Correlation between body mass index and faecal microbiota from children

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Abstract

Childhood obesity is an increasing problem at the global level and considered as a risk factor for obesity development and the associated co-morbidities in adult life. In this study, the occurrence of Bacteroides fragilis group, Clostridium spp., Bifidobacterium spp. and Escherichia coli in 84 faecal samples from 30 obese, 24 overweight and 30 lean children was verified by culture technique and quantitative determination by quantitative PCR. In addition, Lactobacillus spp. and Methanobrevibacter smithii were also analysed. A correlation between the body mass index (BMI) and these bacteria was sought. Bacteroides vulgatus, Clostridium perfringens and Bifidobacterium adolescentis were most prevalent in all samples evaluated by culture-method. The B. fragilis group were found at high concentrations in obese and overweight children when compared with the lean ones (p 0.015). The obese and overweight children harboured higher numbers of Lactobacillus spp. than lean children (p 0.022). The faecal concentrations of the B. fragilis group (r = 0.24; p 0.026) and Lactobacillus spp. (r = 0.44; p 0.002) were positively correlated with BMI. Bifidobacterium spp. were found in higher numbers in the lean group than the overweight and obese ones (p 0.042). Furthermore, a negative correlation between BMI and Bifidobacterium spp. copy number (r = −0.22; p 0.039) was observed. Our findings show some difference in the intestinal microbial ecosystem of obese children compared with the lean ones and a significant association between number of Lactobacillus spp. and B. fragilis group and BMI.

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Keywords: Anaerobic bacteria, body mass index, childhood obesity, Escherichia coli, faecal microbiota, quantitative PCR

Original Submission: 6 May 2015; Revised Submission: 27 October 2015; Accepted: 29 October 2015

Editor: Prof. D. Raoult

Article published online: 10 November 2015

Introduction

Obesity is a consequence of the massive fat mass expansion corresponding to the body mass index (BMI) >30 kg/m² [1]. The high incidence rate of childhood obesity or overweight is a risk factor for obesity in adult life and it has been found to be associated with co-morbidities such as diabetes mellitus, coronary diseases, respiratory disorders and cancer [2,3].

According to the World Health Organization, obesity prevalence is increasing among adults, adolescents and children worldwide, and it is being considered as a public health problem [3]. The aetiology of obesity is complex and involves environmental, genetic, endocrine and neural factors [4,5]; and recently, many studies have associated obesity development with a specific profile of gut microbiota [6–8].

The gut microbiota enables enzymatic digestion of nondigestible polysaccharides producing absorbable monosaccharides; and it activates lipoprotein lipase on intestinal epithelium, which causes rapid absorption of glucose and fatty acids, contributing to the fat mass expansion and weight gain [9,10].
Reports based on quantitative methods and pyrosequencing show differences in the faecal microbial composition of obese individuals displaying high levels of Firmicutes and a lower proportion of the phyla Bacteroidetes, Actinobacteria, Proteobacteria and Archaea [11–13].

Species of Lactobacillus, Escherichia coli and Staphylococcus aureus are found to be associated with obesity, whereas Bifidobacterium spp. and Methanobrevibacter smithii are found in high concentrations in lean individuals [14–16]. Intestinal microbiota composition in adults and infants has been extensively evaluated; however, there are few studies analysing intestinal microbiota composition in individuals older than 2 years [10,17]; and the obtained reports are controversial [4,5].

In this study, the occurrence of the Bacteroides fragilis group, Clostridium spp., Bifidobacterium spp. and E. coli in obese, overweight and lean children was verified using a culture-based technique. These bacterial species are representative of resident members of the intestinal microbiota and their viability is of interest to determine their presence in this ecosystem. In addition, a determination by real-time PCR was also performed to quantify these bacteria, and also Lactobacillus spp. and M. smithii because of their frequent association with weight gain. Finally, a correlation between bacterial quantification and BMI was established.

Materials and Methods

Children and sample collection

Faecal samples were obtained from healthy children at the Institute of Children (Hospital das Clinicas) and private and municipal schools of Sao Paulo city, SP, Brazil. Demographic and clinical data (date of birth, weight, height, gender, length/weight of birth, birth delivery and clinical history) were recorded using a standardized questionnaire. Children were grouped based on Z-score as follows: lean children (≥−2 and < +1), overweight children (≥ +1 and < +2) and obese children (≥ +2). The Z-score was calculated using WHO Anthro Plus Software, taking gender, age, weight and height into consideration [18,19]. All children were 3–11 years old without diarrhoea, and none of them had undertaken antibiotic therapy in at least the 3 months prior to the sample collection. Faeces were collected in sterile universal collecting vials and immediately stored at −80°C until use.

