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REVIEW ARTICLE

Food additives, contaminants and other minor components: effects on human gut microbiota—a review

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Abstract Gut bacteria play an important role in several metabolic processes and human diseases, such as obesity and accompanying co-morbidities, such as fatty liver disease, insulin resistance/diabetes, and cardiovascular events. Among other factors, dietary patterns, probiotics, prebiotics, synbiotics, antibiotics, and non-dietary factors, such as stress, age, exercise, and climatic conditions, can dramatically impact the human gut microbiota equilibrium and diversity. However, the effect of minor food constituents, including food additives and trace contaminants, on human gut microbiota has received less attention. Consequently, the present review aimed to provide an objective perspective of the current knowledge regarding the impacts of minor food constituents on human gut microbiota and consequently, on human health.

Keywords Antibiotics · Bacteroidetes · Dietary emulsifier · Firmicutes · Food additive · Gut microbiota · Non-nutritive sweetener · Proteobacteria

Introduction

Humans have approximately 10 times as many microorganisms (approximately 100 trillion) within their gastrointestinal tract (GI) than the number of somatic cells (10 trillion cells) within their body [16, 28, 42]. Indeed, the gut microbiota (GM) contributes to health and disease in humans, being sometimes referred to as the "forgotten organ" [27].

The GM play an important role in a number of human diseases, such as obesity [3, 51], diabetes [15, 94], [56, 74, 89, 114], cardiovascular diseases [38, 116], metabolic syndrome [27, 39, 68], non-alcoholic fatty liver disease [2, 38, 70], and in several psychiatric disorders [10, 45], which gut microorganisms produce a large number of bioactive compounds that can influence human health [6]. Some (such as vitamins) are beneficial, but other products can be harmful [28]. Additionally, the GM interacts with the immune system, providing signals to promote the maturation of immune cells and the normal development of immune functions [11, 35]. In this context, GM microbes contribute to maintaining the integrity of the intestinal epithelium, preserving cell-to-cell junctions, promoting epithelial repair following injury, and in the regulation of enterocytes turnover [103]. Thus, imbalance in GM can result in a pro-inflammatory luminal environment that could contribute to the progression of low chronic inflammation and metabolic disorders [38].

The association between the GM and non-transmissible chronic diseases have been widely investigated [33]. Among them, the link between the human GM and obesity has received great attention [51]. Thus, the modulation of the GM can have beneficial effects to controlling obesity, and several mechanisms that may contribute to microbiota-induced susceptibility to obesity and metabolic diseases have been proposed [80]. Changes in dietary patterns, and specific functional foods, prebiotics or probiotics intake, have the potential to favorably influence host metabolism by targeting the GM and may be a useful approach for the management of obesity and other adverse metabolic conditions [80]. Various non-nutritional factors, such as stress, age, exercise or climatic

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conditions, can also dramatically affect the human GM diversity and equilibrium [28, 63, 68]. Additionally, the ability of minor food components, or additives and chemical contaminants, to modulate specific components of the GM has been acknowledged. However, the attention paid to the effect of these minor food constituents, food additives and trace contaminants on the GM has received less attention. Consequently, the present descriptive aimed to provide an objective perspective of the current knowledge surrounding the effects of these minor foods constituents on the human GM, and, consequently, on human health.

Composition and evolution of human gut microbiota

There is a continuum increase in the number of bacterial cells occurring in the human gut that ranges from 10^1 to 10^3 bacteria per gram of contents in the stomach and duodenum, from 10^4 to 10^7 in the jejunum and ileum, culminating in 10^{11}–10^{13} in the colon, particularly in the distal part [2], were around 300–500 different species live [42]. The GM also varies in composition depending on the location along the GI and axial depth (mucosal versus luminal) [49]. Globally, the microbial mass in the intestine represents about 1 kg or more body weight and is essential to human metabolic functions [90].

Out of 53 known bacteria phyla on earth, only five to seven phyla (predominantly Firmicutes and Bacteroidetes, comprising 90% of the total) usually colonize the human gut [107]. Firmicutes (the most predominant phyla in people living in developed countries) encompasses mostly Gram-positive bacteria with a DNA that has a low G + C content but also include Gram-negative bacteria. The Gram-negative bacteria are mainly represented by the *Bacteroides* genus in the human gut [87]. The relative proportions of these two dominant phyla vary and can be influenced by a range of factors, but most people have similar proportions of each [28]. Lesser (but also important) contributions from members of the Cyanobacteria, Proteobacteria, Actinobacteria, Fusobacteria and Verrumicrobia phyla comprise the rest of the microbiota [90].

Bacteroidetes, *Faecalibacterium, Bifidobacterium* and *Eubacterium* are numerically the most important genera among GM and may account for more than 60% of the bacteria present in human stool, but their relative abundance is highly variable across individuals [28, 101]. *Clostridium*, *Enterobacteriaceae* and *Streptococcus* are also important genera but less numerous [28].

One metagenomic analysis suggested that the GM of each human is typified by one of three enterotypes, with each enterotype featuring distinct dominant groups of microbes [6], namely *Bacteroides*, *Prevotella* and *Ruminococcus*. However, subsequent studies, including those of The Human Microbiome Project, have been unable to provide conclusive evidence that supports this concept [55, 57].

