NEC NUTRIMENTUM ET CURAE

Nutr Cur 2022; 01: e114
DOI: 10.57625/nec.2022.13

Food additives as non-conventional modulators of gut microbiota: health implications

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ABSTRACT

Every day, billions of individuals consume variable amounts of food additives contained in most ultra-processed foods, even though these substances are considered non-nutritive. The market for these compounds is growing, as well as the incidence of non-communicable diseases (NCDs). Recent evidence suggests the detrimental role of food additives on gut microbiota and homeostasis as crucial players in the onset of NCDs. This review summarizes the main findings about this hot topic dissecting the most recent studies justifying the growing scientific concern about these non-nutritive substances. At least four categories of food additives have been put under the spotlights: artificial sweeteners, emulsifiers, food colorants, and preservatives. Human studies on a large scale are warranted to confirm preclinical results.

KEYWORDS

OBESITY
DIABETES
INFLAMMATORY BOWEL DISEASES
COLITIS
PROCESSED FOODS
FOOD ADDITIVES
GUT MICROBIOTA
DYSBIOSIS

INTRODUCTION

The burden of non-communicable diseases (NCDs) – obesity, dyslipidemia, diabetes, cardiovascular diseases, autoimmune disorders, and cancer – has dramatically changed the medical scenario of the last decades and is going to overwhelm the sustainability of the global health service in the next few years[1].

Among the risk factors of NCDs, tobacco use, physical inactivity, and the harmful use of alcohol are considered the most important. Recently, special attention has been devoted to the modern Western-type diet (WD)[2]. This dietary pattern is characterized by an excessive caloric intake, refined sugars, salt, and saturated fats, along with a sedentary lifestyle[3]. A constant element of WD is the consumption of so-called "processed foods". Processed foods are foods that have been altered in any way during preparation. Not all processed foods are unhealthy (i.e., natural cheese, bread or pasta). However, "ultra-processed foods" may contain many non-nutritive industrial chemical compounds, called "food additives", often added to improve stability, shelf-life, taste, and texture to the original alimentary source[4]. These properties are functional for the commercial use of food products on a large scale.

The use of food additives in the human diet has spread in the last decades[5]. The first additive used were salt and refined sugars. However, due to the growing consumer demand for long-lasting and non-caloric satisfying products, the food processing industries started adding more flavors, emulsifiers, sweeteners, stabilizers,

colorants, fat replacers, and preservatives. In 2020, the food additives market globally was valued at USD 26.2 billion[6], in 2021 reached USD 37,91 billion, and it is estimated to reach a valuation of 55,53 billion by the end of 2027[7]. The growing increase of this market is also due to the emerging demand of China, the Middle East, Africa, the Indian Subcontinent, Southeast Asia, and Central and Eastern Europe because of their rising living standards, increased urbanization and globalization of the food chain.

The primary basis for approving the use of these agents by national and international food safety authorities is the non-existence of safety issues. Recent scientific evidence – both preclinical and human studies – associated the onset of NCDs with the consumption of some of these compounds. These harmful associations could be partly explained by the alteration of gut microbiota composition and functions.

In this review, we will summarize the main evidence about the role of food additives in gut microbiota modulation and its potential health implications.

MATERIALS AND METHODS

A literature search was performed using the electronic database Medline (via PubMed) from inception to 1st October 2022. The following search terms were used: "artificial food additives", "artificial sweeteners", "polyols", "acesulfame potassium", "aspartame", "saccharin", "sucralose", "cyclamate", "neotame", "emulsifiers", "carboxymethyl cellulose", "polysorbate 80", "food coloring agents", "food preservatives", "benzoic acid", "sodium benzoate", "titanium dioxide", "sodium nitrite", "food additive", as well as "non-communicable diseases", "diabetes", "cardiovascular diseases", "hypertension", "cancer", "kidney disease", "obesity", "metabolic syndrome" as well as "gut microbiota" and "microbiome".