Analyses of the gut microbiota

Bacterial isolation. Fresh faeces were streaked onto selective media Bacteroides Bile Esculin agar (BBE) for the B. fragilis group, Cycloserin Cefoxitin Fructose agar (CCFA) supplemented with 5% blood for Clostridium spp., Bifidobacterium modified agar (using as base Reinforced Clostridium Medium, dextrose, and L-cysteine) for Bifidobacterium spp. and MacConkey agar for E. coli. The streaked plates (BBE, CCFA and Bifidobacterium agar) were incubated in anaerobic conditions for 5 days and MacConkey agar was incubated in aerobic conditions for 24 h at 37°C. To assure proper bacterial identification, four characteristic colonies for each microorganism were subcultured on blood or Luria–Bertani agar. Bacterial DNA from the B. fragilis group, Bifidobacterium spp. and E. coli was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. The Bacteroides fragilis group was identified by multiplex-PCR assays [20]; and Bifidobacterium spp. [21] and E. coli [22] by conventional PCR using 16S rRNA primers. Species of Clostridium were identified using an API 20A kit (BioMérieux, Rio de Janeiro, RJ, Brazil).

Faecal DNA extraction. Total DNA was obtained from the collected faeces by using a QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer’s instructions. DNA concentrations were determined by spectrophotometer (NanoDrop 2000, Thermo Scientific, Wilmington, USA), and 10 µL of each DNA sample was checked for integrity on 1% agarose gel. DNA samples were stored at −80°C until use.

Bacterial quantification by real-time PCR

The PCR assays were performed using 16S rRNA genes species-specific sequences (Table 1). DNA amplifications were performed in final volumes of 20 µL containing: 2× SYBR® Green PCR Master Mix (GoTaq qPCR Master Mix, Promega Corporation, Sao Paulo, Brazil), 5 µM of each primer and 2 ng of faecal DNA. Amplification reactions were performed in a Rotor Gene 6000 (Corbett Life Science, Mort lake, NSW, Australia) programmed as follows: initial denaturation at 95°C for 10 min; 40 cycles of 95°C for 15 s and annealing temperature suitable for each primer pair for 60 s.

Statistical analyses

The clinical parameters were analysed by Kruskal–Wallis (Dunn), analysis of variance (Tukey) and chi-square tests. A comparison of quantitative bacterial detection among lean, overweight and obese children was performed with Kruskal–Wallis test. Possible correlations between BMI and quantitative bacterial detection were evaluated by Spearman’s test (r). Taking into account possible confounders like age and gender, a model to identify bacteria whose presence was closely related with BMI was obtained using multiple linear regressions. Variables included in the model were: concentrations of all bacteria (values in log10), age and gender. A further logistic regression method based on the three weight categories (lean = 1; overweight = 2; obese = 3) was performed. Overweight and obese
groups were compared with the lean group, which was used as control. For all statistic analyses, significance levels of 5% were obtained with the GRAPHPAD PRISM version 6.0 for Windows (GraphPad Software, La Jolla, CA, USA), and BIOESTAT 2009 version 5.3.5 as statistical packages.

**Results**

Of the 84 children enrolled in this study, 30 were obese, 24 were overweight and 30 were lean. No statistically significant differences were observed regarding gender (p 0.27), weight at birth (p 0.63), length at birth (p 0.25) and birth-delivery mode (p 0.63). Significant differences for age (p 0.008) and BMI (p <=0.001) were observed (Table 2).

The children harboured at least one species of the Bacteroides fragilis group, Clostridium spp., Bifidobacterium spp. or E. coli by culture-method. All obese children harboured species of the Bacteroides fragilis group and E. coli, 22 (77.3%) Clostridium spp. and 23 (76.6%) Bifidobacterium spp. Among the 24 overweight children, 20 (83.3%) harboured B. fragilis group, 17 (70.8%) Clostridium spp., 15 (62.5%) Bifidobacterium spp. and 21 (87.5%) E. coli. Among the 30 lean children, 21 (70%) harboured species of the B. fragilis group, 16 (48%) Clostridium spp., 18 (60%) Bifidobacterium spp. and 28 (93.3%) E. coli. Bacteroides vulgatus, Clostridium perfringens, Bifidobacterium adolescentis and E. coli were prevalent in all three groups (Table 3).