The development of the human GM is a large and complex process that begins during the fetal age [46]. Recent studies have reported that microbial contact is initiated throughout the course of fetal development and continues thereafter in an accelerated manner [31, 36, 46]. The diversity of the GM in the infant gut is initially very low, and the GM are generally aerotolerant, as the gut initially contains oxygen, however, after birth, they are replaced by anaerobes that are typically found in the adult GM [27]. The GM alters considerably from birth to 6 months, when the GM appears to be relatively similar to the childhood-type population [36]. At this age, one of the most important factors contributing to the formation of the GM is the type of lactation [31, 63]. The bacterial composition begins to converge toward an adult-like GM by the end of the first year of life and fully resembles the adult GM by 2.5–3 years of age [31, 65, 125]. In terms of ecological succession, the *Bifidobacterium*-dominated GM of the infant changes over time into the Bacteroidetes- and Firmicutes-dominated GM of the adult, which can be affected by several factors [6, 49, 55, 112]. Among dietary factors, it was observed that subjects consuming a diet particularly rich in animal protein and fat (such as the typical Western diet) were associated with the *Bacteroides* enterotype, whereas the GM of subjects ingesting more carbohydrates were dominated by the *Prevotella* enterotype [125]. An increase in the phylum Firmicutes and a decrease in the Bacteroidetes (mainly expressed as the Firmicutes/Bacteroidetes ratio; FBR) were associated with obesity in some occasions, but this feature is still not consistent [6]. Additionally, an increase of Actinobacteria in obese individuals was also reported [6]. These changes are probably not a mere consequence of obesity but could be involved or a cause faster obesity pathogenesis [28, 68].

Once the GM has reached maturity, it remains mostly stable until old age, although some differences can be found in the GM of the elderly from that of young adults [27]. Particularly, Bacteroidetes phyla and *Clostridium* genus predominate in the GI of elderly people compared to higher proportions of Firmicutes in young adults [63]. Elderly people are also noted to have significant decreases in *Bifidobacterium* [63]. Young adults have variability in community composition than for old age and vary greatly among individuals, ranging from 3 to 92% for Bacteroidetes and 7–94% for Firmicutes [23, 27]. These findings could be related to the greater number of morbidities associated with the elderly and the complex repertoire of drugs used to treat them that are likely to affect the microbiota [23].

Impact of the human gut microbiota on human health

The GM equilibrium is essential for several physiological functions associated with impact on human health, affecting

almost all organ systems that contribute to metabolic control [41]. Thus, the GM modulates appetite and food intake [42, 125], absorption of nutrients from the gut, hepatic steatosis, inflammation, triglyceride accumulation in adipose tissue [14], and fatty acid oxidation in skeletal muscle and the liver [125] and synthesis of vitamins. However, there is still limited knowledge on the exact mechanisms by which the GM affects human metabolism.

The GM express the enzymatic machinery to process otherwise non-digestible carbohydrates, such as fructooligosaccharides, galactooligosaccharides and inulin, and thus, release monosaccharides that can be used by the host for metabolic purposes [63]. In addition to the conversion of complex carbohydrates into absorbable substrates, the GM also benefits the human host by producing SCFAs, with great impact in the colonic epithelial cells maintenance, and vitamins, like vitamin K, as well as some water-soluble B vitamins, such as biotin, cobalamin, folic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine [2].

The GM also influences the host health status through the enzymatic transformation of bile acids, natural detergents with novel signaling functions including regulation of cholesterol synthesis and absorption, modulation of inflammatory responses, and energy homeostasis [19]. Moreover, the GM synthesizes amino acids, influences iron absorption, and it is involved in the conversion of dietary polyphenolic compounds and in the bile acid biotransformation process [63]. The intestinal microbiota is able to transform potentially carcinogenic compounds, such as N-nitroso compounds and heterocyclic amines, and to activate bioactive compounds including phytoestrogens [104].

Globally, although the healthier GM is not yet fully established, it is well known that the richness and diversity of bacterial species in the human gut may be an indicator of wellbeing, and consequently, alterations in GM can affect multiple health issues [49]. In this context, compositional and functional alterations in the GM have been linked to malnutrition [109], obesity and adiposity-related diseases [68, 95], cardiovascular events [12, 76], type 2 diabetes [64], inflammatory bowel disease [85], colorectal cancer [127], neurodevelopmental disorders [52] and aging-related disturbances [59, 76]. Considering the increasing global incidence of many of these conditions, changes in the lifestyle and diet in the post-industrialization/westernization era have been argued to contribute to their emergence by shifting the GM ecology [125].

Knowledge of the effects of specific microbial phyla is still limited. However, the presence of Firmicutes, from diverse families, namely *Clostridiales*, *Erysipelotrichaceae*, *Ruminococcaceae*, *Eubacteriaceae*, and *Lachnospiraceae* have been shown to be associated with healthy populations [63]. Additionally, certain bacterial genus such as *Bacteroides*, *Bifidobacterium*, *Clostridium* clusters XIVa/IV, *Eubacterium*, *Faecalibacterium*, *Roseburia* or *Lactobacillus* and even specific species, such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* or *Roseburia intestinalis*, have been shown to prevent health disorders such as obesity or diabetes, or to improve immunity and inflammatory status [49, 51, 55, 63].

Effect of minor food compounds on the human gut microbiota

Although dietary patterns have an important effect on the human GM, the individual effects of minor food compounds have received less attention than conventional diets, with different proportions of macronutrients. Micronutrients are pivotal for several health-related functions, like energy metabolism, cellular growth and differentiation, and organ and immune function [9]. A diet low in micronutrients, but not necessarily low in energy, is frequent in populations of low-income countries, but may also be present in poverty-affected settings in middle- and high-income countries [9]. It is estimated that more than three billion people worldwide suffer from various types of micronutrient deficiencies (predominantly vitamin A, iron and zinc), with the majority being women and children [9]. Vitamin A can modulate the immune response of the intestine by interactions with immune cells of modulation of the microbiota [11]. Iron deficiency or anemia is related to a depletion of *Lactobacillus* in women [7].

Some reports have shown that prophylactic doses of Zn in various animal models increased the presence of Gram-negative facultative anaerobic bacterial groups, the colonic concentration of short chain fatty acids (SCFAs), as well as overall species richness and diversity [96]. Likewise, others have found a gut microbiota enriched in members of the phylum Firmicutes, specifically Lactobacillus, following ZnO administration [101]. Moreover, even mild zinc deficiencies can profoundly impact growth and development, as well as block immune differentiation and maturation [96]. Supplementation with high levels of zinc has been shown to result in an increase of *Lactobacillus* in the GM of weaned pigs [108]. Using chicks as a model, one study recently demonstrated that zinc deficiency results in a remarkable change in the microbiota, with metabolic changes, such as decreased SCFAs output [96].

