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Composition of gut microbiota in obese and normal-weight Mexican school-age children and its association with metabolic traits

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Summary

Background: Childhood obesity is a serious public health problem in Mexico. Adult gut microbiota composition has been linked to obesity, but few studies have addressed the role of gut microbiota in childhood obesity.

Objectives: The aim of this study is to compare gut microbiota composition in obese and normal-weight children and to associate gut microbiota profiles with amino acid serum levels and obesity-related metabolic traits.

Methods: Microbial taxa relative abundance was determined by 16S rRNA sequencing in 67 normal-weight and 71 obese children aged 6–12 years. Serum amino acid levels were measured by mass spectrometry. Associations between microbiota composition, metabolic parameters and amino acid serum levels were tested.

Results: No significant differences in phyla abundances or *Firmicutes*/*Bacteroidetes* ratios were observed between normal-weight and obese children. However, *Bacteroides eggerthii* abundance was significantly higher in obese children and correlated positively with body fat percentage and negatively with insoluble fibre intake. Additionally, *Bacteroides plebeius* and unclassified *Christensenellaceae* abundances were significantly higher in normal-weight children. Abundance of both these species correlated negatively with phenylalanine serum levels, a metabolite also found to be associated with obesity in Mexican children.

Conclusions: The study identified bacterial species associated with obesity, metabolic complications and amino acid serum levels in Mexican children.

Keywords: Amino acids, gut microbiota, Mexican children, obesity.

Introduction

Obesity is a major health problem worldwide. In Mexico, overweight and obesity affect 72.5% of the adult population and 33.2% of school-age children (1). Childhood obesity is associated with metabolic complications including dyslipidaemia and type 2 diabetes, and up to one-half of obese children are expected to become obese adults (2). Understanding of the factors involved in the pathophysiology of this disease is therefore crucial.

The adult gut microbiota is known to deeply impact host metabolic processes. Over the last decade, it has emerged as a potential contributor to obesity

and metabolic traits (3). Early gut microbiota studies suggested that *Firmicutes*/*Bacteroidetes* ratio was higher in obese individuals (4). However, later studies failed to confirm this finding (5). Other studies have reported decreased gut microbial diversity in individuals with obesity and metabolic complications (6,7). Interestingly, abundance of certain bacterial species have recently been associated with high levels of aromatic and branched-chain amino acids (BCAA) (8,9), both linked to obesity and insulin resistance (10).

Information on gut microbiota composition and function in children remains limited (11,12), and its association with amino acid serum levels in childhood obesity is unknown. The purpose of this study was therefore to evaluate differences in gut microbiota composition of obese and normal-weight (NW) Mexican school-age children and to test whether these differences are also associated with amino acid serum levels and obesity-related metabolic traits.

Materials and methods

Study population

We recruited 67 NW and 71 obese unrelated children (80 boys and 58 girls), aged 6–12 years from a summer camp for children of Mexican Health Ministry employees. Exclusion criteria included recent weight lost >10% of body weight, antibiotic treatment and the occurrence of diarrhoea or acute gastrointestinal illness 3 months prior to recruitment. The study was approved by the Ethics Committee of participant institutions: Instituto Nacional de Medicina Genómica and Hospital Infantil de México. The parents or guardians of each child signed the informed consent form for participation, and all children assented to participate.

Anthropometric and clinical parameters

Anthropometric parameters, blood pressure and body fat mass percentage (BF%) were measured following standardized procedures, as previously described (13). Obesity was defined as body mass index (BMI) ≥95th percentile, whereas NW was defined as BMI between the 15th and 75th percentiles for age and gender based on Centers for Disease Control and Prevention (CDC) reference data (14).

Dietary records

A semi-quantitative food frequency questionnaire previously validated for the Mexican population was answered by parents to estimate each child's dietary intake over the previous year. This instrument includes 116 food items (15). Average daily energy and nutrient intake was computed through the EVALUATION SYSTEM OF NUTRITIONAL HABITS AND NUTRIENT INTAKE software. Only 65 of the 138 recruited children delivered fully answered food frequency questionnaires and were thus considered for diet analysis (25 NW and 40 obese).