Bacterial quantitative analyses by real-time PCR revealed the occurrence of the B. fragilis group and Lactobacillus spp. in the children and it was higher in obese and overweight children than in lean ones (p <0.05) (Table 4).

Lower quantitative values (number of copies) for Bifidobacterium spp. were observed in obese and overweight children compared with the lean ones (p 0.042) by real-time PCR. The PCR quantification of Clostridium Cluster I, M. smithii and E. coli in the three groups of children did not show significant differences (Table 4).

By using Spearman’s test a positive and significant correlation between the high concentration of Lactobacillus spp. (r = 0.44; p 0.002) or B. fragilis group (r = 0.24; p 0.026) with BMI was observed. The concentration of Bifidobacterium spp. was high in the lean group; a negative correlation between this microorganism and BMI (r = −0.22; p 0.039) was verified. For Cluster I, E. coli and M. smithii showed no association with BMI (Fig. 1).

A significant correlation between the increased BMI values associated with quantification of Lactobacillus spp. (r = 0.51; p 0.006) and M. smithii (r = 0.38; p 0.042) in obese children was found. In overweight and lean groups no significant correlation between evaluated microorganisms with BMI was observed (see Supplementary material, Figs S1, S2 and S3).

Differences in gender were observed in Bifidobacterium spp. copy number in obese children. Specifically, obese girls had

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**TABLE 1. 16S rRNA oligonucleotides used to detected bacterial groups or species, and real-time PCR conditions**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Oligonucleotides</th>
<th>Tm (°C)</th>
<th>Amplicon (bp)</th>
<th>Strain</th>
<th>References</th>
</tr>
</thead>
</table>
| *Bacteroides fragilis* | F: GAG GAA GGT CCC CCA CAT TG  
R: TCC TTC ACG CTA CTT GGC TG | 60 | 113 | B. fragilis  
ATCC 25285 | This study |
| *Clostridium Cluster I* | F: ATG CAA GTC GAG CGA KG  
R: TAT GGC GTA TTA ATC TTC CTT T | 60 | 120 | C. perfringens  
ATCC 1312 | [34] |
| *Lactobacillus* spp. | F: AGC AGT AGG GAA TCT TCC A  
R: ATT YCA CCG CTA CAC ACG | 60 | 380 | L. acidophilus  
ATCC 4356 | [35] |
| *Bifidobacterium* spp. | F: GGC TGG TTA ACA CAT GCA AGT C  
R: CAC CGG TTT CCA GGA GCT ATT | 60 | 125 | B. bifidum  
ATCC 15696 | [35] |
| *Escherichia coli* | F: AGA AGC TGG CTC TTT GCT GA  
R: CTT TGG CCT TGC GAC GAT AT | 60 | 120 | E. coli  
ATCC 25922 | [36] |
| *Methanobrevibacter smithii* | F: AGG TAC TCC CAG GGT AGA GG  
R: GCC TCT ACC GTC AGA ATC G | 59 | 92 | M. smithii  
ATCC 35061 | This study |

*Bacteroides fragilis group: B. fragilis, B. vulgatus, B. uniformis, B. eggerthii, B. thetaiotaomicron, B. ovatus and B. caccae.*  

**TABLE 2. Demographic parameters obtained from the evaluated children**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obese (n = 30)</th>
<th>Overweight (n = 24)</th>
<th>Lean (n = 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>8 ± 9.6</td>
<td>8 ± 9.2</td>
<td>6 ± 9.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>19:11</td>
<td>14:10</td>
<td>13:17</td>
<td>0.273</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>27.12 ± 5.9</td>
<td>19.47 ± 1.62</td>
<td>16.06 ± 1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (x-score), mean ± SD</td>
<td>3 ± 1.6</td>
<td>1.68 ± 0.33</td>
<td>0.19 ± 0.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth weight (kg), mean ± SD</td>
<td>3.3 ± 0.7</td>
<td>3.15 ± 0.56</td>
<td>3.14 ± 0.68</td>
<td>0.632</td>
</tr>
<tr>
<td>Birth length (cm), mean ± SD</td>
<td>48.3 ± 3.4</td>
<td>47.3 ± 3.2</td>
<td>46.2 ± 3.8</td>
<td>0.253</td>
</tr>
<tr>
<td>Birth-delivery mode, (cesarean/vaginal delivery)</td>
<td>20:10</td>
<td>14:10</td>
<td>18:12</td>
<td>0.792</td>
</tr>
</tbody>
</table>