Various other dietary constituents, including various compounds belonging to polyphenols, also nourish colonic microbes [28]. Polyphenols are secondary metabolites found abundantly in a wide variety of foods, such as fruits, vegetables, herbs, seeds and cereals, and in beverages, such as coffee, tea, cocoa and wine [84]. The beneficial activities of polyphenols on the prevention of cancer and cardiovascular disease and, specifically, on the GM have been widely investigated in recent years [28, 39, 84]. Most polyphenols pass

through the SI without being absorbed, thus encountering the GM, which colonizes the colon [84]. Once reached the colon the interaction polyphenols-GM results in a two-way mutual reaction. First, polyphenols are biotransformed in vivo by some GM bacteria, increasing their bioavailability, and thus increasing their effects on human wellbeing [39]. Second, polyphenols modulate the composition of the GM mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria [39, 40, 42, 84]. Several phenolic compounds have been recognized as potential antimicrobial agents with bacteriostatic or bactericidal effects, and have various effects on bacterial species or genus [42, 76, 84]. About 90% of the dietary polyphenols escape digestion and absorption in the SI [116, 118] and can have a significant influence on the microbial populations and their activities [69, 77, 119], but our understanding of the microbial bioconversion processes is still limited [69].

Flavonols [69], quercetin [37, 54], catechin and puerarin [54], anthocyanins [50, 52], ellagitannins [74], resveratrol [94], *trans*-resveratrol [37] and pterostilbene [41] are all reported to impact the GM. Quercetin supplementation resulted in an altered composition of the GM at different taxonomic levels, including the FBR and inhibiting the growth of bacterial species associated with diet-induced obesity, such as *Erysipelotrichaceae*, *Bacillus*, and *Eubacterium cylindroides* [37]. In other recent work, it was demonstrated that different types of flavonoids can modulate the growth of different phyla and genus from GM [63].

Li et al. [73] demonstrated that ellagitannins can stimulate the growth of several bacterial genera with beneficial properties for human health, such as *Akkermansia muciniphila, Butyrivibrio, Escherichia, Lactobacillus* or *Prevotella*. Proanthocyanidins from grape seed can increase *Lachnospiraceae*, *Clostridiales*, *Lactobacillus* and *Ruminococcacceae* in female pigs [22]. In another research, Qiao et al. [94] found that resveratrol ameliorated the dysbiosis in the GM of mice induced by a high-fat diet. Specific effects included an increase in the FBR, significant inhibition of the growth of *Enterococcus faecalis*, and increased growth of *Lactobacillus* and *Bifidobacterium*. Flavonols can also increase the relative abundance of *Bifidobacterium* and *Lactobacillus* at the expense of potentially pathogenic bacteria, notably *Clostridium histolyticum* [77]. In a recent work, it was demonstrated that pterostilbene (a dimethoxy resveratrol derivative) supplementation in rats exerted protective antiobesity effects, improved insulin sensitivity and modified GM by decreasing Firmicutes and increasing Verrucomicrobia. Regarding specific genus, pterostilbene supplementation increased mucin-degrading bacterial members, such as *A. muciniphila* and *Odoribacter* spp. [41].

Besides polyphenols, other minor compounds such as conjugated linoleic acid [17], L-carnitine [61], choline [2], sphingomyelin [76] or ellagitannins [69] have been reported to modulate the GM and consequently, impact human health. Thus, Chaplin et al. [17] found that conjugated linoleic acid increased *A. muciniphila* levels, that was associated with several beneficial associations with metabolism [16]. It was also reported that L-carnitine, present in red meats, can be metabolized to trimethylamine and trimethylamine oxide, and increase atherosclerosis risk [66]. Colonic bacteria can hydrolyze choline to form dimethylamine and trimethylamine, which are precursors of dimethylnitrosamine [2], a potent hepatotoxin, and carcinogen.

Mice fed diet with 0.25% sphingomyelin showed a higher relative phylogenetic abundance of the predominately Gram-positive Firmicutes phylum and significantly lower numbers of the Gram-negative Bacteroidetes phylum and some intestinal pathogens [83]. Milk sphingomyelin supplemented mice had a significantly relative abundance of the beneficial bacteria *Bifidobacterium*, and higher relative abundance of *Bacteroides*, one of the few microbes that synthesize and utilize sphingolipids [83]. A summary of some previously published works regarding effects of food minor compounds in human GM is displayed in Table 1.

Effects of food additives on human gut microbiota

An important change in human diets since the mild-twentieth century is the increasing consumption of food additives that are incorporated into almost all processed foods, often to aid stability, shelf-life, taste, and texture improvement, particularly in processed foods [19]. The primary basis for approving the use of these agents is the notion that they do not cause acute toxicity at concentrations reasonably greater than their approved concentrations. However, only few prospective interventional human studies address the possible impact of additives on the human GM, presumably due to difficulties in allocation of cohorts of healthy individuals who have not been previously exposed to food additives, and the need for robust stratification of potentially confounding factors, such as genetics, lifestyle and dietary patterns [19]. Consequently, researchers have turned to animal models to study the effect of food additives on the GM. Recent studies have demonstrated that the consumption of non-nutritive sweeteners (NNS) and dietary emulsifiers (DEs) can alter the GM, resulting in intestinal disturbance and inflammation, favoring the development of the metabolic syndrome [19, 26] (Table 2).