Blood sampling, biochemical and metabolite analyses

Blood samples of 5 mL were drawn after 8–12 h of fasting. Serum biochemical and amino acid analyses were carried out as previously described (13). Insulin sensitivity was estimated with the homeostasis model assessment for insulin resistance (HOMA-IR). Insulin resistance was defined as a HOMA-IR ≥3.4, a cut-off previously used in Mexican children (16).

Stool sampling and DNA extraction

The parents of all participants were asked to collect the stool samples at home and to place the samples in a sterile plastic container. Samples were refrigerated at home and transported to the research facility within 12 h after collection in a cooler with ice packs. All samples were received at the research facility in the early morning; 200-mg aliquots were made and stored at −70 °C. DNA was extracted from 200 mg of faeces using the QIAamp® DNA Stool Mini Kit (Qiagen, Inc.; Hilden, Germany). DNA was eluted in a final volume of 200 μL and stored at −20 °C.

Sequencing of 16S rRNA and data analyses

The V4 hypervariable region was amplified using 515F and 806R primers, as suggested by the Earth Microbiome Project (17), and sequenced using an Illumina MiSeq 2 × 250 platform. Sequences were analysed using QIIME 1.8.0. (18). A full description of 16S rRNA sequencing is provided in the Supporting Information.

Statistical analyses

All statistical analyses were performed using R statistical package. Microbial relative abundance differences between obese and NW children were evaluated with the Mann–Whitney *U*-test. Differences in operational taxonomic unit (OTU) enrichment between groups were tested using the DESeq2 algorithm that corrects for varying sequencing depths (19). Spearman's correlation coefficients between microbial relative abundance and both BMI percentile and BF% were estimated. Correlations with BF% were adjusted for age and gender. Hochberg false discovery rate (FDR)-adjusted q-values <0.05 were

considered significant (20). Afterwards, the presence/absence of the species whose abundance was found to be associated with obesity was further tested for association with obesity using a chi-squared test with Haldane's correction, when appropriate. A full description of statistical analyses is provided in the Supporting Information.

Analysis in an independent cohort: We then analysed the gut microbial dataset from 279 NW (BMI < 25 kg m^{-2}) and 130 obese (BMI ≥ 30 kg m^{-2}) subjects from the TwinsUK population (http://qiita.microbiome.me; Study ID: 2014, (21)). One of each pair of twins was randomly selected. For cases with more than one faecal sample, data from the first sample were used. Association with obesity was tested for the three species found to be associated with obesity in our study using Mann–Whitney *U*-tests. Statistical significance was set as $p < 0.05$.

Results

Anthropometric and biochemical characteristics of the study population are shown in Table S1. As expected, all obesity-related anthropometric variables were statistically different between NW and obese children. With the exception of high-density lipoprotein cholesterol levels, values for all biochemical parameters were higher in obese children, although the differences were not significant in all cases. Food frequency questionnaire analysis showed no significant differences in total energy, macronutrient or fibre intake between obese ($n = 40$) and NW ($n = 25$) children (Table S2). Metabolomic profiling revealed that BCAA leucine and valine as well as aromatic amino acids phenylalanine and tyrosine were significantly elevated in obese children (Table S3).

Gut microbiota richness and diversity

Faecal DNA sequencing resulted in a total of 20.7 million high-quality reads (mean 150 614, and range 68 925–258 723). After filtering low-abundance OTUs, the mean number of OTUs was 150.11 ± 2.28 per sample. Richness and alpha diversity (observed species and Shannon index) were not significantly different between obese and NW children (Fig. 1a). However, the Shannon index showed a negative correlation with HOMA-IR, alanine transaminase (ALT) activity, serum insulin and uric acid levels (Table S4). Moreover, microbial diversity was significantly lower in insulin-resistant children in the entire sample ($p = 0.017$, Fig. 1b). Interestingly, this difference remained significant when analysing only obese children ($p = 0.014$, Fig. 1c).

