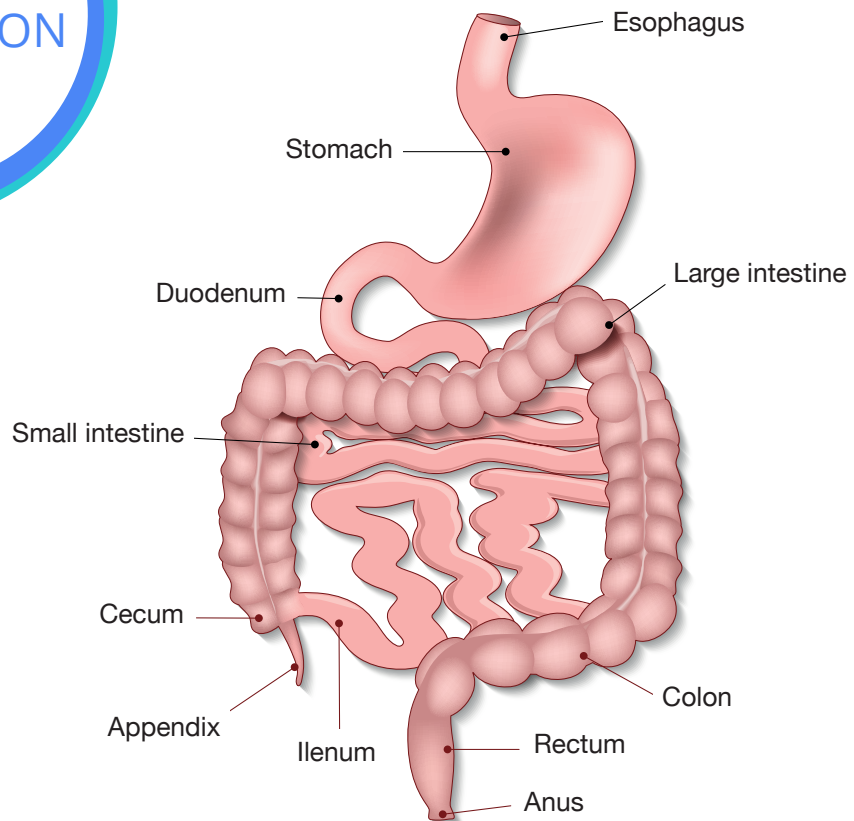


Summary of IBS Studies

DE SIMONE FORMULATION



Irritable Bowel Syndrome (IBS) is a functional syndrome of the gastrointestinal system characterized by a series of chronic or relapsing symptoms in the absence of any detectable organic cause.

Abdominal pain is the major symptom of IBS and it is strongly linked to bowel movements and related changes.

De Simone Formulation (DSF) Professor Claudio De Simone, a pioneer in the field of intestinal microflora recognized internationally, invented a probiotic mixture (8 strains, 450 billion bacteria) which has been the subject of more than 170 publications and is integrated in the international treatment guidelines for some serious medical indications.

A member of the American College of Gastroenterology, Claudio De Simone is a retired Professor of Infectious Diseases of the University of Aquila (Italy), specialized in Gastroenterology, Allergology and Clinical Immunology. His interest in the human microbiota goes back more than 25 years when the understanding of the importance of the role of the intestinal flora was in its infancy.

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Diagnostic criteria

According to Rome IV criteria, the diagnosis of Irritable Bowel Syndrome can be established in the presence of abdominal pain occurring at least one a week during the past three months with at least 2 of the following parameters:

1. associated to defecation; and/or
2. associated to a modification of the frequency of the stools; and/or
3. associated to a modification of the aspect (consistency) of the stools.

Such symptoms should have started at least 6 months beforehand. The sub-types of IBS are transit-predominant: IBS-C (constipation), IBS-D (diarrhea) IBS mixed and non classified.

Epidemiology

IBS is common in the industrialized countries affecting 15-20% of the population, particularly young people (50% of patients < 35 years old). It is more common in women with a ratio of 2:1 compared to men. Only 30-40% of patients with IBS contact their doctors about it. Everyone has a 30-50% chance of being affected by IBS at some point during their life.

