Short Communication

Faecal microbiota composition in vegetarians: comparison with omnivores in a cohort of young women in southern India

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(Submitted 27 July 2011 – Final revision received 12 October 2011 – Accepted 26 October 2011 – First published online 20 December 2011)

Abstract

The effect of vegetarian diets on faecal microbiota has been explored largely through culture-based techniques. The present study compared the faecal microbiota of vegetarian and omnivorous young women in southern India. Faecal samples were obtained from thirty-two lacto-vegetarian and twenty-four omnivorous young adult women from a similar social and economic background. Macronutrient intake and anthropometric data were collected. Faecal microbiota of interest was quantified by real-time PCR with SYBR Green using primers targeting 16S rRNA genes of groups, including: Clostridium coccoides group (Clostridium cluster XIVa), Roseburia spp.–Eubacterium rectale, Bacteroides–Prevotella group, Bifidobacterium genus, Lactobacillus group, Clostridium leptum group (Clostridium cluster IV), Faecalibacterium prausnitzii, Ruminococcus productus–C. coccoides, Butyrivibrio, Enterococcus species and Enterobacteriaceae. The groups were matched for age, socio-economic score and anthropometric indices. Intake of energy, complex carbohydrates and Ca were significantly higher in the omnivorous group. The faecal microbiota of the omnivorous group was enriched with Clostridium cluster XIVa bacteria, specifically Roseburia–E. rectale. The relative proportions of other microbial communities were similar in both groups. The butyryl-CoA CoA-transferase gene, associated with microbial butyrate production, was present in greater amounts in the faeces of omnivores, and the levels were highly correlated with Clostridium cluster XIVa and Roseburia–E. rectale abundance and to a lesser extent with Clostridium leptum and F. prausnitzii abundance and with crude fibre intake. Omnivores had an increased relative abundance of Clostridium cluster XIVa bacteria and butyryl-CoA CoA-transferase gene compared with vegetarians, but we were unable to identify the components of the diet responsible for this difference.

Key words: Gastrointestinal microbiota: Energy harvest: Clostridium cluster XIVa: Diet

The advent of the molecular characterisation of intestinal microbiota has occasioned an increased interest in the relationship between diet, microbiota and nutrition. Unabsorbed dietary carbohydrates form the major energy source for the colonic microbiota. In turn, the bacteria ferment these to SCFA which are absorbed and enter the portal venous system, and contribute to energy in the host. Changes in gut microbiota have been described in association with obesity and have been linked to increased energy harvest(1–3). Vegetarian diets have been promoted for specific conditions including induction of weight loss and improvement in insulin resistance(4,5). The influence of a vegetarian diet on faecal microbiota remains speculative. In an early study based on quantitative culture, consumption of meat increased the counts of Bacteroides, Bifidobacterium, Peptococcus and anaerobic Lactobacillus species(6). On the other hand, a recent study using molecular techniques has reported that young adults had a predominance of Clostridium cluster IV bacteria in faecal microbiota compared with elderly individuals, with omnivores among the young adults having marginally higher proportions of these bacteria than vegetarians(7).

Rural populations in southern India consume a largely vegetarian diet. There are two factors that determine whether these populations eat any meat. The first relates to socio-economic circumstances as a result of which meat forms a part of the diet only once or twice per week. The second is religious persuasion leading to total avoidance of meat intake in significant sections of these populations. These dietary patterns are most distinct among women in these populations. The present study examined a cohort of young women from a rural
background studying in a women's college in southern India, and reports the dietary intakes, nutritional status and faecal microbiota of this cohort, with particular reference to the differences between omnivores and vegetarians.

Methods

A total of thirty-two vegetarian and twenty-four non-vegetarian young women (aged 18–27 years), all from a very similar socio-economic background, were recruited from resident students in a women's college in Tamilnadu. The study was preceded by focus group discussions in the college with the teachers and students where the purpose of the study was explained and students were invited to participate. Participants were excluded if they had consumed antibiotics within the last 1 month or had gastrointestinal symptoms at the time of the study. A 24 h diet recall, together with an FFQ of commonly used foods ingested over the previous 3 months, was administered by a trained dietitian. Food quantities were measured according to a standard set of cups and spoons, and the daily intake of relevant nutrients was calculated from standard tables for the composition of Indian foods. Height and weight were measured and BMI was calculated. The socio-economic score was calculated according to the method of Kuppuswami modified for 2007. Each participant provided a sample of freshly passed stool in cups and spoons, and the daily intake of relevant nutrients was measured according to a standard set of values. The reference universal amplicon was used to examine their correlations (Pearson) with dietary components and with one another, as well as for linear regression. Two-tailed P values less than 0.05 were considered to be statistically significant.

