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The influence of some dietary components on intestinal microbiota

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Summary. The gut microbiota is the set of symbiotic microorganisms that resides in our intestine. The interaction between the gut microbiota and the mucosal immune system can be altered as a result of shifts in the composition or metabolic activity of the intestinal microflora. Recent studies establish that diet is one of the main involved factors in determining the microbial composition of the gut suggesting its role as external factor able to promote the onset of specific diseases by disrupting the immune homeostasis. Starting from the evidence that the 57% of the gut microbiota's entire variation are due to dietary alterations this review aims at providing an overview of the positive impact of some dietary components on gut microbiota composition.

Key words: dysbiosis, gut microbiota, diet, inflammatory bowel disease, IBD

Introduction

The human gut microbiota is a densely populated bacterial community with approximately about 100 trillions of bacteria and more than 500 different species in the colon that can reach a concentration or density of 9×10^{13} units (1). It is a good example of mutualistic relationship since the mammalian hosts provide a uniquely suited environment in return for physiological benefits provided to them by their gut microbiota (2). Examples of the latter include the fermentation of indigestible carbohydrates to produce short chain fatty acids (SCFA) that are utilized by the host, the biotransformation of conjugated bile acids, the synthesis of certain vitamins, the degradation of dietary oxalates, the hydrolysis of urea by urease activ-

ity, the participation in host nitrogen balance, and the modulation of the mucosal immune system. Mainly, the microbiota exerts physiological functions that include the growth inhibition of pathogenic bacteria, the absorption of nutrients and minerals, the regulation of the intestinal immune system and the signals of gut cell renewal, the synthesis of vitamins and amino acids, and the degradation of protein compounds (3). The dietary components are the energy sources that support the microbial community of the large intestine. The anaerobic metabolism by the microbial community in the colon produces SCFA, CO₂, H₂ and CH₄ (4). SCFAs, in particular, play a direct role in the maintenance of intestinal health and in the onset of disease. They originate from the bacterial fermentation of polysaccharides, oligosaccharides, and glycoprotein precursors

and are produced in different amounts in the different tracts of the intestine. SCFAs influence the gut environment and the host as regulators of gene expression and cell differentiation and anti-inflammatory agents (5,6). They are also fundamental for mucous membrane growth control and regulate the proliferation of colonic epithelial cells. The butyrate, in particular, enhances the growth of a specific group of bacteria such as *Lactobacilli* and *Bifidobacteria*, which play a very important role in the physiology of colonocytes (7).

In this review we introduce the preliminary findings reported in literature in order to maintain a healthy resident gut microbiota. Prior to this, however, a brief description of the microbiota and its alteration is given.

Dysbiosis

The term “dysbiosis” was originally coined by Metchnikoff to describe altered pathogenic bacteria in the gut (8). This condition leads to qualitative and quantitative changes in the intestinal flora, its metabolic activity and its local distribution (9). The dysbiosis hypothesis states that the modern diet and lifestyle, as well as the use of antibiotics, have led to the disruption of the normal intestinal microflora. These factors result in alterations in bacterial metabolism, as well as the overgrowth of potentially pathogenic microorganisms. It is believed the growth of these bacteria in the intestines results in the release of potentially toxic products that play a role in many disease conditions (8), including irritable bowel syndrome (IBS) (10) and inflammatory bowel disease (IBD) (11,12) as well as more systemic conditions such as rheumatoid arthritis (13,14). Different studies have shown, in particular, reduced diversity of the gut microbiota in IBD patients (15–18). The most consistent observations of altered composition of the gut microbiota are a reduction in *Firmicutes* and an increase in *Proteobacteria* (15,16,19,20).

The reduced diversity of the gut microbiota observed is largely due to a decline in the diversity of *Firmicutes*. Among *Firmicutes*, has been reported a decrease in the *Clostridium leptum* groups, especially *Faecalibacterium prausnitzii* (16,21,22).

Methods

This review aims to provide an up-to-date overview of the influence of some dietary components on intestinal microbiota composition.

Literature search

Relevant articles were identified through two different search methods: (1) a PubMed search to identify related papers and books, using the following keywords: gut microbiota, dysbiosis, diet, cereal, coffee, pomegranates, wine, resveratrol, inulin, broccoli, banana, chocolate, inflammatory bowel disease; (2) a check of the reference lists of original studies or reviews for additional publications.

Study selection

The title and abstract of each study identified in the search was screened to determine the study's eligibility for full review. The full-text report was retrieved if the study investigated or potentially investigated the effects of some dietary components on gut microbiota. Only human studies published in English were included.