The details of the search string are described in Table 1. The studies were carefully screened by titles and abstracts. The full texts of the relevant articles have been analyzed. The studies included animal and original human articles and had to be written in English to be considered. Duplicate studies, letters, case reports, abstracts, and studies written in languages other than English were excluded. A hand-searching of eligible studies was carried out to check the reference lists and find additional references.

RESULTS

Gut microbiota, Gut Barrier and NCDs

The gut microbiota harbors up to 100 trillion microorganisms, including bacteria, yeasts, viruses, and parasites. The "gut microbiome" accounts for over 3 million genes (the human genome consists of only 23.000 genes), encoding for thousand metabolites related to multiple functions such as metabolism, immunity, inflammation, and neurotransmission. Most of their function is still unveiled[8].

Bacteria are the most represented microorganisms, taxonomically divided into hundreds and perhaps thousands of species. The first two phyla, Firmicutes and Bacteroidetes, represent almost 90% of human gut microbiota, followed by Actinobacteria, Proteobacteria, and Verrucomicrobia. Going more in-depth in the taxonomic hierarchy, they are furtherly classified into class, order, family, genera, and species. Apart from phyla, the most studied categories are the species (such as *Bacteroides*, *Feacalibacterium*, *Bifidobacterium,* etc.)[8].

Pieces of evidence associated some NCDs, such as obesity, with an elevated ratio between Firmicutes and Bacteroidetes[9]. The exact reason is still debated, and studies are controversial[10]; hypothetically, an excess of Firmicutes in an obesogenic microbiome would increase the capacity for energy harvesting and fat storage[9]. Interestingly, in humans undergoing caloric

Table 1. Search strategy performed on 1st October 2022.

Medline	Search terms	Results
#1	(artificial AND food AND additives) OR (artificial AND sweeteners) OR polyols OR (acesulfame AND potassium) OR aspartame OR saccharin OR sucralose OR cyclamate OR neotame OR emulsifiers OR (carboxymethyl AND cellulose) OR (polysorbate AND 80) OR (food AND coloring AND agents) OR (food AND preservatives) OR (benzoic AND acid) OR (sodium AND benzoate) OR (titanium AND dioxide) OR (sodium AND nitrite) OR (food AND additive)	156,052
#2	(non-communicable AND diseases) OR diabetes OR (cardiovascular AND diseases) OR hypertension OR cancer OR tumor OR (kidney AND disease) OR "obesity" OR (metabolic AND syndrome)	3,076,317
#3	(gut AND microbiota) OR microbiome	118,575
#4	#1 AND #2 AND #3	1,199