It is the most frequent pathology to be found in gastroenterology and represents a major social impact considering the total yearly expenses per patient in terms of drugs, examinations and lost working days.

Pathogenesis

The mechanisms underlying IBS have not been clarified yet. Several factors may contribute to the observed symptoms

- Alteration of the intestinal motility
- Visceral hypersensitivity
- Alteration of the bacterial intestinal microflora
- Post-infectious syndromes
- Diet
- Behavioral disorders

Some investigators have focused their research on the relationship between the activities related to the Central Nervous System (stress, anxiety, depression....) and the functional activities of the intestine, through the enteric nervous system (Fig. 1 – Gut-Brain Axis).

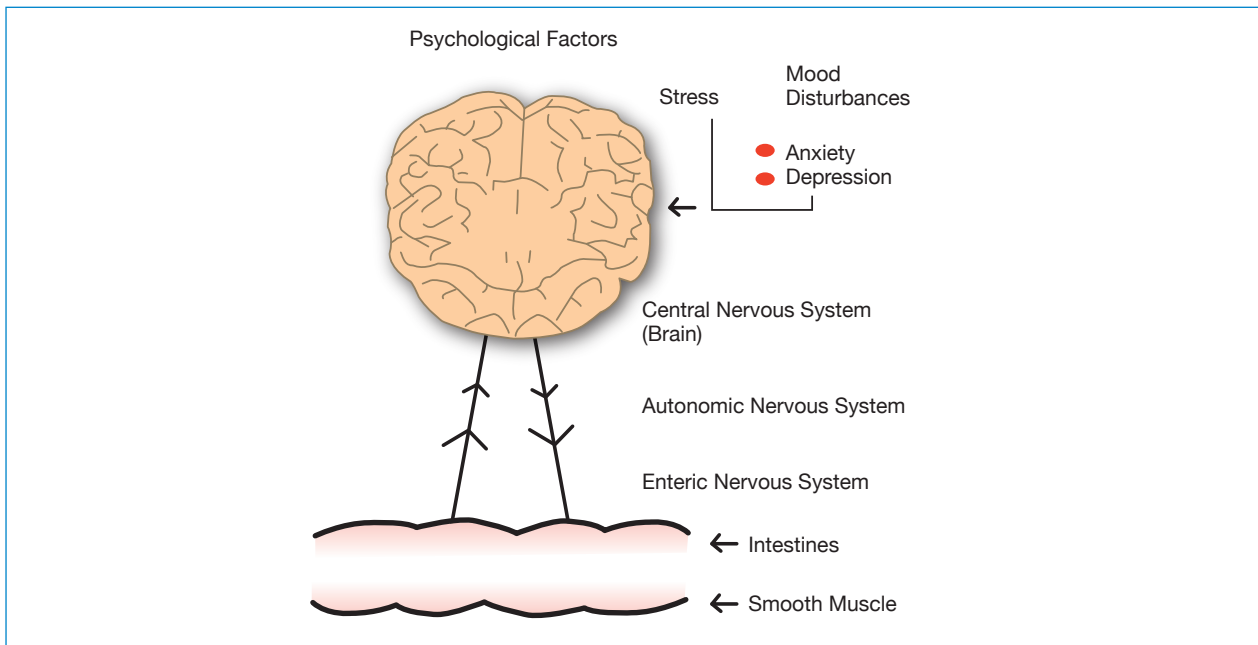


Fig. 1 Gut-Brain axis

IBS symptoms may be triggered by an abnormal intestinal motility or by an exacerbated visceral sensitivity or by both occurrences.

Moreover, numerous studies have shown that IBS may be related to an alteration of the intestinal bacterial microflora:

- Madden JA et al have found an increase of facultative microorganisms and a decrease in numbers of lactobacilli and bifidobacteria in feces samples of patients with IBS. They further suggested that the food intolerance occurring during IBS, may be associated to an abnormal food fermentation in the colon, following destruction of the normal microflora.
- Malinen et al have showed a lower quantity of *Lactobacillus spp* in feces samples of patients with diarrhea-predominant IBS compared to healthy subjects, while patients with constipation predominant IBS showed an increase of *Veillonella spp*.