Association of gut microbiota composition with obesity and diet

The most abundant phylum in NW and obese children was *Bacteroidetes* (67.5% and 69.4%, respectively) followed by *Firmicutes* (27.8% in NW and 26% in obese children) and *Proteobacteria* (3.4% in NW and 3.5% in obese children) (Fig. 2). No significant differences were observed in the *Firmicutes*/*Bacteroidetes* ratio ($p = 0.656$). FDR-adjusted differences from phyla to genus level did not reach statistical significance.

However, at specie level, three taxa with mean relative abundance in the whole sample ranging from 0.4% to 2% where differentially enriched (Table S5). The relative abundance of *Bacteroides eggerthii* was significantly higher in obese than in NW children ($q = 0.004$), while *Bacteroides plebeius* was more abundant in NW than in obese children ($q = 0.046$). Interestingly, the relative abundance of an unclassified species of the *Christensenellaceae* family was significantly increased in NW children but had borderline significance after FDR correction ($p = 0.003$, $q = 0.061$) (Table 1). Global OTU level analysis showed that *B. plebeius* OTU 187751 and *Christensenellaceae* OTU 325254 were the most enriched OTUs in NW children, while *B. eggerthii* OTU 178253 was the most enriched in the obese group (Table S6).

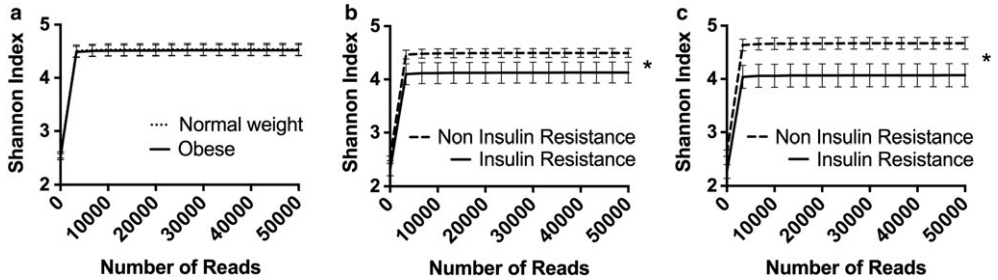

Figure 1 Rarefaction curves comparing gut microbiota diversity by obesity status (a), insulin resistance status in the whole sample (b) and insulin resistance status only in obese children ($n = 71$) (c) *p value <0.05.

