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Does our microbiota eat with or without gluten?

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The microbiota can be regarded as a "functional organ" since it is similar among individuals. Some people have the same amount of bacterial genes involved in various metabolic pathways [1]. Like all our organs, the microbiota performs essential functions. Its proper functioning, on which our state of health depends, is related directly to maintaining balance among the microbes that compose it (condition of aerobiosis). The factors that can lead to an intestinal imbalance ecosystem (dysbiosis) are many: diet, infectious diseases, meeting with pathogens, medications, and lifestyle. Several studies recently recognised the fundamental role of microbiota in the pathogenesis of many pathologies, including celiac disease (CD).

CD [2] is an immune-mediated condition triggered by the ingestion of gluten. In genetically predisposed subjects, it causes inflammation of the small intestine and atrophy of the intestinal villi with consequent malabsorption [3] of nutrients and extraintestinal symptoms. In particular, in the predisposed subjects, ingestion of gluten determines the activation of tissue T lymphocytes, which recognise peptides resulting from the enzymatic digestion of gliadin. Gliadin deamidated by tissue transglutaminase binds to molecules DQ2/DQ8 of the antigen-presenting cells (APC) and activates CD4 T lymphocytes located in the lamina of the intestinal mucosa. Activated CD4s reach the intestinal submucosa, stimulating the production of pro-inflammatory cytokines interferon-γ (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α), which, in turn, determine apoptosis and cell detachment of enterocytes and lymphocytes hyperproliferation and atrophy of villi.

Recently, a factor directly involved in the etiopathogenesis of CD has been identified: mutation of the gene that codes for an intercellular adhesion protein, zonulin, with a regulatory action on enterocytes tight junctions. The mutated form of this protein binds to a specific receptor on the intestinal epithelium. It triggers a cascade of biochemical reactions that cause a reduction in adhesion between enterocytes and increased permeability of the intestinal barrier (leaky gut syndrome). Consequently, some molecules and/or substances, which, in physiological conditions, would be confined to the luminal side of the intestinal epithelium, manage to cross it, triggering a series of autoimmune reactions in tissues. The fact that the 30% of the world population is a carrier of susceptibility genes for celiac disease and that only 2–5% of these

individuals are affected by it, however, suggests that the existence of other factors is able to contribute to the onset of disease, such as intestinal dysbiosis. Each of us has a specific microbiota, defined as an individual human enterotype, which depends on our background (natural or artificial breastfeeding, use of antibiotics, lifestyle, where we live such as city or countryside, etc.). However, in people who are not yet celiac but are predisposed to celiac disease (haplotype DQ2 and DQ8), microbiota would have different characteristics from the typical patient. In whom, instead, the disease has already developed, microbiota changes further with an increase in pro-inflammatory bacteria. Therefore, based on these observations, new suggestions have been proposed concerning pathogenesis models of CD, particularly on the role played by the intestinal microbiota. One specific genetic makeup of the host and environmental factors could promote colonisation of pathobionts and reduce symbionts, consequently causing dysbiosis. Such dysbiosis can interrupt immunological homeostasis and intestinal integrity, thus contributing to the pathogenesis of CD. In this sense, microbiota could be responsible for proteolytic activity (capable of generating gluten-related toxic and immunogenic peptides) or affect the integrity of the intestinal barrier or have immunomodulating properties through a dense network of pro- and anti-inflammatory factors.

In vitro studies showed how varied *Bifidobacterium* species can prevent the formation of toxic peptides of gliadin during digestion [4] and reduce the dysfunction of the intestinal barrier and intercellular junctions [5, 6]. Conversely, *Escherichia coli* and *Shigella* help increase the translocation of gliadin peptides into intestinal loops [5]. These results suggest that intestinal microbiota composition can influence tolerance to gluten by the host.

The importance of eubiosis in ensuring gluten tolerance and reducing pro-inflammatory effects was demonstrated by *in vivo* studies in germ-free mice, i.e., wholly deprived of normal bacteria residing in the body. Indeed, these animals fed for about two months with a gluten diet developed moderate damage to the child intestine (villous atrophy) and have many intraepithelial lymphocytes. Also, with germ-free mice carriers of the DQ8 variant, a much stricter gluten [7, 8] pathology was developed. In reverse, if these mice were colonised with a bacterial flora that included *Proteobacteria* (*Helicobacter* and *Escherichia coli*), they developed the gluten-mediated disease and, if treated early after birth with antibiotics, which is responsible for an overgrowth of Proteobacteria, developed a severe form of CD [8, 9]. These animal models show that answers to gluten are very different according to intestinal microbiota composition.

Other studies support the relationship between the development of CD and eating habits. Recent Swedish work has found an incidence of CD four times higher among those born in the decade 1985–95 than those born in previous years and the two years 1996–97. This increase was associated with changes in nutritional guidelines (introduction of gluten after six months instead of 4 and increase in average consumption of gluten under two years), finding in the child diagnosed with a high CD presence of “Rod-shaped” bacterial strains in the small intestine and a relative reduction of *Lactobacilli* and *Bifidobacteria* [9, 10].

In celiac patients, the persistence of the specific gastrointestinal symptoms is frequently found, despite a gluten-free diet (GFD). These data contrast with clinical explanations that relate to a state of inflammation induced by gluten with gastrointestinal symptoms of CD. Studies on microbiota have shown how many celiac patients continue to harbour an altered microbiota despite a GFD and that those patients complain more than other gastrointestinal disorder patients [11]. A study confirms the reduction of *Bifidobacteria* and the increase of *Bacteroides* and *Enterobacteria* in celiac patients with a GFD [12]. All of these data suggest that the microbiota may play a role in the etiopathogenesis of celiac disease through the following mechanisms:

1) modulation of digestion of gluten peptides, with the formation of both toxic and digestible peptides of different meanings according to the species;

2) regulation of intestinal permeability through the release of zonulin;

3) promoting the regulation of intestinal immunity through activation of anti-inflammatory peptides.

