Microbiota modulation by diet in humans: prebiotics, fibres and other compounds.

ABSTRACT

Based on the relevance of the intestinal microbiota on health, this article is focused in the effect of diet, and its components, in modulating the activity of the colonic flora. For this purpose, we described briefly previous evidences regarding the effects of different prebiotics on the microbial balance, from the classics as fructo-oligosaccharides (FOS), galact-oligosaccharides (GOS) or inulin to the most innovative as resistant starch. In addition, it will be highlighted the importance of other compounds associated with fibre intake, as polyphenols, whose prebiotic/antimicrobial effects remains to be elucidated. Finally, some perspectives for future research in this subject were pointed up, which we believe may represent a considerable advance in knowledge of this topic.

INTRODUCTION

With the increased commercialization of functional foods, which has occurred in the last decades, foods with probiotics, prebiotics or both have been used by most of the population. The human gastrointestinal tract (GIT) harbours a very complex and dynamic microbial community which, in number, exceeds by an order of magnitude the number of host cells (1). Different microorganisms and levels are found throughout the gastrointestinal tract, as corresponds with the different ecological niches present from mouth to colon; the stomach and upper bowel being sparsely populated whilst the colon is heavily colonized. The process of establishment of this microbiota starts at birth and later develops depending on interplay between genetics, environment and diet. This microbiota plays an important role in human health not only due to its participation in the digestion process, but also for its critical functions on the development of the gut and the immune system. Indeed, it has been demonstrated that this bacterial colonization of the intestine is needed for the development of oral tolerance (2) and for the establishment of the mucosal barrier and the maintenance of intestinal homeostasis (3). The microbiota is also needed for a proper morphological development of the intestine (4) and it regulates host metabolism (5). In addition, it plays an essential role for an adequate immune development (6).
On the other hand, the human gastrointestinal tract is one of the larger surfaces of exposure to the outer environment being an important area of exchange of information with the outer environment. There are more lymphoid cells associated with the gastrointestinal mucosa than with the spleen, peripheral lymph nodes and blood taken together, more than an 80% of the B-cells in the body are gut-associated, making the gut the biggest immune organ (7). Taking into account these considerations, it is obvious that the intestinal microbiota provides the most important contact with the environment for the host and a barrier against harmful food components and pathogenic bacteria.

The new molecular techniques have enormously increased our knowledge and helped to determine the community structure of the gut microbiota. They have allowed identifying three human microbiota enterotypes and assessing the effect of diet (8). Determining the microbiome of healthy as compared with disease individuals has allowed identifying aberrancies related to several human diseases and alterations related with different life stages, such as senescence or prematurity. However, most of our microbiota data today are derived from results obtained from faecal samples and, therefore, represent the microbiota present in the colonic lumen. Studies on other intestinal locations, such as the mucosa, are much limited although differences appear to exist (9) and alterations related with disease have also been observed (10).

**EVIDENCES OF STUDIES WITH PREBIOTICS IN HUMANS**

A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health (11). Candidate prebiotics must fulfil the following criteria (12): (a) resistance to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption; (b) substrate of fermentation by intestinal microorganisms belonging to the human microbiota; (c) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The most studied prebiotic compounds are non-digestible oligosaccharides such as inulin, fructo-oligosaccharides (FOS) and galact-oligosaccharides (GOS), among others.

A large part of research activity had been focused on the selection of compounds able to modify the microbiota intestinal in the colon. However, nowadays there is increased evidence that prebiotics may induce beneficial physiological effects along to the gastrointestinal tract (GIT) as well as at systemic level (13). These include, amongst others: -Selective growth of beneficial intestinal microbiota and improvement the intestinal functions. Gut microbiota is a key player in health and wellbeing with a composition in which beneficial microorganisms dominate over potentially harmful ones. Bifidobacteria and Lactobacilli constitute the traditional target of prebiotics, but other microorganisms such as *Faecalibacterium prausnitzii* also have ability to degrade them. Prebiotic consumption has been reported to improve intestinal functions like regularity, intestinal barrier or competitive exclusion of pathogens. -Immunomodulatory properties. Prebiotics may influence the immune system directly or indirectly through changes in the microbiota that may affect the immune response of the host. Prebiotics may also bind to...
receptors of immune cells and participate in the modulation of different immune parameters. -
Stimulation of mineral absorption. Prebiotics help to increase Ca bioavailability by reducing
intestinal pH as consequence of short chain fatty acids (SCFA) production and extending the site
of mineral absorption towards the large intestine. The absorption of others minerals, such as Mg or
Fe, have also been suggested to be improved too. -Reduction of risk of disease. Prebiotics
reduce risk of intestinal infections by competitive exclusion, reducing intestinal pH, etc.
Compounds with prebiotic properties have also been reported to promote the endocrine
function of the gut, favouring the regulation of lipid metabolism, glycemia and insulin resistance;
and food intake and satiety (14).