Foods that can modulate the intestinal microbiota exerting positive effects on health

Some preliminary studies suggest that dysbiosis is caused by genetic and environmental factors, rather than being a consequence of inflammation.

Zhang et al. (23) reported that dietary alterations are responsible for 57% of the gut microbiota's entire variation, whereas genetic background explains only 12%. Moreover, changes in dietary factors cause both quantitative and qualitative shifts in the supply of substrates available to the intestinal microbiota (24). On the basis of these observations, it becomes clear that dietary factors can lead to the prevention of dysbiosis that seems to be the prelude to the onset of several diseases such as inflammatory bowel diseases. Keys' studies (25) have showed the nutritional value of the Mediterranean Diet that thanks to the high supply of

fibres and antioxidants favours the prevalence of saccharolytic bacterial species responsible of the short-chain fatty acids production, while Western Diet promotes the shift towards a proteolytic profile which leads to dysbiosis (26). Although each adult's gut shows a unique microbiome community with a stable structure (core), diet can modulate resident and traveler microbes altering the balance towards a potentially healthier microflora (27). Emerging evidences have already showed that a very low-carbohydrate, high-fat (LC) diet compared with a high-carbohydrate, high-fibre, low-fat (HC) diet leads to reductions in fecal output, concentrations of butyrate and total SCFA and counts of *bifidobacteria* (28).

Furthermore, long-term intake of fiber can reduce the risk of developing Crohn's disease (29). This could be explained considering that fibers derived from fruits are soluble and so inhibit the translocation of *E. Coli* across Peyer's patches maintaining the intestinal barrier function. Some fruits are able to activate also the aryl hydrocarbon receptor that can mediate protection against environmental antigens activating dioxin- or xenobiotic-response element sequences.

Several non-digestible carbohydrates, such as glucans, galactans, arabinoxylans (AX), resistant starch (dextrin), can be fermented by the gut microbiota leading to a switch of microflora's composition (30,31). Significant studies demonstrate that AX added to a high-fat diet led to an increase in *bifidobacteria*, particularly *Bifidobacterium animalis* subsp. *lactis*. AX also restored the cecal content of Gram-negative *Bacteroides-Prevotella* spp. and Gram-positive *Roseburia* spp. bacterial populations to control levels, which were decreased following a high-fat diet. Moreover, many human studies demonstrated that fructose oligosaccharides have a bifidogenic effect on human colonic endogenous flora (32-34). Additional data showed that inulin-type fructans have the same effect on humans (35-36). By means of these, the growth of *bifidobacteria*, was measured in the stool of human volunteers fed varying amounts of prebiotics. Stool slurries were incubated with a specific prebiotic and changes in *bifidobacteria* were quantitated, the results were mixed and a dose-response curve could not be obtained (37). The bifidogenic effect could be explained by the fact that prebiotics are preferential substrates for *bifidobacteria* and interact with other bac-

teria causing environmental changes, such as changes in luminal pH and other factors.

Starting from these assumptions, we report a table (Table 1) indicating some foods and beverages with potentially positive impact on individual's health.

Chicory, artichokes, Jerusalem artichokes, garlic, onion, asparagus

In vivo human studies have confirmed both the fermentability and the bifidogenic effect of chicory fructooligosaccharides. It was detected not only a significant increase in the *bifidobacteria* population but also a profound modification of the composition of the fecal flora (31).

In a double-blind, randomized, placebo-controlled, parallel-group study with forty-five volunteers was showed that Jerusalem artichoke inulin or chicory inulin can increase the number of *bifidobacteria* and reduce *bacteroides/prevotella* (38).

Daily consumption of a very-long-chain inulin extracted from globe artichoke confirmed a pronounced prebiotic effect on the human faecal microbiota composition (39).

A significative study based on in vitro batch fermentation system with human fecal microbiota evaluate the prebiotic activity of inulin. It was evidenced that Fermentation of inulin resulted in a significantly greater ratio of *Lactobacillus* or *Bifidobacteria* to *Enterobacteria* strains (40).

On the basis of these preliminary evidences all the foods with high oligofructose and inulin contents such as Jerusalem artichokes, garlic, onion and asparagus may help to maintain an healthy intestinal microenvironment thanks to their prebiotic activity.

Pomegranates and wine

It has been postulated that some phenolic compounds can readily contribute to modulation of the gut bacteria (41-43) since they are poorly absorbed in the upper part of the gut and can reach the colon to be fermented by bacteria. For example, polyphenols present in pomegranates (ellagitannins and anthocyanins) have important antioxidant and anti-inflammatory bioactivities (44). One study demonstrated that exposure of gut

Table 1. Foods and beverage that may have a positive impact on individual's health.