restriction, the abundance of Bacteroidetes increases, whereas that of Firmicutes decreases, irrespectively of diet type[11]. At the species level, the effect of strains on weight gain in humans and animals could be species-specific. Indeed, a comparative meta-analysis showed that the genus of *Lactobacilli*, *L. fermentum* and *L. ingluviei* are significantly associated with weight gain, whereas *L. plantarum* is associated with weight loss[12].
Irrespective of the hypotheses, the colonization of germ-free wild-type mice with a gut microbiota-derived from obese donors results in a significant increase in total body fat, still on the same diet[9]. Indeed, an obesogenic microbiome could reduce the expression of fasting-induced adipocyte factor, an inhibitor of lipoprotein lipase, thus increasing the storage of triglycerides in adipose tissue and the liver. Other involved mechanisms are the stimulation of peptide YY production, which inhibits gut motility allowing gut microbes to digest more polysaccharides, and the inhibition of phosphorylated adenosine monophosphate-activated protein kinase levels yielding to the reduction of β-oxidation in muscle[13].
Patients affected by NCDs often host a dysbiotic microbiome in terms of reduced richness. A low richness of the human gut microbiome highly correlates with metabolic markers, such as overall adiposity, insulin resistance, and dyslipidemia; moreover, individuals with a low bacterial richness have a more pronounced inflammatory response than those with high bacterial richness[14].
Gut microbial dysbiosis in NCDs may be also species-specific. Indeed, a recent systematic review[15] of observational studies identified a decreased abundance of *F. prausnitzii*, *Roseburia*, *Dialister*, *Flavonifractor*, *Alistipes*, *Haemophilus,* and *Akkermansia muciniphila* and increased abundance of *Lactobacillus*, *Streptococcus*, *Escherichia*, *Veillonella* and *Collinsella* in patients affected by glucose intolerance and newly diagnosed type 2 diabetes mellitus than in healthy individuals.
Among species, *A. muciniphila* – a mucin-degrading bacterium belonging to the phylum of Verrucomicrobia – plays a pivotal role in the onset of NCDs. In obese patients, subjects with higher *A. muciniphila* gene richness express a healthier metabolic status (fasting glucose, waist-to-hip ratio, and subcutaneous adipocyte diameter) than ones with a lower richness. The baseline *A. muciniphila* abundance also correlated with a significant improvement in insulin sensitivity markers and other clinical parameters after caloric restriction in the same subjects[16].
Gut dysbiosis may also influence gut permeability. The gut barrier is a multilayered functional and anatomical unit aiming to prevent the passage of microbial antigens and toxins while preserving the capacity to absorb nutrients. The intestinal mucus layer plays a key role as the interface between host and microbes since its breakdown leads to gut bacterial encroachment[4,17]. The leakage of the mucus and epithelial layers elicits the inception of an inflammatory response in the gut mucosa and the passage of microbial components such as the lipopolysaccharides (LPS) in the bloodstream, generating a pro-inflammatory condition called "endotoxemia". The endotoxemia generates a low-grade inflammatory response at the basis of the onset of NCDs such as obesity, insulin resistance[18], metabolic liver diseases[19], autoimmune[20,21], and neurodegenerative disorders[22]. The increase in gut permeability could be related to the blooming of pro-inflammatory strains such as Proteobacteria and Desulfovibrionaceae that break the gut mucus layer through metabolic pathways, including bacterial hydrogen sulfide biosynthesis[20,21]. A pro-inflammatory microbial signature has also been associated with the onset of colorectal cancer[23].

Food additives, Gut Microbiota and NCDs

The prevalence of NCDs has dramatically increased in the last four decades since globalization and westernization of diet have spread in the industrialized world. To date, at least three systematic reviews and meta-analyses[24-26] associated the consumption of ultra-processed foods (food containing a wide range of food additives) with chronic NCDs. In the next paragraph, we will dissect the evidence for each of the most used food additives classes regarding their relationship with gut microbiota.

Artificial Sweeteners

Non-caloric artificial sweeteners (NAS) are characterized by a strong sweetening flavor without calories. They are mainly found in soft drinks, snack foods, sugar-free candies, and dairy products. The first evidence of the association between the use of NAS and glucose intolerance was reported by Suez et al in Nature[27]. The authors demonstrated that the chronic consumption of NAS (saccharin, sucralose, aspartame) induced glucose intolerance in mice through compositional and functional alteration of the intestinal microbiota (over-representation of *Bacteroides* and under-representation of Clostridiales). This metabolic effect could be transferrable to germ-free mice upon fecal microbiota transplantation and could be reverted by antibiotic therapy. Also, they found a positive correlation between NAS consumption and metabolic-syndrome clinical features (increased weight and waist-to-hip ratio, higher fasting blood glucose, glycosylated