Nowadays, most processed foods contain one or more DEs in order to seek for specific textures. Some authors have suggested that DEs may be a factor resulting from industrialization that has resulted in a reduction of GM diversity, altered host-microbiota interactions and, consequently, have contributed to the increased incidence of metabolic syndrome and other inflammatory diseases in industrialized societies [19, 27]. Two DEs, namely carboxymethylcellulose and polysorbate 80, have

Table 1 Recent works regarding the effects of micronutrients on gut microbiota (GM)

Reference	Models	Micronutrients	Supplementation dosage	Main conclusion
[7]	Observational study (8 anemic and 26 normohemic females)	Iron	–	Fecal *Lactobacillus* were significantly lower in anemic women
[17]	Pigs	Conjugated linoleic acids (CLA)	6 mg of CLA/day was given to mice consuming both a normal-fat diet and a high-fat diet	CLA supplementation exerted a prebiotic action on *Bacteroidetes/Prevotella* and *Akkermansia muciniphila*. However, it was not able to override the negative effects of a high-fat diet on *Bifidobacterium* spp.
[22]	Pigs	Proanthocyanidins	Diet containing 1% (w/w) of grape seed extract daily for 6 days	Dramatic increase in fecal *Lachnospiraceae*, *Clostridiales*, *Lactobacillus* and *Ruminococcacceae*
[37]	Rats	Polyphenols	*Trans*-resveratrol (15 mg/kg body weight/day], quercetin (30 mg/kg/day) or a combination of both polyphenols at those doses	Quercetin attenuated the *Firmicutes/Bacteroidetes* ratio and inhibited the growth of *Erysipelotrichaceae*, *Bacillus* and *Eubacterium cylindroides*. *Trans*-resveratrol supplementation alone or in combination with quercetin scarcely modified the GM
[41]	Rats	Pterostilbene	15 mg/kg body weight and day for 6 weeks	Pterostilbene decreased Firmicutes levels and increased Verrucomicrobia, *A. muciniphila* and *Odoribacter* spp.
[50]	In vitro model of human gut	Malvidin-3-glucose, gallic acid and a mixture of anthocyanins	Gallic acid (150 mg/L and 1000 mg/L), malvidin-3-glucoside (20 mg/L and 200 mg/L), and enocianin (4850 mg/L and 48,500 mg/L)	All the anthocyanins tested significantly enhanced the growth of *Bifidobacterium* spp. and *Lactobacillus*−*Enterococcus* spp.
[54]	In vitro model of the human gut	Flavonoids (quercetin, catechin, puerarin)	Each flavonoid at 0.15 g/L	Catechin and puerarin presented different activities on regulating the GM, but all increased GM diversity
[66]	Mice	L-carnitine	250 mg	L-carnitine supplementation significantly altered cecal microbial composition, markedly enhanced synthesis of trimethylamine/trimethylamine oxide, and increased atherosclerosis
[72]	Uncontrolled study including 22 healthy human volunteers	Ellagitannins	Pomegranate extract at 1000 mg/day for 4 weeks	Ellagitannins from pomegranate stimulated *A. muciniphila, Butyrivibrio, Enterobacter, Escherichia, Lactobacillus* and *Prevotella* and inhibited *Collinsella*
[77]	Case-controlled study including 22 healthy human volunteers	Flavonols	Dark chocolate at 50 g/day for 1 week	Cocoa flavonols increased the relative abundance of *Bifidobacterium* and *Lactobacillus* at the expense of potentially pathogenic bacteria, notably the *C. histolyticum* group
[83]	Mice	Sphingomyelin	High-fat diet with 0.25% of milk sphingomyelin added 45% Kcal as fat	Decrease in Gram-negative bacteria, such as Bacteroidetes or Tenericutes phyla and increase in Gram-positive bacteria, such as Firmicutes and *Actinobacteria* phyla
[94]	Mice	Resveratrol	200 mg/kg per day	Resveratrol increased GM dysbiosis induced by a high-fat diet, with an increase in the FBR, *Lactobacillus* and *Bifidobacterium* growth and a significant decrease in *Enterococcus faecalis*
[96]	Chicken	Zinc	Zinc oxide supplementation at 42 μg/g or 2.5 μg/g	The zinc-deficient group had a significantly lower cecal microbial diversity

Table 1 (continued)

Reference	Models	Micronutrients	Supplementation dosage	Main conclusion
[108]	Pigs	Zinc	57 (low) or 2425 (high) mg/kg zinc oxide for 5 weeks	Pronounced reductions were observed for *Enterobacteriaceae* and the *Escherichia* group as well as for *Lactobacillus* spp. and for three of five studied *Lactobacillus* spp.
[119]	Case-control study including 22 healthy human volunteers	Cocoa flavonols	High-cocoa flavonol (HCF) group received 494 mg cocoa flavonols/day), while low-cocoa flavanol and low-cocoa flavanol group received 23 mg cocoa flavonols/day, for 4 weeks	Consuming the HCF drink for 4 weeks significantly increased the *Bifidobacterium* and *Lactobacillus* populations but significantly decreased the *Clostridia* counts in fecal samples

demonstrated to promote bacterial overgrowth in the murine SI and facilitate translocation of bacteria across a model gut epithelia [19]. Additionally, they reduced the mucus layer thickness and were involved in the onset of intestinal inflammation, obesity, and diabetes. These effects were also associated with an increased food intake, from still unknown origin [27]. A previous study, also carried out in mice, showed as polysorbate 80, enhances the translocation of *E. coli* across M-cells [35]. An increase in numbers of *E coli* have been found in association with Crohn's mucosa [99]. There are studies showing that the *E. coli* translocation can increase in 59 folds. Thus, this emulsifier may contribute to the impact of dietary factors on Crohn's disease pathogenesis [99].

