EXTENDED ABSTRACT

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The role of the colon microbiota in the Mexican children obesity

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Abstract
Obesity is an epidemic malady in Mexico with genetics roots in the chromosomal human genome, however nowadays it has also been recognized the role of the human microbiome in the development of this condition. Normally, the colon microbiota produces short chain fatty acids (SCFA) by fermentation of undigested carbohydrates, and changes in the bacterial diversity of the gut of Mexican children may affect SCFA absorption contributing to the development of obesity. Normal-weight (n=81), overweight (n=29), and obese Mexican children (n=80) aged 9-11 were selected. V3-16S rDNA libraries were prepared by PCR from fecal DNA and high-throughput sequenced. After QIIME analysis of data, a metabolic profile was done using PICRUSt. The bacterial genera *Blautia* spp., *Faecalibacterium* spp., *Roseburia* spp., *Lachnospiraceae* spp., and *Coprococcus* spp. were significantly increased in overweight and obese children. Principal component analysis revealed association of these bacteria with increased BMI and triglycerides in overweight and obese children. Functional profile prediction showed that fatty acids and lipid biosynthesis functional genes were significantly more abundant in obese children. From this data, we conclude that a particular colon microbiota imbalance in overweight and obese children, increases fatty acid and lipid production. These changes are in agreement with the increase of BMI and triglycerides in overweight and obese children.

List of abbreviations: Microbiota, Triglycerides, Obesity, Mexican children, BMI, Short chain fatty acids.

Introduction

In the current decade, obesity has been one of the leading public health problems in the world in both developing and developed countries (James WPT, 2008). Overweight and obesity complications is the 5th-leading cause of all deaths, with more than 3 million annual deaths attributed to it (World Health Organization., 2008). Obesity is a process that usually begins in childhood and adolescence, which is set by an imbalance between energy intake and energy expenditure. It is also a prominent risk factor for the development of chronic diseases, such as type 2 diabetes and cardiovascular diseases (Wild & Byrne., 2006). Prevalence of obesity has increased over the past three decades in all countries in the world. The prevalence of obesity and overweight in Mexican school children aged 5–11 is also high: one child in four is overweight (Latnovic & Rodriguez Cabrera., 2013).

In the human body, there is a microbial abundance of 10^1–10^3 cfu/ml in stomach, 10^1–10^3 cfu/ml in duodenum, 10^4–10^7cfu/ml in ileum, and 10^{11}–10^{12} cfu/ml in colon (O'Hara & Shanahan., 2006) with important bacterial members. Among that, colon microbiota has a higher colonization and a higher diversity. The colon microbiota provides additional metabolic energy through fermentation of undigested carbohydrate fibers, being this metabolic activity more relevant for the development of obesity. The main metabolic products of colon microbiota are short chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, which can be utilized for *de novo* lipid or glucose synthesis (Eckburg *et al*., 2005). Alteration in the levels of SCFAs in obesity might be due to

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dysbiosis in the colon microbiota. So it is essential to explore the distal colon community members and SCFAs level to comprehend its role in development of obesity. In a previous published report, we have described the role of microbial community profile and its metabolites of normal weight, overweight and obese Mexican children, in association to obesity (Murugesan *et al.,* 2015). In this study, we show the association of triglycerides levels, and BMI values with the abundance of some distal colon bacterial members in Mexican obese children.

Materials and methods

Selection of Children

Unrelated healthy 190 children of 9-11 years were selected and classified into Normal weight (n=81), overweight (n=29) and obese (n=80) phenotypes based on their Body Mass Index (BMI) profile. After 12 h fasting blood samples were collected for biochemical studies such as glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides. Values were measured in mg/dL using an ILab 350 System (García-Cuartero *et al*., 2007)

16S rDNA libraries preparation for high throughput sequencing

Total Genomic DNA was extracted from 250 µL 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen). The quantity and quality of purified DNA, was assessed by 260/280 absorbance using a NanoDrop Lite Spectrophotometer (Thermo Scientific), and electrophoretic fractionation in 0.5% agarose gels, stained with Midori Green (Cat. MG02, Nippon Genetics) and recorded using BIO-RAD Molecular Imager Gel DOC XR+Imaging System. For preparation of DNA libraries, proper amplicons of approximately 281 bp, including the V3 polymorphic region of 16S rDNA, were amplified using previously reported forward and reverse primers (Murugesan *et al*., 2015). The thermocycler program was 5 min at 95° C; 25-cycles [15 s, 94° C; 15 s, 62° C; 15 s, 72° C] followed by 10 min at 72° C. Amplification was carried out using GeneAmp PCR System 2700 Thermocycler (Applied Biosystems).

