Dietary polyphenols and their effect on the gut microbiota and human health

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Abstract

Problem presentation: Over the past decade the oxidative stress, caused by reactive oxygen species (ROS) has been recognized as a key factor in the development of various diseases e.g. diabetes, cardiovascular diseases and neurodegenerative disorders. Antioxidant support, which can also be provided with proper nutrition, can reduce the negative effects of oxidative stress and have a positive effect on our health. Plant foods are a rich source of biologically active compounds, among them many polyphenols are very important. Due to their well-known antioxidant properties, polyphenols are associated with a number of physiological mechanisms that have protective effects on various organs, including the gastrointestinal tract. Polyphenols and their metabolites help to maintain a healthy gut primarily through microbiota modulation. They have prebiotic-like effects, they can stimulate the growth of beneficial and inhibit the growth of pathogenic bacteria. In addition, the gut microbiota plays an important role in the metabolism of polyphenols, the production of active metabolites and their bioavailability. Therefore, the interaction between dietary polyphenols and gut microbiota can be of significant benefit to human health. *Purpose:* The aim of this review is to summarize the data on the protective role of dietary polyphenols and their metabolites on human health in general with an emphasis on gut microbiota modulation. *Conclusions:* Current research indicates that there is a positive relationship between dietary polyphenols and the healthy composition of the gut microbiota. Therefore, the promotion of a diet rich in plant foods should also be considered as an important element affecting the health of the working population.

Keywords: dietary polyphenols, gut microbiota, human health

Introduction

Dietary polyphenols represent a large class of naturally occurring chemical compounds characterized by the presence of multiple phenol structural units. As secondary metabolites, they are found widely in plant foods providing colour, flavour and astringency, and defence against exogenous stresses, like reactive oxygen species (ROS), ultraviolet radiation (UV) and plant pathogens. Due to the electron-donating phenolic groups polyphenols are well known antioxidants that prevent stress-related cellular and extracellular damage. In humans, they have been found to possess important biological activities, including anti-inflammatory, anticarcinogenic and antimicrobial activities (Zhang, 2015). Several epidemiologic studies have shown that consumption of food rich in polyphenols has beneficial effects on human health. Their antioxidant and anti-inflammatory properties have preventive effects on different chronic diseases such as cardiovascular diseases, diabetes, obesity, neurodegenerative disorders and cancer (Fraga et al., 2019; Li et al., 2014). Most polyphenols pass through the small intestine without being absorbed, thus encountering the gut microbiota (Ozdal et al., 2016). This has led to the development of a two-way mutual relationship between polyphenolic compounds and gut microbiota. First, polyphenols are biotransformed by gut microbiota that results in the increased bioavailability of their metabolites. The microbiota is responsible for the extensive breakdown of the original polyphenolic structures into low-molecular-weight phenolic metabolites that can be easily absorbed and may actually be responsible for the health effects derived from polyphenol-rich food consumption (Cardona et al., 2013). Second, polyphenols modulate the composition of the gut microbial community mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria. The last is supported by their prebiotic properties enriching the beneficial bacteria (Valdés et al., 2015). Therefore, the interactions of dietary polyphenols and gut microbiota may impact human health.

Polyphenols characterization and bioavailability

Polyphenols are classified into a range of structurally related groups, with over 9000 different structures identified in various plant species. This heterogeneous group of molecules, divided into four main classes according to their chemical structure: flavonoids (including flavonols, flavanols, flavanones, flavones, anthocyanidins, chalcones, dihydrochalcones, dihydroflavonols and isoflavones), lignans, stilbenes and tannins. Phenolic acids (hydroxybenzoic, hydroxycinnamic, hydroxyphenylacetic, hydroxyphenylpropanoic and hydroxyphenylacetic acids) are also frequently included in this category (Abbas et al., 2017).

Most dietary polyphenols exist as polymers or in glycosylated forms, in which one or more sugar moieties are bound to phenolic or a hydroxyl group at the C-3 position (Manach et al., 2004). The basic structure of flavonoids, meaning the structure of the aglycon form, and which type of sugar moiety is at-

tached strongly affect their bioavailability. Bioavailability is a crucial factor in determining their biological activity *in vivo* (Manach et al., 2005).