As a result of the many negative health conditions associated with the intake of excessive sugar, there has been an upsurge in the consumption of NNS as an alternative [105, 111]. NNS are synthetic compounds that are several hundredfold sweeter than sucrose. Thus, they can be used in small amounts with negligible added caloric value. Some NNS are excreted unchanged from the mammalian body, and are, therefore, considered metabolically "inert" [105, 111]. Theoretically, NNS would only aid in weight loss if compensatory sugar intake did not occur. However, the common perception that NNS may promote weight loss by reducing calories is misguided because it was reported that consumption of saccharin-sweetened liquids might increase overall food intake [1, 105]. Furthermore, positive correlations between NNS consumption and increased body mass index in children and adolescents have been described in several observational studies [43, 105].

The effects of NNS on the GM could be due to the bacteriostatic effects of the NNS, such as saccharin, sucralose, aspartame and stevia [1, 86, 110, 111]. Data from studies in animals [1, 86] and from a small study in human subjects [110] suggests that the bacteriostatic effects of NNS are not limited to the microbial inhabitants of the mouth, but extend to those in the gut, thereby affecting the host metabolic phenotype and disease risk [89]. Pioneer work showed that 12 weeks of exposure to Splenda significantly altered the GM composition by decreasing beneficial bacteria and was associated with weight gain in rats [1]. In another work, it was confirmed and extended these findings by identifying a microbe-mediated mechanism by which NNS might influence metabolism [110], inducing higher glucose intolerance, mediated by alterations in the GM. Consistent with previous findings, it was showed that 8 weeks of aspartame exposure in a dose equivalent to human subjects consuming 2–3 diet soft drinks per day, perturbed the GM and resulted in elevated fasting glucose levels and impaired insulin tolerance in rats [1, 86].

Other additives reported to significantly alter the GM are essential oils (EOs), which were used to prevent the growth of pathogenic bacterial species that are generally more sensitive to EOs than most commensal bacteria [113]. It was

Table 2 Recent works regarding the effects of food additives on gut microbiota (GM)

Reference	Models	Additive	Supplementation dosage	Main conclusion
[1]	Rats	Splenda	100, 300, 500, or 1000 mg/kg for 12 weeks	Total anaerobes, Bifidobacterium, Lactobacillus, Bacteroides, clostridia, and total aerobic bacteria were significantly decreased. No significant changes were found in the *Enterobacteriaceae*
[19]	Mice	Carboxymethylcellulose and polysorbate-80	1% of each emulsifier for 12 weeks	Reduction in the microbial diversity, Bacteroidales, Verrucomicrobia phyla (particularly *Akkermansia muciniphila*) and enriched mucosa-associated inflammation-promoting Proteobacteria
[29]	Rats	Aspartame	Chow and high-fat feed added with 0.4 g/100 mL of aspartame in water for 8 weeks	Increase in total bacteria associated with aspartame addition, and reductions in *Lactobacillus* and *Bacteroides*
[32]	Pigs	Saccharin	Diet supplemented with 0.015% saccharin + neoesperidin dihydrochalcone	Saccharin + neoesperidin dihydrochalcone dramatically increased the cecal population abundance of *Lactobacillus*
[86]	Rats	Aspartame	5–7 mg/kg/day for 8 weeks	Aspartame increased total bacteria, Enterobacteriaceae and *Clostridium leptum* in the feces, and attenuated the increase in Firmicutes/Bacteroidetes ratio
[97]	In vitro trial	Sucralose	1.1–11 mg/kg	Sucralose had little effect on *E. faecalis* and *C. sordellii*, while there was a concentration-dependent inhibition of the growth of *Bacteroides*, *B. fragilis* and *B. uniformis*
[110]	Mice	Saccharin	0.1 mg/ml in water for 11 weeks	Saccharin induced an increase of the Bacteroidetes and reduction in Firmicutes
[113]	In vitro model of the human gut	Thymol, nerolidol, eugenol, methyl isoeugenol and geraniol	100–500 mg/kg	Thymol and geraniol suppressed pathogens, such as *C. difficile*, with no concern for beneficial colonic bacteria in the distal gut

demonstrated that EOs (mainly thymol), selected for their effectiveness against gut pathogens (*C. difficile*) did not have significant effects on the abundance of *F. prausnitzii*, which plays an important anti-inflammatory role in the gut [113]. In particular, EOs may have potential use as an adjunct to chemotherapeutic agents used to treat colorectal cancers [89]. Patients receiving chemotherapy for cancer treatments suffer from gastrointestinal disturbances due to damage to the mucosal cells of the GI and disrupt the gut ecological balance. Consequently, chemotherapy increases the risk of bacterial infections, such as the overgrowth of *C. difficile* [113] and decreases beneficial microbial populations such as *Bifidobacterium*, *Lactobacillus*, *Veillonella* and *F. prausnitzii*. Consequently, EOs might be exploited as prophylactic agents and as adjuncts in chemotherapy to protect commensal bacteria, including *Bifidobacterium* spp. and *F. prausnitzii* [126].

Toxic compounds produced by gut microbiota metabolism

In addition to their action on certain populations of the GM, some compounds can be metabolized by gut microorganisms and exert potentially toxic effects to their consumers. A summary of previously published work, describing toxic compounds produced by its metabolization by GM, is reported in Table 3.

In particular, alcohol can be metabolized by bacteria to aggravate their intrinsic negative effects. Thus, oral bacteria, such as *Streptococcus*, have the capacity to convert ethanol in wine to acetaldehyde, which is an in vitro and in vivo genotoxin and a recognized human carcinogen [15]. Furthermore, the GM is suggested to play an important role in alcohol-induced liver injury, apparently through dysbiosis of the intestinal ecosystem caused by alcohol intake [15].

Other example was reported that the occurrence of renal injury in infants and children exposed to melamine-tainted milk in China could also be attributed to the metabolism of the GM [60]. Certain gut bacterial species, like *Klebsiella terrigena*, can convert melamine to cyanuric acid, which then forms complex precipitates that lead to kidney stone formation and causes renal toxicity [60].

