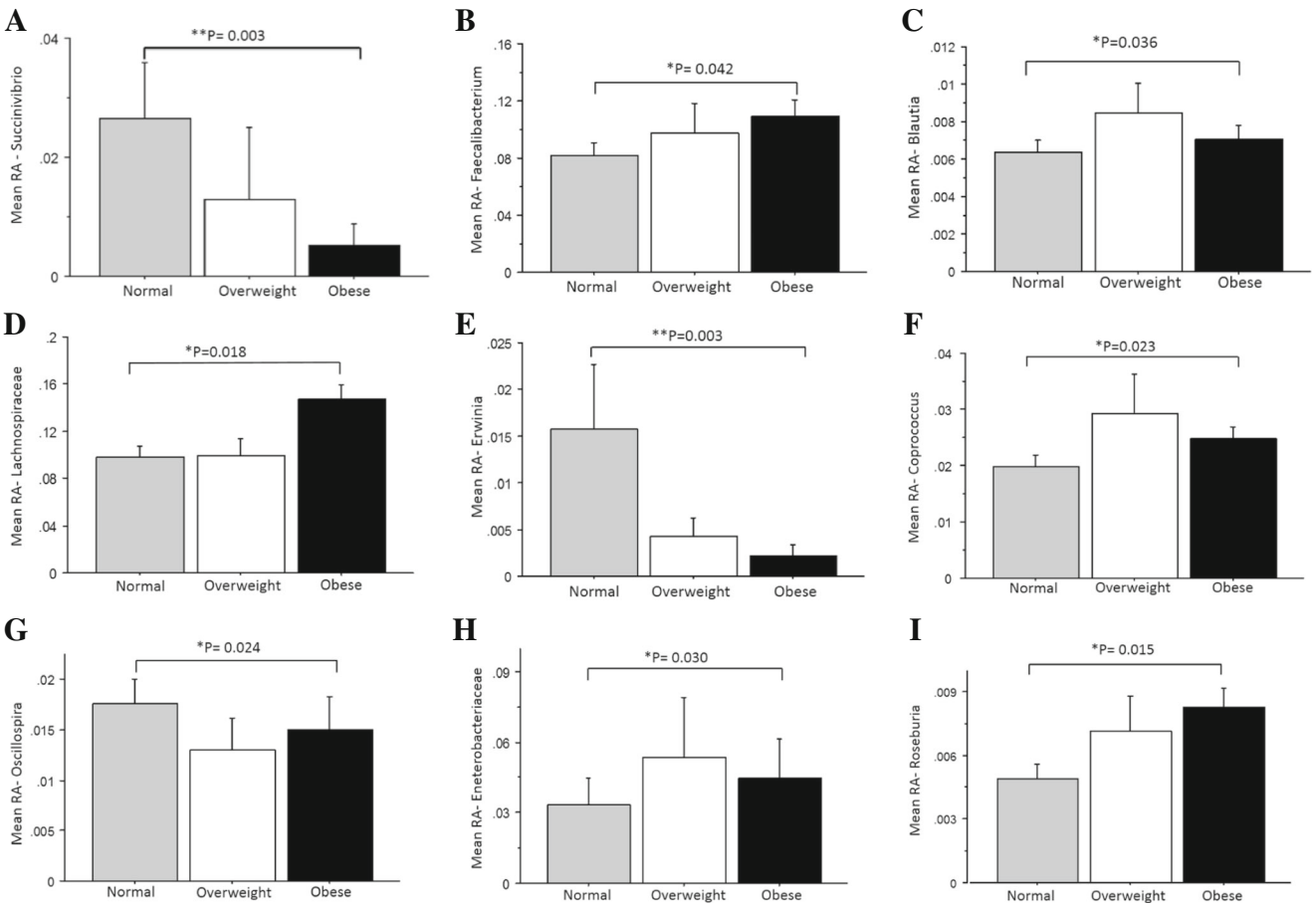

Fig. 3 Relative abundance of particular bacteria in normal, overweight, and obese Mexican children. The histograms show the relative abundance of nine major bacterial genera found in feces from the normal weight (n=81), overweight (n=29), and obese (n=80) Mexican children in the study. Metagenomics analysis was done as described in the Materials and methods section. Data are shown as: normal weight children, *gray bars*; overweight children, *white bars*; and obese children, *black bars*. The *error bars* represent mean ± standard error. *p<0.05, **p<0.01. *y*-axis, relative abundance (RA) of each OTU; *x*-axis, phenotypic categories. **a** Genus *Succinivibrio* sp.; **b** genus *Faecalibacterium* sp.; **c** genus *Blautia* sp.; **d** family Lachnospiraceae; **e** genus *Erwinia* sp.; **f** genus *Coprococcus* sp.; **g** genus *Oscillospira* sp.; **h** family Enterobacteriaceae; **i** genus *Roseburia* sp.

normal weight children [65]. Another work correlating the abundance of *Bacteroidetes* and *Firmicutes* in association with obesity in school children from Kazakhstan revealed a significant decrease in *Bacteroidetes*, but no significant difference was found for *Firmicutes* in the stool of obese girls [66]. In Egypt, it has been revealed that, for normal and obese children, as well as adults, the abundance of both phyla *Bacteroidetes* and *Firmicutes* significantly increased in the obese group [67]. The particular features found in the bacterial diversity of Mexican children associated to obesity might be due to special aspects of the human genome found in Mexicans, which is clearly different to the genetic background of other world populations [68, 69]. In the future, the drawing of definite conclusions on the importance of various bacterial groups associated to obesity should take into account parameters such as diet, genetic background, environment, and overall fitness in order to understand the definite role of colon microbiota.

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Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The authors declare that all procedures were performed in accordance with the ethical standards approved by the Local Ethical Committee Board of Health from the Instituto Mexicano del Seguro Social R-2011–1402 1402–10, Mexico City, and with the Helsinki Declaration revised in 2000.

Informed consent The authors declare that informed consent was signed by all parents and children in accordance with the Helsinki Declaration revised in 2000.

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