The bioavailability of dietary polyphenols is, in general, low. Small amounts of their intake (about 5-10 %) may be absorbed in the small intestine, mainly those with monomeric and dimeric structures. The released aglycones enter the enterocytes by passive diffusion. Once absorbed, polyphenols reach the liver through the portal circulation. Here, they undergo biotransformation via phase I (oxidation, reduction and hydrolysis) and phase II (conjugation) reactions. These transformations produce water-soluble conjugated metabolites (glucuronide, sulphate and methyl derivatives) which are released in the systemic circulation for subsequent delivery to organs and excretion by the urine. More complex polyphenols, especially oligomeric, and polymeric structures, reach the colon almost unchanged, where they are metabolized by the gut microbiota together with conjugates excreted into the intestinal lumen through the bile. Here, they undergo microbial enzyme transformations, including C-ring cleavage, decarboxylation, dehydroxylation, and demethylation. The result is the generation of less complex compounds such as phenolic acids and hydroxycinnamates (Corrêa et al., 2019).

Polyphenols and gut microbiota modulation

The human gut is an ecosystem of around 10^{13} – 10^{14} bacterial cells, participating in several metabolic functions that the host cannot fulfil by itself. Microbiota that colonize the distal regions of the colon represent the highest concentration of microorganisms found in human body, as well as the most diverse. A harmonious balance in their composition has been associated with maintaining health and a higher life expectancy accompanied by a satisfactory quality of life (Nicholson et al., 2012). The mechanisms by which the phenolic compounds modulate the gut microbiota still remain to be elucidated, but may involve direct and indirect interactions. Phenolic compounds could directly stimulate or inhibit bacterial growth. Inhibition is closely related to the antimicrobial properties of these compounds and stimulation presumably associated with the capacity of the bacteria to metabolize them (Ettxeberria et al., 2013). It could be said that polyphenols possess a selective bacteriostatic or bactericidal effect, inhibiting the growth of a wide range of potentially pathogenic bacteria and slightly affecting or even promoting the beneficial microbial population.

Some microbiota members are preferred to others due to efficacy they have shown in ameliorating the gut ecosystem with positive effects at the local and systemic levels. For this reason, most studies have focused on the effects of polyphenols on *Bifidobacterium* and *Lactobacillus*, which have been observed to contribute to human health at different levels (Gibson, 2008). They enhance gut barrier function, stimulate the host immune system, prevent diarrhoea or allergies, contribute to activation of provitamins, and modulate lipid metabolism (Burcelin et al., 2012; Gibson, 2008). However, there are other bacterial species associated with negative implications, such as *Clostridium dif-*

ficile, which has been associated with inflammatory bowel disease (Rastall et al., 2005). Therefore, it is of crucial importance to understand the inhibitory or stimulatory effect of phenolic compounds on beneficial or pathogenic bacteria. The influence of phenolic compounds on gut microbiota is summarized in details by Ozdal et al. (2016).

In vitro cell culture studies were performed by different polyphenol type substances. Among flavonols tested on six bacteria species (*Bacteroides galacturonicus*, *Lactobacillus* spp., *Enterococcus caccae*, *Bifidobacterium catenulatum*, *Ruminococcus gauvreauii*, and *Escherichia coli*) quercetin showed a dose-dependent inhibitory effect on the growth of all analysed bacterial species, whereas this effect was weaker for rutin (Duda-Chodak, 2012). In another study quercetin supplementation resulted in an altered composition of gut microbiota at different taxonomic levels, including the relative *Firmicutes:Bacteroidetes* ratio and inhibiting the growth of bacterial species associated with diet-induced obesity such as *Erysipelotrichaceae*, *Bacillus* spp., and *Eubacterium cylindroides* (Ettxeberria et al., 2015).

Many different polyphenols were demonstrated to influence the growth of human gut bacteria and their adhesion to enterocytes. Accordingly, naringenin promoted the growth of *Lactobacillus rhamnosus*, commensal *E. coli*, along with inhibition of two pathogens, *Staphylococcus aureus* and *Salmonella* Typhimurium. In general, the Gram-positive enteropathogen *S. aureus* was the most sensitive to naringenin, while the Gram-negative pathogen *S. Typhimurium* and the commensal bacteria *E. coli* were likely to be similar in their sensitivity to naringenin (Parker et al., 2008). The impact of naringenin and hesperetin was tested on six bacteria species (*Bacteroides galacturonicus*, *Lactobacillus* sp., *E. caccae*, *B. catenulatum*, *R. gauvreauii*, and *E. coli*) and inhibited the growth of almost all analysed bacteria (Duda-Chodak, 2012).

Isoflavones are transformed by gut microbiota, although there are few studies regarding the effect of isoflavone supplementation on gut microbiota composition. The investigated isoflavones (e.g. daidzein and genistein) induced a decrease in bacterial growth (Kawabata et al., 2013). The consumption of flavanol-rich foods containing epicatechin and catechin may support gut health through their ability to exert prebiotic actions (Tzounis et al., 2008). The flavan-3-ol modulates microbiota composition and inherent catabolic activity, inducing changes that could affect the bioavailability and potential bioactivity of these compounds (Cueva et al., 2013).