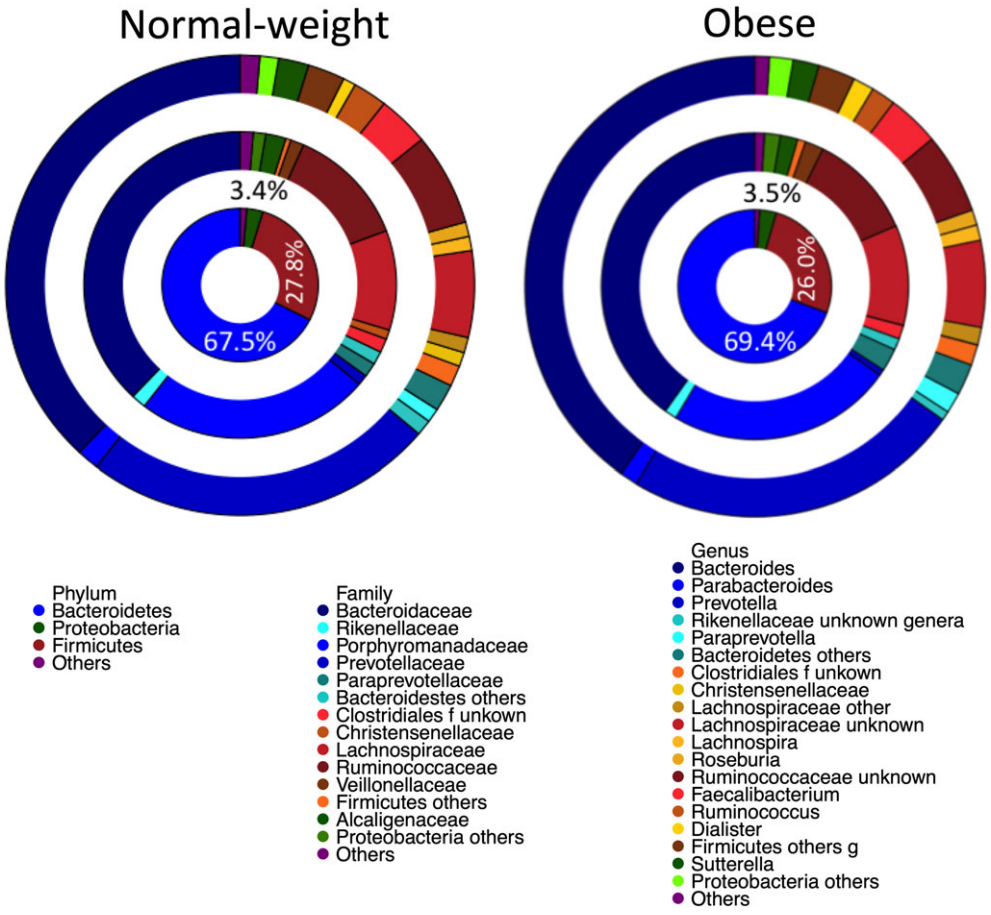

Figure 2 Gut microbiota composition in normal-weight and obese children. The innermost ring shows composition at phylum level; the middle ring shows composition at the family level and the outermost ring at the genus level. No significant differences were observed between normal-weight and obese children. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 Relative abundance and presence/absence (P/A) of bacteria species associated with obesity

	Normal weight		Obese			
Taxa	Relative abundance	P/A	Relative abundance	P/A	q^*	p^{**}
Bacteroides eggerthii	0.124	45/22	0.955	71/0	0.004	<0.0001
Bacteroides plebeius	2.915	66/1	1.014	67/4	0.046	0.670
Unclassified						
Christensenellaceae	0.991	18/49	0.001	7/64	0.061	0.017

*Comparison of relative abundance between normal-weight and obese children, false discovery rate corrected.
**Comparison of presence/absence of species between normal-weight and obese children.

Because associations of gut microbiota with obesity can vary with gender, we performed a gender-stratified analysis of *B. plebeius*, unclassified *Christensenellaceae* and *B. eggerthii* abundances. The same differences for the three species remained significant in boys, while in girls, only *B. eggerthii* abundance remained significantly lower in the NW group (p = 0.008), and the difference in *B. plebeius* abundance reached borderline significance (p = 0.055). Additionally, the global gender-stratified species analysis identified the genus *Odoribacter* as significantly more abundant in NW (median = 0.396 [0.199–0.733]) than in obese girls (median = 0.136 [0.051–0.278], [q = 0.04]).

Bacteroides eggerthii and unclassified *Christensenellaceae* were not found in 15.9%

and 81.8% of the total study sample, respectively. We thus evaluated the association of the presence/absence rather than abundance of these species with obesity. Interestingly, the 22 children with undetected *B. eggerthii* belonged to the NW group; 15 of which (68%) had detectable unclassified *Christensenellaceae***.** The presence of *B. eggerthii* was associated with obesity ($p < 0.001$), while the presence of the unclassified *Christensenellaceae* species was associated with lower risk ($p = 0.011$) (Table 1). In addition*, B. eggerthii* abundance showed a positive and significant correlation with BMI percentile ($r = 0.362$, $q = 0.001$), while unclassified *Christensenellaceae* correlated negatively with BMI percentile ($r = -0.269$, $q = 0.030$). *B. plebeius* showed a negative correlation with BMI percentile but lost significance after FDR correction ($r = -0.224$, $p = 0.008$, $q = 0.115$). Moreover, only these three species were found significantly correlated with BF% ($r = 0.465$, $q = 2 \times 10^{-6}$ for *B. eggerthii*; $r = -0.351$, $q = 0.001$ for unclassified *Christensenellaceae*; and $r = -0.263$, $q = 0.042$ for *B. plebeius*; Table S7).

