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Research Article

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Differences in gut microbiota composition between healthy children, obese children and children with type 1 diabetes

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ABSTRACT

Obesity is viewed as one of the important public health problems of our times, and the velocity of propagation is highest in children. However, the composition of the intestinal bacterial flora may be a third element involved in the development of excess weight. The intestinal flora has links with intermediary metabolism and inflammation and may be involved in the pathogenesis of diabetes (types 1 and 2), metabolic syndrome and obesity. The aim of the present study was to characterize the composition of fecal flora in obese children, children with type 1 diabetes as compared with healthy children and to determine the possible relationship of fecal flora of children with the body mass index and glycemic level. Our microbial's results the intestinal flora of obese children and diabetes's children type 1 is different than others children who are with predominance of Lactobacillus and Enterococcus to obese children. A tripling of Staphylococcus bacteria was registered in obese children case by contribution to witnesses' children. They also have high of Candida yeasts proportions. Significant correlations were found between the presence of these yeasts and BMI of obese children ($p < 0.05$). All times Enterobacteriaceae are elevated in obese but not significant. The ratiois significantly higher between obese children and Healthy ($p<0.0005$)

Key words: Children, Diabetes, Fecal flora, Obesity.

INTRODUCTION

Obesity is viewed as one of the important public health problems of our times, and the velocity of propagation is highest in children [1]. The prevention of obesity during early childhood is critical because the high risk of becoming obese adults [2,3]. Moreover, obese children and adolescents develop serious co morbidity, including type 2 diabetes, metabolic syndrome, non-alcoholic fatty liver disease and sleep-disordered breathing [4-6].Obesity results from the accumulation of excess adipose tissue; however, it is not a single disorder but a heterogeneous group of conditions with multiple causes. Major causes of the increasing prevalence of obesity include behavioral and environmental factors, such as excessive consumption of energy-dense foods and a sedentary lifestyle [7]

The cause of obesity is multifactorial and complex, resulting from the interaction between genetic determinants / epigenetic, environmental, behavioral and socio-psychological with high interindividual variability. However, the composition of the intestinal bacterial flora may be a third element involved in the development of excess weight. The intestinal flora has links with intermediary metabolism and inflammation and may be involved in the pathogenesis of diabetes (types 1 and 2), metabolic syndrome and obesity. [8]

Ley et al [9] demonstrated, in rodents, obesity may be associated with qualitative changes in the intestinal flora. They have shown that obese animals had twice less Bacteroidetes and a proportional increase in Firmicutes .From identically, the same group of researchers confirmed these data in humans [9]. Some studies suggest that the characteristics of the bacterial ecosystem in infancy may influence the incidence of obesity much later in life [10,11].

Very recently it has been demonstrated that the incidence of type 1 diabetes in non-obese mice was also influenced by the intestinal flora. Wen and colleagues have demonstrated that exposure of non-obese mice immunodeficient and germ in the intestinal flora normally present in humans reduced the incidence of type 1 diabetes [12]

EXPERIMENTAL SECTION

We studied the fecal flora of 54 children with an average age of (10.25 ± 2.22 years), the children are divided into three groups, healthy children and normoponderaux (H) n = 19; Children of obese (Ob) n = 19; Type 1 diabetic children (D1) n = 16). All these are studied in public schools during the year (2010-2011), in the city of Mascara (western Algeria). Parents have been informed and gave written informed consent. Exclusion criteria of the children selected for the study: acute or chronic infectious diseases were among the exclusion criteria and the existence of an intestinal disease or problems that may affect the intestinal flora (diarrhea, constipation).All children were not under antibiotic treatment or taking any other drugs known to influence the fecal microbiota composition for at least three months prior to sampling.

1. Anthropometric measurements

Anthropometric measurements (weight, Height) were measured in all children by regularly checked equipment; measuring rod fixed SECA (France; precision mm); electronic scales TERRAILLON (France; 100g accuracy). Each child was weighed and measured standing barefoot and lightly dressed. Body mass index (BMI), which corresponds to the formula weight divided by the square of Height [kg / m^2] was used to determine obesity according to references the *International Obesity Task Force* (IOTF 2000) [13]

2. Laboratory measurements

Blood glucose was measured fasting with a meter (ACON Laboratories, San Diego, USA). For children with a fasting blood glucose value ≥1,2 g / L by the meter are selected again to take a blood sample from the vein in order to perform the blood glucose in the laboratory by a young method enzyme (Spinreact, Spain). Diabetes was defined according to the criteria of the American Diabetes Association (ADA) by a fasting glucose ≥1,2 g / L [14].