Results

In the present study, thirty-two vegetarians and twenty-four omnivores were enrolled in the study. There were no significant differences in age, height, weight, BMI or socio-economic score between the two groups (Table 1). Total energy intake, complex carbohydrates and Ca were significantly higher in the omnivorous group than in the vegetarian group (Table 1).

Faecal levels of the C. coccoides–E. rectale group (Clostridium cluster XIVa bacteria) were higher in the omnivores (relative difference: median 0.08 108, interquartile range 0.04 229–0.12 477) compared with the vegetarian group (median 0.03 158, interquartile range 0.00 912–0.06 229; P ¼ 0.004). This was contributed by significant increases in Roseburia–E. rectale (P ¼ 0.025), whereas R. productus–C. coccoides and Butyrivibrio were not increased (Fig. 1). All the other microbial groups tested were similar between the two groups (Fig. 1). Interestingly, butyryl-CoA-CoA-transferase gene levels were significantly higher in the omnivore group compared with the vegetarian group (P ¼ 0.019, Fig. 1). Log-transformed levels of the gene were significantly correlated with C. coccoides–E. rectale (Pearson r ¼ 0.607, P < 0.0001) and Roseburia–E. rectale (Pearson r ¼ 0.777, P < 0.0001) but not with R. productus–C. coccoides. Transferase gene levels were negatively correlated with fat intake (r ¼ −0.288, P ¼ 0.032) and showed a trend for a positive correlation with crude fibre intake (r ¼ 0.231, P ¼ 0.089). C. coccoides–E. rectale and R. productus–C. coccoides abundance correlated with crude fibre intake (r ¼ 0.33, P ¼ 0.014; r ¼ 0.30, P ¼ 0.022, respectively). Linear regression with butyryl-CoA-CoA-transferase as the dependent variable and various microbial communities, crude fibre and fat as independent variables showed that Roseburia–E. rectale (P ¼ 0.000) and C. coccoides–E. rectale
Faecal microbiota of vegetarians

Table 1. Demographics, anthropometric data and dietary intakes of the study participants (Median values and interquartile ranges)

<table>
<thead>
<tr>
<th></th>
<th>Vegetarians (n 32)</th>
<th>Omnivores (n 24)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19</td>
<td>18–19</td>
<td>19</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155</td>
<td>154–157</td>
<td>155</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>48</td>
<td>45–53·7</td>
<td>53·5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20·0</td>
<td>18·3–22·6</td>
<td>21·9</td>
</tr>
<tr>
<td>Socio-economic score</td>
<td>20</td>
<td>17–22</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary intakes</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/d)</td>
<td>5380</td>
<td>4807–6112</td>
<td>5983</td>
</tr>
<tr>
<td>Simple carbohydrate (g/d)</td>
<td>29·5</td>
<td>17·5–36·5</td>
<td>25·5</td>
</tr>
<tr>
<td>Complex carbohydrates (g/d)</td>
<td>225</td>
<td>192–239</td>
<td>246</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>30</td>
<td>26–33</td>
<td>32</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>21·5</td>
<td>19–25·5</td>
<td>20·5</td>
</tr>
<tr>
<td>Non-haem Fe (mg/d)</td>
<td>8</td>
<td>6·7–9</td>
<td>8·68</td>
</tr>
<tr>
<td>Ca (mg/d)</td>
<td>195</td>
<td>138–235</td>
<td>250</td>
</tr>
<tr>
<td>Fibre (g/d)</td>
<td>2·90</td>
<td>2·24–3·30</td>
<td>2·95</td>
</tr>
</tbody>
</table>

* P values from Mann–Whitney U tests for the assessment of significant differences between the median values.

(P=0.044) were the only variables that independently correlated with log abundance of the transferase gene.

Discussion

The present study evaluated differences in faecal microbiota that could be related to a vegetarian or omnivore diet in a well-matched population of young women in southern India, and found increases in Clostridium cluster XIVa bacteria in the omnivore group compared with the vegetarian group. This was accompanied by an increase in the genes coding for a key enzyme involved in butyrate production.

