

Short Communication

Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children

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Gut bacteria contribute to energy conservation in man through their ability to ferment unabsorbed carbohydrate. The present study examined the composition of predominant faecal microbiota in obese and non-obese children. The participants (n 28) aged 11–14 years provided fresh faecal samples and completed a dietary survey consisting of 24 h diet recall and a FFQ of commonly used foods taken over the previous 3 months. Faecal bacteria were quantitated by real-time PCR using primers targeted at 16S rDNA. Of the participants, fifteen (seven female) were obese, with median BMI-for-age at the 99th percentile (range 97 to > 99) while thirteen participants (seven female) were normal weight, with median BMI-for age being at the 50th percentile (range 1–85). Consumption of energy, carbohydrates, fat and protein was not significantly different between the obese and non-obese participants. There was no significant difference between the two groups in faecal levels of *Bacteroides-Prevotella*, *Bifidobacterium* species, *Lactobacillus acidophilus* group or *Eubacterium rectale*. Levels of *Faecalibacterium prausnitzii* were significantly higher in obese children than in non-obese participants ($P=0.0253$). We concluded that the finding of increased numbers of *F. prausnitzii* in the faeces of obese children in south India adds to the growing information on alterations in faecal microbiota in obesity.

Microbiota: *Faecalibacterium*: Obesity: Colon: Butyrate-producing bacteria

Obesity and its attendant consequences are a major cause of ill health in developed countries, and a growing problem in the developing world⁽¹⁾. Obesity can physiologically be attributed to any or all of a combination of increased intake of energy, more efficient absorption of ingested energy, or reduced energy expenditure. The intestine and colon are host to trillions of bacteria, which are largely anaerobic and survive by metabolising unabsorbed dietary constituents⁽²⁾. It is estimated that 10–15% of dietary sugar and starch is not absorbed in the small bowel; fermentation by colonic luminal bacteria to SCFA which are absorbed and metabolised serves to salvage energy⁽³⁾. Individuals with a colectomy weigh on average 4 kg less than age- and height-matched healthy individuals with similar energy intake⁽⁴⁾. It is estimated that the colon contributes to 5–10% of energy requirements in residents of Western countries^(5,6). There are theoretical reasons to believe that the colon may contribute significantly more to energy conservation in countries in Asia and Africa where there is a high intake of starchy foods⁽⁷⁾.

The intestinal microbiota of obese individuals may be more efficient at extracting energy from a given diet than the flora of lean individuals^(8,9). Compared with lean mice, obese mice had fewer bacteria belonging to the division Bacteroidetes,

and more bacteria belonging to the division Firmicutes⁽¹⁰⁾. Human studies have consistently reported increases in Firmicutes in obese adults compared with normal individuals^(8,11,12), whereas alterations in *Bacteroides-Prevotella* were variable. The present study, undertaken in a developing world rural setting, set out to examine the nature of the dominant faecal microbiota in obese children compared with their normal peers.

Methods

The participants were recruited from three private schools. After focus group discussions, children were invited to participate in the study. Obesity was defined as BMI exceeding the 97th percentile for that age using WHO reference growth charts⁽¹³⁾. For each obese participant, a non-obese counterpart in the same class was invited to serve as a control. Anyone who had taken antibiotics within the previous month was excluded.

Socio-economic status was graded according to the modified Kuppuswami scale⁽¹⁴⁾. A 24 h diet recall and a FFQ of commonly used foods taken over the previous 3 months were used to calculate macronutrient intake from food

Abbreviation: LPS, lipopolysaccharide.

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composition tables for Indian diets⁽¹⁵⁾. Standard cups and spoons were used to assess meal sizes. Freshly passed specimens of faeces were collected in plastic containers, transported to the laboratory on ice and stored at -70°C to be processed in batches. Faecal DNA was extracted using the QIAamp DNA stool mini kit (QIAGEN GmbH, Hilden, Germany) and quantitative PCR was carried out using genus- and group-specific primers targeting 16S rRNA genes (rDNA) as described in our earlier publications^(16,17).

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Ethics and Research Committees of the Christian Medical College, Vellore, which reviewed the protocol and the consent forms. Written informed consent was obtained from all participants and their parents.

Statistics

All values are shown as median (interquartile range). Comparisons between groups were done using Mann–Whitney tests and two-tailed *P* values less than 0.05 were taken as statistically significant.

Results

There were twenty-eight participants included in the study, of whom fifteen (seven female) were obese and thirteen (seven female) non-obese. Their demographic characteristics are shown in Table 1. Dietary intakes of energy and of macronutrients did not differ significantly between the two groups of participants (Table 1).

The quantitative bacterial studies showed no significant differences in the levels of the *Bacteroides–Prevotella* group, *Eubacterium rectale*, *Bifidobacterium* group (Fig. 1) or *Lactobacillus acidophilus* group (data not shown) between

the study groups. However, levels of *Faecalibacterium prausnitzii* were significantly higher in the obese compared with the non-obese participants ($P=0.0253$) (Fig. 1).