Another group of compounds that can be metabolized by the GM and cause harmful effects are contaminants, such as drugs, heavy metals or environmental chemicals [25]. An interesting study showed how the GM has the ability to inactivate drugs delivered into the intestine, with the potential to

Table 3 Recent works regarding foods that can become toxic by the metabolism of gut microbiota (GM)

Reference	Models	Food/substance	Dosage	Main conclusion
[15]	Mice	Alcohol	10% v/v in drinking water for 7 days, plus an additional oral gavage of 5 mg/kg on day 7	The GM plays an important role in alcohol-induced liver injury, apparently through dysbiosis of the intestinal microbial ecosystem caused by alcohol intake
[20]	Mice	Mixture of polychlorinated biphenyls (PCBs) congeners	150 μmol/kg for 2 days	PCBs decreased the levels of Proteobacteria and induced substantial changes in the gut microbiome, which may then influence their systemic toxicity
[62]	Rats	Chlorpyrifos	1 mg for 30 days	Chronic, low-dose exposure to chlorpyrifos was found to induce dysbiosis in the microbial community with the proliferation of *Bacteroides* sp. and decreased levels of *Lactobacillus* and *Bifidobacterium* spp.
[92]	Mice	Arsenic	Cecal content of mice was added with 0, 200, 1000 and 2000 μg/kg arsenic	Thioarsenicals were found in soluble and particulate fractions of the reaction mixtures, suggesting interactions with anaerobic microbiota
[106]	In vitro trial	Glyphosate	0.05, 0.15, 0.075, 0.3, 0.6, 1.2 and 2.4 mg/ml for 5 days	Reduction of beneficial bacteria, such as some *Bifidobacterium* spp. or *Lactobacillus* spp. that could disturb the normal gut bacterial community, whereas limited effect was shown on the intestinal pathogens
[120]	In vitro model of human gut	Polycyclic aromatic hydrocarbons (PAHs)	Hypothetical soil ingestion of 5 g/day	PAHs biotransformation potency of colon microbiota suggests that the current risk assessment may underestimate the risk from ingested PAHs
[122]	Mice	Nitric oxide (NO)	Daily intrarectal bolus treatment with an NO donor in two doses + 4% dextran sodium sulfate	NO-producing microorganisms in the gut lumen should be considered a modulating process during colitis
[124]	Mice	Nitrogen compounds	Diet supplemented with 1.0% betaine, 1.0% choline, 0.12% trimethylamine *N*-oxide or 1.0% dimethylbutanol for 3 weeks	Mice fed diets supplemented with trimethylamine species (choline or trimethylamine oxide) showed increased peritoneal macrophage cholesterol content and raised plasma levels of trimethylamine oxide
[129]	Rats	2,3,7,8-tetrachlorodibenzofuran	24 μg/kg for 5 days	Dietary 2,3,7,8-tetrachlorodibenzofuran altered the GM by shifting the Firmicutes/Bacteroidetes ratio. The cecal content was enriched with *Butyrivibrio* spp. but depleted in *Oscillibacter* spp. These changes in the GM were associated with altered hepatic lipogenesis, gluconeogenesis, and glycogenolysis
[60]	Rats	Melamine	0.2 mg/kg	Melamine is converted to cyanuric acid in vitro by *Klebsiella terrigena* cultured from normal rat feces. Rats colonized by *K. terrigena* showed exacerbated melamine-induced nephrotoxicity

generate toxic compounds, like hydrogen sulfide [104]. The gut normally converts luminal hydrogen sulfide to thiosulfate, which can be further oxidized to tetrathionate. High concentrations of hydrogen sulfide severely inhibit cytochrome C oxidase, blocking mitochondrial activity [104, 122]. Regarding effects of heavy metals by the GM, Pinyayev et al. [92] reported that anaerobic microbiotas of the mouse cecum convert arsenate into oxyarsenicals and thioarsenicals. Additionally, it was reported that exposure to mercury altered the bacterial community in the gut of a terrestrial isopod (*Porcellio scaber*) [20].

Contaminants may be poorly absorbed after ingestion, and subsequently can reach the distal SI and caecum by peristalsis. Additionally, environmental chemicals (or their metabolites) may also be excreted in the bile [25]. There is increasing evidence that chronic exposure to environmental chemicals through the diet, particularly persistent organic pollutants, may promote the development of obesity and type 2 diabetes

in humans, even without inducing dysbiosis [25]. Of particular interest is the role of the aryl hydrocarbon receptor, which is bound and activated by a variety of persistent organic pollutants including coplanar polychlorinated biphenyls and halogenated aromatic hydrocarbons [129]. For instance, it was recently reported that a persistent organic pollutant, 2,3,7,8-tetrachlorodibenzofuran, can dramatically alter the GM by shifting the FBR, increasing *Butyrivibrio* spp. and decreasing *Oscillibacter* spp. These changes in the GM were associated with altered BA metabolism and subsequent host metabolic disorders as a result of an altered hepatic lipogenesis, gluconeogenesis, and glycogenolysis [25].

Conversely, the GM can regulate the expression of cytochrome P450 enzymes, which are involved in the metabolism of a variety of environmental chemicals [24]. Polycyclic aromatic hydrocarbons are among the most widespread organic pollutants and can be transformed by the GM to estrogenic metabolites [120]. Furthermore, it has been shown that the rat and human GM could regenerate benzo(a)pyrene from its hepatic conjugate, reversing the endogenous detoxification process, which is of potential toxicological relevance [24]. Choi et al. [20] reported that after exposure to polychlorinated biphenyls in mice, the most striking change in the intestinal microbial profiles was a decrease in bacterial species.