3. Analysis of fecal microbiota

3.1. Sample collection

Fresh fecal samples were obtained from 54 chilren ; were collected at home by their parents in sterile boxes closed and labeled. The transmission was made immediately to the laboratory for analysis via coolers (+4 ° C). The stools were then stored at (-80 ° C) until analysis. [15] One gram of feces was suspended in 9 ml of sterile physiological saline. Then decimal dilutions to 10-8 were performed in the same liquid. From these, the following media were seeded: Nutrient Agar (GN) for isolation of total aerobic and anaerobic flora; Eosin Methylene Blue (EMB) and Hektoen for *Enterobacteriaceae*; Man Rogosa Sharpe (MRS) for *Lactobacillus*; Bile Esculin azide (BEA) for *Enterococcus*; Chapman for *Staphylococcus* and finally middle Sabouraud chloramphenicol and actidione for *yeast*. The plates were incubated at 37 ° C for 1 to 4 days, anaerobic (total anaerobic flora, Lactobacillus) and aerobic (*Enterobacteriaceae, Enterococcus, Staphylococcus*, *yeasts*). The enumeration of bacterial colonies is expressed in CFU (colony forming units) per gram of stool [16].

3.2. Firmicutes/Bacteroidetes ratios

An estimate of the total amount of Firmicutes was obtained by adding values obtained from Gram positive bacteria (*Enterococcus; Staphylococcus* and *Lactobacillus*). For Firmicutes / Bacteroidetes reports, calculations were obtained for each individual using the CFU [17].

4. Statistical analysis

All results were conducted en duplicate and were expressed as mean ± standard deviation.

The collected data were entered using Excel (Microsoft Office 2010) and analyzed by the software (STATISTICA 7) and graphics are presented by Excel (Microsoft Office 2010). An analysis of variance was applied. Comparisons of means between groups of children were performed by the Student test. The correlations between variables (different microbial groups), BMI and blood glucose levels were calculated by the correlation test (Spearman Rank). The statistical significance was ≤ 5%.

RESULTS AND DISCUSSION

Anthropometric variables and clinical characteristics

Anthropometric variables and clinical characteristics of children enrolled in this studyare shown inTable 1. The anthropometric and biochemical variables of the healthy children and those with diabetes are shown in Table 2. Apart from the levels of BMI and weight, which were significantly higher in obese children, no other significant differences were seen between the groups in the anthropometric and clinical characteristics.

Table 1: Anthropometric variables and clinical characteristics in of children enrolled in this study (means ± SD)

	Healthy children	Obese children	Children with type 1 diabetes	*p*
	N=19	N=19	N=16	
Male/Female	11/8	11/8	6/10	
Age (years)	9,16 ± 1,93	11,68 ± 1,87	9,91 ± 2,86	0,4
Body mass index (kg/m^2)	16,87 ± 2,81	29,64 ± 3,66	19,33 ± 3,1	<0,001[a]
Weight (kg)	25,67 ± 7,45	56,58 ± 12,15	28,59 ± 12,07	<0,001[b]
Height (m)	1,19 ± 0,17	1,39 ± 0,12	1,18 ± 0,23	0,06
Diabetes start (years)	/	/	5,42 ± 3,62	/
Diabetes duration (years)	/	/	4,88 ± 3,25	/

*a : Values are significantly different between*Healthy children *vs* Obesechildren.
b : *Values are significantly different between*Healthy children *vs* Obesechildren.
Values are presented as means ± SD. Values are significantly different for P ≤0.05.