Discussion

Following the demonstration in mice of characteristic alterations in the faecal flora of obese compared with lean animals and the transferable nature of the obese phenotype by transplanting the flora to germ-free mice⁽⁸⁾, several human studies have been undertaken to confirm the presence and nature of alterations in the faecal microbiota in obese individuals. Studies in adults have consistently reported increases in Firmicutes in obese adults compared with normal or lean individuals^(8,11,12). In a very recent study, Turnbaugh *et al.*⁽¹⁸⁾ examined the faecal microbiota of mono- and dizygotic twin pairs concordant for obesity or leanness, and found that obesity was associated with significantly fewer Bacteroidetes and significantly more Actinobacteria but no significant difference in Firmicutes⁽¹⁸⁾. The present study reconfirmed, in a different a setting as possible compared with earlier studies, that there were increases in the population of *F. prausnitzii* (prominent carbohydrate-fermenting bacteria) in the gut of obese children. At the time of study, energy and macronutrient intake was similar in both groups.

E. rectale–Clostridium coccoides, *Bacteroides–Prevotella* and *F. prausnitzii* are the dominant phylogenetic groups in the faecal microbiota⁽¹⁹⁾. Members of the *Bacteroides–Prevotella* group play important roles in the hydrolysis and fermentation of dietary fibre, producing acetate and propionate⁽²⁰⁾. Butyrate, a physiologically important SCFA, is produced by several bacteria, important among which are the *E. rectale–C. coccoides* group and *F. prausnitzii*^(21,22). *F. prausnitzii*, belonging to the *C. leptum* group of bacteria, is a Firmicute and a highly functionally active member of the intestinal microbial flora⁽²³⁾. It has been identified as one

Table 1. Demographic characteristics and macronutrient intake of the children studied* (Medians and ranges or interquartile ranges (IQR))

	Obese			Non-obese		
	Median	Range	IQR	Median	Range	IQR
Age (years)	13	10–15		12	10–14	
Sex (<i>n</i>)						
Male		8			6	
Female		7			7	
Weight (kg)	65	48–94		43	33–71	
Height (cm)	154	146–169		157	136–177	
BMI for age (percentile)†	99	97– > 99		50	1–85	
Socio-economic class‡	II	I–III		II	I–III	
Total energy intake (kJ/d)	8243		6841–9542	8424		6439–8571
Carbohydrate intake (g/d)	317		285–370	303		255–360
Unrefined	270		242–307	259		219–301
Refined	30		20–52	28		14–51
Sugar	16		12–21	15		11–20
Protein intake (g/d)	51		39–62	50		38–55
Fat intake (g/d)	44		34–64	52		37–64

* Other than weight and BMI percentile, none of the differences between the two groups was statistically significant.

† BMI-for-age percentile value derived from WHO reference growth charts⁽¹³⁾.

‡ Socio-economic scores were used to derive a socio-economic class⁽¹⁴⁾ and was calculated based on the education and occupation of the head of the household and the monthly family income adjusted for 2007. Socio-economic classes I, II and III represent upper, upper-middle and middle classes, respectively, on a scale of I–V.