hemoglobin, and glucose tolerance test) in humans. In this cohort, they found significant positive correlations between NAS consumption and multiple taxonomic entities – including the Enterobacteriaceae family, the Deltaproteobacteria class, and the Actinobacteria phylum. Further animal studies[28,29] confirmed the role of NAS at low doses in inducing insulin resistance and glucose intolerance through gut dysbiosis. Moreover, mice treated with saccharin showed gene expression of inducible nitric oxide synthase (iNOS) and TNF-α (a critical inflammatory cytokine) in the liver[30]. Recently, Shil and Chichger[31] tested different concentrations of saccharin, sucralose, and aspartame in an *in vitro* model of intestinal epithelium (Caco-2 cells) and gut bacteria (*E. coli* NCTC10418 and *E. faecalis* ATCC19433). They showed that sweeteners differentially increase the ability of bacteria to form a biofilm, to adhere to, invade and kill the host epithelium. Sánchez-Tapia et al[32] showed that sucralose (dissolved in water to a concentration of 1.5%) increased the Firmicutes/Bacteroidetes ratio, reducing microbial richness (α-diversity). In a more recent study, Zheng et al confirmed the role of sucralose in altering the gut microbiome in mice also at lower doses – up to 0.3 mg/mL, corresponding to the acceptable daily intake (ADI) of 5 mg/kg body weight/day for the human consumption. They found an abundance increase of *Allobaculum* – positively correlated with diabetes –, the potential pathogens *Tenacibaculum*, *Ruegeria*, and *Staphylococcus,* and a reduction of the eubiont abundance (*Lachnoclostridium* and *Lachnospiraceae*)[33] in mice treated with a low dose of the sweeteners. On the other hand, many human studies investigating the effect of specific NAS (aspartame, steviol glycoside, saccharin, and sucralose) on body weight and adiposity showed no significant impact on these outcomes, suggesting that their effect on glucose metabolism could be different from energy harvesting[34].

Emulsifiers

Emulsifiers are amphiphilic molecules used to facilitate processing or improve processed foods' texture and shelf-life[35]. They are used in industrial foods such as sauces, puddings, margarine, and ice-creams.

To date, the most studied emulsifiers are carboxymethylcellulose (CMC) and polysorbate-80 (P80). CMC-treated IL-10 gene-deficient mice reported an increase in total bacteria abundance in the ileum compared with control mice, with a decrease in *E. rectale* in the ileum and jejunum and an increase in Bacteroides[36]. A pioneer animal study published by Chassaing et al in Nature in 2015 showed that both CMC and P80 act like detergent molecules in the gut, reducing the intestinal mucus thickness at relatively low concentrations and allowing bacterial translocation across the epithelium. Consequently, wild-type mice developed low-grade inflammation and obesity/metabolic syndrome, while predisposed mice developed clear colitis. Such evidence associated these health impairments with microbiota encroachment, altered species composition, and increased pro-inflammatory responses[37]. Two years later, the same group assessed the direct impact of CMC and P80 on the microbiota through the mucosal simulator of the human intestinal microbial ecosystem (M-SHIME): this *in vitro* simulator showed a decrease in the abundances of Proteobacteria and Firmicutes and an increase of Bacteroidetes levels under the action of CMC[38].

Similarly, Furuhashi et al[39] showed an increase of Gammaproteobacteria and sulfide-producing bacteria *Proteus* spp. and a reduction of the α-diversity (microbial richness) in the small intestine after 8-week administration of P80 in mice; P80 pretreatment also exacerbated the indomethacin-induced colitis, an effect abolished by the antibiotic pretreatment. This is in line with the results of Swidsinski et al[36], showing bacterial overgrowth and intestinal inflammation in mice treated with CMC. Sandall et al[40] went further, proposing a low emulsifier diet in patients with stable Crohn's disease.

In 2022, Chassaing et al[41] published a randomized controlled human trial regarding the impact of CMC on healthy individuals. They found that adding CMC to an additive-free diet increased postprandial abdominal discomfort and altered gut microbial composition reducing its diversity. Moreover, they reported a reduction in beneficial metabolites such as short-chain fatty acids (SCFAs) and free amino acids and an increased microbiota encroachment into the normally sterile inner mucus layer in some individuals[41].

Carrageenan and glycerol monolaurate are other emulsifiers mainly found in ultra-processed foods such as dairy products, spices, juice and protein drinks, soy products, and baked goods. Carrageenan has been investigated as a potential risk factor for colitis onset in C57BL/6 J mice. At least two studies[42,43] correlated carrageenan-induced colitis with changes in gut microbiota composition, specifically an increased abundance of *A. finegoldii* and *B. acidifaciens* and a decreased abundance of *A. muciniphila*. Glycerol monolaurate (GML) significantly changed the α-diversity with a decrease in *A. muciniphila* and *L. luteus*, and an increase in *B. acidifaciens* and *E. coli* in low-fat diet-fed mice[44]. However, a recent study[45] of mice fed on diets supplemented with GML at different doses for 4 months showed compositional gut microbiota variations without inducing systemic