**Abbreviations:** BMI, body mass index; SD, standard deviation.

*Overweight and obese = lean.  
Overweight and obese = lean.  
Analysis of variance and Tukey’s test.  
Chi-square test.*
By linear regression analysis, bacterial concentrations (quantitative PCR) were correlated with BMI and a significant association between Lactobacillus spp. (coefficient 0.718; 95% CI 0.024–1.412; p = 0.043), B. fragilis group (coefficient 3.731; 95% CI 0.765–6.758; p = 0.015) and age (coefficient 1.109; 95% CI 0.680–1.537; p < 0.001) was observed (see Supplementary material, Table S1). In a logistic regression the variables eligible for the final model were Bifidobacterium spp., Lactobacillus spp., B. fragilis group and age. The logistic regression analysis showed that Lactobacillus spp. and age are significantly associated with overweight; B. fragilis group and age are associated with obese children; and Bifidobacterium spp. were associated with lean children (Table 6). These results confirm the findings obtained by univariate analysis.

## Discussion

Studies have shown that the gut microbiota composition is associated with diet, host genetic, socio-economic status, lifestyle and others diseases, including allergy, obesity and type 2 diabetes mellitus [23–25]. Exogenous factors such as vaginal or caesarean delivery, breastfeeding and administration of antibiotics in infants can affect the intestinal microbial diversity; however, it is not clear if these factors might have an obesogenic effect [26]. In this study, no differences were found between delivery mode, weight and length at birth and BMI, indicating no association between these factors and weight gain in the evaluated children.

Qualitative culturing was used to identify and characterize strains with specific traits, and to determine their viability in the intestinal ecosystem. Culture-base technique is a ‘gold standard’ for isolation of selected bacterial group and it can help to

### Table 3. Bacterial species identified in faeces from 30 obese, 24 overweight and 30 lean children by culture method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Obese (n = 30)</th>
<th>Overweight (n = 24)</th>
<th>Lean (n = 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. vulgatus</td>
<td>24 (80)</td>
<td>10 (41.6)</td>
<td>16 (53.3)</td>
<td>0.011</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>3 (10)</td>
<td>2 (8.3)</td>
<td>3 (10)</td>
<td>0.055</td>
</tr>
<tr>
<td>L. volatilis</td>
<td>3 (10)</td>
<td>5 (20.8)</td>
<td>1 (3.3)</td>
<td>0.116</td>
</tr>
<tr>
<td>C. cocaceae</td>
<td>0</td>
<td>2 (8.3)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>B. uniformis</td>
<td>0</td>
<td>2 (8.3)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>B. stercoris</td>
<td>0</td>
<td>0</td>
<td>2 (6.6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Clostridium spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. perfringens</td>
<td>11 (36.6)</td>
<td>8 (33.3)</td>
<td>6 (20)</td>
<td>0.333</td>
</tr>
<tr>
<td>C. doxiadiforme</td>
<td>4 (13.3)</td>
<td>1 (4.1)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>C. glycolicum</td>
<td>0</td>
<td>1 (4.1)</td>
<td>1 (3.3)</td>
<td>ND</td>
</tr>
<tr>
<td>C. innocuum</td>
<td>4 (13.3)</td>
<td>5 (20.8)</td>
<td>2 (6.6)</td>
<td>0.308</td>
</tr>
<tr>
<td>C. saccharolyticus</td>
<td>0</td>
<td>0</td>
<td>1 (3.3)</td>
<td>ND</td>
</tr>
<tr>
<td>C. paraputri</td>
<td>0</td>
<td>1 (4.1)</td>
<td>2 (6.6)</td>
<td>0.908</td>
</tr>
<tr>
<td>C. sordelli</td>
<td>0</td>
<td>1 (4.1)</td>
<td>1 (3.3)</td>
<td>ND</td>
</tr>
<tr>
<td>C. difficile</td>
<td>1 (3.3)</td>
<td>1 (4.1)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>C. sphenogalilis</td>
<td>0</td>
<td>1 (4.1)</td>
<td>1 (3.3)</td>
<td>ND</td>
</tr>
<tr>
<td>C. uniformis</td>
<td>1 (3.3)</td>
<td>1 (4.1)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>C. tertium</td>
<td>1 (3.3)</td>
<td>0</td>
<td>1 (3.3)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Bifidobacterium spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. adolescentis</td>
<td>19 (63.3)</td>
<td>13 (54.1)</td>
<td>15 (50)</td>
<td>0.569</td>
</tr>
<tr>
<td>B. infantis</td>
<td>15 (50)</td>
<td>5 (20.8)</td>
<td>8 (26.6)</td>
<td>0.048</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>30 (100)</td>
<td>21 (70)</td>
<td>28 (93.3)</td>
<td>0.152</td>
</tr>
</tbody>
</table>