Food / Beverage	Effective components	Effect	Mechanism of Action	Ref.
Whole grains (eg. barley, rice, wheat)	Insoluble fibers	Feed the intestinal microbial population contributing to its equilibrium	Increase populations of bifidobacteria and lactobacilli, Increase of ferulic acid concentrations in blood	Costabile et al.,2008 Christensen et al,2013 Connolly et al,2010
Broccoli, savoy cabbage, Brussels sprouts	Glucosinolates	Feed the intestinal microbial population	Changes in bacterial metabolic activity	Li et al. 2009
Coffee	Melanoidins	Feed the intestinal microbial population	Increase the population of Bifidobacterium spp. and in some cases increase the metabolic activity of Bifidobacterium spp.	Jaquet et al.,2009 Nakayama et al,2013
Pomegranates, wine	Phenolics	Modulate the intestinal microbiota population	Exposure of by-product with faecal bacteria enhanced the growth of Bifidobacterium spp. and Lactobacillus spp. and in addition increased concentrations of short chain fatty acids Wine phenolics inhibit detrimental bacteria and stimulate beneficial bacteria, in particular Lactobacteria and Bifidobacteria	Basu & Penugonda,2009 Bialonska et al.,2010 Biasi et al.,2014 Queipo-Ortuno et al. 2012
Chicory, artichokes, Jerusalem artichokes, garlic, onion, asparagus	Inulin	Can feed selectively some good bacteria (e.g. Bifidobacteria, Lactobacilli)	Induce an increase in the number of bifidobacteria in human faecal flora Transgalactosylated disaccharide increased the number of bifidobacteria and lactobacilli	Kleessen et al,2007 Costabile et al,2010 Jung et al,2015
Banana	Fructooligosaccharides	Modulate the intestinal microbiota population	Increase in Bifidobacterial levels	Mitsou et al,2011
Chocolate, wild berries, strawberries,	Polyphenolics	Can feed selectively some good bacteria (e.g. Bifidobacteria, Lactobacilli)	Increase in the number of Bifidobacteria and Lactobacilli	Tuohy et al,2012 Tzounis et al,2011 Etxeberria et al,2013

bacteria to pomegranate by-products enhanced the total growth of *Bifidobacterium* spp. and *Lactobacillus* spp. without influencing the *C. coccoides* - *E. rectale* and the *Clostridium histolyticum* groups (45). Some researchers suggest that pomegranate oligomers composed of gallic acid, ellagic acid and glucose units may account for the enhanced growth of specific bacteria associated with beneficial effects.

It has been hypothesized that wine phenolics may act as prebiotics and that their activity may derive from their ability to interact with microbes' growth, both by inhibiting detrimental bacteria and by stimulating beneficial bacteria in the gut, in particular *Lactobacteria* and *Bifidobacteria* (46,47).

A group of research investigated also the effect of polyphenols derived from fruits and showed that they are able to inhibit the growth and adhesion of pathogens (*S. typhimurium*) to a human gut cell line and to enhance the proliferation and adhesion of a probiotic, *L. rhamnosus* (29).

Broccoli, Brussels sprouts

In a randomized, crossover, controlled feeding study fecal samples were collected from 17 participants at the end of 2 14-d intake periods: a low-phytochemical, low-fiber basal diet and a high cruciferous vegetable diet. It was evidenced that cruciferous vegetable consumption can alter human gut bacterial community (48).

Coffee

Jaquet and co-workers (49) illustrate a means of modulating the human microbiota through coffee consumption. Sixteen healthy adult volunteers consumed a daily dose of three cups of coffee during 3 weeks. Fecal samples were collected before and after the consumption of coffee, and the impact of the ingestion of the product on the intestinal bacteria as well as the quantification of specific bacterial groups was assessed using nucleic acid-based methods. Data obtained showed that the population of *Bifidobacterium* spp., a bacterial group of reputed beneficial effects, increased after the 3-week test period. The same results were obtained by a Japanese study in 2013 (50).

Whole grain wheat

The efficacy of whole-grain (WG) wheat compared with wheat bran (WB) alone was evaluated in a double-blind, placebo-controlled crossover study (51). Thirty-one healthy subjects were randomized in two groups and were fed either WG wheat breakfast cereal (48 g/d), or WB breakfast cereal (48 g/d) as placebo for 3 weeks. After a 2-weeks washout phase volunteers were then crossed over to the other breakfast cereal treatment for another 3 weeks. Fasting blood, 24h urine and single stool samples were collected before and after treatment with the cereals, and changes within the gut microbiota and its metabolic output in terms of SCFA profiles and plasma. It was demonstrated that is possible to beneficially modulate the gastrointestinal microbiota and their activities. WG and WB have a differential impact on the microbial ecology of the human gut. Moreover, WG breakfast cereal reveal a prebiotic mode of activity since they have been associated with increased populations of *bifidobacteria* and *lactobacilli* compared to WB. These results suggest that it would be possible to plan dietary interventions able to ensure the maintenance of a balanced microflora.