To determine whether dietary macronutrients and fibre are associated with relative abundance of these three obesity-associated bacteria, we performed correlation analyses in a subgroup of 65 children. After adjusting for total energy intake and age, only *B. eggerthii* showed a negative correlation with insoluble fibre intake ($r = -0.307$, $p = 0.014$; Table S8).

Association of obesity-related bacterial species with metabolic parameters and circulating amino acids

After adjusting for BMI percentile, only unclassified *Christensenellaceae* abundance showed a negative and significant correlation with lipid traits (total cholesterol and Apo B levels, $r = -0.187$, $p = 0.029$ and $r = -0.242$, $p = 0.008$, respectively) and a negative and significant correlation with gamma glutamyl transpeptidase (GGT) ($r = -0.183$, $p = 0.03$). Interestingly, *Odoribacter* correlated negatively with HOMA-IR ($r = -0.267$, $p = 0.002$) and insulin levels ($r = -0.259$, $p = 0.002$).

Regarding host serum amino acid levels, *B. eggerthii* abundance correlated negatively with citrulline ($r = -0.184$, $q = 0.037$), while *B. plebeius* and unclassified *Christensenellaceae* abundances correlated negatively with phenylalanine serum levels ($r = -0.176$, $q = 0.045$ and $r = -0.178$, $q = 0.044$; respectively). Unclassified *Christensenellaceae* abundance was also negatively correlated with arginine ($r = -0.203$, $q = 0.021$), methionine ($r = -0.184$, $q = 0.037$) and ornithine ($r = -0.280$, $q = 0.001$).

Gut microbiota functional analysis

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (Picrust) analysis was used to predict functions encoded in the genomes of the gut microbiota. The analysis identified 240 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, but no significant differences in predicted abundance of these pathways between NW and obese children were observed. In addition, no significant correlations with obesity phenotypes (BMI percentile, %BF and waist circumference) or insoluble dietary fibre were found ($q < 0.05$). Because previous studies have consistently associated dietary fibre with serum lipopolysaccharide (LPS) levels (22), we sought whether LPS pathways were nominally associated with this dietary component. Interestingly, nominal inverse correlations between insoluble fibre intake and abundance of predicted genes for LPS biosynthesis ($r = -0.325$, $p = 0.009$) and LPS biosynthesis proteins ($r = -0.319$, $p = 0.010$) were observed. Interestingly, these pathways also showed a positive correlation with HOMA-IR ($r = 0.178$, $p = 0.038$ and $r = 0.173$, $p = 0.044$, respectively).

Discussion

The present study aimed to find differences in gut microbiota composition between NW and obese Mexican children. Abundance of phyla and *Firmicutes*/*Bacteroidetes* ratio showed no significant differences between groups. However, at the species level, *B. eggerthii* abundance was significantly higher in obese children, whereas *B. plebeius* and unclassified *Christensenellaceae* abundance were higher in NW subjects.

Previous studies have related higher gut microbial diversity with a healthier metabolic status (6,7). Although we did not find higher microbial diversity in NW than in obese children, we observed lower diversity in children with insulin resistance, regardless of their obesity status. Given the high prevalence of type 2 diabetes in the Mexican population (1), this finding in children could be of epidemiological relevance and should be further analysed in longitudinal studies.

