

# Microbioma, dieta, disturbi gastroenterici e disbiosi

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## SUMMARY

Microbiome, diet, gastrointestinal disease and dysbiosis

Intestinal microbiota is the set of microorganisms (bacteria, fungi, archaea, protozoa, and viruses) present inside the gut; bacteria are of fundamental importance because they help maintain gut homeostasis by competing with pathogens, by regulating energy metabolism and also producing immunomodulatory substrates such as short-chain fatty acids (i.e. acetate, propionate, butyrate). Shifts in microbial communities are due to disease, drug administration, diet, etc., and in recent years, the study of gut microbiome (DNA-based techniques) has better defined/characterized this relationship in many cases. The link between microbiome (dysbiosis) and health and disease is very complex and still far to be completely understood. We wanted to underline the importance of diet in modulating gut microbiome, possibly a useful tool also in term of disease prevention, and of microbial-microbial signaling. Even more we wanted to stress that it will be fundamental to better characterize dysbiosis, cause this may guide treatment decisions.

## KEY WORDS

fecal microbiome, diet, dysbiosis, microbial signaling

## INTRODUCTION

Intestinal microbiota is the set of microorganisms (bacteria, fungi, archaea, protozoa, and viruses) present inside the gut; mammals' guts contain roughly  $10^{10}$ - $10^{14}$  microorganisms. Bacteria are of fundamental importance because they help maintain gut homeostasis by competing with pathogens, by regulating energy metabolism and also producing immunomodulatory substrates such as short-chain fatty acids (i.e. acetate, propionate, butyrate). Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria are the most common ones (around 99%); the composition differs between species.<sup>(7,23)</sup>

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### Diet and dysbiosis

With regard to diet, evidence demonstrates that it could (variably) influence microbiome composition. A recent study in dogs showed that fiber supplementation (beet pulp), for a fourteen-day period, modified the Firmicutes:Fusobacteria ratio with an increase of the former.<sup>(15)</sup> Nevertheless, the administration of a fiber enriched diet in eighteen dogs did not significantly alter the fecal bacterial populations in the previous study of Simpson et al.,<sup>(20)</sup> although the fecal concentration of ammonia, sulfide and indole decreased, as did the fecal pH, while concentrations of acetic, propionic, and butyric acid increased.<sup>(20)</sup> Also potato fiber supplementa-

tion altered the Firmicutes:Fusobacteria ratio, leading to an increase of Firmicutes and a decrease of Fusobacteria; such a fiber also showed probiotic potential due to the increased proportion of *Faecalibacterium* and fecal short-chain fatty acid concentration.<sup>(17)</sup> Similarly, the increase of protein and/or carbohydrates in a diet was also able to modify the fecal microbiome. More precisely, it has been shown that high-protein and high-carbohydrate diets, both lead to a reduction of bacterial diversity and of *Clostridium* cluster XIVa (fam. *Lachnospiraceae*), while the higher protein level led to an increase of order *Fusobacteriales*, while carbohydrates increased the *Clostridium* cluster XVIII (order *Erysipelotrichales*).<sup>(6)</sup> A recent study also showed the importance of a raw meat-based diet in modifying the gut microbiome, with a reduction of Fusobacteria and Proteobacteria and of *Faecalibacterium*, *Lactobacillus*, and *Prevotella*.<sup>(2)</sup> The influence of diet on the microbial population has also been recently studied in cats. A first study showed that even in the absence of significant changes, the administration of diets supplemented with 4% cellulose, or fructooligosaccharides (FOS), or pectin led to a higher percentage of Actinobacteria in the FOS group than in the other two groups, and to higher percentages of Firmicutes and Proteobacteria in the pectin group, where total bacteria also increased.<sup>(1)</sup> Subsequently, the effect of a high-protein low-carbohydrate diet was also tested in cats (kittens) and, if compared to a moderate-protein moderate-carbohydrate diet, the former diet resulted in a higher percentage of Actinobacteria (also *Clostridium*, *Faecalibacterium*, *Ruminococcus*, and *Blautia*

were higher), and a lower percentage of Fusobacteria, with more *Bifidobacterium* present if fed on the latter diet.<sup>(6)</sup> Finally, a raw meat diet has also been tested in cats; in the study, a chicken-based extruded diet and raw whole chicks were administered to cats, resulting in higher percentages of *Faecalibacterium* and Recently, it has also been hypothesized that the different consistency of the diets (dry vs wet), with two slightly different macronutrient profile diets, resulted in higher percentages of Actinobacteria and *Lactobacillus*, and lower percentages of Fusobacteria and Proteobacteria in cats fed the dry diet.<sup>(3)</sup> *Succinivibrio* in animals fed on the former diet (extruded), while cats fed the raw diet presented with higher percentages of unidentified Lachnospiraceae, *Peptococcus* and *Pseudobutyrvibrio*. Interestingly, *Lactobacillus* and *Bifidobacterium* were not found in the feces of cats fed raw diet.<sup>(11)</sup>

### Dysbiosis and Gastrointestinal (GI) disease

A very important question is whether, and to what extent, the dysbiosis is the cause or the effect of the GI disease and, consequently, how to interpret a dysbiosis. It is well known that GI diseases are variably associated with dysbiosis. A recent study showed important differences between the fecal microbiome of healthy dogs, dogs with acute non-hemorrhagic, and acute hemorrhagic diarrhea, and dogs with controlled and uncontrolled IBD; interestingly, during diarrhea potentially positive bacteria that are producers of short-chain fatty acid are commonly decreased.<sup>(22)</sup> However, only few studies showed a direct link between dysbiosis and GI disease. In TNF<sup>delta</sup>ARE mice (a model for CD-like [Crohn's disease] ileitis) it has been shown that the transplantation of disease associated microbiota led to CD-like ileitis in genetic susceptible mice housed under germ-free conditions, and the severity of the ileitis appeared associated with compositional and functional dysbiosis.<sup>(19)</sup> Similarly, it was also shown that the dysbiosis associated with IBD could lead to an altered bile acids (BA) metabolism, with a decrease of secondary BA, that in some cases showed an in vitro anti-inflammatory activity, suggesting that the dysbiosis could be associated with an increased inflammatory response in IBD patients.<sup>(5)</sup>