In postmenopausal women following a 12-week energy-restricted dietary intervention with whole-grain or refined wheat was evidenced a prebiotic effect in the first group (52). In particular, for the first time the impact of oat grain on the microbial ecology of the human gut was reported and was highlighted its ability of increasing *Bifidobacterium* population (53).

Banana

Thirty-four healthy women having Body Mass Index (BMI) 24-30 kg/m, age 19-45 years, no history of gastrointestinal disease and no antibiotic and other medication participated in a controlled trial (54). All women were asked to maintain their usual dietary habits for 60 days and they were randomly assigned to consume twice a day a pre-meal snack, either one medium banana, or one cup of banana-flavoured drink or one cup of water (control group). Stool samples were collected at baseline, on days 30 and 60 of intervention for enumeration of total anaerobes, *bifidobacteria* and *lactobacilli* by plate count techniques, as well as

for pH and short chain fatty acids (SCFAs) measurement. Gastrointestinal symptoms were also recorded. Mean bifidobacterial levels were increased only in the banana group both at 30 and 60 days of intervention. Daily consumption of bananas representing therefore an healthy eating behaviour, which may induce bifidogenesis in women experiencing body weight problems.

Chocolate, wild berries, strawberries

In a controlled, double-blind, randomized clinical trial, researchers compared the outcomes of consuming a high-cocoa vs. a low-cocoa flavanol drinks (55). Their results show a significant increase in certain gut microbes such as *Bifidobacteria* and *Lactobacilli*. On the other hand, a significant decrease in *Clostridia*, which are a class of the *Firmicutes*, was noted. According to the same study, these changes in bacterial population was accompanied by changes in other biological markers such as triglyceride and C-reactive protein concentration.

Additional studies have showed that polyphenol-rich chocolate and certain fruits induced an increase in fecal *bifidobacteria* (56,57).

Discussion

Some foods may contribute to the development of exacerbations in several diseases (i.e. inflammatory bowel diseases) via modulation of the immune system and the intestinal microbiota. In particular, the intestinal microbiota has a key role in this phase as reported by recent findings, showing that diversion of the fecal stream and treatment with antibiotics can improve the disease activity. Different studies evidenced a different composition and a less bacterial diversity in inflamed vs. non-inflamed mucosal samples of patients with active and inactive IBD (37). Even though, causative bacterial species are still unknown. Recent work has suggested that individuals can be classified into 'enterotypes' based on the abundance of key bacterial taxa in gut microbial communities. However, the generality of enterotypes across populations, and the existence of similar cluster types for other body sites, remains to be evaluated (38). This emerging evidence will lead

to clarify if individuals with a *Bacteroides*-predominant gut microbiota may have a higher incidence of diseases due to the Western diet. Once the possible increased risk has been ascertained, it would be reasonable to direct towards long-term dietary interventions able to induce a switch towards a *Prevotella*-predominant microbiota. It should be taken into account the hypothesis that the mechanism of action of dietary factors do not involve only a change in the microbiota composition but rather influence its metabolism leading to the production of metabolites such as the gas trimethylamine that may cause the development of disease. In our work we suggest some dietary components that may be able to maintain a well being state preventing the perturbation of microbiota homeostasis. A balanced diet characterized by a high consumption of specific type of vegetables and fruits along with a moderate intake of meat may have beneficial effects on individual's long-term health. Anyhow further studies are needed to better understand the interaction between diet and intestinal microbiota in the hope of finding new therapeutic or preventive strategies.

Conclusions

On the basis of a bacterial mediated immune regulation it become reasonable trying to maintain a healthy environment in the gut. These evidences has raised the possibility that some form of modulation of the intestinal microbiota may constitute a potential therapeutic basis for the disease. Given the plasticity of microbiota, it is presumable that dietary interventions could positively affect health status, either by keeping a physiological gut flora or by helping to reverse a dysbiosis condition. Some preliminary findings indicate that foods such as pomegranates, chicory, whole grains, chocolate can be able to modulate the composition and metabolic activity of intestinal microbial population. Further studies are required to confirm the mechanisms of action of these foods on microbiota and to better understand the pathways by which food interacts with microbiome in order to design highly personalised nutritional strategies.

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