Associations of gut microbiome composition with obesity

Although obesity was initially related to increased *Firmicutes/Bacteroidetes* ratio (4), these findings have not been confirmed in more recent studies (5). These inconsistencies could be explained by methodological differences or population differences such as host genetics, geography and diet (23). The lack of

differences in *Firmicutes*/*Bacteroidetes* ratio between NW and obese Mexican children observed here is consistent with the findings of a previous study in children from Central Mexico (11). In contrast, significant differences were observed at the species level, as *B. eggerthii* abundance correlated negatively with BMI percentile and BF%. To our knowledge, these correlations have not been previously reported. Until recently, *B. eggerthii* was part of the *Bacteroides fragilis group*. Interestingly, a previous study associated high *B. fragilis* abundance with increased future risk of obesity in infants aged 3 weeks to 1 year (24). *B. eggerthii* has also been identified as a colitis-promoting species in an animal model (25). Together, these findings suggest a role for *B. eggerthii* in promoting obesity and inflammation; however, the mechanisms underlying weight gain or adiposity remain unclear.

Bacteroides plebeius and unclassified *Christensenellaceae* were significantly more abundant in NW than in obese children. In consistency, a study in the Dutch population reported that the relative abundance of *B. plebeius* et rel. group was fourfold higher in non-obese than in obese adults, showing a significant negative correlation with BMI (26). Moreover, unclassified *Christensenellaceae* was the only taxon showing a significant negative correlation with BMI percentile in the present study. This is in line with previous studies showing increased *Christensenellaceae* abundance in subjects with BMI $< 25\ kg\ m^{-2}$ (21,27). Importantly, germ-free mice transplanted with faecal samples incubated with *Christensenella minuta*, a cultured member of the *Christensenellaceae* family, gained significantly less weight than mice treated with unamended stool, demonstrating that *C. minuta* can directly contribute to the host phenotype (21). However, how *B. plebeius* and *Christensenellaceae* may affect BMI of the host is unknown.

To determine whether the associations of unclassified *Christensenellaceae, B. plebeius* and *B. eggerthii* abundance and obesity found here are reproducible, we analysed the data from unrelated subjects from a large population of adult UK twins where higher *Christensenellaceae* family abundance was found in lean subjects (21). In this analysis, unclassified *Christensenellaceae* and *B. plebeius* were significantly more abundant in NW than in obese subjects ($p = 2.5 \times 10^{-5}$ and $p = 0.004$, respectively), while *B. eggerthii* showed higher abundance in obese than in NW subjects ($p = 0.036$). Thus, the differential abundance of these three species is not exclusive from Mexican children, as it was found to be associated with obesity in adults from another ethnic group.

Diet is known to influence gut microbiota composition (3). We did not find significant dietary differences in NW and obese children; however, insoluble fibre intake negatively correlated with *B. eggerthii* abundance. This is consistent with previous findings in an animal model, where a Mexican pre-Hispanic diet based on high-fibre grains and seeds decreased the abundance of *B. eggerthii* 29-fold and diminished LPS circulating levels by 52% (28). Altogether, these findings may be useful to design dietary intervention studies, which could eventually lead to translational dietary recommendations in humans.

Associations with metabolic traits and amino acid serum levels

Unclassified *Christensenellaceae* was the only species associated with host lipid traits, which is in line with other studies (7,9,27). Although further research is required, this finding highlights the potential role of gut microbiota-targeted interventions for the prevention of dyslipidaemia.

Gut microbiota has been recently found to be an important contributor to the increased serum BCAA and aromatic amino acid levels in obesity and its metabolic complications (8,9). In consistency with these studies, we observed significantly higher serum levels of BCAA (valine and leucine/isoleucine) and aromatic amino acids (phenylalanine and tyrosine) in obese children. Interestingly, phenylalanine serum levels were found to have a negative and significant correlation with both *B. plebeius* and unclassified *Christensenellaceae* abundance. Although these correlations have not been previously reported, *Christensenellaceae* family abundance was negatively correlated with isoleucine and valine serum levels in Finnish adults (9).