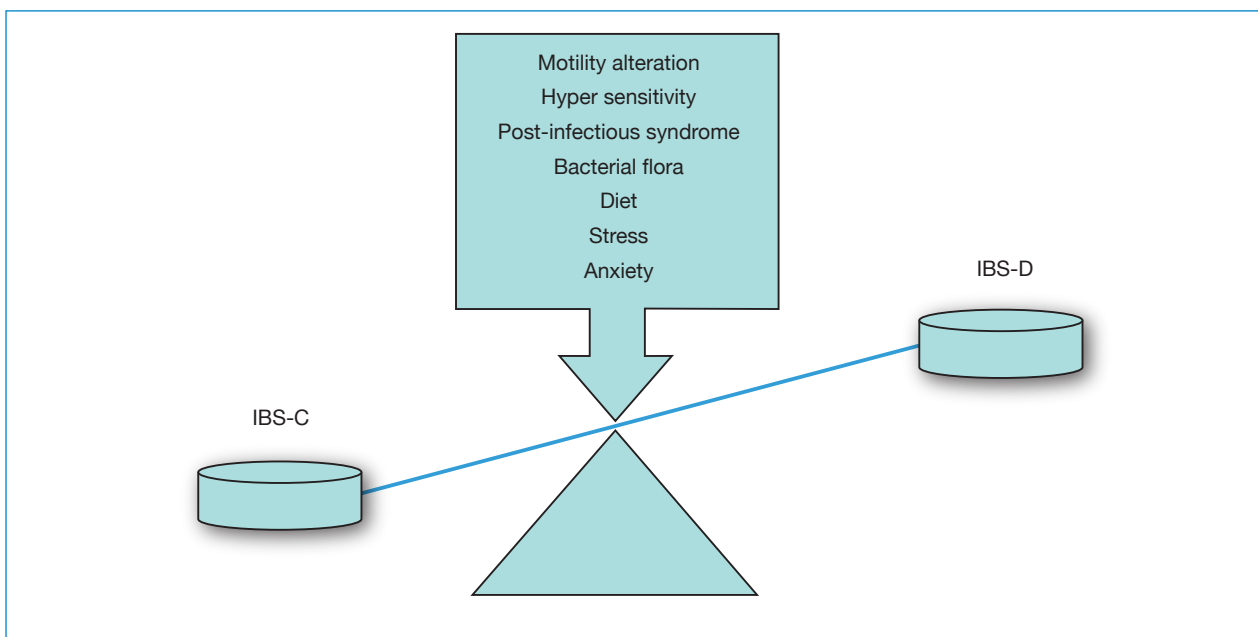


Fig. 2 - Diarrhea-predominant IBS (IBS-D) or constipation-predominant IBS (IBS-C)

Symptoms

Pain is the major symptom and it can be localized or diffuse to all the abdomen. It starts with food intake and is only alleviated by defecation or gas emissions. Others symptom are:

- Gas
- Flatulence
- Abdominal distention
- Bloating

Patients with IBS can be affected with others associated disorders such as heartburn, dyspepsia, headache, asthenia, sleep disorders, urinary disorders but they are never affected with fever, persistent diarrhea, rectal bleeding, anemia or weight loss.

Therapy

In absence of any definite pathogenesis, the therapy aims essentially at the symptoms:

- Pain** The use of antispasmodic agents is recommended. In case of persistent pain, low-dose antidepressants may be prescribed.
- Flatulence** In order to alleviate the sense of bloating and flatulence in IBS patients, there is a widespread use of agents for gas absorption (charcoal, simethicone), however they do not always give the expected results.
- Constipation** A diet rich in fibers (improves evacuation, although it may often increase pain and abdominal distention) is recommended as well as the use of laxatives.
- Diarrhea** The patient is advised to reduce the intake of fibers and fermented foods. They may be recommended drugs that reduce intestinal motility (loperamide) or poorly absorbable antibiotics (rifaximin)

It is interesting to note that 30% of patients with pain will have a regression of their symptoms after placebo administration.