Intestinal dysbiosis affects CD’s aetiology and the persistence of gastrointestinal symptoms in some celiac patients; a better qualitative and quantitative characterisation of the “celiac microbiota” would make

it possible to develop therapeutic strategies and nutritional approaches aimed at modifying the intestinal microbiota composition.

The diet and/or the possible integration of pre and probiotics could promote the recovery of gluten tolerance, constituting a fundamental approach therapeutic for celiac disease. It has been shown that taking probiotics would favour the restoration of intestinal microbiota homeostasis in 30% of celiac patients. The GFD reduces but does not resolve irritable bowel syndrome (IBS)-like symptoms (dyspepsia, diarrhoea, and abdominal pain). Two Italian studies reveal that two types of probiotics (multi-row compositions respectively of 8 and 5 probiotic strains) are capable of colonising the intestine [13, 14], hydrolysing some fragments of gluten gliadin [13], and reducing IBS-like symptoms according to gastrointestinal symptoms rating scale (GSRS) score [14].

The hydrolysis of gliadin could also help to reduce the abnormal secretion of zonulin, which, as already mentioned, constitutes an additional risk factor for developing CD. Reducing "toxic" peptides deriving from the enzymatic digestion of gluten would increase gluten tolerance and improve the subject's quality of life. A study [15] conducted on 64 individuals (26 celiac patients with a diet containing gluten, 18 with GFD, and 20 healthy subjects) evaluated whether a specific intestinal bacterial flora composition could be associated with gastrointestinal symptoms of celiac disease and if two particular strains of *Bifidobacteria* were capable of improving the clinical picture. Such research confirmed:

1) the active role of the intestinal microbiota in the onset of pro-inflammatory phenomena typical of CD;

2) the beneficial effect exerted by two species of *Bifidobacteria* (*Bifidobacterium longum ES1* and *Bifidobacterium bifidum ES2*) in inflammatory picture caused by gluten.

Many patients correlate some gastrointestinal disorders to the type of food they eat, holding it responsible for increased gas production, abdominal distension, visceral hypersensitivity, and changes in motility. They have recently aroused intense interest in low-content diets of poorly absorbable carbohydrates and sugar alcohols [fructooligosaccharides (FOS), oligosaccharides, sugar alcohols]. These carbohydrates, also found in gluten, are fermented by intestinal bacteria resulting in gas production and increased fluid secretions in the intestinal lumen. To describe these short-chain carbohydrates, poorly absorbable, the acronym FODMAP was coined (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols). Several studies have shown how a low FODMAP diet can improve symptoms gastrointestinal in patients with irritable colon syndrome. However, such dietary approaches should be considered cautiously because of potential adverse effects on the intestinal microbiome and plasmatic calcium levels.

Compared to a regular diet, patients controlled at four weeks on the FODMAP diet showed a reduction in the concentration of *Bifidobacteria*, *Faecalibacterium prausnitzii*, and *Clostridium*, and unchanged levels of *Bacteroides*, *Prevotella*, *Eubacterium rectale*, *Clostridium coccoides*, *Lactobacillus* and *Enterococcus*. Interestingly, even patients with non-celiac gluten sensitivity benefited from a FODMAP diet, improving gastrointestinal symptoms. However, a reduction of *Bifidobacteria* and an increase of *Lachnospiraceae* were observed in these patients. Evidence, therefore, demonstrated that A low-FODMAP diet might cause marked changes in microbiota composition. Further studies are, however, necessary to understand whether these alterations are harmful to not celiac patients and if these effects persist for a prolonged time.

An interesting study [16] on 60 healthy volunteers, divided into groups of a low or high gluten intake (2 g/day *vs.* 18 g/day) for eight weeks has been published. After a 6-week washout period with a standard diet (containing on average 12 g/day of gluten), groups were reversed for the same length of time. A low-gluten diet has registered changes in microbiota composition, such as a decrease in species belonging to *Bifidobacterium*, *Eubacterium halli*, *Anaerostipes hadrus*, *Blautia wexlerae* and an increase in some species of *Lachnospiraceae* and *Clostridioides*. Also, the low-gluten diet has decreased metabolic pathways associated with the degradation and/or assimilation of carbohydrates from bacterial functionality.

Ultimately, the researchers analysed the impact of a reduction in gluten on the immune system. One was observed reduction of IL-1β (cytokine produced in response to bacterial infections) compared to healthy patients who ate gluten, suggesting a decrease in the selective inflammatory response. In contrast, no change was observed in permeability and intestinal inflammation markers (faecal calprotectin, citrulline).

In conclusion, a low-gluten diet [17–22] followed with healthy individuals involves a change in intestinal microbiota composition and functionality, an alteration of the fermentation process with a reduction of intestinal gases, and finally, a partial effect on the immune system.

Abbreviations

CD: celiac disease

FODMAP: fermentable oligosaccharides, disaccharides, monosaccharides, and polyols

GFD: gluten-free diet

Declarations

Author contributions

GM and MM conceived the paper; AC, PG and GF collected the data; LDR and ADL revised the paper. All authors contributed to manuscript revision, read and approved the submitted version.

Conflicts of interest

The authors declare they have no conflicts of interest.

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