In an attempt to explore the functional role of the gut microbiota in obesity phenotypes and dietary components, we inferred bacterial metabolic pathways. However, no associations with KEGG pathways were significant after FDR correction. Interestingly, the nominal inverse association of insoluble fibre intake with LPS synthesis pathways is consistent with previous observations that fibre intake decreases LPS circulating levels (22,29). This suggests that increased fibre intake reduces the abundance of LPS-synthesizing bacteria, preventing metabolic endotoxaemia and consequently obesity-associated complications, such as insulin resistance (Fig. S1).

Certain limitations of the study should be addressed. First, the sample size was reduced; although other studies with similar sample sizes have reported significant differences in microbiota taxa of obese

children (11,12). In addition, it is known that gut microbiota analysis by 16S rRNA sequencing can introduce bias by primer specificity (23). However, the V4 region used here shows the greatest similarity to community profiles determined by shotgun sequencing (30). Finally, the functional prediction analyses of gut microbiota composition did not lead to mechanistic insights related to the pathophysiology of obesity and its complications. Metagenomic approaches are needed to determine whether microbiota functionality contributes importantly to obesity and its metabolic complications.

In summary, the present study identified differences in the abundance of *B. eggerthii, B. plebeius* and unclassified *Christensenellaceae* in NW and obese Mexican children. Our results confirm the previous findings of a correlation between the *Christensenellaceae* family and BMI and suggest that *B. eggerthii* is a novel taxon related to obesity and higher adiposity in Mexican children. Interestingly, insoluble fibre intake correlated negatively with *B. eggerthii* abundance. In addition, the abundance of these obesity-associated species was associated with amino acid serum levels. Understanding the structure and function of the gut microbiota in children may be useful to design dietary intervention studies, which could eventually lead to translational dietary recommendations in humans.

Conflict of Interest Statement

The authors declare no conflict of interest.

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B. L.-C. and S. C.-Q. conceived the experiments. B. L.-C., S. M.-R. and L. M.-K. analysed the data. B. L.-C., S. M.-R., R. V.-V., F. S.-M., L. L. L.-M. and A. O.-L. performed the experiments. P. L.-M., H. V.-R., J. V.-B., I. I.-G., M. M.-V., A. C.-R., T. V.-M. and C. A.-S. contributed to data collection and database generation. All authors were involved in writing the manuscript. We thank Alfredo Mendoza for his technical assistance and Alejandro Rodriguez for the graphics. This project was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) grant SALUD-2013-01-202859.

REFERENCES

1. Shamah-Levy TC-NL, Rivera-Dommarco J, Hernández-Ávila M. Encuesta Nacional de Salud y Nutrición de Medio Camino. Mexico, 2016.
2. Serdula MK, Ivery D, Coates RJ, Freedman DS, Williamson DF, Byers T. Do obese children become obese adults? A review of the literature. *Prev Med* 1993;22:167–77.
3. Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annu Rev Med* 2011; 62: 361–380.
4. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; 444: 1022–1023.
5. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett* 2014; 588: 4223–4233.
6. Le Chatelier E, Nielsen T, Qin J, *et al*. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; 500: 541–546.
7. Lim MY, You HJ, Yoon HS, *et al*. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. *Gut* 2016; 66: 1031–1038.
8. Pedersen HK, Gudmundsdottir V, Nielsen HB, *et al*. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016; 535: 376–381.
9. Org E, Blum Y, Kasela S, *et al*. Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort. *Genome Biol* 2017; 18: 70.
10. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat Rev Endocrinol* 2014; 10: 723–736.
11. Murugesan S, Ulloa-Martinez M, Martinez-Rojano H, *et al*. Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. *Eur J Clin Microbiol Infect Dis* 2015; 34: 1337–1346.
12. Riva A, Borgo F, Lassandro C, *et al*. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in *Firmicutes* populations. *Environ Microbiol* 2017; 19: 95–105.
13. Moran-Ramos S, Ocampo Medina E, Gutierrez-Aguilar R, *et al*. An amino acid signature associated with obesity predicts 2-year risk of hypertriglyceridemia in school-age children. *Sci Rep* 2017; 7: 5607.
14. Kuczmarski RJ, Ogden CL, Guo SS, *et al*. CDC growth charts for the United States: methods and development. *Vital Health Stat* 2000; 11: 1–190.
15. Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, Willett W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. *Salud Publica Mex* 1998; 40: 133–140.
16. Juarez-Lopez C, Klunder-Klunder M, Medina-Bravo P, Madrigal-Azcarate A, Mass-Diaz E, Flores-Huerta S. Insulin resistance and its association with the components of the metabolic syndrome among obese children and adolescents. *BMC Public Health* 2010; 10: 318.
17. Caporaso JG, Lauber CL, Walters WA, *et al*. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 2012; 6: 1621–1624.
18. Caporaso JG, Kuczynski J, Stombaugh J, *et al*. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; 7: 335–336.