Other environmental chemicals, for example, pesticides or herbicides, can also exert increased harmful effects on human health via the action of the GM [57, 98]. Indeed, chronic exposure to chlorpyrifos, an organophosphate insecticide commonly used to treat fruit and vegetable crops and vineyards has been shown to induce dysbiosis of the GM in both human and rats and was associated with the proliferation of *Bacteroides* sp. and decreased levels of *Lactobacillus* sp. and *Bifidobacterium* sp. [62]. Glyphosate, the most widely used herbicide worldwide, has been shown to have important effects in poultry GM [109]. The sensitivity to glyphosate is dependent on the bacterial strain. Some typical pathogens, such as *Salmonella* or *Clostridium*, are highly resistant, whereas beneficial bacteria, like *Lactobacillus* spp. or *Bifidobacterium* spp. are moderately or high susceptible. No trials were performed using human models, but if it were demonstrated that glyphosate acts similarly in human GM, this would be of a toxicological relevance [106].

Specific effects of antibiotics on the human gut microbiota

Several drugs can modulate the GM. Although it was reported that other pharmacological treatment can alter the GM [44, 71], the drugs that primarily play the most significant action on the GM are antibiotics [26, 78, 100, 102]. Antibiotics are one of the most prescribed drugs in human medicine, particularly in pediatrics and neonatal nursing in developed countries [47, 67]. The effect of these drugs on the human GM, both during and after the treatment has been widely investigated in recent years, although it is not yet fully understood [87].

Interestingly, although the effects of therapeutic doses of antibiotics employed in human medicine have been widely investigated in recent years, the effects on GM of antibiotics residues present in foods at trace concentrations, derived from veterinary medicine, have received little attention [18, 21, 82, 98]. Only a few investigations focused on the effects of low concentrations of antibiotics on the GM [30, 34, 121]. This is surprising because antibiotics are the most widely used drugs in the livestock industry in the world [8] and their residues can reach humans through animal feeds, vegetables and surface waters [98]. Paradoxically, while humans are interested in modulating their microbiota to aid in weight loss, producers of animal feed have used antibiotics for decades to increase the weight gain of the animals. Antibiotics in livestock production are incorporated in animal feed either as growth promoters in countries where such use is allowed [30, 73, 98] or as prophylactic or therapeutic agents in the European Union and other countries where antibiotic use as growth promoters is banned. Importantly, these antibiotic effects are not limited to oral administration, but may also be present and, therefore, have effects on microbiota when administered parenterally [31] (Table 4).

As a general rule, it was reported that antibiotic intake in mice increased adiposity [3, 18, 30, 67, 81, 117], and thus favored the development of obesity and type II diabetes [79], besides affecting normal metabolic activity, hormonal and immune development. However, antibiotic treatment does not always display adverse effects on the GM of experimental animals. Indeed, in some instances, antibiotic treatment improved the insulin response in Bio-Breeding diabetes-prone rats [13].

Antibiotics exert very different actions on the individual groups that constitute the GM. Overall, for a variable period after antibiotic treatment ceases, the microbiota usually regains its original composition. However, some bacterial species have been reported to irreversibly disappear in certain individuals [27]. This can influence the health of the host, particularly if the bacterial group that is suppressed affects a physiological health-related function [27].

Cho et al. [21] found a significant increase in the FBR as a result of the administration of beta-lactams and vancomycin. An increase in this ratio, as explained previously in this review, is associated in diverse studies with obesity and other metabolic disorders. Other authors [30] found significant decreases in the taxa associated with beneficial health properties, such as *Lactobacillus* spp. and *Bifidobacterium* spp. and significant increases of *Enterobacteriaceae* family that includes many genres considered potentially pathogenic. Other authors [102] treated mice with antibiotics, such as amoxicillin, metronidazole,

Table 4 Effects of antibiotics and concentrations on the gut microbiota (GM)

Reference	Models	Antimicrobial	Dosage	Main conclusion
[3]	Prospective trial in 28,354 mother-child days for 7 years	Different antibiotics	Several antibiotics and doses depending on the type of disease and patient characteristics	Early exposure to antibiotics increased the risk of being overweight in later childhood by decreasing the diversity of the GM
[5]	Prospective trial in 27 preterm infants and 13 full-time babies	Different antibiotics	Several antibiotics and doses depending on the type of disease and patient characteristics	Prematurity and perinatal antibiotic administration caused lower percentages of *Lactobacillaceae* or *Bacteroidaceae* and increased *Enterobacteriaceae on* GM
[21]	Mice	Penicillin, vancomycin, tetracycline or vancomycin + penicillin	Subtherapeutic dosages at 1 μg/g body weight per day	Antibiotic treatment induced significant changes in GM, increased adiposity and modified lipid metabolism and cholesterol
[30]	Mice	Penicillin	Subtherapeutic dosages	Modified the GM and induced long-term changes in the metabolism of the host, inducing obesity
[34]	Prospective trial in 3 people before and after antibiotic treatment	Ciprofloxacin	1 g/day for 5 days	Ciprofloxacin treatment reduced the GM diversity, with significant effects on 1/3 of the bacterial taxa
[48]	Observational study in 74 infants	Ampicillin and gentamicin	Various dosages and treatment and durations	Infants who received 5–7 days of antimicrobials in the first week had an increased relative abundance of *Enterobacter* and lower bacterial diversity in the second and third weeks of life
[58]	Prospective trial on 6 patients	Clarithromycin + metronidazole	250 mg + clarithromycin and 400 mg metronidazole	Antibiotic treatment affected the GM by decreasing Actinobacteria and this disturbance on the GM persisted after 4 years
[79]	Prospective study in 12 males	Vancomycin, gentamicin and meropenem	500 mg vancomycin, 40 mg gentamicin and 500 mg meropenem	Antibiotic treatment caused significant shifts in the GM. Nevertheless, the changes observed did not have important effects on glucose metabolism
[81]	Retrospective cohort study of 74,946 children with asthma and /or allergies	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Exposure to antibiotics during the first year of life was associated with an increase in the body mass index of 5–8-year-old children
[87]	Prospective study in patients with no digestive diseases	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Both fluoroquinolones and beta-lactam reduced the GM diversity in more than 25% of the patients
[91]	In vitro model of the human gut	Ampicillin + sulbactam and cefazolin	Ampicillin + sulbactam on first days and intravenous cefazolin during 14 days	Antibiotic treatment caused a marked decrease in Bacteroidetes and increase in Firmicutes
[100]	Mice	Vancomycin	100 mg/L in drinking water	Different antibiotics had specific effects on the GM
[102]	Mice	Vancomycin or streptomycin	200 mg/L in drinking water	Vancomycin caused a loss in Bacteroidetes, which were largely replaced by Firmicutes, Paenibacillaceae, Verrucomicrobia (specifically *Akkermansia*), and *Enterobacteriaceae*. In contrast, streptomycin increased the Bacteroidetes, particularly *Porphyromonadaceae* and *Bacteroidaceae*
[115]	Observational study in 96 males	Vancomycin plus other antibiotics	Different doses depending on the type of disease and patient characteristics	Vancomycin plus gentamicin treatment increased the risk of obesity in men. High levels of *Lactobacillus* were found, possibly related to the use of vancomycin as a growth promoter
[121]	Calves	Ampicillin, ceftiofur, penicillin and oxytetracycline	0.005, 0.01 and 0.3 mg/ml and 0.1 g/ml, respectively from birth to weaning	Antibiotic residues resulted in discriminate GM communities, although they did not result in disruption of the taxonomic levels above the genus
[123]	Randomized controlled trial in 20 obese males	Vancomycin	500 mg for 7 days	Vancomycin reduced fecal microbial diversity with a decrease in Gram-positive bacteria (mainly Firmicutes) and a compensatory increase in Gram-negative bacteria (mainly Proteobacteria)
[128]	Mice	Tetracycline and ampicillin	50 mg/kg or 2 mg/kg (for tetracycline) and 30 mg/kg (for ampicillin)	Antibiotic oral administration had important effects on the selection and extent of antibiotic resistance genes