Massive DNA semiconductor sequencing

Amplicons were high-throughput sequenced as previously described (Murugesan *et al*., 2015). In our case, emulsion PCR was carried out using Ion OneTouchTM 400 Template Kit v2 DL (Thermo Fisher Scientific) according to the manufacturer's instructions. The sequencing was done using the Ion 318 v2 Chip 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

Sequencing data were analyzed using Ion Torrent PGM software, Torrent Suite v4.0.2 as previously reported (García-Mena *et al*., 2016). Demultiplexed sequencing data were analyzed using QIIME v.1.9.0 pipeline. Closed reference operational taxonomic units (OTUs) were determined at the 97.0 % similarity level using the UCLUST algorithm. Chimeras were detected, and removed from the datasets using the Chimera Slayer. Sequence alignments were done against the Greengenes core set (DeSantis *et al*., 2006).

Prediction of functional profiling of microbial communities

Predictive Functional Profiling of children microbiota was analyzed by using PICRUSt (Langille *et al*., 2013). This was done by picking OTUs against Greengenes database. The output file was further analysed using Statistical Analysis of Metagenomic Profiles (STAMP) software package (Parks *et al*., 2014).

Results and discussion

SCFA concentration in feces and Biochemical markers in blood samples, including glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides levels have been measured for the participant children of this study project (Murugesan et al., 2015). We found lower fecal SCFA concentration, especially lower concentrations of butyric and propionic acids in obese children compared to normal-weight children; in addition, we observed increased levels of serum triglycerides in obese children with respect to normal weight children.

Sequence data analysis at genera level revealed that, there is not a severe dysbiosis in the microbiota profile among the three studied phenotypes (Fig. 1). However, there were remarkable particular differences in the bacterial abundance among these phenotypes. Nine bacterial genera were found to be significant.

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Succinivibrio spp., *Erwinia* spp., *Oscillospira* spp., were significantly higher in normal weight than overweight and obese children. *Blautia* spp., *Coprococcus* spp., and *Enterobacteriaceae* were significantly higher in overweight children than normal weight and obese children. On the other hand, *Faecalibacterium* spp., *Roseburia* spp. *Lachnospiraceae* were significantly higher in obese children (Fig.1).

Association of triglycerides level with microbiota

A principal component analysis among serum triglycerides, BMI, and bacterial abundance revealed that particular bacterial abundances in overweight and obese Mexican children are associated with the increase in triglycerides level through their clustering among them (Fig. 2). Bacterial genera such as *Succinivibrio* spp., *Erwinia* spp., and *Oscillospira* spp. were associated with a decrease in BMI and triglycerides level. Normal weight children showed a completely distinct pattern and did not cluster, with overweight and obese group (Fig. 2A). *Blautia* spp., *Coprococcus* spp., *Enterobacteriaceae, Faecalibacterium* spp., *Roseburia* spp. and *Lachnospiraceae* abundances were associated with increase in triglycerides with increase in BMI. Overweight and obese children showed same profile pattern and were clustered into same group (Fig. 2B, 2C).

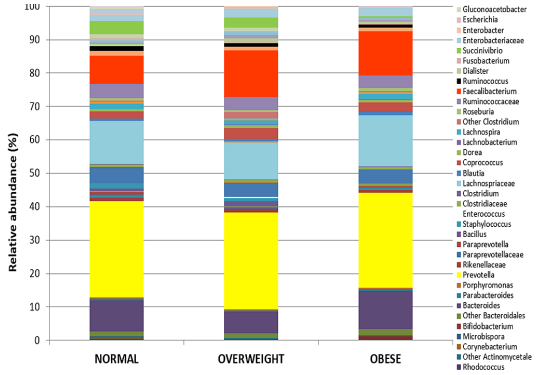

Fig 1. Relative abundance of bacterial genus in Normal, Overweight and Obese Mexican children. The Figure 1 shows a graphic display of abundant genera observed by high-throughput DNA sequencing of V3 16S rDNA libraries prepared from extracted genomic DNA as described in Materials and methods. The Y-axis shows % of relative abundance, and the X-axis indicates the abundance for each phenotypic condition. Each taxonomic category is shown by a different color.

Predicted functional profiling of Microbiota

Functional metabolic pathway profile of children microbiota was predicted against KEGG pathway as described in Materials and methods. Fatty acids and lipid biosynthesis proteins pathways were found to be significantly elevated in obese phenotype than Normal-weight children (Fig. 3). This might explain the capability of obese children microbiota to extract energy from undigested carbohydrate fibers, producing more SCFAs, increasing triglycerides in obese children.