IBS and Probiotics

The intestine is colonized by more than 500 different bacterial species, 40 of these are predominant and made of aerobic and anaerobic bacteria, both gram positive and gram-negative such as: Bacterioides spp., Bifidobacteria spp., Lactobacilli spp., Eubacteria spp. and Probionobacteria spp.

In the colon, these bacteria reach a concentration of 10^{10} - 10^{12} per ml of fecal contents.

It has now been scientifically proven that the intestinal microflora, and in particular bifidobacteria and lactobacilli, play a significant role in the:

- reduction of the intraluminal pH through to the production of lactic acid, acetic acid and other organic acids;
- production of nutrients (short chain fatty acids, arginine, glutathione, vitamins etc.) necessary for a correct functioning of the intestinal mucosa;
- stimulation of the immune system, in particular the macrophage activity, cytokine production, natural killer activity, lymphocyte proliferation, IgA production;
- competition with pathogenic microorganisms for nutrients and for adhesion to the intestinal epithelium;
- production of bacteriocines;
- modulation of the inflammatory response

The intestinal microflora plays a significant metabolic function both from a nutritional point of view as well as for the maintenance of an efficient intestinal mucosal barrier. Together with the local and systemic immune response, these functions are fundamental for the protection of the organism.

The De Simone Formulation (the DSF) is a high-concentration probiotic preparation containing 450 billion bacteria per sachet of the following strains:

- 4 strains of lactobacilli (*L. acidophilus*, *L. paracasei*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*,)

- 3 strains of bifidobacteria: (*B. breve*, *B. infantis*, *B. longum*)
- 1 strain of *Streptococcus thermophilus*.

The De Simone Formulation (DSF) is able to colonize the gut and modulate the intestinal microflora. It is the probiotic with the strongest scientific evidence (60 original clinical papers, over 170 original publications) and it is the only probiotic mentioned in the Guidelines of the major international Gastroenterology Associations, in particular:

- *American College of Gastroenterology*
- *British Society of Gastroenterology*
- *Deutsche Gesellschaft für Verdauungs und Stoffwechselrekrankungen*
- *European Crohn and Colitis Organization*

The efficacy of the DSF was scientifically proven in Inflammatory Bowel Diseases (ulcerative colitis, pouchitis). Further studies have also confirmed its efficacy in chronic liver diseases (steatosis, steatohepatitis, NAFLD, etc.).

IBS and the De Simone Formulation

Considering the acknowledged importance of bacterial overgrowth in this pathology, the De Simone Formulation may represent a new therapeutic approach to IBS, thanks to its ability to colonize and balance the intestinal microflora.

The DSF relieves abdominal bloating in adult IBS patients and benefit was demonstrated in double-blind placebo-controlled trials:

- Bazzocchi et al, in an open label study showed that patients with IBS who were administered the DSF, 900 billion daily dose, had a reduction in colon motility by manometric evaluation. They also observed reduced bowel movements and flatulence.
- In a study of 25 patients receiving 8 weeks of treatment, Kim et al 2003 showed that the DSF 1 sachet daily dose (450 billion of bacteria), for a period of 8 weeks, was more effective than placebo to reduce flatulence and bloating in patients with IBS experiencing significant reductions from baseline in bloating (mean pre- to post-treatment in VAS score -13.7; $p=0.046$).
- Kim et al. 2005 showed, in a study of 48 patients receiving 4 ($n=31$) or 8 ($n=17$) weeks of treatment, 46% of patients receiving the DSF reported satisfactory relief of bloating during at least half of their treatment period vs 33% in the placebo group.