19. McMurdie PJ, Holmes S. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* 2014; 10 e1003531.
20. Benjamini Y, Hochberg Y. Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J Roy Stat Soc B Met* 1995; 57: 289–300.
21. Goodrich JK, Waters JL, Poole AC, *et al*. Human genetics shape the gut microbiome. *Cell* 2014; 159: 789–799.
22. Ghanim H, Batra M, Abuaysheh S, *et al*. Antiinflammatory and ROS suppressive effects of the addition of fiber to a high-fat high-calorie meal. *J Clin Endocrinol Metab* 2017; 102: 858–869.
23. Lozupone CA, Stombaugh J, Gonzalez A, *et al*. Meta-analyses of studies of the human microbiota. *Genome Res* 2013; 23: 1704–1714.
24. Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN. Intestinal microflora and body mass index during the first three years of life: an observational study. *Gut Pathogens* 2011; 3: 8.
25. Dziarski R, Park SY, Kashyap DR, Dowd SE, Gupta D. Pglyrp-regulated gut microflora *Prevotella falsenii*, *Parabacteroides distasonis* and *Bacteroides eggerthii* enhance and *Alistipes finegoldii* attenuates colitis in mice. *Plos One* 2016; 11: e0146162.
26. Verdam FJ, Fuentes S, de Jonge C, *et al*. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity* 2013; 21: E607–E615.
27. Fu J, Bonder MJ, Cenit MC, *et al*. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circ Res* 2015; 117: 817–824.
28. Avila-Nava A, Aguilar-López M, Sanchez-Tood M, *et al*. Pre-hispanic Mexican diet reduced dysbiosis and inflammation mediated by LPS in diet induced obesity. *FASEB* 2017; 31: 965.23.
29. Cani PD, Geurts L, Matamoros S, Plovier H, Duparc T. Glucose metabolism: focus on gut microbiota, the endocannabinoid system and beyond. *Diabetes Metab* 2014; 40: 246–257.
30. Brooks JP, Edwards DJ, Harwich MD Jr, *et al*. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. *BMC Microbiol* 2015; 15: 66.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. This figure summarizes the main differences observed in gut microbiota composition between NW and obese Mexican children. Upper panel: Relative abundance of unclassified *Christensenellaceae* and *Bacteroides plebeius* was higher in NW children, while *Bacteroides eggerthii* abundance was higher in obese children. Lower panel: High insoluble fiber intake was associated with lower abundance of genes for lipopolysaccharide (LPS) synthesis, and less insulin resistance.
Table S1. Anthropometric and biochemical characteristics of the study population
Table S2. Energy and nutrient intake of the study children
Table S3. Metabolite serum levels of the study groups
Table S4. Correlations between Shannon Index and clinical parameters
Table S5. Relative abundance of species identified by 16S sequencing
Table S6. OTUs differentially enriched by obesity status
Table S7. Correlation between relative abundance of species with BMI percentile and Body Fat Percentage
Table S8. Correlations between relative abundance of obesity associated species with nutrients consumption