The two groups of participants were very well matched, being within a narrow age range, of the same sex, all from the same geographical area and all accustomed to food prepared in a typical southern Indian style. The vegetarians in this group consumed small amounts of cows’ milk on a daily basis, in keeping with traditional practices in southern India, and were not vegans. Vegetarian diets are associated with lower BMI, lower overall cancer rates and a lower risk of death from IHD(20). In the present study, BMI of the participants in the omnivorous group was significantly higher than that of the participants in the omnivore group. The intake of Ca, normally quite low among women in India, was particularly low in the vegetarian group compared with the omnivore group.

Dietary preferences play a major role in shaping the intestinal microbiota. Unabsorbed dietary carbohydrate, consisting of amylase-resistant starch and NSP, is the major energy source for the microbiota. The latter, in turn, helps the host by salvaging otherwise ‘lost’ energy through fermentation of unabsorbed carbohydrate and production of SCFA which contribute to energy in the human host. There are only a few studies of the faecal microbiota of vegetarians in the molecular era. A recent study has used molecular (PCR-denaturing gradient gel electrophoresis) analysis of the faecal microbiota of vegetarians and omnivores but failed to show significant differences in faecal microbiota; however, the authors noted a tendency for Clostridium cluster IV bacteria and F. prausnitzii (belonging to the same cluster) to be found more frequently in the faeces of omnivores compared with vegetarians(7).

In the present study, the faecal microbiota contained significantly greater numbers of the C. coccoides–E. rectale group (belonging to Clostridium cluster XIVa) in omnivorous women compared with vegetarian women. Bacteria belonging to Clostridium clusters XIVa and IV are noted for their ability to ferment carbohydrate to SCFA. SCFA, especially butyrate, have physiologically important effects on the colonic epithelium and on host metabolism. Dominant butyrate-producing bacteria including Roseburia species, E. rectale, Ruminococcus species and Butyrivibrio species(21) belong to Clostridium cluster XIVa. Using appropriate primer pairs, we have shown that the faecal microbiota of omnivores was enriched with Roseburia–E. rectale compared with vegetarians, whereas R. productus–C. coccoides and Butyrivibrio were not significantly different between the two groups. Butyryl-CoA-CoA-transferase is an enzyme involved in butyrate synthesis by the microbiota(18,21), and its gene levels correlated with the levels of both the Roseburia–E. rectale group and cluster XIVa bacteria as a whole, possibly indicating that these microbial groups are the predominant butyrate-producing bacteria in this population. Quantitative PCR was the only tool used to assess the abundance of subgroups and showed an increased relative abundance of Clostridium cluster XIVa bacteria (C. coccoides–E. rectale) generally and specifically of the Roseburia–E. rectale group within this cluster. Although differences in the abundance of the subgroups may be compensated by other microbial groups that provide the same metabolic capacity, we also found a difference in metabolic capacity (i.e. in butyryl-CoA-CoA-transferase gene numbers) for producing butyrate, which suggests that the difference in subgroups between vegetarians and omnivores is relevant. The reason for the increased abundance of...
Roseburia–E. rectale in omnivores was not clear. Although crude fibre intake and fat intake correlated with the abundance of specific microbial communities in the group as a whole, they did not explain the differences between vegetarians and omnivores. It is possible that dietary constituents not measured in the study or complex nutrient–microbiota–microbiota interactions are responsible for this observation.

In conclusion, comparison of the faecal microbiota of lacto-vegetarian and omnivorous young women showed that Clostridium cluster XIVa bacteria and butyrate-producing bacteria were significantly more abundant in the faecal microbiota of omnivores. Identifying the reason for this phenomenon may require more detailed approaches involving metagenomic and metabonomic analysis.
Acknowledgements

J. K. was supported by a Senior Research Fellowship from the Indian Council of Medical Research. This study was supported by grants from the Department of Biotechnology, New Delhi. J. K. was responsible for the conception, design, sample analysis, data analysis and writing of the manuscript; R. S. D. was responsible for participant recruitment and dietary analyses; R. R. M. was responsible for conception and participant recruitment; B. S. R. was responsible for the conception, design and writing of the manuscript. The authors have no conflicts of interest to report.

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