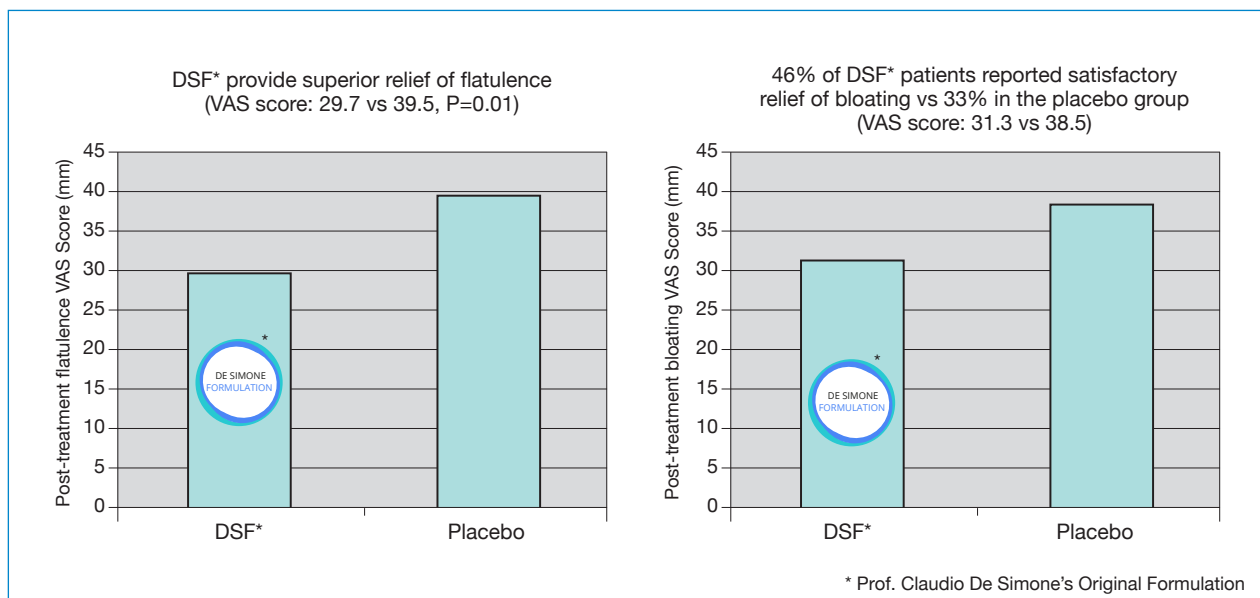


Fig 3 and 4 - Kim et al 2005 flatulence and bloating scores in the probiotic and in the placebo groups after 8 weeks of supplementation

- Guandalini et al., in a double-blind crossover study conducted with 59 pediatric patients (age between 4 and 18) proved that the DSF was superior to placebo ($p < 0.05$) in the evaluation of the Subject's Global Assessment of Relief (SGARC). This specific probiotic mix also proved more efficient than placebo on pain/abdominal discomfort ($p < 0.05$) and significantly improved the Family Assessment of life disruption parameters ($p < 0.01$).

