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# Effects of a gluten-free diet on gut microbiota and immune function in healthy adult humans

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**Diet is a major environmental factor influencing gut microbiota diversity and functionality, which might be relevant to subjects following dietary therapies. Celiac disease (CD) is an enteropathy caused by an aberrant immune response to cereal gluten proteins and the only therapy is the adherence to a gluten-free diet (GFD). In this context, a preliminary study was conducted to establish whether the GFD in itself could modify the composition and immune properties of the gut microbiota. The trial included 10 healthy subjects (30.3 years-old), which were submitted to a GFD over one month. Analysis of fecal microbiota and dietary intake indicated that numbers of healthy gut bacteria decreased, while numbers of unhealthy bacteria increased parallel to reductions in the intake of polysaccharides after following the GFD. Fecal samples of subjects under a GFD, which represent an altered microbiota, also exerted lower immune stimulatory effects on peripheral blood mononuclear cells than those of subjects on a regular gluten-containing diet. This addendum presents further discussion on the rationale behind these findings, limitations of the study and possible consequences of dietary counselling in the care process of celiac disease patients.**



**Key words:** gut microbiota, gluten-free diet, celiac disease, immunity, probiotics, polysaccharides, prebiotics

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## Relationship between the Gluten-Free Diet and the Gut Microbiota

The human intestinal tract harbors a collection of beneficial bacteria (symbionts/mutualists) that perform an array of functions, including the provision of attributes not encoded in the human genome.[1] This ecosystem is greatly influenced by the diet, which constitutes a major environmental factor driving bacterial diversity.[2,3] Therefore, long-term dietary practices for treating food-related diseases might affect the composition of the resident microbiota and, thereby, its functional relationships with diverse host organs and tissues. In particular, intestinal bacteria constitute a constant challenge of antigens to their host that modulate mucosal immunity and the primary line of defence against antigens acquired orally. Celiac disease appeared as a result of dietary changes associated with the development of the agriculture and cereals cultivation.[4] This is a chronic enteropathy caused by an aberrant immune response to cereal gluten proteins and, still the only therapy for the patients is to exclude the gluten from the diet. Although the adherence to a strict gluten-free diet (GFD) usually leads to the remission of the major clinical symptoms, nutritional deficiencies and health complications are often reported in treated patients.[5-7] In addition, the microbiota of patients under a GFD is not completely restored in comparison with that of healthy subjects.[8-10] In this context, we published a preliminary study to establish whether the GFD in itself could lead to modifications on the composition and immune properties of the gut microbiota.[11] This study included 10 healthy subjects (30.3 years-old), who were following a GFD over one month by replacing the gluten-containing foods they usually ate with certified gluten-free foods (with no more than 20 parts per million

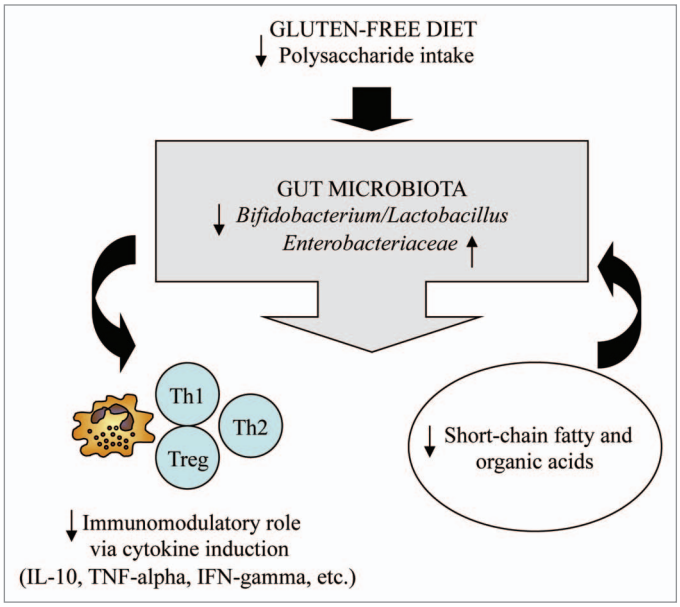


**Figure 1.** Schematic representation of the possible interactions between the gluten-free diet and the gut microbiota.

of gluten). Analyses of fecal microbiota and dietary intakes, indicated that populations of generally regarded healthy bacteria decreased (Bifidobacterium, *B. longum* and Lactobacillus), while populations of potentially unhealthy bacteria increased parallel to reductions in the intake of polysaccharides (from 117 g to 63 g on average) after following the GFD. In particular, increases were detected in numbers of *E. coli* and total Enterobacteriaceae, which may include opportunistic pathogens.[12] This evidence suggests a disruption of the delicate balance between the host and its intestinal microbiota (dysbiosis), which might favor the overgrowth of opportunistic pathogens and weaken the host defences against infection and chronic inflammation via possible alterations in mucosal immunity.[13]