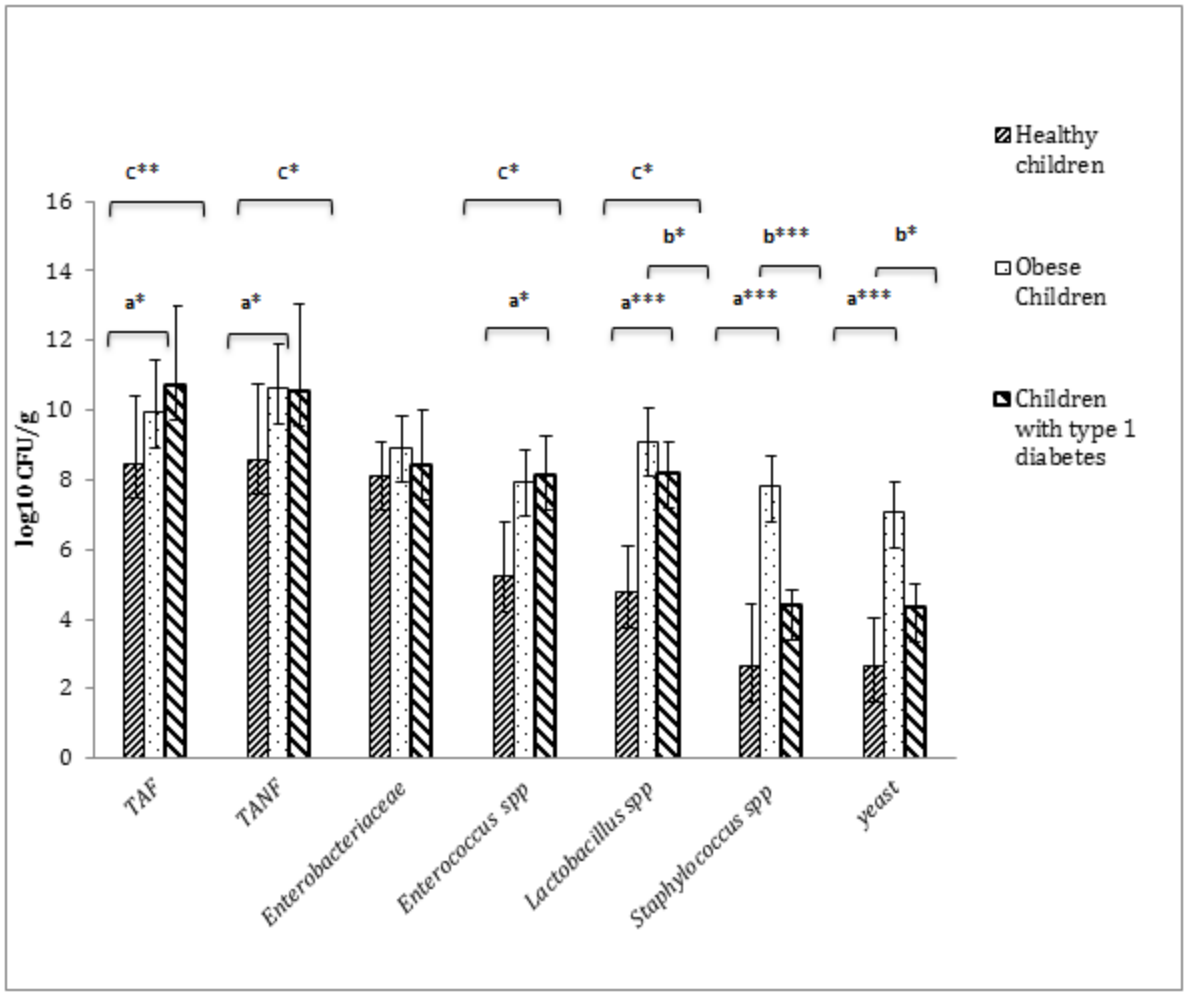

Figure 1 : Quantitative and qualitative distribution of the different microbial groups in selected children (Healthy children, Obese Children, Children with type 1 diabetes)
TAF:Total aerobic flora; TANF:Total anaerobic flora
a:Healthy children vs obese children ;
b:obese children vs children with type 1 diabetes;
c : Healthy childrenvs children with type 1 diabete
** p≤0.05. **p≤0.005. ***p≤0.0005.*