**ND,** without sufficient positive samples to perform the chi-square test.

**a**Prevalence reflects the number of positive samples by culture-based technique.

**b**Chi-square test was applied.

By linear regression analysis, bacterial concentrations (quantitative PCR) were correlated with BMI and a significant association between Lactobacillus spp. (coefficient 0.718; 95% CI 0.024–1.412; p = 0.043), B. fragilis group (coefficient 3.731; 95% CI 0.765–6.758; p = 0.015) and age (coefficient 1.109; 95% CI 0.680–1.537; p < 0.001) was observed (see Supplementary material, Table S1). In a logistic regression the variables eligible for the final model were Bifidobacterium spp., Lactobacillus spp., B. fragilis group and age. The logistic regression analysis showed that Lactobacillus spp. and age are significantly associated with overweight; B. fragilis group and age are associated with obese children; and Bifidobacterium spp. were associated with lean children (Table 6). These results confirm the findings obtained by univariate analysis.

## Discussion

Studies have shown that the gut microbiota composition is associated with diet, host genetic, socio-economic status, lifestyle and others diseases, including allergy, obesity and type 2 diabetes mellitus [23–25]. Exogenous factors such as vaginal or caesarean delivery, breastfeeding and administration of antibiotics in infants can affect the intestinal microbial diversity; however, it is not clear if these factors might have an obesogenic effect [26]. In this study, no differences were found between delivery mode, weight and length at birth and BMI, indicating no association between these factors and weight gain in the evaluated children.

Qualitative culturing was used to identify and characterize strains with specific traits, and to determine their viability in the intestinal ecosystem. Culture-base technique is a ‘gold standard’ for isolation of selected bacterial group and it can help to

### Table 4. Bacterial prevalence and quantification verified in faeces of obese, overweight and lean children by quantitative PCR

<table>
<thead>
<tr>
<th>Bacteria/organism</th>
<th>Obese (n = 30)</th>
<th>Overweight (n = 24)</th>
<th>Lean (n = 30)</th>
<th>Total (n = 84)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. adolescentis</td>
<td>30 (100)</td>
<td>24 (100)</td>
<td>30 (100)</td>
<td>84 (100)</td>
<td>ND</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>29 (96.6)</td>
<td>24 (100)</td>
<td>27 (90)</td>
<td>80 (95.2)</td>
<td>ND</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>30 (100)</td>
<td>23 (95.6)</td>
<td>30 (100)</td>
<td>83 (98.8)</td>
<td>ND</td>
</tr>
<tr>
<td>Methanobrevibacter smithii</td>
<td>20 (66.6)</td>
<td>21 (87.5%)</td>
<td>27 (90%)</td>
<td>68 (80.9%)</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>Quantitative determination (log_{10} copies/g faeces)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>9.5 (9–9.6)</td>
<td>9.1 (8.9–9.5)</td>
<td>8.9 (8.7–9.7)</td>
<td>9.1 (8.9–9.7)</td>
<td>0.015</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>4 (2.8–6.3)</td>
<td>4 (3.5–6.2)</td>
<td>4.4 (3.7–6.2)</td>
<td>4.1 (3.1–6.3)</td>
<td>0.702</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.3 (6.8–8.5)</td>
<td>7.7 (6.6–9.2)</td>
<td>7.5 (6.9–9.4)</td>
<td>7.5 (6.9–9.4)</td>
<td>0.672</td>
</tr>
<tr>
<td>Methanobrevibacter smithii</td>
<td>4.1 (0–8.4)</td>
<td>4.5 (3.9–8.6)</td>
<td>4.3 (3.8–8.8)</td>
<td>4.4 (3.7–8.8)</td>
<td>0.262</td>
</tr>
</tbody>
</table>

**ND,** without sufficient positive samples to perform the chi-square test.

**a**Values noted as number (percentage), chi-square test.

**b**Data were presented as median (interquartile range; IQR); differences among three groups were compared using Kruskal–Wallis test (Dunn post-test).