## Influence of the Gluten-Free Diet on Immune Properties of the Gut Microbiota

Cytokine production by peripheral blood mononuclear cells (PBMCs) stimulated with fecal samples of healthy individuals before and after the GFD was also evaluated to establish the possible relationships between the stimulus of the gut microbiota and the host immune function under this dietary practice.[11] PBMC cultures were considered a good in vitro model for such studies since monocytes of the intestinal mucosa are known to be constantly replenished by blood monocytes.[14] Immunostimulatory properties of feces, which up to 50% can be represented by bacteria, were remarkably reduced as a consequence of the GFD, inducing a significantly lower production of pro-inflammatory cytokines and chemokines (TNFα, IFNγ and IL-8) and anti-inflammatory cytokines (IL-10) in PBMCs than those collected before the GFD. It seems that GFD led to a generalized reduction of bacterial-induced cytokine production as a result of the generalized reduction of the total large intestinal bacterial load, as detected in patients under a gluten-free diet.[8] The fact that the GFD led to reductions in total Bifidobacterium and *B. longum* numbers could also explain the reductions in the ability of fecal samples to stimulate IL-10 production, since strains of this genus and species might preferentially stimulate IL-10 secretion.[15,16]

## Rationale Behind the Effect of GFD on the Gut Microbiota

The composition of the gut microbiota is susceptible to the influence of the diet and, especially, to the quality and quantity of ingested carbohydrates.[17-19] The reductions in polysaccharide intake associated with the GFD could explain the observed changes in the microbiota, since these dietary compounds usually reach the distal part of the colon partially undigested, and constitute one of the main energy sources for commensal components of the gut microbiota.[20] The genome of these bacteria encodes many enzymes specialized in the utilization of non-digestible carbohydrates, which provide these bacterial groups a competitive advantage over potentially pathogenic bacteria to colonize the intestine.[2,21] Thus, the genome sequence of *B. longum* subsp. *longum* showed that more than 8% of the annotated genes were involved in carbohydrate and polysaccharide metabolism,[21] which could explain the reduction of its levels after the GFD. It also seems feasible that when the growth of beneficial bacteria is not supported due to a reduced supply of their main energy sources other bacterial groups, which can be opportunistic pathogens, can overgrowth leading to intestinal dysbiosis. Within the gut ecosystem, the microbiota acts as a metabolic organ whose survival and composition is determined by a dynamic process of selection and competition. For example, survival of the commensal bacterium *Bacteroides thetaiotaomicron* has been demonstrated to be influenced by both the bacterial community composition and nutrient availability in a mouse model.[22] In addition, products generated from polysaccharide fermentation, such as butyric acid, could play a role in this competitive process by generating a hostile environment for instance for enterobacteria. In fact, intake of complex dietary carbohydrates (e.g., dietary fiber) has been shown to influence both microbial colonization and fermentation variables in the mammalian gut. Thus, high intake of dietary fiber resulted in a greater short-chain fatty acid concentration in (e.g., acetic and butyric acids), and lower *Escherichia coli* counts in piglet intestine, while an opposite trend was shown with low fiber intake.[23]

## A Possible Role of Dietary Counselling in Celiac Disease Patients

Although this preliminary study has limitations, including number of participants

and the short duration of the intervention, the changes in the microbiota found in healthy subjects following a GFD were to some extent similar as those detected previously in patients after compliance with a long-term GFD. In particular, reductions in Bifidobacterium plus Lactobacillus populations relative to Gram-negative bacteria (Bacteroides and *E. coli*) were detected in untreated CD children and, particularly, in CD patients treated with a GFD.[8] These findings indicate that this dietary therapy may contribute to reducing beneficial bacterial counts and increasing enterobacterial counts, which are microbial features associated with the disease[8,10] and, therefore, it would not favor completely the normalization of the gut ecosystem in treated CD patients. On the other hand, the immune suppressive effects associated with the microbiota of subjects under a GFD may be partly beneficial for CD patients, which are prone to a Th1-biased immune response, but may also imply a defect of their defence and regulatory mechanisms against harmful bacteria and chronic inflammation. This sets up a scenario where individuals under a GFD would be more susceptible to overgrowth of harmful bacteria and infections, which might be associated with unpleasant symptoms and increased health risks. Moreover, the findings suggest that dietary counselling aimed at promoting polysaccharide and probiotic intake could be considered in the care process of treated patients in the future.

In the light of these preliminary results, further studies on the nutritional quality of the GFD and its effects on gut ecology and health in a larger population group and over longer periods are warranted. Although the evidence supports the hypothesis that dietary counselling and interventions with complex polysaccharides (prebiotics) or probiotics could benefit the health status of CD patients, this should be confirmed by specific human intervention trials in the target population group.

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