Comparative analysis of microbial groups between different groups of children

Quantitative and qualitative distributions of various microbial groups in children selected for the analysis are shown in (Figure 1). The bacteria belonging to the genus Enterococcus are significantly more likely in obese children and children with diabetes (type I) by contribution to child healthy (log_{10} 7.95 ± 0.89 CFU / g; log_{10} 8.12 ± 1.1 CFU / g *vs* log_{10} 5.23 ± 1.56 CFU / g, respectively; $p = 0.023$) but no difference was found between obese and diabetic. Lactobacillus spp are also more likely in obese and diabetic by contribution to child witnesses (log_{10} and 9.09 ± 1.00 CFU / g log_{10} 8.18 ± 0.92 CFU / g *vs* log_{10} 4, 77 ± 1.31 CFU / g ; $p = 0.0004$). Tripling the number of bacteria of the Staphylococcus genus was also recorded in obese children and diabetic contribution to child witnesses (log_{10} and 7.81 ± 0.90 CFU / g vs 3.41 ± 0.46 log_{10} 2 , 64 ± 1.81 CFU / g , $p < 0.001$). In addition, two groups of children (obese and diabetic) have high proportions of Candida yeasts in their intestinal microbiota by contribution to child healthy (log_{10} and 7.07 ± 0.84 log_{10} 4 42 ± 0.67 CFU / g vs log_{10} 2.64 ± 1.41 CFU / g; $p < 0.01$). Enterobacteriaceae were slightly higher in obese but the difference was not statistically significant compared to control children (log_{10} 8.93 ± 1.59 CFU / g vs log_{10} 8.11 ± 0.96 CFU / g; $p = 0.06$).

Correlation between BMI and the composition of the faecal flora in obese children

A positive and significant correlation was found between the rate of *Staphylococcus*spp (r = 0.56; p = 0.01), *yeast* (r = 0.66; p = 0.002) and BMI.

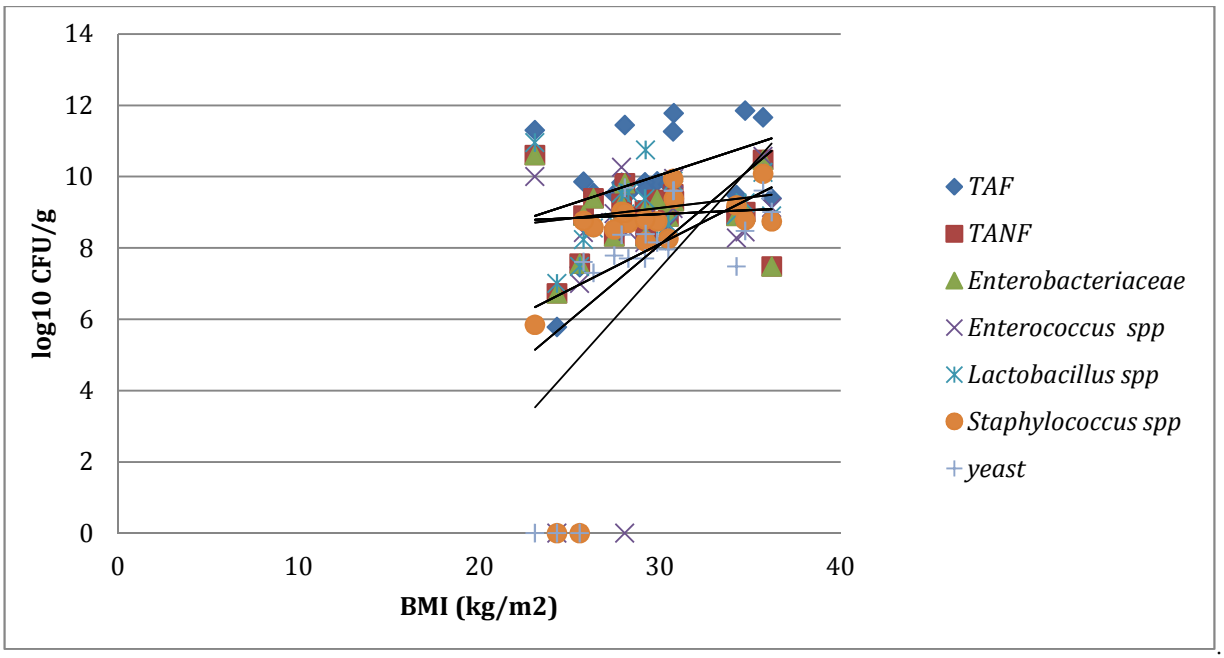

Figure 2 :Correlation between BMI and the composition of the faecal flora in obese children

Correlation between blood glucose levels and the composition of the faecal flora in children with diabetes (type I).