**p < 0.05** indicated significant differences as compared with the lean group.

**p < 0.05** indicated significant differences compared with the obese and overweight groups.
FIG. 1. Correlation between bacterial copies detected by quantitative PCR and body mass index (BMI) in total children (n = 84). Spearman correlation test (r): (a) Lactobacillus spp. (r = 0.44; p = 0.002), (b) Bacteroides fragilis group (r = 0.24; p = 0.026), (c) Bifidobacterium spp. (r = -0.22; p = 0.039), (d) Cluster I (r = 0.00; p = 0.966), (e) Escherichia coli (r = -0.12; p = 0.254) and (f) Methanobrevibacter smithii (r = -0.05; p = 0.599).
The obese children showed high numbers of viable bacteria compared with the overweight and lean children. However, the obese children showed low bacterial diversity when compared with the lean and overweight children, similar to that observed by Karlsson et al. [5]. Interestingly, overweight and lean children harboured similar numbers of bacterial isolates, and these results might be explained because of the transition stage from lean to overweight.

The B. fragilis group were detected by quantitative PCR in higher concentrations in obese and overweight children than in lean children, and it showed a significant correlation with BMI and weight gain. It is known that the B. fragilis group is predominant in the intestinal resident microbiota. Obese or overweight children eat a diet rich in carbohydrates, which can be used by the host to store as fat and by intestinal Bacteroides to produce short-chain fatty acids, increasing a risk for development of obesity [12,35]. Previous studies on children found no positive association between the Bacteroides level and BMI [16,17,24,27]. These differences in the results can be ascribed to the different methodologies used in the studies.

In accordance with previous findings [16,28], in this study, Lactobacillus spp. were present in higher concentrations in the obese and overweight subjects; and a positive correlation between this microorganism and BMI was observed. Some studies correlated the species Lactobacillus reuteri with BMI in adults [8,15], though we have not evaluated this species, the continual findings of Lactobacillus spp. indicate a strong association between this genus and obesity, not only in adulthood but also during infancy.

Moreover, gender differences in the levels of Lactobacillus spp. were observed in the obese and overweight children but not in the lean group (Table 5), which is in disagreement with Xu et al. [27], and Mueller et al. [29], whereas the cause of this difference is unclear. Differences observed in girls and boys regarding the qualitative intestinal microbiota, may be due to hormonal, endocrine, behavioural and socio-economic factors; whichever, this has not been, evaluated herein.

TABLE 5. Univariate analysis of the bacterial association with body mass index levels by gender

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obese children (n = 30)</th>
<th>Overweight children (n = 24)</th>
<th>Lean children (n = 30)</th>
<th>Total (n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides fragilis group</td>
<td>(n = 11)</td>
<td>9.1 (9.9–6.5)</td>
<td>10.1 (10.9–4.5)</td>
<td>13.2 (13.9–7.7)</td>
</tr>
<tr>
<td>Clostridium Cluster I</td>
<td></td>
<td>10.1 (10.9–4.5)</td>
<td>12.1 (12.9–6.6)</td>
<td>17.2 (16.9–8.7)</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>(n = 11)</td>
<td>7.4 (7.8–4.2)</td>
<td>9.4 (9.9–6.5)</td>
<td>12.4 (12.9–8.7)</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>(n = 10)</td>
<td>6.1 (6.3–7.2)</td>
<td>6.4 (6.7–6.8)</td>
<td>8.7 (8.9–7.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>(n = 10)</td>
<td>7.1 (6.7–8.8)</td>
<td>8.1 (8.8–6.7)</td>
<td>10.1 (10.9–8.4)</td>
</tr>
<tr>
<td>Methanorevibacter smithii</td>
<td>(n = 16)</td>
<td>4.5 (8.6–8.4)</td>
<td>5.6 (8.6–6.7)</td>
<td>7.7 (8.6–7.0)</td>
</tr>
</tbody>
</table>

Data were presented as log10 median (interquartile range; IQR); differences among three groups were compared using Kruskal–Wallis test (Dunn post-test). P < 0.05.