However, only some of these proposed beneficial effects of prebiotics are backed by human
studies. These human studies and RCTs have been performed to evaluate some potential health
benefits of prebiotics including; effects on microbiota, immune system, mineral absorption and
gastrointestinal disorders (Table 1).

<table>
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<tr>
<th>Author</th>
<th>Mechanism</th>
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<tr>
<td>Microbiota</td>
<td>Inulin, FOS and GOS are the compounds more tested in human trials able to change the gut flora composition. AXOS increase the bifidobacteria, decrease the proteolitic fermentation and increase the butyric levels in healthy adults.</td>
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<td>Roberfroid et al. (2010)</td>
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<td>Immune system</td>
<td>GOS increased phagocytosis, NK cell activity and IL-10; and decreased IL-1, IL-6 and TNFα, in healthy elderly volunteers.</td>
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<td>Vulevic et al. (2008)</td>
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<td>Mineral absorption</td>
<td>Calcium absorption was increased by prebiotic consumption in healthy adolescents and postmenopausal women.</td>
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<td>Roberfroid et al. (2010)</td>
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<td>GI disorders</td>
<td>Benefits in treating active of pouchitis, Crohn’s disease and ulcerative colitis and a reduction on colon cancer risk markers by prebiotics</td>
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<td>Casellas et al. (2007), Lindsay et al. (2006), Friedman et al. (2000), Clark et al. (2012)</td>
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Mixture GOS/FOS reduced significantly the frequency of atopic eczema in babies with risk of allergy, and this effect was maintained during two years.


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Table 1 Effects of prebiotic products on human health.

FIBRE INTAKE AND SHORT CHAIN FATTY ACIDS

Dietary fibre includes non-starch polysaccharides, oligosaccharides, lignin and resistant starch, compounds that reach the colon without being absorbed in a healthy human gut. Increased consumption of dietary fibre is widely recommended in occidental societies, to improve health. Generous intake of fibre reduces risk for developing some chronic diseases like coronary heart disease, stroke, hypertension, diabetes or obesity. In addition, due to its indigestible nature, these compounds may exert some beneficial effects on the gastrointestinal tract. In this sense, insoluble fibre, like cellulose and lignin, is only fermented to a limited extent in the colon and contributes to increase faecal bulk, enhance gut motility and reduce transit time, while soluble fibre with high fermentability, such as pectin and resistant starch, are associated with the production of the volatile SCFA, together with lactate, CO₂, methane and hydrogen. The main SCFA are, in this order, acetate, propionate and butyrate, in a molar ratio of approximately 3:1:1, respectively, in the proximal and distal colon. In the last years, a great number of actions have been attributed to SCFA, whose concentration has been associated with reduced risk of some diseases, such as the irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease and cancer (Figure 1). Numerous studies have pointed butyrate as a key factor in the maintenance of colon health, being the principal energy source for colonocytes, enhancing normal colonic cell proliferation or exerting antioxidant and anti-inflammatory properties, this has led to consider it as important preventive factor of colon cancer. O’Keefe et al., compared the excretion of SCFA in three healthy populations with different nutritional characteristics and different risk level of colon cancer: Native Africans (low risk), African Americans (high risk) and Caucasian Americans (high risk). They found that acetate, propionate and butyrate were higher in Native Africans than in the other two groups. This observation was attributed, by the authors, to their diet rich in whole-grain cereals and resistant starch. It is thought that butyrate excretion is mainly due to the fermentation of resistant starch, and there are some studies that confirm this hypothesis. In a population study carried out by Segal et al., was showed that total SCFA and butyrate excretion levels were greater in native Africans than those in Europeans living locally, despite of the higher intake of total dietary fibre in the latter. This could be due to an increased consumption of resistant starch in the Africans. Some dietary intervention works have supported these observations in epidemiological studies. Jenkins et al., in a study conducted in 24 healthy subjects, found higher levels of faecal butyrate with a diet rich in resistant starch.
However, other authors found that a high intake of resistant starch, not only increases butyrate levels, but also total SCFA and acetate. Based on these results, it seems obvious that resistant starch is the main source for butyrate synthesis, however further studies are needed to deepen this association.

Propionate has been related with hepatic lipogenesis regulation, decrease serum and hepatic cholesterol and triglycerides levels, and appetite control. Tabernero et al., analysing the in vitro fermentability of different fibre sources, found that cereal fibre was the main contributor to propionate production. Cereals are rich in β-glucans and arabinoxylans, soluble fibre types which have been associated with propionate synthesis by other authors. These scientific evidences could lay the groundwork for the blood lipid modulation by the diet. In this way, the American Heart Association recommends an increased intake of carbohydrates, especially complex carbohydrates and fibre to prevent cardiovascular disease. Finally, acetate, as well as propionate, is involved in lipid metabolism, being the primary substrate for cholesterol synthesis. Bridges et al., by a dietary intervention found that acetate serum levels were higher when subjects were fed with a diet rich in oat-bran, food rich in fermentable fibres. These evidences confirm that, not only is important the type of fibre but also the source of this fibre is a factor to consider.