Negative correlation was recorded between microbial groups and blood glucose levels in diabetic children but is statistically significant only in the case of the total anaerobic floraTANF (r = - 0.33; p = 0.001); *Enterobacteriaceae*(r = - 0.08); *Enterococcus spp*(r = - 0.30); *Lactobacillus spp*(r = - 0.29); *Staphylococcus spp*(r = - 0.35) and yeast (r = - 0.38).

Firmicutes/Bacteroidetes *ratio*

For the Firmicutes/Bacteroidetes ratio, we observed significantdifferences between obese children and children with type 1 diabetes (0.9 and 0.8, respectively ; p≤0.05) and between Healthy children and children with type 1 diabete (0.5 and 0.8, respectively ; p≤0.005) (Figure 4). The ratiois significantly higher betweenHealthy children and obese children(0.5 and 0.9, respectively ; p≤0.0005)

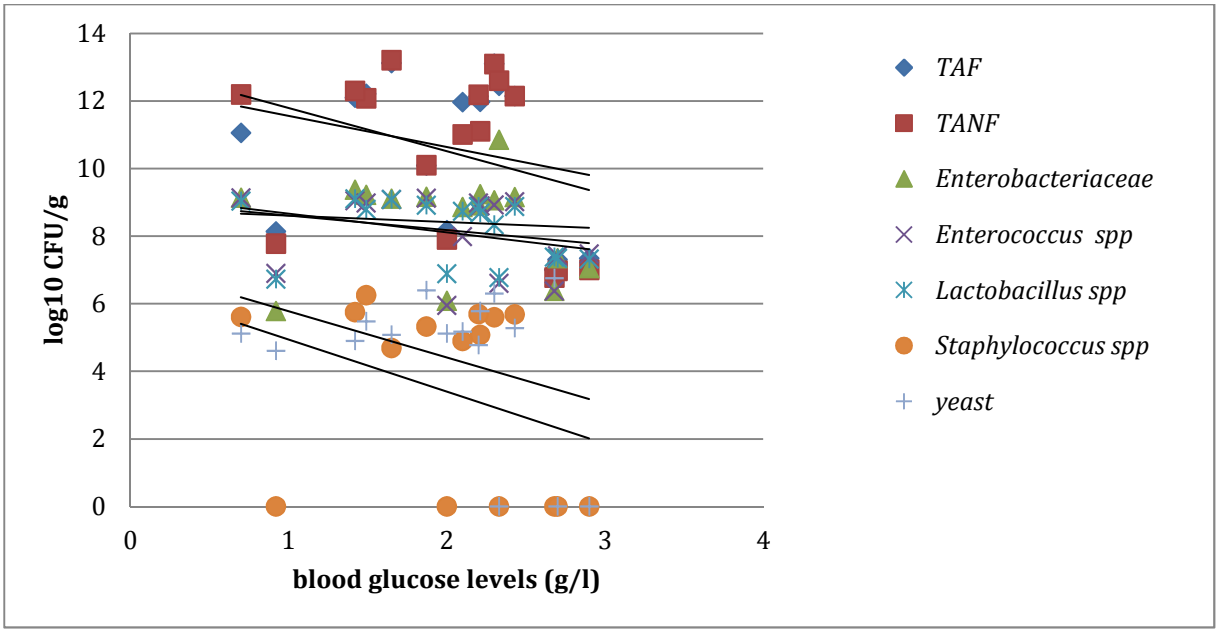

Figure 3 : Correlation between blood glucose levels and the composition of the faecal flora in children with diabetes (type I)