inflammation and without impacting glucose and lipid metabolism. The dosages 400 and 800 mg kg-1 GML improved the richness of *Barnesiella*, whereas a high dosage of glycerol monolaurate (1600 mg kg-1) significantly increased the abundances of *Clostridium XIVa*, *Oscillibacter* and *Parasutterella*. These results demonstrate that the dose effect should be systematically considered in animal and human studies, although in the daily life, the packaging of ultra-processed food does not specify the dose of these substances.

Food Colorants

Food colorants are mainly added to cheeses, sauces, skimmed milk, ice-creams, pastries, sweets, chocolates, and chewing-gum[46]. Among them, titanium dioxide (TiO_2) raised health concerns. TiO_2 (also named E-171) is a brightening agent in food products and is one of the most studied colorants. In recent years, the impact of the oral consumption of TiO_2 on gut microbiota has been studied *in vitro* in mice and humans. In mice treated with TiO_2, a significant increase in Firmicutes[47] and a decrease in Bacteroidetes[48], *Lactobacillus*, and *Bifidobacterium*[49] were reported compared with controls. Moreover, the abundance of *Barnesiella*, a beneficial anaerobic bacterium belonging to the Porphyromonadaceae family of the Bacteroidetes phylum, was significantly affected by TiO_2 exposure[48]. TiO_2 nanoparticles were also detected in the immune cells of the Peyer's patches and regulatory T cells involved in rat inflammatory responses[50]. Specifically, after TiO_2 exposure, the stimulation of immune cells isolated from Peyer's patches showed an increase in Th1 interferon-gamma (IFN-γ) secretion and Th1/Th17 inflammatory response[50]. *In vitro*, TiO_2 is trapped by intestinal mucus even if *in vivo* does not impair mucin O-glycosylation and SCFAs synthesis[51]. Furthermore, possible associations between TiO_2 exposure and the development of intestinal diseases and colorectal cancer were found in rodents[52]. The effect of TiO_2 as a food additive on human health is still under debate. However, according to the European Food Safety Authority (EFSA), from 2021, TiO_2 is no longer considered safe when used as a food additive due to its genotoxicity[53] and from 2022 is banned as a food additive in the European Union (EU)[54].

Preservatives

Food preservatives are added to delay degradation in food products by inhibiting the growth of bacteria, fungi, or antioxidants and the oxidation of food constituents. Although the benefits and safety of artificial preservatives are debated among food scientists and toxicologists, little is known about the effect of food preservatives on the microbiota. A mixture of sodium benzoate, sodium nitrite, and potassium sorbate consumption by mice colonized with a human microbiome highlighted an overgrowth of Proteobacteria and a decrease in Clostridiales[55]. Interestingly, the proportions of gut bacteria with anti-inflammatory properties, such as *C. tyrobutyricum* or *L. paracasei*, were significantly decreased while pro-inflammatory bacteria, such as *B. thetaiotaomicron* or *E. faecalis,* increased[56]. Interestingly, a recent review discussed some issues concerning the safety of their applications, including the possibility of allergies and immunosuppressive effects from benzoate, the formation of carcinogenic nitrosamines from nitrites, and interaction sorbate with nitrite in the stomach, which consequently can be resulted in the production of a series of genotoxic compounds[57]. Thus, although that sodium benzoate and sodium nitrite are considered safe, the synergic effects induced by the concomitant consumption of several different food additives need to be assessed.

CONCLUSIONS

Food additives are non-nutritive molecules added to commonly used processed food for industrial purposes. The most recent scientific evidence raised concerns about safety issues regarding a possible role in the pathogenesis of NCDs. Many studies – *in vitro* or on animal models – have been conducted confirming harmful effects on the gut microbiota and gut barrier; however, these results need to be confirmed by further homogeneous animal studies. Moreover, large studies are warranted to confirm their pathogenetic role in humans, even if ethical questions should be posed.

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

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