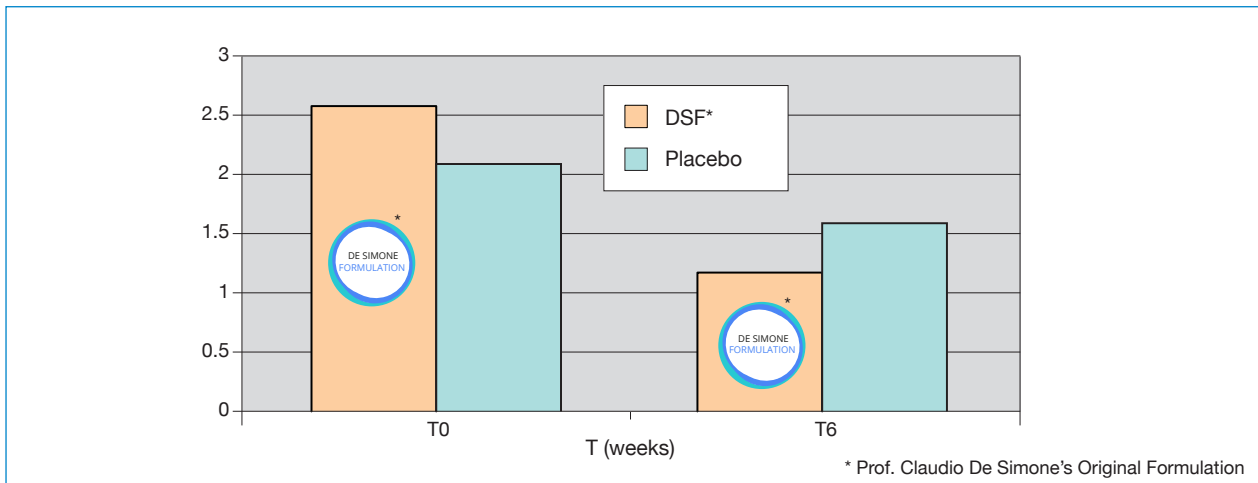


Fig. 5 Guandalini S et al Abdominal bloating score rated on a scale of 0 to 4

- Michail et al. conducted a double-blind randomized placebo controlled study in 24 diarrhea predominant IBS patients who received the DSF or placebo for 8 weeks. The gut microbiota was not changed but the patients taking the probiotic showed improvement in specific Gastrointestinal Symptom Rating Scale-IBS scores as well as a favorable change in satiety subscale.
- Parkes et al conducted a study in 41 patients, 20 affected with constipation predominant IBS (IBS-C) and 21 affected with Slow Transit Constipation (STC). Administration of the DSF significantly increased the average weekly spontaneous complete bowel motion SCBM (95% CI 0.5 to 1.4 $p < 0.0001$) and improved quality of life score (31.4 vs 22.9, $p < 0.0001$).

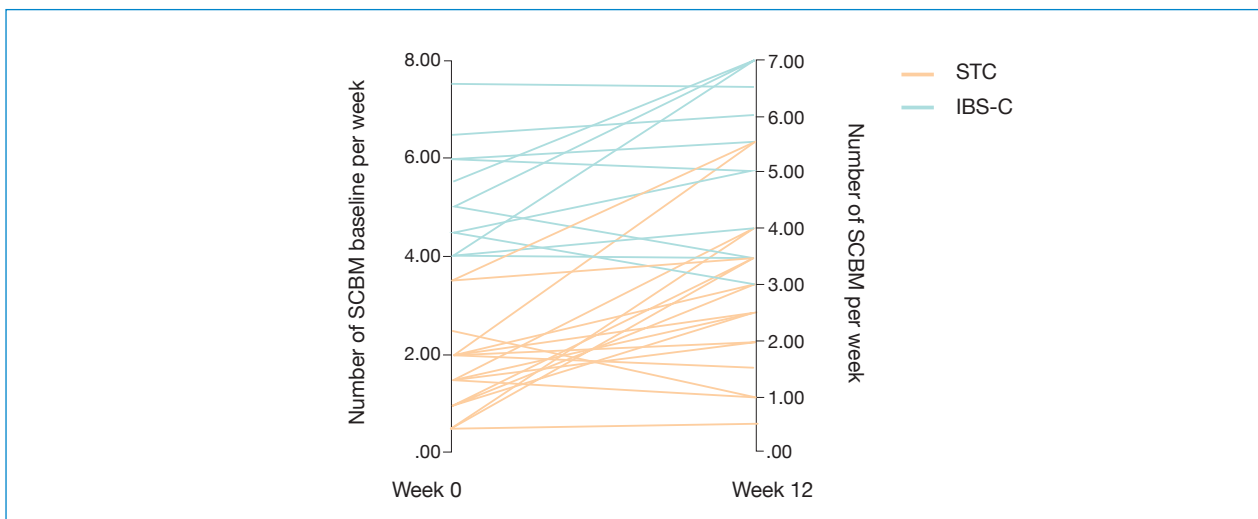


Fig. 6 – Parkes et al.