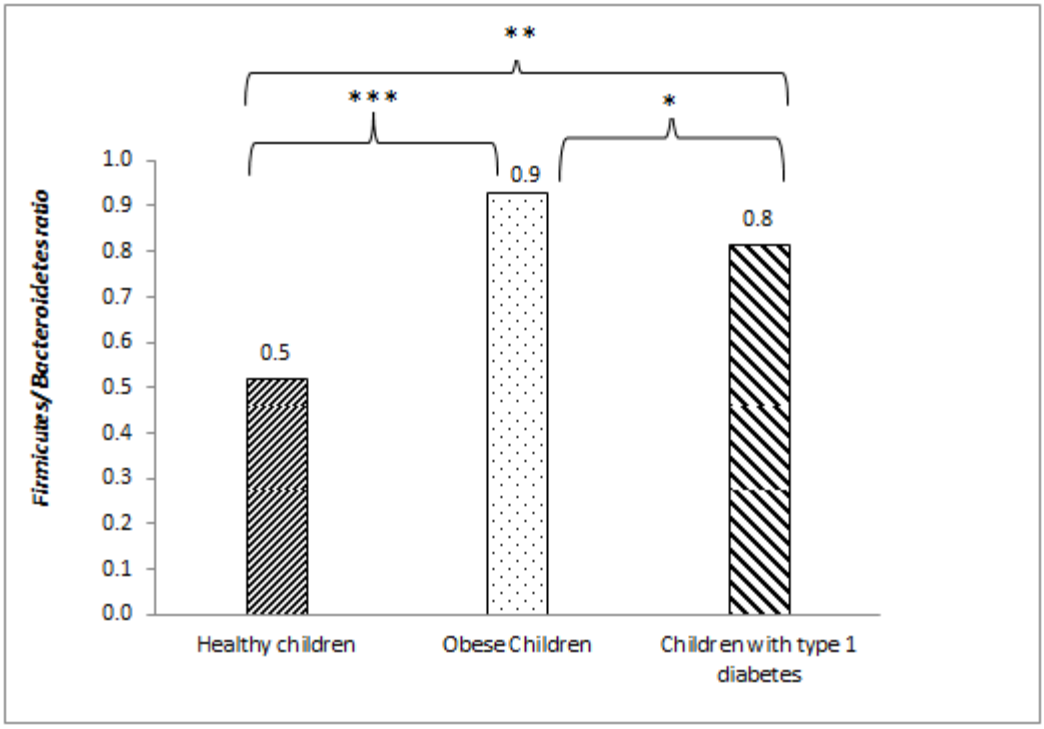

Figure 4: Firmicutes/Bacteroidetes ratio in the three children's groups (Healthy children, Obese Children, Children with type 1 diabetes)
***G+**: Gram positive bacteria*
***G-**: Gram negative bacteria*
** p≤0.05. **p≤0.005. ***p≤0.0005.*

Our results show that the intestinal flora of obese children and children with diabetes (type 1) is significantly different than the control children. This flora was characterized by the presence of elevated levels of *Enterococcus*, *Lactobacillus*, *Staphylococcus* and yeast. The difference is remarkable especially in obese children. The abundance of *enterococci* has already been demonstrated in obese Chinese, but the role of these bacteria in the development of obesity remains unknown. [18] Lactobacillus were in turn positively associated with obesity in several studies [18, 19] .The results observed in the diabetic group, however, are at odds with the work of Murri et al., Have shown that children with diabetes Type 1 have low levels of Lactobacillus [20] .Remely et al. demonstrated in type 2 diabetic patients have higher proportions of lactic acid bacteria with an abundance of *Enterococcus*, *Lactobacillus* and

Streptococcus. [21] Our study also reported positive correlations between BMI and quantities of Staphylococcus and Candida in obese children. However, several published studies suggest that elevated levels of *S. aureus* in obese patients may be associated with an inflammatory process and storage of fat. These results have been confirmed in children predisposed to overweight [10] .The coagulase-negative staphylococci are now recognized as the first colonizers of the intestine of newborn babies, regardless of the mode of delivery. [22] In addition, most children are colonized with S. aureus during the first months of life via the skin of parents and colonization remains long-term. [23] Many strains of S. aureus produce toxins that can act as antigens responsible pro-inflammatory potential [22-24]. The origin of this inflammatory process, however, remains poorly documented. Various studies show the power involvement in the development of postprandial inflammation [25]. Obesity is therefore dependent pathology not only the genome but also eating habits and / or physical activity of the subjects.

CONCLUSION

The results of our study show the quantitative and qualitative difference in the composition of the intestinal flora of child witnesses, obese and diabetic type1. High levels of Staphylococcus and Candida were recorded in obese and diabetic children with significant correlations with BMI in obese children.

The rates of Enterococcus and Lactobacillus are also high in obese and diabetic children. Further studies are needed to understand the relationship between the microbiota and these metabolic diseases and better consider the role and origin of the inflammatory process associated to them by the dosage markers of intestinal inflammation and microbial metabolites at an age early in order to prevent and / or correct these parameters before reaching adulthood.

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