SCFA’s synthesis not only depends on diet, but also another factors like number and types of bacteria in the colon and gut transit time. These compounds can, in turn, modulate intestinal microbiota. In this sense, it has been seen that total SCFA in general and butyrate in particular promotes the growth of Lactobacilli and Bifidobacteria species, which have been related with an improved health.

OTHER COMPOUND: PHYTO-COMPOUNDS

Polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin (as fruits, vegetables, tea and coffee, red wine, chocolate and olive oil). Apart from its role as antioxidants, the consumption of polyphenols has been suggested to have diverse benefits such as improved gut health. There are few studies in the literature analysing the effect of polyphenols on modulation of human microbiota and most of the research in this area comes from intervention studies performed with isolated of different selected polyphenols or polyphenol-rich foods or beverages. Recent studies carried out in animals and human volunteers revealed that polyphenol-rich food such as red wine, tea or cocoa produces modifications in intestinal bacteria populations. While Queipo-Ortuño et al. have recently published changes in the concentration of Proteobacteria, Fusobacteria, Firmicutes and Bacteroidetes in humans after consuming alcoholic beverages (red wine and gin) and de-alcoholized red wine, animal studies have found a lower proportion of Clostridium and Lactobacilli in polyphenol-treated rats respect control ones. By other hand, the effects of cocoa flavanols remain controversial: an increase in Lactobacilli and Bifidobacteria has been described in humans, but evidences in animal studies showed a decrease of Bacteroides, Clostridium and Staphylococcus. Although
the differences in the research that analyses the relationship of polyphenols with the microbiota make them difficult to compare, the evidence seems strong enough to take into account the intake of these phyto-compounds in assessing the effect of probiotics and prebiotics on intestinal flora. It appears that the stimulatory or antimicrobial effect of polyphenols depends largely on the microbial group. Both, the effect on decreasing pathogenic bacteria as increasing the beneficial ones, they could contribute in the maintenance of a balanced microbiota and, hence, in our health.

DIETARY PATTERNS AND MICROBIOTA

Most of the previous research about diet and microbiota addressed the effect of single food components on different groups of bacteria. There are solid evidences that prebiotics, fibre and other dietary compounds affect bacterial populations that live in our colon. However, the interrelation between diet and intestinal microbiota is very complex in the extent to which bacteria are affected by the compounds that reach the colon from the diet but also they produce changes in some of these compounds, so analyse both a bidirectional relationship. The complex interaction between the different compounds consumed as a part of a whole diet, together with the effect of other factors as antibiotic use or stress makes difficult to clarify this association. Furthermore, the effect of one or more isolated compounds on bacterial populations is not extrapolated to the context of a diet. The combined intake of foods in a diet may modify the effect of the individual components on the microbiota due to the existence of synergies and antagonisms between them. In this sense, Mediterranean diet has been proposed in recently studies as a protector risk factor of colon cancer, whilst Western diet characterized by high intake or red and processed meat and refined carbohydrates, have the opposite effect. Some authors propose that the protective effect of Mediterranean diet is due the combination of food sources with fibre and the intake of other compounds such as polyphenols associated (24). At this point, many questions remains in the air, How much time is necessary to modify the intestinal microbiota changing food intake?, Are the amounts of fibre, prebiotics and phyto-compounds that we consume in a normal diet capable to impact on our flora or it would be necessary the use of supplements?. In this regard, despite of the lack of studies in humans, works conducted in animals showed an increase in Firmicutes and a decrease in Bacteroidetes phylum by consequence of being fed with a Western diet (33). This ratio Firmicutes/Bacteroidetes is often used in humans as an indicator of microbial balance.

CONCLUSION AND PERSPECTIVES OF FUTURE

It is well demonstrated that the intestinal microbiota constitutes an important factor for the health and well-being. The rationale for developing strategies aiming at the modulation of the colonic microbiota derives from this demonstration on the importance of microbiota on host’s health and these microbiota aberrancies constitute clear targets for the future development of dietary interventions directed to correct them. Now, it is time to take advantage of this omics era where
the metagenomics is allowing us to know in deep the microbial composition of different human populations and which bacterial groups constitute targets for modulating in different human populations. To this regard, dietary manipulation represents a potential tool for the rational and directed manipulation of the human intestinal microbiota in the context of the health and disease.

REFERENCES AND NOTES


Authors: ADRIANA CUERVO 1, SILVIA ARBOLEYA 2, MIGUEL GUEIMONDE 2 AND SONIA GONZÁLEZ 1*.

1 Department of Functional Biology, University of Oviedo. Facultad de Medicina, C/Julián Clavería s/n, 33006, Oviedo, Asturias, Spain.

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Figure 1 Diet, microbiota and health effects.