### Dysbiosis: the concept of "quorum sensing" abrogation and *interneicine* production in GI disease

Within a prokaryotic species, secretion of soluble chemicals is used by individual bacterial cells to communicate with one another, inducing coordinated gene regulation when environmental conditions are appropriate (*quorum sensing*). A subset of these small diffusible molecules that mediate intraspecies signaling can be perceived by other strains of bacteria, allowing interspecies communication. Thus, the normal microbiota produces a carefully balanced combination of interspecific and intraspecific chemical signals that could suppress pathogenic invaders, as well as optimize the composition and numbers of appropriate members of the microbiota. A dysbiotic microbiota might also involve some degree of *interneicine* dysfunction, a deleterious combination of chemical signals that disorder the microbiotal community structure. A dysbiotic microbiota is an ecological disorder of the bacterial community; the concept is often associated with the pathogenesis of IBD.<sup>(18,21)</sup> Germ-free mice have increased susceptibility to a variety of enteric pathogens, an observation that led to the concept of "colonization resistance."<sup>(25)</sup> This presumptive role of the microbiota in suppressing encounters with overt pathogens is likely multifactorial. The normal microbiota may compete for access to adhesive sites on the epithelial surface or stimulate increased mucin production. SCFAs may be bacteriostatic for a subset of bacterial species, either directly or by reducing pH. Some

members of the microbiota also generate bacteriocins, small peptide molecules with microbicidal or microbistatic properties.<sup>(4)</sup> In addition, there is increasing interest in the effects of microbial-microbial signaling on the overall equilibrium of optimal microbiotal ecosystems.<sup>(10)</sup>

The mammalian gut is equipped with both innate and adaptive immunity to tolerate a "correct" microbiota and realize its benefits, maintaining surveillance over the microbiota and controlling its number and composition. The detailed mechanisms by which host non-adaptive immunity monitors and responds to the presence of microbes has been extensively reviewed.<sup>(9,14)</sup> The immune system, broadly defined, is entrusted to modulate bacterial numbers and perhaps diversity, whereas the resident prokaryotes have their own means to deliberately modify host processes. This mutual crosstalk often involves reciprocal manipulation of growth, survival, and inflammatory controls and is another dimension of the gut microbe relationship, which when disturbed can result in a spectrum of intestinal disorders. The general paradigm holds that the gut is equipped with pattern recognition receptors (PRRs), an operational term for transmembrane or intracytoplasmic receptors that are defined by the ability to specifically recognize and bind distinctive microbial macromolecular ligands designated as microbial-associated molecular patterns (MAMPs). PRRs include the trans-membrane *Toll-like receptors* (TLRs), which scan the extracellular space, whereas the *Nod-like receptors* (NLRs) guard the intracellular cytoplasmic compartment. Nonpathogenic prokaryotes, including natural commensals and those with proposed probiotic function, are able to suppress eukaryotic inflammatory signaling pathways and inflammatory effector functions; these suppressive effects are mediated either by intact viable organisms or by secreted products.<sup>(12)</sup> An additional facet of the "negotiated settlement" that occurs between host and microbe is adaptive immunity, only present in vertebrates, specifically the gut/mucosal arm of the adaptive immune system, which provides humoral and cell-mediated immunity against ingested antigens and luminal organisms. Adaptive immunity features the selective ability to respond to or ignore individual, specific antigens based on past encounters. Thus, the mucosal immune system can develop tolerance to ingested (or gut resident) antigens; repeat or continual exposure to the same stimulus does not elicit the immune response that it does in a naive animal. The occasional dysregulation of this arrangement, such as during the pathogenesis of IBD and perhaps other immune and metabolic disorders, may be the price paid for an extended metabolic ability (and other still uncharacterized benefits) provided by a normal microbiota.

Stimulation of immune development can be mediated via recognition of bacterial capsular polysaccharides. A specific polysaccharide (polysaccharide A) product of *B fragilis* has been identified that is recognized by dendritic cells and serves to stimulate development of regulatory T lymphocytes (*Tregs*) with the ability to attenuate pathogen- or chemical-induced colitis.<sup>(13)</sup> These data indicate that specific carbohydrate moieties on symbiotic bacteria can initiate suppressive regulatory effects on effector lymphocytes. This cytoprotective effect of a bacterial product acts via the adaptive immune system, rather than the more ancient PRR-based innate/inflammatory signaling and cellular cytoprotective pathways.

A microbiota in ecological collapse could permit emergence of autochthonous bacteria that blur the distinction between symbiont and pathogen, exemplified by *Clostridia difficile* proliferation following vancomycin treatment, which can result in pseudomembranous colitis.<sup>(16,24)</sup> Together, these data illustrate the important role of the microbiota in protection

from enteric pathogens and the compositional and functional concept of dysbiosis in causing the GI tract pathology.

## CONCLUSIONI

The link between microbiome (dysbiosis) and health and disease is very complex and still far to be completely

understood, but growing evidences reveal pieces of this puzzle. We wanted to underline the importance of diet in modulating gut microbiome, possibly a useful tool also in term of disease prevention, and even more to stress that it will be fundamental to better characterize dysbiosis, cause this may guide treatment decisions.

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