- Wong et al in 2015 conducted a double blind placebo controlled study on 42 patients taking the DSF or placebo, 4 capsules for 6 weeks and observed a significant decrease of abdominal pain duration and distension intensity together with improved rectal distension pain thresholds. Such improvements correlated with a rise in morning melatonin levels suggesting a possible mechanism of action of the probiotic on melatonin production, thus modulating IBS symptoms in individuals with normal circadian rhythm.

- Kim et al in 2015 investigated the short term treatment with DSF (30 patients, 2 sachets per day for 2 weeks) vs control patients in functional constipation and improved clinical symptoms, including Bristol Score, stool consistency, Complete Spontaneous Bowel Movement and abdominal bloating were confirmed.

Taking into consideration the proven clinical efficacy and the safety of the product, may certainly become a complement of choice for these long-term patients.

Why is it important to have good intestinal functions

Until recently, the intestine was believed to play a minor role in the human physio-pathology and its main function was to absorb nutrients and eliminate all refuse from the organism. In the light of current available knowledge, the intestine can be considered as a real organ where a main part of the immune system is located and where many fundamental metabolic functions take place. The intestinal microflora plays a primary role in the good functioning of the intestine, in particular in the synthesis of Short Chain Fatty Acids, of vitamins (K, B1, B6, B12, PP, folic acid, pantothenic acid, etc.), in the digestion of carbohydrates, lipids and protides, in the transformation of biliary acids, of cholesterol and estrogens and finally, thanks to competition mechanisms, allows to antagonize the adhesion of bacteriocines and pathogenic microorganisms to the intestinal mucosa.

The intestinal ecosystem is a combination of five elements that interact among themselves: the bacterial flora, the temperature, pH level, intestinal epithelium and the immune system. A correct interaction between these elements will determine a correct functioning of the whole intestine. If any function is modified due to fermentative or decomposition processes due to different types of food or refined sugars ingested, or following antibiotic treatment, or alteration in the immune system, the balance will be compromised and lead to alteration of the bacterial flora creating a dismicrobism.

There is now strong scientific evidence to prove that a good and healthy intestine has positive consequences on the functioning of the organism as a whole. To reach a general state of well-being, it is important to consider the human body as a complex, global system in which all organs are closely linked and interconnected together and in which the intestine certainly plays a role of major importance.

References

- Bazzocchi G, Gionchetti P, Almerigi PF, et al. **Digest Liver Dis 2002; 34, S48-53.**
- Brigidi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. *Res Microbiol* **152**:735-471 (2001)
- Guandalini S, Magazzù G, Chiaro A, La Balestra C, Di Nardo G, Gopalan S, Sibal A, Romano C, Berni Canani R, Lionetti P, Setty M. **JPGN 2010;51, 24-30**
- Kim HJ, Vazquez Roque MI, Camilleri M, Stephens D, Burton DD, Baxter K, Thomforde G & Zinsmeister AR. *Neurogastroenterol Motil* **17**:1–10 (2005)
- Kim HJ, Camilleri M, Mc Kinzie S, Lempke MB, Burton DD, Thomforde GM, Zinsmeister AR. *Alim Pharmacol Ther* **17**:895-904 (2003)
- Kim S., Choi S., Park K., Park M., Shin J., Lee T., Jung K., Koo H., Myung S., and Constipation Research group of Korean Society of Neurogastroenterology and Motility. *J Neurogastroenterol Motil*, 2015 Jan 1;21(1):111-20.
- Madden JA, Hunter JO. A. **Br J Nutr , 2002 Sep; 88 Supp 1: 567-72**
- Malinen E, Rinttila T, Kajander et al. *Am J Gastroenterol* 2005, 100:373-82.
- Michail S. and Kenche H. *Probiotics & Antimicro. Prot.* **3**:1-7 (2011)
- Parkes GC, Chatoor D, Emmanuel A. *Gut* 2011;60:A163 doi:10.1136/gut.2011.239301.345
- Wong R., Yang C., Song C., Wong J., Ho K. *Dig Dis Sci.* 2015 Jan; 60(1):186-94.