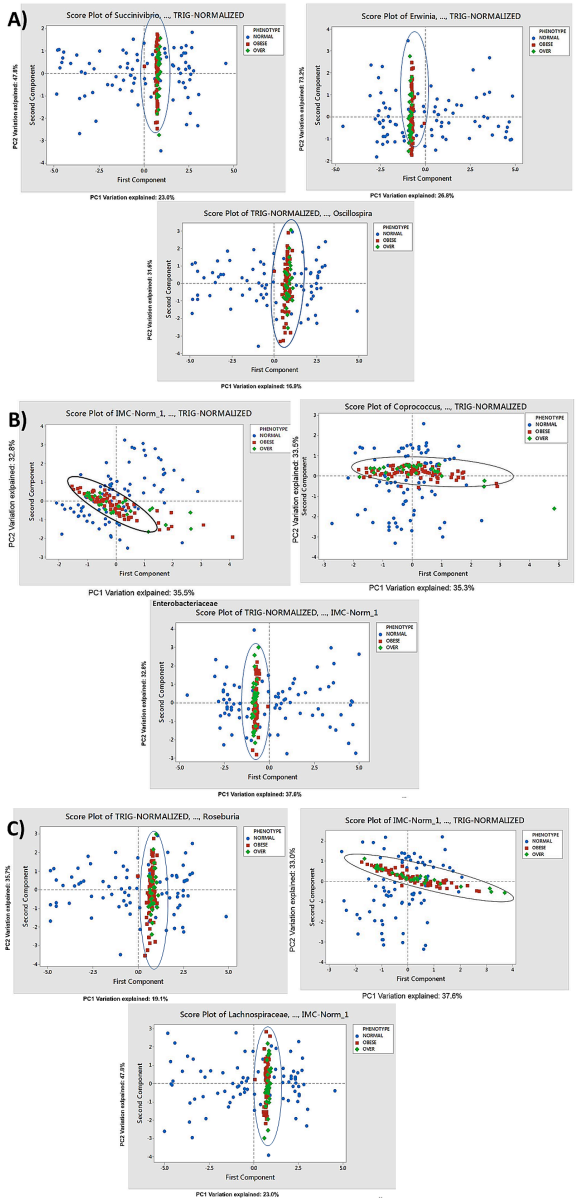

Figure 2. Principal Component Analysis. (**A)** *Succinivibrio* spp., *Erwinia* spp., *Oscillospira* spp., with BMI, and triglycerides. (**B**). *Blautia* spp., *Coprococcus* spp., *Enterobacteriaceae* with BMI, and triglycerides. (**C**) *Faecalibacterium* spp., *Roseburia* spp., *Lachnospiraceae*, with BMI, and Triglycerides level.

Conclusions

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A prominent increase in the level of triglycerides in the studied overweight and obese children, was observed compared to normal weight children, this increase may be due to higher mucosal absorption of the SCFAs produced by colon microbiota in overweight and obese children. The distal colon microbiota of obese children had higher abundance of *Fecalibacterium* spp., *Coprococcus* spp., *Lachnospiraceae* family, and *Roseburia* spp. from the Firmicutes phylum with the capacity to extract high extent of energy from undigested fiber. We conclude that a change in the abundance of these bacteria in the colon bacterial communities of overweight and obese children is associated with the increase in fatty acid and lipid production, causing an increase in the BMI and triglycerides level in overweight and obese children.

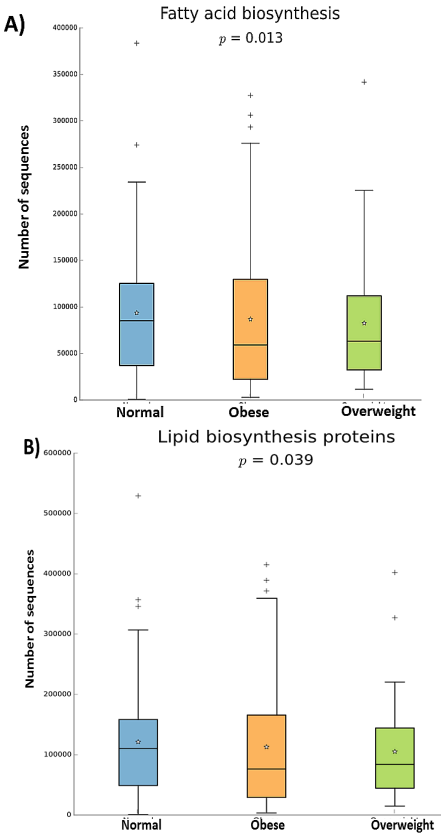

Figure 3. Functional divergence between Normal, Overweight and Obese Mexican children. **(A)** Genes in Fatty acid biosynthesis pathway (**B**) Lipid biosynthesis proteins pathways were significantly different between normal, overweight and obese Mexican children.

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