cefoperazone, and a combination of all three. As a result, the Proteobacteria and, in particular, the *Enterobacteriaceae*, become dominant in the intestinal tract of the treated mice, accounting for 73% of the total microbiota. Two weeks after ceasing the antibiotic treatment, the microbiota of these animals recovered a relatively low proportion of Proteobacteria (5.77%), although it remained considerably more abundant than the percentage of the total microbiota representing this phylum in untreated mice (1.2%).

Indeed, although Proteobacteria usually represent about 15% of the intestinal microbiota, they accumulate more than 35% of the antibiotic resistance genes contained in the microbiome. In contrast, despite representing 31% of the total microbiota, *Bacteroidetes* accumulate only 6% of the antibiotic resistance genes [53]. Hence, it is highly feasible that an antibiotic treatment can cause fewer declines in the population of Proteobacteria (or even increase, occupying the space left by other bacterial groups more sensitive to the action of the antimicrobial) than *Bacteroidetes*, for instance. Similarly, it is also reasonable that once the Proteobacteria reach a high proportion within the microbiota, before gradually declining, its population will be maintained at high levels compared to prior to the administration of the antimicrobial.

Another study developed in experimental animals showed that after treatment with cefoperazone (a broad-spectrum antibiotic), there was a significant loss of microbial diversity, without recovery, even at 6 weeks post therapy [4, 47]. In another research work [102], in which mice were given vancomycin or streptomycin in their drinking water, no significant changes regarding the action of streptomycin were found, while vancomycin was associated with significant variations in both the bacterial load and diversity. An almost total removal of Bacteroidales and a marked enrichment of *Lactobacillus* were observed.

However, humans have a greater variation in diet and lifestyle than experimental mice, which introduces factors affecting the recovery of metabolic disturbances or susceptibility to weight gain [30]. Hence, the influence of antibiotics on the GM of humans, particularly children, has been studied. Children are often the most exposed to antibiotic treatments within the human population and typically experience the greatest effects [47]. Indeed, some reports suggest that exposure to antibiotics within the first 6 months of life predisposes the individuals to a significant increase in body mass in later life [3, 80, 117]. However, other authors found conflicting results, suggesting important differences according to the antibiotic regimens, the routes of administration, the choice of methods of statistical analysis, or other poorly controlled factors [47].

Antibiotic treatments can also significantly alter the microbiota composition of the adult GI, causing a decrease in the microbial diversity to between one-quarter to a third of the pre-antibiotic state [47]. However, in this stage of life, the GM is relatively strong and, in most instances, recovers after several weeks of ceasing the antibiotic treatment [87]. However, other studies have shown that after cessation of treatment, the microbiota requires several months to fully recover [34, 58, 75, 87]. However, in some cases, it has even demonstrated that some bacterial groups eliminated by an antibiotic treatment not reappear again in several years after discontinuation of treatment [27, 31, 123]. These effects can be more severe in elderly people, in whose GM is less diverse compared to younger adults and a more unstable balance that can easily lead to the emergence of various pathologies [23, 93].

It has also been shown that upon contact with antibiotics, the GM is perhaps the most accessible reservoir of genes encoding antibiotic resistance due to their high density within the gut ecosystem, which can have important consequences for human wellbeing [31]. The GI is also an open system, which incorporates everyday bacteria from the environment [88]. These incoming bacteria often possess antibiotic resistance genes, and besides being a potential risk to the host, because these resistance encoding genes can be transferred to the host.

Conclusions

The vast majority of experimental evidence supporting the effects of food minor components and contaminants on GM has been generated in animal (especially mice) models. However, mice and humans differ in their microbiota composition, immune function, diets, and metabolism and the results obtained in mice are not totally extrapolable and valid to humans. Thus, interventional studies in humans are also needed, although are seriously limited by ethical concerns. In this sense, the use of in vitro models of the human gut enables investigating the effects of minor compounds (even those dangerous for humans) without health risks and ethical concerns. In view of the results explained in the present work, there are a large variety of food minor components, additives and chemical contaminants that can dramatically affect GM. Thus, there is a profound need for more in-depth investigations into the effects on the human GM of the cited compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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