Anthocyanins and their metabolites may stimulate beneficial members of the gut microta community. Interestingly, malvidin-3-glucoside mixed with other anthocyanins exhibited a synergistic effect in promoting beneficial microbes. *In vitro* incubation of phenolic gallic acid in a fecal slurry reduced a group of potentially harmful bacteria such as *Clostridium histolyticum* without any negative effects on beneficial bacteria. In addition, it significantly reduced *Bacteroides* spp. growth and enhanced both the total bacterial number and the abundance of *Atopobium* spp. (Hidalgo et al., 2012). In another

study, the influence of hydroxycinnamic acids such as caffeic acid, chlorogenic acid, o-coumaric acid, p-coumaric acid on the growth of a probiotic microbe (*L. rhamnosus*), a commensal (*E. coli*) and two pathogenic bacteria (*S. aureus*, *S. Typhimurium*) was investigated. They compared the MIC values of all polyphenols tested and observed that flavonols, isoflavones and glycosides have low antibacterial activity, while phenolic acids were found to be at an intermediate level. On the other hand, the flavanone and flavanol had high antibacterial activity (Parkar et al., 2008).

The effect of hydrolysable tannins (ellagitannins) on the growth of intestinal bacteria is inadequately characterized, and generally their antimicrobial potential has been assessed *in vitro*. It was observed that pomegranate by-products and punicalagins inhibited the growth of pathogenic *Clostridia* and *S. aureus*. Interestingly, probiotic lactobacilli and bifidobacteria were generally not affected by ellagitannins (Bialonska et al., 2009). In this experiment, pomegranate extract was able to increase the total bacterial number, enhancing the growth of *Bifidobacterium* spp., *Lactobacillus* and *Enterococcus* groups, while no effect was observed for the *C. histolyticum* group (Bialonska et al., 2010).

Resveratrol, the representative of stilbenes, increased lactobacilli and bifidobacteria as well as diminished the increase of enterobacteria in *in vivo* studies (Larrosa et al., 2009) not representative from a dietary point of view. Our aim was to ascertain whether resveratrol can exert anti-inflammatory activity *in vivo* at an attainable dietary dose. Rats were fed with 1 mg of resveratrol/kg/day (a human equivalent dose). The results of another study showed that resveratrol ameliorates the dysbiosis in the gut microbiota induced by the high-fat diet, specific effects include an increase in the *Bacteroidete:Firmicutes* ratio, significant inhibition of the growth of *Enterococcus faecalis*, and increased growth of *Lactobacillus* and *Bifidobacterium* (Qiao et al., 2014).

The main limitation of the presented studies is that the information obtained from *in vitro* studies about the role of individual phenolic compounds on gut microbiota cannot be directly extrapolated to what occurs in the physiological context of the gut ecosystem. Of those performed, most were focused on a single polyphenol molecule and selected bacterial populations. Most phenolic fractions and pure phenolic compounds have been analysed without considering the bioavailability and the chemistry of phenolic compounds in the colon. Human and animal intervention studies involved very high doses of individual phenolic compound, or high amounts of foods rich in phenolic content, neither of which represents the regular diet (Ozdal et al., 2016). Therefore, there is a lack of adequate *in vivo* studies which are needed to understand the effect of phenolic compounds on gut microbiota. Human intervention studies will provide the best models for studying the effect of phenolic compounds on gut microbiota modulation. There may be a highly variable response to phenolic compounds according to the differences in gut microbiota composition. Future studies should provide answers about the inter-individual differences in

gut microbiota while studying the effect of phenolic compounds on gut microbiota modulation from the immunological point of view.

Conclusions

Dietary polyphenols have increasingly interested the scientific community due to their proposed health benefits. Possible beneficial effects of polyphenols are determined by their bioavailability where the gut microbiota have an important role. Phenolic compounds are biotransformed into their smaller metabolites by gut microbiota, which contributes to increased bioavailability. At the same time, phenolic compounds can alter the gut microbiota community, resulting in a greater abundance of beneficial microbes, and a consequent increase in bioavailability. Despite the results provided and published over recent years, future studies including human trials will give more confirmatory results about the efficacy of phenolic compounds at the gut level. These results may lead to the design of dietary recommendations not only to suppress or reduce symptoms in disease but also to provide the healthy population with simple tools to promote the maintenance of health.

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