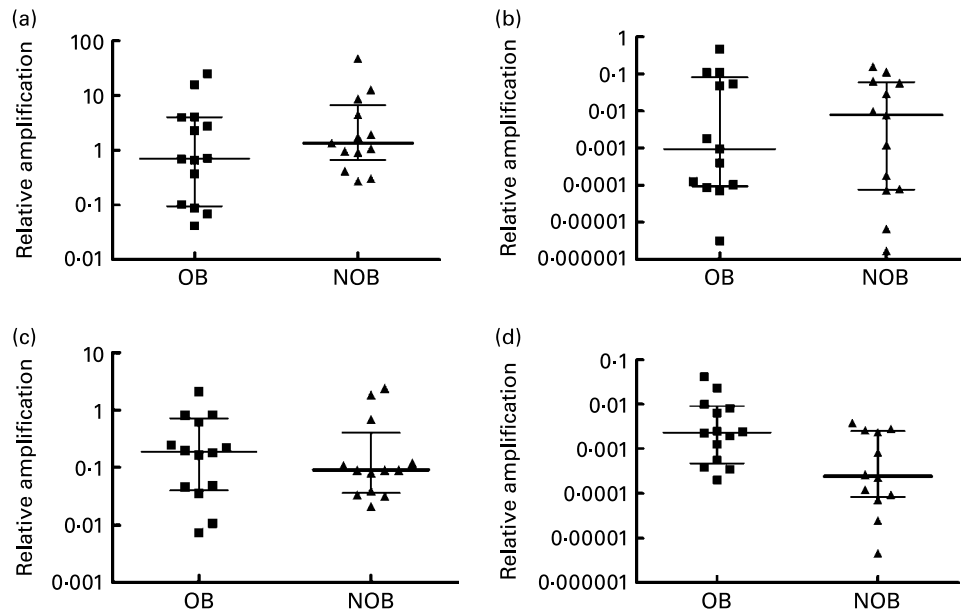


Fig. 1. Quantitative PCR of different bacterial groups from the faeces of obese (OB) and non-obese (NOB) participants: (a) *Bacteroides-Prevotella-Porphoryomonas*; (b) *Bifidobacterium*; (c) *Eubacterium rectale*; (d) *Faecalibacterium prausnitzii*. Values are shown relative to amplification of a conserved segment of 16S rDNA, and the bars represent medians and interquartile ranges. The only statistically significant difference between the two groups was with respect to *F. prausnitzii*, which were significantly higher in the faeces of the obese children ($P=0.0253$; Mann-Whitney test).

of the key functional members of the microbiome that most influence host metabolism⁽²⁴⁾, and is responsible for a significant proportion of fermentation of unabsorbed carbohydrate. In many tropical populations, up to 20% of dietary carbohydrate, including that in such foods as rice, maize, banana and potatoes, is unabsorbed because of the presence of NSP and amylase-resistant starch⁽²⁵⁾. It is conceivable that the presence of *F. prausnitzii* in greater numbers in obese children leads to increased energy salvage from unabsorbed carbohydrate that would not otherwise contribute to dietary energy intake. Interestingly, it has been demonstrated that *F. prausnitzii* were significantly reduced in frail elderly individuals as well as in patients with chronic idiopathic diarrhoea and malnutrition^(26,27). It is of interest that dietary carbohydrate restriction results in reductions of *E. rectale* and other butyrate-producing Firmicutes in the faeces of obese individuals^(12,28). SCFA such as acetate, propionate and butyrate are ligands for the G-protein-coupled receptors Gpr41 and Gpr43 on colonic epithelial cells, the activation of which result in the release of gut-derived hormones such as peptide YY which affects energy harvest from the diet⁽²⁹⁾. Although the specific microbial classes or genera involved in the effect are not yet known, the establishment of an intestinal microbiota in germ-free mice has been shown to suppress epithelial cell production of fasting-induced adipocyte factor resulting in increased lipoprotein lipase activity in adipocytes and promoting storage of energy as fat⁽⁹⁾. Yet another mechanism, involving regulation of systemic inflammation, has been proposed for the connection between intestinal microbiota and obesity. Lipopolysaccharide (LPS) is a key constituent of gut bacteria and plays a central role in innate immune responses in the gut. It is also absorbed systemically presumably through intercellular junctions and this process can be regulated by factors that control intestinal permeability.

Obesity has been shown to be associated with increases in intestinal permeability and in plasma LPS in mice fed a high-fat diet; similar increases in plasma LPS were noted in *ob/ob* mice ingesting normal chow⁽³⁰⁾. On the other hand, the plasma LPS rise and the metabolic changes associated with obesity were lacking in *ob/ob* CD14^{-/-} mice that were non-responsive to LPS. Furthermore, antibiotic treatment of the *ob/ob* mice led to decreases in adipose tissue inflammatory markers and metabolic markers of obesity⁽³⁰⁾. These microbiota-dependent changes may be mediated via release of glucagon-like peptide⁽³¹⁾. *F. prausnitzii* is known to be reduced in the faecal microbiota of patients with Crohn's disease^(32,33), and is thought to be protective against intestinal inflammation by secreted metabolites able to block NF- κ B activation and IL-8 secretion. The intestine normally exists in a state of controlled inflammation, but a relationship between the degree of background mucosal inflammation and obesity has not been explored.

Assigning causality to the association between changes in faecal microbiota and obesity in humans is difficult. One approach is to examine the faecal microbiota at birth and to follow up these children later. Kalliomaki *et al.*⁽³⁴⁾ analysed the faecal microbiota at ages 6 and 12 months in children that were later at age 7 years identified as being overweight or of normal weight⁽³⁴⁾. They found that children who were overweight at age 7 years had significantly fewer bifidobacteria and significantly more staphylococci in the stool during infancy. The present study does not make any conclusions regarding causality, and does not indicate whether *F. prausnitzii* is a mere marker or whether it is causally linked to obesity. Accrual of data from different regional and ethnic groups can possibly improve our understanding of the role of the faecal microbiota in obesity. Studies are also required to identify the mechanisms whereby different constituents of

the microbiota regulate body weight. Eventually this understanding may allow the use of therapeutic manipulations of the flora to combat obesity.

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R. B. was responsible for the quantitative analysis and supervision of the study; G. G. was responsible for recruitment, consenting and sample collection; J. K. and A. M. S. C. were responsible for the diversity studies and analysis; J. H. was responsible for dietary analysis; and B. S. R. was responsible for overall conception, supervision and manuscript finalisation.

The authors have no conflicts of interest to declare.

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