*p < 0.05, indicates significant differences between girls and boys in obese and total group. No significant differences between girls and boys were found in the either overweight group or the lean group.

p > 0.05 indicates significant differences compared with the lean group.

ELUCIDATE THE HOST—MICROBIOTA INTERACTION IN OBESITY DEVELOPMENT [7,15].

In addition to qualitative culturing, real-time PCR was also used for a better determination of bacterial number in each group of children. These results were used to determine a possible association of bacterial number with BMI for each group. Species-specific primers used in quantitative PCR have shown good reproducibility, sensitivity and specificity; however, significant differences between culture and PCR have also been observed for Lactobacillus spp. by Million et al., [15]. In this study, PCR showed much more sensitivity than culture to detect selected species of B. fragilis group, Bifidobacterium, Clostridium and E. coli.

The obese children showed high numbers of viable bacteria compared with the overweight and lean children. However, the obese children showed low bacterial diversity when compared with the lean and overweight children, similar to that observed by Karlsson et al. [5]. Interestingly, overweight and lean children
Species of *Bifidobacterium*, especially *B. adolescentis*, were recovered more from obese and overweight children compared with lean but showed inverse numbers in real-time PCR analysis. In addition, a negative and significant correlation between *Bifidobacterium* spp. levels and BMI was observed; suggesting that this genus may not be associated with weight gain.

*Bifidobacterium* spp. and *Lactobacillus* spp. are considered beneficial bacteria in the human intestinal microbiota, but gut colonization by these lactic acid bacteria are easily influenced by diet. Probiotics have been used to increase the weight gain in animals for decades, therefore the oral administration of specific probiotics might be a possible factor involved in obesity development in humans [15].

*Clostridium* cluster I was observed in children but with no statistical difference in the number of copies. Zuo et al. [30], by using bacterial cultivation and counting technique, found lower numbers of *C. perfringens* in obese adults. In our study, similar percentages for *C. perfringens* and *Clostridium* cluster I were found in lean, overweight and obese children. Certainly, the role of these microorganisms in the intestinal microbiota of obese subjects needs further investigation.

*Enterobacteriaceae* is a family of gram-negative commensal bacteria; mainly *E. coli* is predominant in the intestinal ecosystem during childhood [31]. As expected, most of the children showed a high prevalence of *E. coli* as determined by culture and molecular techniques. In contrast to a previous study by Karlsson et al. [5], our study found an inverse correlation with BMI, suggesting no association with increased BMI. Considering the mean age of the obese children, our results are in accordance with the data reported by Million et al. [8], who also detected lower bacterial loads in obese adults.

Methanogenic archaea are important because they are involved in the removal of H2 from the mammalian gut. *Methanobrevibacter smithii* is the most common archaea found in the human intestinal microbiota [32,33]. As the lean children showed high numbers of *M. smithii* compared with overweight and obese children, the presence of this microorganism might not be associated with obesity, in accordance with other reports [8,12].

In addition, our results suggest that the quantitative changes of species of the *B. fragilis* group and *Lactobacillus* spp. are associated with obesity; although no association with birth delivery mode or weight at birth was observed. The better knowledge of the intestinal microbiota composition in obese, overweight and lean individuals and its interaction with host endocrine factors could help elucidate obesity development.

**Acknowledgements**

The authors thank Mrs Marcia H. Fukugaiti for her technical support and Mrs Rosana Duarte Prisco for statistical analyses. Strains ATCC and DNA were kindly donated by Dr Sydney M. Finegold from Veterans Affairs, West Los Angeles Medical Center, Los Angeles, CA, USA; Dr Jacques R. Nicoli from University Federal of Minas Gerais, MG, Brazil, and Dr Ruchi Mathur Department of Medicine, Cedars-Sinai Medical Center, California. During the course of this work AI was supported by FAPESP fellowship (2012/10659-7). This study was supported by the grants: CNPq No. (158799/2012-7) and FAPESP (2013/1739-9).

**Appendix A. Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.10.031.

**References**


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